Fertilizers

Components, Uses in Agriculture and Environmental Impacts

Fernando López-Valdez
Fabián Fernández-Luqueño
Editors

Biotechnology in Agriculture, Industry and Medicine

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BIOTECHNOLOGY IN AGRICULTURE, INDUSTRY AND MEDICINE

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FERNANDO LÓPEZ-VALDEZ
AND
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PREFACE

The purpose of this book is to provide the state of the art of some important topics on subsistence agriculture, modern agronomy, and technological improvements which have sharply increased yields from cultivation, with special emphasis on the sustainable management and environmental impact of commercial and nontraditional fertilizers. We are really sure that this book provides cutting-edge knowledge in both theoretical and applied aspects of fertilizer management for undergraduate and postgraduate students, researches, and other professionals in agricultural disciplines. This book is divided in five sections: **Section I** (*Traditional fertilizers*), where you can find two chapters about mineral fertilizers such as nitrogen and phosphorus, and their effects on the environment (atmospheric pollutants and dispersion in water bodies). The **Section II** (*Organic fertilizers*) presents two chapters about fishery wastes and biochar as an important alternative of fertilization and their effects on soil fertility and crop productivity. In the **Section III** (*Biofertilizers*), we provide two interesting chapters that compare the bio- and chemical fertilization with the biofertilizers as complements to chemical or organic fertilization. **Section IV** (*Non-conventional fertilizers*), where you can find interesting themes on silicon fertilizers, Nanofertilizers, and Non-traditional ameliorants, as others important sources of agricultural fertilizers. Finally, **Section V** (*Improving fertilizer applications*), we select this important topics as a complement for fertilizer application, that include topic as the modified natural rubber as controlling release of fertilizers, the effect of mulch materials on crop yield, and the radiological impact of fertilizers.

Certainly, this book is a valuable contribution to the agricultural sciences and it would not have been possible without the invaluable contribution, immeasurable acknowledge and recognized expertise from the authors.

The Editors

SECTION I. TRADITIONAL FERTILIZERS

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Chapter 1

MINERAL NITROGEN FERTILIZERS: ENVIRONMENTAL IMPACT OF PRODUCTION AND USE

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ABSTRACT

The industrialized agro-ecosystem is formed by technological and environmental subsystems, interacting strictly among one another to guarantee high quantitative and qualitative productivity requested by the market. Efficient management, at the moment, requires a large use of fossil fuels that often severely affect the environmental subsystem.

Therfore, the agro-ecosystem produces a high environmental impact, both at the local and global scale. Mineral nitrogen fertilization represents one of the main agricultural practices with a high emission of pollutants in the atmosphere, soil and water. This practice is necessary to guarantee high crop yields in spite of soil nitrogen depletion, which is linked to the progressive degradation of the soil organic matter. The environmental loading of nitrogen mineral fertilizers is due to the activities of the technological subsystem (production, transport, application), to the alteration of some soil microbial processes and to the excess supply not absorbed by the cultivated crops.

The present chapter will review the environmental impact of nitrogen fertilization by analyzing the effects caused by the interactions of the technological and environmental subsystems. The environmental loads associated with the technological subsystem will be described by means of Life Cycle Assessment (LCA) approach. The Life Cycle Assessment is a method which is able to quantify the environmental aspects and potential impacts associated with a product, process, or activity throughout its entire cycle of life: from extraction of raw materials, through production, use and maintenance, to decommissioning at the end of life. The procedure is in accordance with the ISO 14040 and ILCD Handbook.

Moreover, the main environmental effects of the nitrogen fertilizers application on soil environmental subsystems will be described. In detail, the state of art of the effect of

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nitrogen application on soil microbial activities, responsible for the production of atmospheric pollutants (carbon dioxide, methane, nitrogen compounds) and nitrogen dispersion in water bodies, will be presented. Mitigation strategies for both sub-systems suitable for reducing the environmental impact in the use of nitrogen fertilizers will be also reported.

Keywords: Mineral nitrogen fertilizers, urea, ammonium nitrate, calcium ammonium nitrate, LCA, environmental impact

INTRODUCTION

The agro-ecosystem is an ecosystem which has been modified by man to produce food, raw material and energy. Several forms of management have been developed in the course of time and in different regions of the planet. They caused different modifications in the original ecosystem metabolism (flux of energy and cycle of matter). One of the main consequences of these modifications is the environmental pollution, at local and global scale. These may be particularly intense for the so-called industrialized agro-ecosystem. The industrialized agro-ecosystem is formed by technological and environmental subsystems, interacting strictly among one another to guarantee high quantitative and qualitative productivity requested by the market. Efficient management, at the moment, requires a widespread use of fossil fuels that often severely affect the environment (Figure 1).

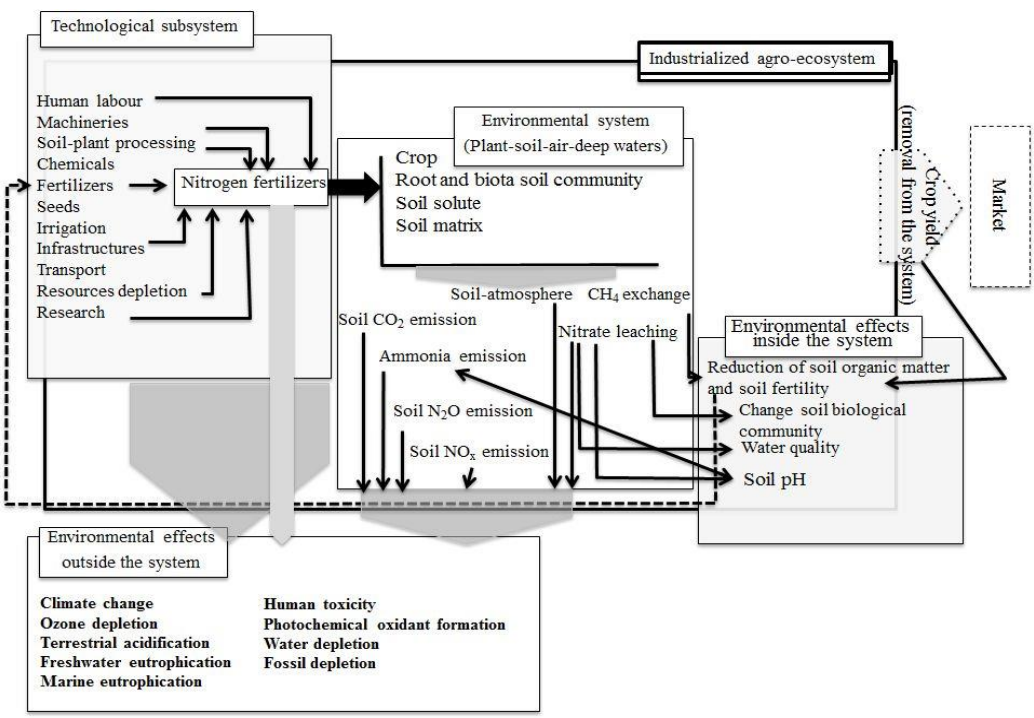

Figure 1. Environmental burdens of the agro-ecosystem due to the interaction between technological system and environmental system.

Therefore, the agro-ecosystem plays a key role in the sustainability of world life, since the exponential growth of global population will lead to an increased food demand. A recent approach to mitigate the dependence of fossil fuel has been the prospect of producing alternative energy from plant biomass. Table 1 shows the global extent of agricultural lands, referred to year 2009, providing the potential role of the agriculture on the environmental damage. At present, more than 1.5 billion ha, about 12% of the world land area, is used for crop production (FAO, 2013). World agricultural production grew on average between 2 and 4% per year over the last 50 years, while the cultivated area (permanent cropland and arable land) grew by only 1% annually. More than 40% of the increase in food production came from irrigated areas, which have doubled in size (FAO, 2013).

Nitrogen removed by the harvesting consequently reduces in the nitrogen available in the soil for the next crop cycle. Loss of nitrogen soil fertility is recovered by means of mineral fertilizers, whose production requires a large amount of fossil fuel (Haber-Bosch process). In addition, human activities increase the input of reactive nitrogen (Nr; defined as all nitrogen compounds other than N_2) into the biosphere that currently exceed the rate of biological N_2 fixation in native terrestrial ecosystems (Galloway et al., 2004). The increase of reactive nitrogen is due not only by fertilizer production, but also by the fossil fuel combustion used to support food and energy demands. High mineral nitrogen fertilization represents one of the main human agricultural practices with high environmental emission of pollutants into the atmosphere, soil and water.

Table 1. Global extent of the agricultural lands referred to the year 2009 (FAO, 2013)

Mainland	Agricultural surface (%)	Agricultural surface (thousand ha)	Arable (%)	Permanent crops (%)	Permanent meadows and pastures (%)
World	37.6	4,889,048	28.3	3.1	68.8
Africa	39.2	1,161,062	19.3	2.5	78.4
Latin America and Caribbean	35.7	722,358	20.7	2.8	76.5
Northern America	25.3	471,290	44.1	2.1	53.8
Asia	53.0	1,638,836	28.9	4.7	66.7
Europe	21.4	472,631	58.8	3.3	37.9
Oceania	49.8	422,870	11.4	0.4	88.2
Melanesia	3.9	2,090	22.2	46.6	31.3
Micronesia	30.4	96	9.4	73.0	27.4
Polynesia	20.3	164	30.7	52.7	19.0

Table 2 reports the global nitrogen fertilizer consumption referred per ha of arable land and permanent crops for 2009. Nitrogen fertilizer consumption is measured by the quantity of nitrogen nutrients used for plant nutrition per unit of arable land. Today, Asian farmers are the major users of fertilizers (Table 2). One-third of the increase in cereal production worldwide and half of the increase in India's grain production during the 1970s and 1980s have been attributed to increased fertilizer consumption (FAO, 2013). It has been estimated that, on average, only about 50% of the N applied as fertilizer is actually available for near-term crop production. Global per capita rates of nitrogen fertilizer consumption per year have risen from 0.2 kg in 1900 to 2 kg in 1950, to nearly 14 kg in 2000 (Smil, 2001).

Table 2. Mainland data of nitrogen fertilizer consumption referred per hectare of arable land and permanent crops for 2009 (FAO, 2013)

Mainland	N (kg ha^{-1})
World	69.3
Africa	11.0
Latin America and Caribbean	49.2
Northern America	58.9
Asia	128.1
Europe	44.1
Oceania	22.6
Melanesia	18.2
Micronesia	0.0
Polynesia	39.4

The environmental loading of the nitrogen fertilizers is due first to the various activities of the technological subsystem (production, transport, application), second to the alteration of some soil microbial processes and third to the excess supply not absorbed by the cultivated crop (Figure 1).

Holistic and systemic approaches are useful in studying complex systems, such as the agro-ecosystem. The present chapter will review the state of art of the environmental impact of nitrogen fertilization by analyzing the effect caused by the interactions of the technological and environmental subsystems. The environmental loads connected to the technological subsystem will be described by means of Life Cycle Assessment (LCA) approach. The Life Cycle Assessment is a method able to quantify the environmental aspects and the potential impacts associated with a product, process, or activity throughout its entire cycle of life. The analysis is done at all stages, from extraction of raw materials, through production, use and maintenance, to decommissioning at the end of life. The procedure is in accordance with the ISO 14040 and ILCD Handbook. LCA has been used to evaluate the cradle-to-grave environmental impacts of agriculture-based products since the late 1990s. As shown in several LCA studies, the agricultural system can be a main contributor to environmental burdens. Among the main concerns are trace gas emissions and leaching losses associated with nitrogen fertilizers usage on agricultural lands. These are due to pedo-climatic conditions and site specific agricultural management (Figure 1). In particular, the agricultural activities linked to mineral nitrogen fertilization contribute considerably to the emissions of greenhouse gases (GHGs) such as CO_2, CH_4, N_2O along with trace gases (NH_3 and NO_x) and NO_3^- leaching. For this reason in an LCA study, direct field emission (DFE) of these compounds has to be as much as it is possible representative of local pedo-climatic conditions and cultivation.

In addition, the purpose of this chapter is to describe in detail the effect of nitrogen application on soil microbial activities responsible for the production of atmospheric pollutants (carbon dioxide, methane, nitrogen compounds) and nitrogen dispersion in bodies of water.

Moreover, mitigation strategies for both sub-systems suitable for reduction of the environmental impact in the use of nitrogen fertilizers will also be reported.

DIFFUSION AND USE OF NITROGEN FERTILIZERS

Nitrogen is the main macro-nutrient affecting plant growth and crop yields, since nitrogen is an important component in plant cells of several structural, genetic and metabolic compounds such as proteins and nucleic acids. It is also a component of energy-transfer compounds, such as ATP (CFF, 2010). Nitrogen can be taken up by plant roots in inorganic forms (also called mineral nitrogen) such as ammonium ion (NH_4^+) and nitrate ion (NO_3^-) (CFF, 2010).

The need for a more efficient and productive agriculture in conjunction with other circumstances originated in northwestern area of Europe in the latter part of the 18th and the first half of the 19th centuries with the discovery of the biological role of chemical elements in plant nutrition and the industrial revolution which stimulated technological innovation and the growth of chemical, mining and transportation industries. These factors gave rise to the development of fertilizer industry.

In the world today more than 110 millions ton per year of nitrogen fertilizers are produced, two-thirds in developing countries that are also the biggest consumers (IFA, 2013).

In fact, while there is a negative trend to the nitrogen consumption in developed countries due to legal restrictions and to the growing success of organic farming, whereas the demand for nitrogen fertilizer in developing countries has increased more then threefold in the last thirty years (Table 3).

Table 3. Production and consumption of nitrogen in developed and developing countries

Developed Countries (ton nutrients)							
	2010	2005	2000	1995	1990	1985	1980
Consumption	31,056.5	28,979.8	29,116.6	29,562.7	35,864.8	39,119.9	36,376.4
Production	37,680.4	37,049.5	36,906.6	38,782.7	36,689.7	46,279.4	44,278.1
Developing countries (ton nutrients)							
	2010	2005	2000	1995	1990	1985	1980
Consumption	73,195.5	63,947.3	52,953.1	48,661.9	40,912.3	30,930.2	24,116.3
Production	71,488.4	60,144.7	49,716.9	43,055.9	35,274.0	26,477.8	18,672.4

Source: IFA, 2013.

The world's population is predicted to be 11 billion by 2100. Eighty-seven percent will live in the developing countries. Africa alone will increase its current population four fold (from 1.1 to 4.2 billion people). As consequence of the population increase will necessarily expand the use of fertilizers in order to meet growing food demand.

Table 4 shows the present-day worldwide consumption of different N-products shared by macro-regions. Data are averages from 2007 to 2011 (IFA, 2013).

The most commonly used N-fertilizer is urea (Table 4). In different regions there are distinct differences in use of N-fertilizer. In Europe, Calcium Ammonium Nitrate (CAN) and Ammonium Nitrate (AN) are the most widespread fertilizer followed by NPK (nitrogen, phosphorus and potassium) compounds. In South America, Oceania and above all in Asia (mainly in China and India) urea consumption is much higher than all other N-products. In North America the consumption of ammonia (or anhydrous ammonia), nitrogen solutions and urea are more or less equivalent and much more used than other compounds.

Because there are significant differences in the uptake of nutrients by different crops, their impact on fertilizer consumption could be higher for certain crops than for others (Figure 2).

Table 4. Consumption of different N fertilizers by regions (average 2007-2011)

Consumption (,000 ton)	A	B	C	D	E	F	G	H
Asia	-	376.8	4,626.00	1,369.0	471.1	44,786.2	4,046.4	15.4
West and central Europe	4.4	2,049.0	212.0	321.4	2,476.2	1,620.0	1,574.4	1,161.8
Africa		598.0	152.0	94.2	134.2	1,439.8	609.6	0.6
Latin America	44.8	605.4	655.4	813.6	68.4	3,454.4	473.0	176.8
East Europe		1,290.0	101.2	118.4	18.8	470.4	284.8	158.2
Oceania	32.2	11.4	180.0	97.6	3.96	782.2	66.8	101.4
North America	3,691.0	282.0	782.0	365.8	17.54	3,287	998.4	3,244.8

(A) Ammonia; (B) Ammonium nitrate; (C) Ammonium phosphate; (D) Ammonium sulfate, (E) Calcium ammonium nitrate; (F) Urea; (G) NPK; (H) Nitrogen solution.

According to estimates of the IFA (IFA – FUBC assessment 2010) nitrogen applied to cereals, amounting up to 57.5 Mt, represents 55.2% of the global N-fertilizer consumption in 2010-11 (18.1% wheat, 16.8% maize, 15.4% rice, 4.8% other cereals). Oil crops contributed 7.3%, cotton 4.3%, sugar crops 3.5% and root & tubers 2.8%. Fruits and vegetables accounted 14.9% of the total, and other crops for 12.0%.

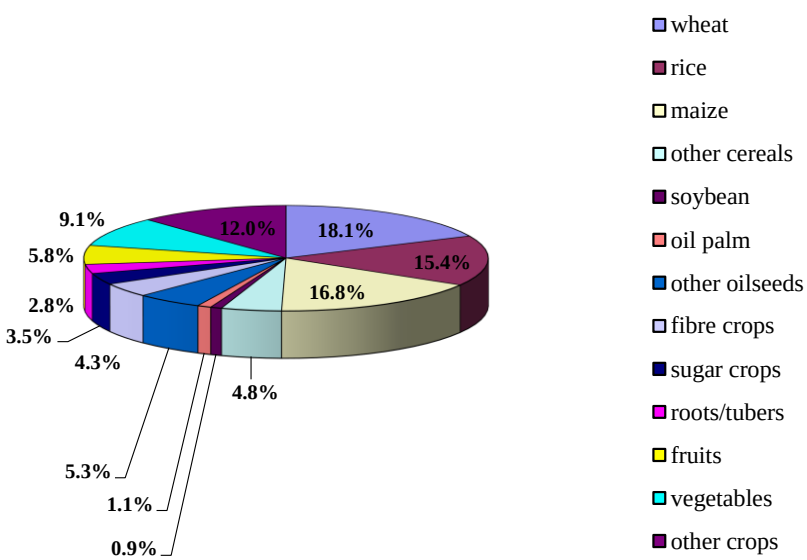

Figure 2. N-fertilizer use by crops at the global level (IFA, 2013).

THE NITROGEN FERTILIZER INDUSTRY

Fertilizer production currently account for about 2-3% of the total global energy consumption. Nitrogen fertilizers are responsible for the majority of this consumption (European Commission, 2006). Most of the energy is required by the fixation of atmospheric nitrogen to manufacture ammonia. Considerable energy is also required for the conversion of ammonia to urea.

The energy required for nitrogen production is fairly evenly distributed around the planet and there is production in every region. However, there is a trend towards increased production in locations where cheap natural gas is available, in areas such as the Middle East and the Caribbean and also in the main regions of consumption, such as South Asia and China.

PRODUCTION PROCESSES

Ammonia

Ammonia (NH_3) is the primary input for the majority of worldwide nitrogen fertilizer production (97% of N-fertilizer is derived from ammonia) (European Commission, 2006).

Ammonia production is largely based on modifications of the process developed by the two German scientists Haber and Bosch in the early years of the 20^{th} century. NH_3 is synthesized from a 3:1 volume mixture of hydrogen and nitrogen at elevated temperatures and pressure in the presence of an iron catalyst (Wood & Cowie, 2004). This process revolutionized the nitrogen fertilizer industry, breaking the former dependence on the relatively rare mineral sources of nitrogen and enabling the development of products with a higher nitrogen content. The first Haber-Bosch plant was opened in 1913, and nitrogen production has been largely dependent on ammonia synthesis ever since (UNEP/UNIDO, 1998).

All the nitrogen used is obtained from the air and the hydrogen may be obtained by either of the following processes:

a) steam reforming of natural gas or other light hydrocarbons (Natural Gas Liquids, Liquefied Petroleum Gas or Naphtha);
b) partial oxidation of heavy fuel oil or coal;
c) coal gasification.

Around 77% of world ammonia production is based on natural gas steam reforming (Figure 3), 14% on coal gasification, mainly in China, and 9% on the partial oxidation of oil products and heavy hydrocarbon fractions, mainly in India and to a lesser degree in China.

The synthesis of ammonia is a very energy demanding process based on an industry wide benchmarking study conducted by IFA the average energy requirements for ammonia production is 36.9 GJ/t, and ranges from 28 to 53 GJ/t of ammonia (Chinese plants were not considered) (Table 5).

A typical heavy-oil-based process uses 1.3 times as much energy as a gas-based process. A coal-based process uses 1.7 times more energy than a gas-based process.

In China and India, the energy demand per ton ammonia produced is very high: 48.8 GJ/ton NH_3 for China and 43.3 GJ/t NH_3. China is the largest producer of ammonia with 30% of global production and India produces another 8% of global production (Table 5) (Kool et al., 2012).

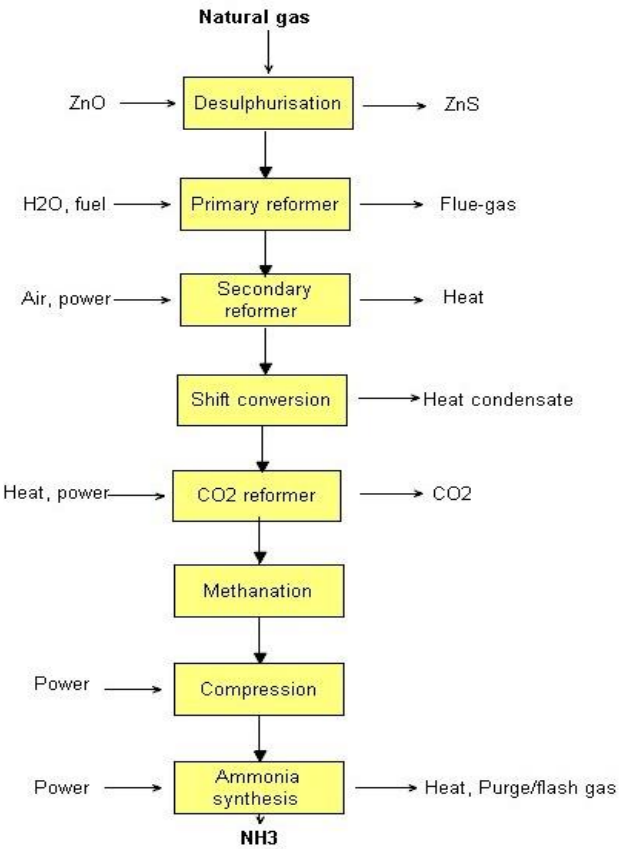

Figure 3. Block diagram of steam reforming of natural gas (EFMA, 2000a).

The CO_2 emission on the average for European ammonia production is 1.82 ton CO_2 eq/t NH_3 (Kongshaug, 1998). From a worldwide survey, Williams and Al-Ansari (2007) calculated a global average emission of 2.07 ton CO_2 eq/t NH_3. Haas & Van Dijk refer to a CO_2 emission from European ammonia production of 1.95 ton CO_2-eq./ton NH_3 (Table 6).

The IFA report on Energy Efficiency and CO_2 Emissions in ammonia production (IFA, 2009), stated that some 36% of the CO_2 produced in 2008 was recovered. About one-third of the CO_2 generated globally was captured for production of urea ($CO(NH_2)_2$). The remaining CO_2 captured by the fertilizer industry (2.2%) was sold into other value chains, e.g., to the oil and gas industry or to the beverage industry.

Table 5. The energy input for production of ammonia

Region	GJ/ton NH_3			Reference
	sources	min	max	
World average	36.9	27.6	53.0	a
	36.6	27.0	58.2	b
	41.5	28.0		c
Europe	34.7			d
	32.1	28.4		e
	35.0	28.0		c
Russia + Central Europe	40.7			c
North America	37.9	28.0		c
China and India	47.6			c
Rest of the world	36.4			

References: a = Williams & Al-Ansari, 2007; b = IFA, 2009; c = IEA, 2007; d = Haas & van Dijk, 2010 and e = Kongshaug, 1998.

Thus, due to consumption of natural gas or other hydrocarbons both for feedstock and for fuel, CO_2 emissions are the major component of GHG budgets for ammonia manufacture.

Table 6. The GHGs emissions, expressed as CO_2 equivalent, for production of ammonia

Region	ton CO_2 eq./ton NH_3			Reference
	Sources	min	max	
World average	2.1	1.5	3.1	a
Europe	1.9			b
	1.8	1.6		c

References: a = Williams & Al-Ansari, 2007 b = Haas & van Dijk, 2010; c = Kongshaug, 1998.

Nitric Acid

Nitric acid is used in the manufacture of ammonium nitrate, calcium nitrate and potassium nitrate. For fertilizer purposes, the acid strength is in the range of 50-65%. Ammonia is vaporized, mixed with air and burned over a platinum/rhodium alloy catalyst to form nitrogen monoxide and water (eq. 1) according the following reactions (UNEP/UNIDO, 1998):

$$4NH_3 + 5O_2 \rightarrow 4NO + 6H_2O \quad (1)$$

Simultaneously, some nitrogen and nitrous oxide are formed (eq. 2 and 3):

$$4NH_3 + 3O_2 \rightarrow 2N_2 + 6H_2O \quad (2)$$

$$4NH_3 + 4O_2 \rightarrow 2N_2O + 6H_2O \quad (3)$$

The nitric oxide is oxidized to nitrogen dioxide, and the latter is absorbed in water to give nitric acid (eq. 4 and 5):

$$2NO + O_2 \rightarrow 2NO_2 \quad (4)$$

$$3NO_2 + H_2O \rightarrow 2HNO_3 + NO \quad (5)$$

the conversion of ammonia to nitric acid is exothermic (heat releasing) and contributes to a considerable release of steam. Two types of processes are currently used: single pressure and dual pressure. In single pressure, the oxidation and absorption stages are conducted at the same pressure (medium between 1.7 and 6.5 bar or high between 6.5 and 13 bar). In the dual pressure process the absorption occurs at a higher pressure than the oxidation.

Nitrous oxide (N_2O) is the most significant GHG associated with the production of nitric acid. The N_2O global warming potential is 298 times greater than that of CO_2 (IPCC, 2007). The amount of N_2O emitted is dependent upon combustion conditions (pressure, temperature), catalyst composition, burner design (EFMA, 2000b) and emission abatement technologies. In Table 7, N_2O emissions of nitric acid plants in different global regions are listed.

Most plants in Europe synthesize nitric acid by the medium pressure technique with average emissions of 6 to 8 kg N_2O per ton HNO_3. Using modern technology, N_2O emissions can be highly reduced. The Best Available Techniques (BAT) produces emissions of only 1.8 kg N_2O per ton HNO_3 (Kongshaug, 1998) or 1.85 kg N_2O per ton HNO_3 (Haas & Van Dijk, 2010).

Table 7. The dinitrogen oxide emissions of nitric acid production

Region	kg N_2O /ton HNO_3			Reference
	Sources	Min	max	
World, low pressure	5.0	4.5	5.5	a
World, medium pressure	7.0	5.6	8.4	a
World, high pressure	9.0	5.4	12.6	a
Developing countries	8.9	4.0	19.0	b
Europe (EFMA)	4.6			c
	7.0	6.0	8.0	d
	7.0	0.01	21.6	e
	6.7	1.8		f
North America, USA	9.0			g

References: a = IPCC, 2006; b = EPA, 2010; c = Haas & van Dijk, 2010; d = Zwiers et al., 2009; e = European Commission, 2006; f = Kongshaug, 1998; g = IEA, 2007.

The greenhouse gas emissions per ton nitric acid varies between 2.8 ton CO_2 eq./ton nitric acid for Europe, North America and rest of the world to 3.6 for China and India (calculated by combining the figures of N_2O emissions, energy use/export and ammonia production) (Kool et al., 2012).

Urea and Urea Ammonium Nitrate (UAN)

Urea has become the most widely used fertilizer in the world. Asia is today the biggest consumer mainly due of its use in flooded rice fields.

Urea fertilizers are produced by reacting liquid ammonia with carbon dioxide at high pressure (European Commission, 2006; EFMA, 2000b):

$2NH_3 + CO_2 \rightarrow NH_2COONH_4 \rightarrow CO(NH_2)_2 + H_2O$.

The steps of the process include (Figure 4):

- solution synthesis where ammonia and carbon dioxide react to form ammonium carbamate which is dehydrated to form urea with a yield on the order of 50-80%;
- solution concentration by vacuum, crystallization, or evaporation to produce a melt; solids formation by prilling (converting a material into a granular free-flowing form) or granulating;
- solids cooling and screening;
- coating the solids; and bagging and/or bulk loading.

The carbon dioxide from urea manufacture is produced as a by-product from the ammonia plant. The design of urea production processes has involved the separation of urea from the other constituents, the recovery of excess NH_3 and the decomposition of carbamate for recycling. The simplest way to decompose the carbamate to form CO_2 and NH_3 requires the reactor effluent to be depressurized and heated. Recycling techniques were developed to recover and recycle some of the NH_3 and CO_2 to the process. The recovery of the gases for recycling was essential to the urea synthesis, in order to optimize raw material utilization since recompression was too expensive, an alternative method was developed. This involved cooling the gases and re-combining them to form a carbamate liquor which was then pumped back into the reactor. A series of loops involving carbamate decomposers at progressively lower pressures and carbamate condensers was used (Total Recycle Process). A consequence of recycling the gases was that their molar ratio in the reactor increased, thereby increasing the urea yield.

Production of urea is usually linked to an ammonia plant, where by-product CO_2 from ammonia synthesis is used as a primary input in urea production. Atmospheric emissions are mainly NH_3 and urea dust. Both arise from the prilling or granulation process. From the prilling tower, emissions should range from 0.5 to 1 kg NH_3/ton and 0.5-1.5 kg urea dust/ton. With granulation, the granulator exit gas is scrubbed and losses can thereby limited to 0.25-0.8 kg NH_3/ton and 0.25-0.4 kg urea dust/ton. NH_3 also escapes from the process absorption vents within a range of 0.2-0.75 kg/ton urea. Liquid effluents are mainly NH_3, CO_2 and urea in a well-managed BAT plant, emissions to water can be limited to 0.0025 kg NH_3 and 0.0005 kg urea per ton of product. From a life cycle point of view there are discrepancies among different authors in the accounting for CO_2 consumption. Kongshaug (1998) and Kuesters and Jenssen (1998) consider that the consumption of CO_2 derived from ammonia production constitutes a net reduction in by-product CO_2 emissions, Davis and Haglund (1999), considering that the CO_2 is only stored for a short time and released upon application of urea fertilizers in the field, did not include this net credit. Data average for Greenhouse Gas Emission Factors for urea production in Europe vary from 420.0 g kg^{-1} of product to 1,848.7 g kg^{-1} (Wood & Cowie, 2004).

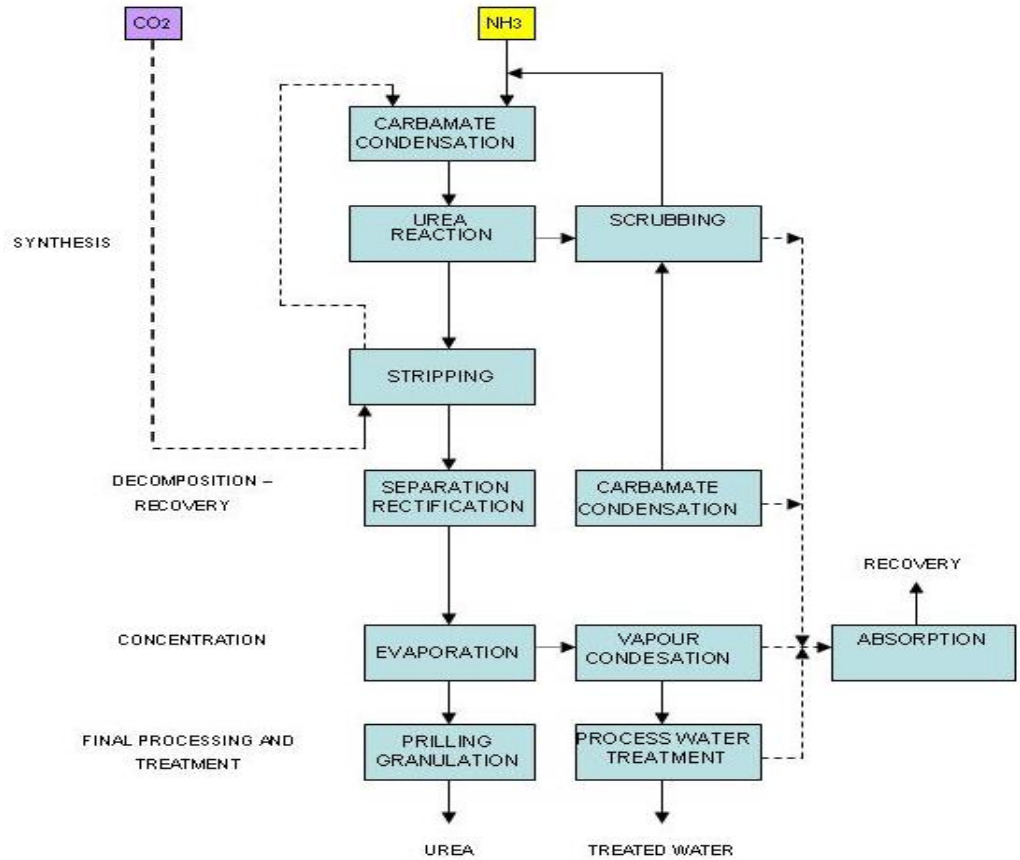

Figure 4. Block Diagram of a Total Recycle NH_3 Stripping Urea Process (EFMA, 2000a).

UAN is produced from urea and ammonium nitrate trough continuous or batch type processes in which concentrated urea and ammonium nitrate solutions are measured, mixed and then cooled. A partial recycling CO_2 stripping urea process is also suitable for UAN solution production. The GHGs emission from UAN production is dominated by N_2O emission because nitric acid is an intermediate product in ammonium nitrate synthesis.

Ammonium Nitrate (AN) and Calcium Ammonium Nitrate (CAN)

Ammonium nitrate and calcium ammonium nitrate are the most used N-fertilizers in Europe. Ammonium nitrate is made by neutralizing nitric acid with anhydrous ammonia (European Commission, 2006; EFMA, 2000a):

$NH_3 + HNO_3 \rightarrow NH_4NO_3$.

The reaction is highly exothermic and proceeds rapidly. The heat produced is often used to generate steam. The steam in turn may be used to evaporate the ammonia. The resulting 80-90% solution of ammonium nitrate can be sold as it is or it may be further concentrated to a 95-99.5% solution (melt) and converted into prills or granules. The manufacturing steps

include: solution formation, solution concentration, solids formation, solids finishing, screening, coating, bagging and/or bulk shipping.

As mentioned previously, the steam produced from the exothermic reaction allows a significant reduction in the energy consumption in AN production. The average consumption in Europe is 0.7 GJ/ton but a modern plant may consume 0.09-0.22 GJ/ton AN.

Calcium ammonium nitrate is obtained from an AN solution by mixing it with dolomite, limestone or calcium-carbonate. Subsequently its production requires a higher energy consumption due to the grinding process of dolomite or other raw materials (European Commission, 2006).

Many studies on the GHG emissions from AN and CAN have reported that the majority of these emission are made up of CO_2 emission from the ammonia synthesis and N_2O emission from nitric acid production. The latter accounted for an estimated 60-78% and 52-61% of total CO_2 emission from AN/CAN respectively (Wood & Cowie, 2004). Emissions arising from processing of intermediate products (i.e., ammonia and nitric acid) into final products (i.e., CAN, AN etc.) were of relatively minor importance (Patyk & Reinhardt, 1996; Davis & Haglund, 1999).

EFFECT OF NITROGEN FERTILIZATION ON SOIL ORGANIC MATTER TURNOVER AND RESULTING SOIL CO_2 EMISSION

Depletion of soil carbon content, recorded over the past 100 years, has been mainly due to conventional tillage practices, which increase soil organic matter (SOM) mineralization rate.

Agricultural soils have lost, compared with native soil, their natural capability to accumulate carbon (C), thereby releasing CO_2 into the atmosphere (Schlesinger, 1984). Depletion of soil carbon content, globally recorded over the past 100 years, has been mainly due to conventional tillage practices, which increase soil organic matter (SOM) mineralization rate.

Matson et al. (1997) reported SOM decline, often by 50% or more. Practices to improve C storage in cropping systems are: (1) improve C capture and (2) decrease SOM respiration. When agronomic practices increase SOM, CO_2 is removed from the atmosphere in the long-term (Lal et al., 1998 and 2003). In the last few decades a belief was developed in the scientific community that agricultural soils have the potential to increase C sinks and reduce CO_2 emissions if better management practices (BMPs) are adopted. Cole et al. (1997) estimated that it would be possible to increase the amount of C stored in the agricultural soils of the planet by 0.44 to 0.88 billion tons annually over a 50-year period.

SOM degradation is the main source of soil CO_2 emission, even if vegetation contributes to the total CO_2 emission with root and rhizo-microbial respiration. For this reason, the total soil CO_2 emission cannot be considered a direct measure of SOM oxidation, in spite of the fact that some studies continue to interpret it in such a manner (Hanson et al., 2000). Therefore, the so-called "basal respiration" refers to the CO_2 evolved by the degradation of SOM in roots-free soil. Three different C pools as sources of CO_2 from soil have been identified (Kuzyakov, 2006): (1) SOM; (2) above and below ground dead plant residues; (3) organic substances released by living roots such as exudates, secretions and sloughed-off root

cells. The last group is frequently described as rhizo-deposits. The soil carbon pools are oxidized by different groups of heterotrophic organisms for their metabolic needs. The most important and active heterotrophs in soil are microorganisms: bacteria, fungi, actinomycetes and protozoans. The activity of this heterotrophic community is affected by different soil environmental factors: SOM quality, nutrient availability, temperature, moisture, redox potential. The quality of SOM is one of the most important factors affecting the decomposition since humified carbon slows down the process since the acquisition of energy from such a substrate is slow. Microbial growth in soil is also limited by available nitrogen (N), since they have to satisfy their stoichiometric requirement (C:N ratio) from the substrate they feed on which has a different C:N ratio. If mineral nitrogen is available the competition for nutrients stimulating SOM decomposition by microorganisms is less intense. In soil there is an active competition between plant roots and microorganisms for mineral nitrogen, root uptake of N raises the competition for nutrient and decreases the microbial growth and metabolism, thereby depressing SOM decomposition (Schimel et al., 1989; Bottner et al., 1999). N fertilization, soil temperature (affected in agricultural systems by spatial and temporal reduction of plant canopy) and moisture (affected in some agricultural systems by irrigation) strongly influence soil CO_2 emission, by modifying the relative contribution of the two components (autotrophic and heterotrophic) (Wiseman & Seiler, 2004; Davidson & Janssens, 2006).

The adoption of better management practices (BMPs) can improve soil organic carbon (SOC) content, enhance soil quality, restore degraded ecosystems, increase biomass production, improve crop yield, and encourage investment in soil resources for soil restoration (Lal et al., 1998). The removal of crop residues does not necessarily induce a rapid decrease of SOM content. For instance, Campbell et al. (1991) showed that the removal of straw over a period of 30 years did not significantly affect the SOM content of an old wheat-wheat-fallow rotation system.

The effect of N fertilization on SOM mineralization and resulting CO_2 emission is very complex since many factors, both natural and/or linked to crop management, are involved in affecting the biological activities responsible of the process. Moreover, experimental design and measurement techniques present some difficulties. For example, it is difficult to discriminate among the different biogenic sources of CO_2; SOM-derived and plant-derived CO_2 is essential to evaluate the real capacity of soil as source or sink of atmospheric CO_2.

Khan et al. (2007) reported a promoted SOM decomposition by N fertilizer in a long-term study (51-year, Illinois). Other experiments (long term studies or experiment with isotopic signature) have shown that fertilizing crops with N results in higher levels of soil C over time (Paustian et al., 1992; Liang et al., 1996; Paustian et al., 1997; Wilts et al., 2004; Jagadamma et al., 2007). After these studies, the positive effect of N fertilization was basically due to the high yield crop which increases the annual input of crop residue to soils, mainly coming from roots and root exudates during growth. The same authors stated that an adequate fertilization contributes to the increase of SOM and does not alter the turnover of native SOM. This conclusion is in contrast with process called “priming effect” (Bingeman et al., 1953) considered as a stimulation of the mineralization of native SOM after the incorporation of fresh organic matter such as green manure, straw, rhizodeposits. This phenomenon has been clearly observed at the rhizosphere scale. For example, Liljeroth et al. (1994) showed in laboratory conditions without mineral nutrient supply, that the rhizodeposition by wheat and maize induced a two-fold increase in the mineralization rate of

pre-existing soil carbon. However, the mechanisms leading to the priming effect remain poorly understood (Kuzyakov et al., 2000). It is commonly believed that the low quality of SOM limits the amount of available energy for soil microorganisms, and in turn the rate of SOM mineralization. Thus, the priming effect is thought to result from an increase in overall microbial activity due to the higher availability of energy and nutrients released from fresh organic matter (Löhnis, 1926; Bingeman et al., 1953; Sørensen, 1974). However, mineral nutrients appeared that induce no or little effect on SOM mineralization (Wu et al., 1993; Shen & Bartha, 1997).

A possible role of added N is a chemically stabilizing C in the soil since N compounds may react with lignin in the process of humus formation, as a mechanism of C stabilization (Paustian et al., 1992). In addition, most SOM stabilizes with a C:N ratio of approximately 10:1 again indicating that if soil C storage is to increase, N is needed (Schulten & Schnitzer, 1997).

At any rate, the positive role of N fertilization in pooling C, may be offset by changing the CH_4 soil-atmosphere exchange and soil N_2O emissions (see later in this chapter).

EFFECT OF N FERTILIZATION ON SOIL-ATMOSPHERE CH_4 EXCHANGE

Methane (CH_4) is considered the second greenhouse gas after CO_2 as atmospheric concentration, despite a short residence time in the atmosphere (about 10 years), since its ability to absorb infrared radiation makes is 20 to 30 times more efficient than CO_2 (Rodhe, 1990). Methane is also involved in changes in the chemical composition of the atmosphere since it is chemically reactive. In particular, it reacts with hydroxyl radicals in the troposphere, thereby reducing its oxidative power and its ability to eliminate pollutants such as chloro-fluoro carbons (CFCs), and it also leads to the production of other greenhouse gases (O_3, CO, CO_2) (Cicerone & Oremland, 1988).

Soil-atmosphere methane exchange is the result of two antagonistic microbial processes, strictly connected and linked by a syntrophic relationship with other transformation processes in the soil. Methane is produced in anaerobic soils or in anaerobic micro-sites of drained soils by methanogens. In aerobic soils, methane produced in anaerobic microsites and methane from air is oxidized into CO_2 by methanotrophs. A soil is considered CH_4 source when the balance between production and consumption is positive; when the balance is negative, the soil is considered a CH_4 sink. Globally, soils most efficient as CH_4 sources are generally those which are anaerobic because they are often submerged or water-saturated. In these cases a significant methanogenic activity develops at intervals: ricefields, landfills, peat soils, (Whalen et al., 1990; Nesbit & Breitenbeck, 1992; Sundh et al., 1995). However, 60 to more than 90 % of CH_4 produced in anaerobic soils is re-oxidized in their aerobic zones, so the balance between CH_4 production and oxidation is usually positive (Le Mer & Roger, 2001). Methane oxidation by aerobic upland soils is rarely higher than 0.1 mg CH_4 m^{-2} h^{-1}. Forest soils are the most active, followed by grasslands and cultivated soils (Le Mer & Roger, 2001).

Methanogenic bacteria are responsible of the complete mineralization of organic matter in anaerobic environments, where sulfate and nitrate concentrations are low, through a fermentation process which produces CH_4 and CO_2 according to the reaction:

$C_6H_{12}O_6 \rightarrow 3\,CO_2 + 3CH_4$.

This transformation requires successive actions by a sequence of populations of micro-organisms that degrades complex molecules into simpler compounds: (1) hydrolysis of biological polymers into monomers (glucides, fatty acids, amino acids) by an aerobic, or facultatively, or strictly anaerobic microflora; (2) acetogenesis from the previous metabolites by a syntrophic or homoacetogenic microflora; (3) methanogenesis from the simple compounds that can be used by methanogens (in particular H_2, CO_2 and acetate ion) which constitutes the last step of the methanogenic fermentation. Methanogenesis, requires strict anaerobiosis and low oxydo-reduction potentials (Eh < –200 mV).

Methanotrophy in soils is known in two forms. The first form is called high affinity oxidation, and occurs at CH_4 concentrations close to that of the atmosphere (< 12 ppm). It appears to be ubiquitous in soils that have not been exposed to high NH_4^+ concentrations (Topp & Hanson, 1991). High affinity oxidation is estimated to contribute 10% of total CH_4 consumption (Topp & Pattey, 1997). The second form of oxidation is a low affinity oxidation occurs at CH_4 concentrations higher than 40 ppm. It is considered as methanotrophic activity in the strictest sense (Jones & Nedwell, 1993). Methane oxidation in methanogenic environments (ricefields, peat soils, landfills, etc.) is a low affinity type.

Methanotrophs use CH_4 as carbon and energy source. Oxygen availability is the main factor limiting their activity.

Different environmental conditions and factors affect the two microbial processes. Gas diffusion and its consequentual relationship to the oxydo-reduction level and CH_4 transfer. This factor is in turn affected by water content and soil texture. In addition, the typical conditions affecting the microbial activities: temperature, pH, Eh, substrate availability, physicochemical properties of soils.

Lowland rice cultivation represents the only major source of CH_4 from established cropping systems; about 40 Tg $year^{-1}$ are emitted from rice soils worldwide (Sass et al., 1999).

The effect of N fertilization on net soil-atmosphere CH_4 emission occurs by means of different actions, even if its effect on CH_4 emissions from rice fields is not well understood (Zuo et al., 2005) and data from literature are sometimes contradictory, depending by the nature of the fertilizer and the quantity applied (Lindau, 1994). Urea application in rice fields is usually reported as a fertilization practice increasing CH_4 emission by increasing rice productivity and soil pH resulting from urea hydrolysis (Wang et al., 1992; Lindau & Bollich, 1993). Bufogle et al. (1998) and references therein reported that CH_4 emissions were higher in ricefields fertilized with urea than those fertilized with ammonium sulfate, since in extreme anaerobic condition sulfate-reducing bacteria compete with methanogenic bacteria. Under such conditions, electron acceptors other than CO_2, especially nitrate and sulfate, may cause bacterial competition which is unfavourable to methanogens and decrease CH_4 production/emission. H_2 and acetate are preferentially used by sulphate-reducing bacteria, sulfate application generally reduces methanogen activity. Ammonium sulfate was frequently reported to significantly reduce (30 to 60%) CH_4 flux from ricefields (Bronson et al., 1997; Cai et al., 1997). Wang et al. (1992) explained that the negative effect on CH_4 production of sulfate addition with N fertilizer was not associated with an increase in soil Eh, so a direct inhibitory effect of sulfate on methanogenesis was assumed. Moreover, indirect ammonium inhibition for methanogenesis, and consequent CH_4 production, was hypothesized to be

accomplished by nitrates produced by its nitrification (Conrad & Rothfuss, 1991; Jugsujinda et al., 1995). On the other hand, ammonium is inhibitory for methanotrophy, reduces CH_4 reoxidation resulting in an increase of net soil emission (Conrad & Rothfuss, 1991). Compared with urea, it has been confirmed that the advantage of using ammonium sulfate reduced CH_4 emission by 50–60% (Kimura et al., 1992; Lindau, 1994).

N fertilizers containing nitrate can also reduce CH_4 production/emission since nitrate application causes a competition for H_2 between denitrifying bacteria and methanogens, favoring denitrifying bacteria. Nitrate is active also in the reduction of CH_4 emission from soil by increasing soil Eh, since it is an oxidant (Jugsujinda et al., 1995; Roy & Conrad, 1999).

Methane consumption in soil, in contrast to CH_4 production, is broadly affected by agricultural N use. Methanotrophic bacteria capable of consuming atmospheric CH_4 are found in most aerobic soils, including arable lands, making the uptake of CH_4 globally important. The global soil sink of CH_4 has been estimated to be about 30 Tg CH_4 $year^{-1}$, corresponding to the same magnitude as the annual atmospheric increase of CH4 (about 37 Tg CH_4 $year^{-1}$) (Robertson et al., 2013). In natural terrestrial ecosystems, CH_4 uptake is limited by its rate of diffusion in soil microsites and by methanotrophic activity (von Fischer et al., 2009). Diffusion is regulated by some physical factors: moisture, temperature, soil structure and CH_4 concentration in the bulk soil atmosphere. Some authors reported that agricultural management reduced soil CH_4 oxidation approximately 70% or more (Mosier et al., 1991; Robertson et al., 2000).

The mechanism responsible of this suppression is largely related to soil N availability as affected by N fertilizers, and other N inputs (Steudler et al., 1989; Suwanwaree & Robertson, 2005). Ammonium is known to competitively inhibit CH_4 mono-oxygenase, the principal enzyme responsible for oxidation at atmospheric concentrations. For this reason ammonium and urea usually inhibit atmospheric CH_4 oxidation, contrary to the nitrate (Le Mer & Roger, 2001). In addition there is a transfer of the CH_4 oxidizing activity towards nitrification and the toxicity of NO_2^- produced (Le Mer & Roger, 2001). Nitrite, the end product of methanotrophic ammonia oxidation, was found to be a more effective inhibitor of CH_4 consumption than ammonium (Schnell & King, 1994).

EFFECT OF N FERTILIZATION ON SOIL N_2O EMISSION

N_2O is a greenhouse gas with a global warming potential 298 times greater than CO_2 whose emission to the atmosphere is mainly from global soils. The increase of N_2O in the atmosphere is due to the human alterations of the global N cycle, with 24% of annual emissions produced by agricultural soils and the application of N fertilizer (Bouwman, 1996 and 2002a; IPCC, 2007). Soils are a key source of N_2O emissions to the atmosphere contributing to about 53% of the global anthropogenic emission (Denman et al., 2007) and are directly related to the combined effect of climate, crop management and soil physical–chemical characteristics affecting microbial driven processes.

Soil N_2O production is the biogenic product of microbial processes of denitrification and nitrification, as affected by physical–chemical characteristics of soil. Bacterial denitrification is a respiratory reduction of nitrate and/or nitrite to gaseous NO, N_2O and N_2, coupled to

phosphorylation electron transport. Many aerobic microorganisms use NO_3^- as electron acceptor to derive energy from organic compounds when oxygen tension is low (heterotrophic denitrification), and, in this process, N_2O is an obligatory intermediate.

Microbial nitrification is the oxidation of ammonium to nitrite and nitrate and, in most soils, autotrophic bacteria are responsible of this process, even though some studies suggest heterotrophic nitrifiers may also contribute to nitrification and N_2O production (Schimel et al., 1989; Anderson et al., 1993). N_2O is not an obligatory intermediate of nitrification process, since when O_2 supply is limited in soil (denitrification by nitrifiers), autothrophic bacteria can produce nitrous oxide by enzymatic reduction of nitrite. Autothrophic ammonium-oxidizing bacteria such as *Nitrosomonas europea* can use NO_2^- as an alternative electron acceptor under anaerobic conditions, thus reducing NO_2^- to N_2O by the nitrite reductase enzyme (Firestone & Davidson, 1989; Groffman, 1991). The production of N_2O may also be caused by other microorganisms, e.g., during dissimilatory reduction of nitrate to ammonium, nitrate reduction to nitrite, and nitrate assimilation (Smith & Zimmerman, 1981; Bleakley & Tiedje, 1982). The mechanism of N_2O production by these bacteria and their role in N_2O production in soil require extensive study.

Typical key soil factors affecting the two microbial processes are: pH, temperature, total and mineral nitrogen content, labile organic matter availability, water content, soil aeration, redox potential. It is fundamentally necessary to distinguish between soil production and soil emission, since the N_2O produced by the two microbial processes does not necessarily leave the soil, depending on soil physical characteristics. Nitrification and denitrification can be active simultaneously in soil, since it is a complex and heterogeneous system with aerobic and anaerobic microsites, which are not homogeneously distributed, and, consequently, N_2O can be evolved via both these processes (Nielsen et al., 1996; Abbasi & Adams, 2000). At the same time, in microsites where anaerobic conditions are extreme, denitrification process can utilize N_2O produced by the same process or by nitrification, thus reducing the gas emission from soil. Davidson (1991) presented a simplified model (hole in the pipe model) to describe the processes affecting production and emission of N_2O from soils. The model identifies three levels of control. Level I is represented by all factors affecting the rates of nitrification and denitrification; level II is for N_2O emissions depending on how soil physical–chemical parameters affect the ratio of end-products via both processes and on how fast; level III accounts for N_2O diffusing to atmosphere through the soil gaseous phase.

Typical soil managements, such as nitrogen fertilization and irrigation, are responsible for large N_2O fluxes. The great application of N fertilizers in traditional croplands, such as NO_3^-, NH_4^+, NH_4NO_3 and urea, is a key controller of microbial processes involved in N_2O evolution from soil (Bremner & Blackmar, 1980; Duxbury et al., 1982; Dambreville et al., 2006). Eichner (1990) listed the following factors affecting fertilizer-derived N_2O emissions: (1; *management factors*) fertilizer type, application rate, application technique, application timing, tillage system, use of other chemicals, crop type, irrigation, and residual N and C from crops and fertilizer and (2; *environmental factors*) temperature, precipitation, soil moisture content, SOC content, soil O_2 status, soil porosity, soil pH, freezing and thawing cycles, and microorganism abundance and activity.

Bouwman (1996) summarized international research results on the release of N_2O from N fertilizers, and developed a generalized fertilizer induced emission (FIE) of 1.25% of the amount of N fertilizer applied. The fraction of applied N actually emitted as N_2O varies widely on a site-specific basis. Even if the 2006 IPCC Guidelines for National Greenhouse

Gases Inventory (IPCC, 2006) the value of emission factor (EF) has been changed from 1.25% to 1%, as a result of new analyses of the available experimental data. For example, Fierro and Forte (2012) reported an emission factor of 0.8% in a conventional tilled, N fertilized and irrigated maize crop under Mediterranean climate conditions. As well as a GHG inventory in Canada reported low N_2O emissions in arid regions, on average of 0.16 and 0.8% of N fertilizer applied was emitted as N_2O, in the Brown-Dark Brown and Grey-Black soil zones, respectively, compared to 1.19% in Eastern Canada (Grant & Wu, 2008). Some studies concluded that emission factors values are appropriate only when N fertilization rates are less than or equal to those required for maximum crop yields, because the percentage of N that is emitted as N_2O becomes more variable at higher N rates, so EFs may increase when the N rate exceeds the crop and soil uptake capacity (McSwiney & Robertson, 2005; Grant et al., 2006; Halvorson et al., 2008). Estimates of N_2O emissions from croplands in the world in 1995 (IFA/FAO, 2001), estimated using IPCC emission factor of 1%, on the total amount of N added (73.4 million t mineral form plus 20.66 million t animal manure) produced 3.50 million t of N lost as N_2O, corresponding to a fertilizer induced of 0.735 million t. In terms of GWP, this is equivalent to 4.65 kg CO_2 per kg of N applied, with an uncertainty range of 1.4 to 14.0.

Representative EFs values are also linked with the experimental design and monitoring techniques used for N_2O fluxes measurements. The length of measurement period and the frequency (and intensity) of measurements were considered as key factors in any local or large-scale estimations of N_2O emissions (Parkin, 2008), since soil N_2O fluxes result from complex interaction among biological, physical and chemical factors, within a large spatial and temporal variability (Clemens et al., 1999; McSwiney & Robertson, 2005; Wagner-Riddle & Thurtell, 1998). Much of the challenge arises from the fact that small areas (hotspots) and brief periods (hot moments) often account for high fluxes. N_2O hotspots in soils involve the interaction among patches of organic matter and physical factors controlling oxygen diffusion in soil, and transport and residence time of N_2O in soil pores. Thus, a series of plant and soil factors, e.g., rooting patterns and soil structure at small (0.1 to 10 m) scales, topography, hydrologic flow paths and geology at larger (> 1 km) scales, need to be considered to understand the spatial distribution of hotspots. Currently, soil N_2O emissions predicting models are calibrated on the basis of spatial variability. However, their reliability to predict temporal variations is seriously undermined due to the very few data available in literature to calibrate these models over time. The hot moments concept has been known since long time but scarcely investigated by continuous monitoring, particularly in the small time scale, since few experiments are based on high-time-resolution measurements systems [dynamic chambers, Tunable Diode Laser (TDL) associated with eddy covariance technique]. The large part of data produced up-to-now, are referred to manual chamber measurements limited in temporal resolution.

The increase of soil N_2O emissions is not only the direct effect of N fertilizers on soil but it is also due to the so-called indirect emissions of N_2O from Nr sprinkled in the environment (NH_3, NO_x, NO_3^-). Volatilized N can affect N_2O emissions because a portion of this N will be deposited on agricultural and non-agricultural soils and in water and be subjected to transformations that may result in N_2O emissions; a portion of the NO_3^- leached or discharged in drainage can also be denitrified and result in N_2O emissions (Del Grosso et al., 2006). Indirect emissions are difficult to estimate because there is an uncertainty in both the amount of Nr that escapes and the portion of N that is then converted to N_2O. IPCC Tier 1

methodologies assume that 0.75% of the N that is leached from cropped systems and 1% of the N that is volatilized and subsequently deposited to downstream ecosystems are emitted later as N_2O (De Klein et al., 2006). This topic is so complex, that is credible that the EFs for leached N and for volatilized and re-deposited N depends on the type of waterway and on the N status (e.g., limiting or non-limiting) of the receiving ecosystem (Beaulieu et al., 2011).

EFFECT OF N FERTILIZATION ON NH_3 VOLATILIZATION

Worldwide, agriculture is the biggest source of ammonia (NH_3) emission coming from ammonium (NH_4^+) contained in fertilizers converted into ammonia and released to the air (Mosier, 2001; Galloway et al., 2004; Erisman et al., 2008). The emitted NH_3 results in nitrogen loads to ecosystems through its atmospheric transportation and deposition, which may impact ecosystems in various ways (Hayashi & Yan, 2010). Ammonia released into the atmosphere, contributes to acidification and the eutrophication of terrestrial and water ecosystems. Its impact acts at local and regional scale, depending by the atmosphere transportation.

Loss of N by ammonia volatilization has ranged from negligible amounts to >50% of the fertilizer N applied, depending upon fertilizer practice and environmental conditions (Peoples et al., 1995). In flooded rice fields, ammonia volatilization can account for 20% to >80% of the total N lost from fertilizer sources (Mosier et al., 1989). The amount and temporal dynamic of NH_3 emission are affected by type of canopy, climatic and soil conditions, typology of fertilizer and management of its application (Huijsmans et al., 2003; Sommer et al., 2004).

Some of the most important factors regulating NH_3 loss are the concentration of ammoniacal-N, temperature, and pH of the soil solution or irrigation water, since all three variables markedly affect the partial pressure of NH_3. The pH in particular affects the equilibrium between ammonium and ammonia, so that the relative concentration of NH_3 increases from 0.1 to 1, 10 and 50% as the pH changes from 6 to 7, 8 and 9, respectively (Freney et al., 1983). Soil pH at values in excess of 8.0 are responsible of high ammonia volatilization (Larney & Olson, 2006; Kim et al., 2008). Urea is rapidly hydrolyzed by urease to NH_3 within one week in soil (Han et al., 2004). The protonation of NH_3 via $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ after urea hydrolysis is the most critical process in the increase of soil pH to values in excess of 8, thereby resulting in an increase in NH_3 volatilization (Fenn & Hossner, 1985).

On the contrary, NH_3 volatilization appeared inhibited by low soil pH, as well as high soil cation exchange (ECEC) even at the low soil pH; and the relatively high nitrification potential (Hayashi et al., 2011). So, the pH-buffer capacity and cation exchange capacity of the soil are factors able to reduce ammonia volatilization.

Rates of NH_3 emissions are also very sensitive to temperature (Montes et al., 2009). As temperatures rise, the relative proportion of NH_3 to ammonium present at a given pH increases, while the solubility of NH_3 in water decreases. This increases the diffusion of NH_3 through the soil, and affects the rate of microbial transformations. Volatilization takes place mainly during the first days after application, and for a longer time the lower the temperatures

are (at dressing), though at a lower rate. The amount of volatilized nitrogen increases with temperature.

Wind speed is another major determinant, controlling volatilization through its effect on mixing in the liquid phase, and the rate of transport of NH_3 away from the air-water or air-soil interface (Denmead et al., 1982; Fillery et al., 1984).

Ammonia loss from surface applications of urea can be reduced by the use of a urease inhibitor which allows urea to move into the soil before hydrolysis. The ammonia then released is retained by the soil. One compound which has been widely tested for its capacity to reduce ammonia loss from urea is the phosphoroamide N-(n-butyl) thiophosphoric triamide (NBTPT) (Byrnes & Freney, 1995). In upland fields Bronson et al. (1989) found that addition of NBTPT markedly reduced ammonia volatilization from loamy sands.

Ammonia loss from cropland tends to be important when anhydrous ammonia or urea fertilizers are misapplied to dry soil, such that the NH_3 that is added as anhydrous ammonia or formed from urea escapes to the atmosphere before it can be dissolved in the soil solution as NH_4^+. Fertilizer misapplication in this way is inefficient and is more likely to occur during extended dry periods (Robertson et al., 2013).

EFFECT OF N FERTILIZATION ON NITROGEN OXIDES (NO_x) EMISSION

Nitrogen oxides (NO_x) are produced by agricultural soils (Karl et al. 2009; Hudman et al. 2010), even if they are a minor but significant source (Robertson & Vitousek, 2009). Production and emissions are typically enhanced by N fertilizers application, precipitation, and elevated temperature (Butterbach-Bahl et al., 2009). Jaeglé et al. (2005) estimated that the emissions from agricultural soils summed to about 14 % of global surface emissions. In most cropped soils NO_x, together with N_2O, NH_3, N_2 and NO_3^-, represent a form of nitrogen compounds emitted when they escape to plant uptake, so a net loss of these compounds indicate a bad N management. Emission of NO_x from agriculture include also residue burning and land clearing (Robertson & Vitousek, 2009).

NO_x (NO and NO_2) carry out, directly and indirectly, several functions in some chemical transformations in the atmosphere. They affect the production of O_3 in the low atmosphere (troposphere), which acts as a greenhouse gas and as a harmful chemical to human health and to plant productivity (Derwent et al., 2008). Ozone (O_3), by reducing plant photosynthesis, leads to a reduction of atmospheric CO_2 sequestration by the plant biomass and resulting in more CO_2 –driven warming (Felzer et al., 2004; Sitch et al., 2007).

Another indirect effect of NO_x is on atmospheric CH_4. The largest removal process of CH_4 is oxidation by the hydroxyl radical (OH), accounting for 88% of the total sink. Emissions of NO_x can increase atmospheric OH and accordingly, decrease CH_4 concentrations (Boucher et al. 2009).

NO_x can lead to increasing O_3 on daily time scales while, on a decadal time, they can lead to decreases in O_3, the net result of these competing effects depends on where the NO_x emissions occur (Collins et al., 2010; Fry et al., 2012). However, the net impact of NO_x on atmospheric radiative property is likely to be cooling, by (1) decreasing the CH_4 atmospheric

concentration, and (2) decreasing atmospheric O_3 formation due to lower CH_4 concentrations (Fuglestvedt et al., 2010).

In addition, both NO_x and ammonia (NH_3) react with atmospheric constituents to form fine particles (aerosols), considered cooling agents (Forster et al., 2007). Eventually, the NO_x compounds are deposited on downstream ecosystems in gaseous, particulate, or dissolved forms, where it undergoes the same fate as other Nr inputs, including potential transformation to N_2O, terrestrial acidification and water eutrophication.

NO from agricultural soil is produced by microbial processes, as by-products of nitrification and products of denitrification, both microbial processes (see previous section) are strongly affected by N fertilization (Mosier et al., 1998; Robertson & Groffman, 2007), more broadly NO emissions are also affected by the same environmental and agronomic factors, including fertilizer application rate and soil moisture. Soil NO can be also produced by chemodenitrification when HNO_2 spontaneously decomposes to NO. NO produced in soils is rapidly oxidized to NO_2 in the atmosphere.

NO_x emissions in some croplands are considered episodic, in some cropped systems the magnitude of NO_x emissions can rival those of N_2O (Matson et al., 1998). Stehfest and Bouwman (2006) estimated that global NO emissions from cropland and grassland are less than half of the global N_2O-N emissions.

LIFE CYCLE ASSESSMENT METHODOLOGY

Life Cycle Assessment (LCA) is a technique used to assess each and every impact associated with all the stages of a process from cradle-to-grave (i.e., from raw materials through materials processing, manufacture, distribution, use, repair and maintenance, and disposal or recycling) (Figure 5). LCA enhances the understanding of how alternative systems compare with each other, but also how different sub-processes in a system affect the overall results (Bauman and Tillman, 2004). The environmental performance of the systems studied is calculated using the LCA-based methodology described in the ISO 14000 series standards (ISO, 2006a,b). The LCA concept consists of four steps: (1) Goal and Scope definition, (2) Life Cycle Inventory (LCI), (3) Life Cycle Impact Assessment (LCIA), (4) Life Cycle Interpretation.

Goal definition and scoping is the phase of the LCA process that defines the purpose and method of including life cycle environmental impacts into the decision-making process. In this phase, the following items must be determined: the type of information that is needed to add value to the decision-making process, how accurate the results must be to add value, and how the results should be interpreted and displayed in order to be meaningful and usable. Therefore, the following items shall be considered and clearly described: the functional unit, the system boundaries, the product system to be studied; the product system boundaries; allocation procedures; types of impact and methodology of impact assessment, and subsequent interpretation to be used; data requirements; assumptions and limitations; initial data quality requirements; type of critical review, if any; type and format of the report required for the study.

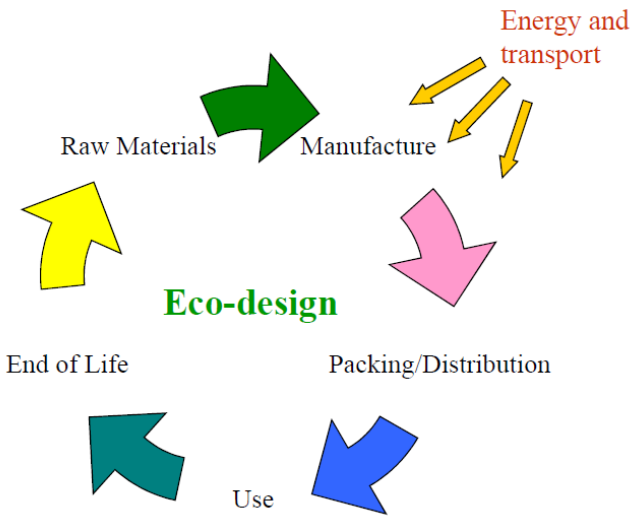

Figure 5. Cradle-to-gate approach to LCA.

Inventory analysis involves data collection and calculation procedures to quantify relevant inputs and outputs of a product system. These inputs and outputs may include the use of resources and releases to air, water and land associated with the system. Interpretations may be drawn from these data, depending on the goals and scope of the LCA. These data also constitute the input to the life cycle impact assessment.

National or regional databases, which evolved from publicly funded projects, provide inventory data on a variety of products and basic services that are needed in every LCA, such as raw materials, electricity generation, transport processes, and waste services as well as sometimes complex products. Several national and international public databases have been released in the past, among them the Swedish SPINE@CPM database (CPM, 2007), the German PROBAS database (UBA, 2007), the Japanese JEMAI database (JEMAI, 2007), the US NREL database (NREL, 2004), the Australian LCI database (RMIT, 2007), the Swiss eco-invent database (Eco-invent, 2007), and the European Reference Life Cycle Database (ELCD) (European Commission, 2007).

The purpose of the Life Cycle Impact Assessment (LCIA) is to provide additional information to help assess the results from the Inventory Analysis in order to better understand their environmental significance. The impact assessment phase consists of different steps: assigning of inventory data to impact categories (classification), modeling of the inventory data within impact categories (characterization), the contribution of the analyzed system to the total extent of the environmental effects in Europe is analyzed (normalization), possibly aggregating the results in very specific cases and only when meaningful (weighting).

Interpretation is the phase of LCA in which the findings from the inventory analysis and the impact assessment are combined together, or, in the case of life cycle inventory studies, the findings of the inventory analysis only, consistent with the defined goal and scope in order to reach conclusions and recommendations. The findings of this interpretation may take the form of conclusions and recommendations to decision-makers, consistent with the goal and scope of the study.

Evaluation of the Environmental Impact by Means of LCA

During the life cycle of mineral nitrogen fertilizers, greenhouse gas emissions may arise through the extraction of resources, the transport of raw materials and products, in fertilizer production processes and during an agronomic phase as N_2O, NO_3^-, NH_3 and NO_x. The fertilizer industry recognizes that it contributes directly and indirectly to the emission of greenhouse gases (GHGs), as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), during production, distribution and use of fertilizers (IFA, 2009). In line with international greenhouse accounting practice (IPCC, 2006), emission factors are expressed as carbon dioxide equivalents using the "global warming potential" (GWP), which determines the relative contribution of a gas to the greenhouse effect (Table 8). In particular, the global GHG emissions can be divided for the following processes: 0.93% from fertilizer production; 0.07% from fertilizer distribution; 1.5% from fertilizer use. Therefore, due to the environmental relevance of the production phase of fertilizers, the evaluation of this contribution to GHGs emissions of agricultural processes by Life Cycle Assessment must be considered (Wood & Cowie, 2004). Several studies have shown that the crop production is based on the use of input flows directly or indirectly dependent upon the global availability of the fossil fuels and other minerals, both non-renewable resources, like fertilizers and diesel oil for agricultural machines (Table 9). The same results was also obtained in case of the biodiesel production from soybean oil in Brazil (Cavalett & Ortega, 2010) and sunflower cultivation in Tuscany (Italy) (Spugnoli et al., 2012; Spinelli et al., 2012 and 2013a), and electricity from willow biomass crop production systems in New York (Heller et al., 2003).

Because production processes are based on fossil fuel, it can be seen in many life cycle assessment studies that the production of nitrogen fertilizers is one of the major contributors to greenhouse gas emissions from agriculture production systems. In particular, the basic component in current industrial nitrogen fertilizer production is ammonia produced by the Haber-Bosch process ($N_2+3H_2 \rightarrow 2NH_3$). The hydrogen originates from natural gas and the nitrogen from air (EFMA, 2000a). Furthermore, during the production of nitrate-based fertilizers, like ammonium nitrate, significant amounts of N_2O are emitted during the production of nitric acid. The N_2O gas emissions from the nitrogen fertilizers feedstocks could generate effects on climate change higher than to those caused by carbon dioxide emissions because of the 298 time greater global warming potential (Forster et al., 2007; Brentrup et al., 2001; Spinelli et al., 2013b). On the other hand, in the urea plant, the carbon dioxide emissions are high with respect to nitrate-based fertilizers plant and carbon dioxide is re-used in the synthesis phase (European Commission, 2006). However, a switch to urea or another non-nitrate fertilizer would lead to higher contributions to acidification and eutrophication (Brentrup et al., 2001).

Table 8. Global Warming Potential relative to CO_2 equivalents for greenhouse gases emissions (Solomon et al., 2007)

Common name	Chemical formula	Global Warming Potential for Given Time Horizon		
		20 - yr	100 - yr	500 - yr
Carbon dioxide	CO_2	1	1	1
Methane	CH_4	72	25	7.6
Nitrous oxide	N_2O	289	298	153

Table 9. Inventory of the main input mass for several agricultural production systems in Europe

Input	Unit	Franzese et al., 2013 (Brazil)		Spinelli et al., 2013b (Italy)	Tsoutos et al., 2010 (Greece)			Halleux et al., 2008 (Belgium)	
		Corn	Soybean	Sunflower	Rapeseed	Sunflower	Soybean	Sugar beet	Rapeseed
Nitrogen fertilizer	kg/ha	81.6	-	114	230	70	70	144	135
Phosphate fertilizer	kg/ha	64	60	92	200	-	-	74	212.5
Potassium fertilizer	kg/ha	127	60	-	90	-	-	74	125
Pesticides	kg/ha	10.2	10.5	0.96	2	1	2.5	2.3	5.3
Diesel	kg/ha	48.1	41.6	117	n.a.	n.a.	n.a.	111.3	101

The acidification impact is caused by the emission of inorganic species like ammonia, nitrogen oxides and sulphur dioxide (Neung-Hwan & Richter, 2004; Kozlowski et al., 2011). Spinelli et al. (2013b) reported that the production of ammonium nitrate was principally involved in eutrophication impact because of high emissions of nitrate. Conversely, the higher direct ammonia emission from the use of urea is mainly responsible for the acidification impact.

Another aspect that should be taken into account is the estimation on the climate change by the use of default value for soil N_2O emissions, usually considered to be 1%, and local emission data of mineral nitrogen applied to soil. Spinelli et al. (2013b) have analyzed the global greenhouse gases effect due to the use of default soil N_2O emission factor (EF) of 1% and the value of 0.8% derived from experimental data of a Mediterranean maize crop (Fierro & Forte, 2012). The mineral nitrogen fertilizers, urea and ammonium nitrate, showed a reduction of 7.8% and 4.9% for the impact of dinitrogen dioxide as greenhouse gases by using the direct field data for the emission factor than default value. Therefore, these results have demonstrated the need to use more precise data for the accounting. In fact, as a result of new analysis of the available experimental data in the 2006 IPCC Guidelines for National Greenhouse Gases Inventory (IPCC, 2006) the value of EF has been changed from 1.25% to 1%, as compared to the 1996 IPCC Guidelines.

As mentioned previously, mineral nitrogen fertilizers showed a higher contribution for all environmental impact categories than other inputs of agricultural production systems. Therefore an environmental analysis of cultivation systems with different chemical forms of nitrogen fertilizers would help to evaluate a reduction of environmental burdens. Brentrup et al. (2001) reported a comparison of sugar beet production by the use of urea ammonium nitrate, calcium ammonium nitrate and urea: urea-based fertilizers showed a higher acidification/eutrophication impact compared to nitrate-based fertilizers. Spinelli et al. (2013b) reported a lesser impact due to the use of urea fertilizer for carcinogenic effects, climate change effects, ecotoxicity and minerals in the sunflower cultivation.

Conversely, use of ammonium nitrate showed limited advantages for the environmental categories of respiratory inorganics, radiation, acidification/eutrophication and fossil fuel consumption.

Direct and Indirect Emissions Estimation

Estimation of indirect emissions from the fertilizer production processes can be extracted from primary data or from the database. For the Eco-invent v3 database, in addition to the tillage, all the relevant up-stream processes involved to the mineral nitrogen fertilizers were taken into account by considering the production technology in the respective country and the relative import shares. Transports of the intermediate products were included as well as the transport of the fertilizer product from the factory to the regional storehouse. Production and waste treatment of catalysts, coating and packaging of the final fertilizer products were not included. Transport specifications of the fertilizer product to the regional department store, which were not included in the reference used for this inventory, were complemented by data given in Patyk & Reinhardt (1997).

Direct field emission in air from the application of nitrogen fertilizers is calculated for ammonia, dinitrogen oxide, nitrogen oxides and carbon dioxide as reported in Nemecek and Schnetzer 2011: (i) to calculate the N_2O emissions from mineral fertilizers and from crop residues an emission factor as a percentage of nitrogen lost as N_2O is used; (ii) to calculate the NH_3 emissions are used different emission factor for fertilizers; (iii) the NO_x emissions are estimated from the emissions of N_2O; (iiii) to calculate the CO_2 emissions is considered the emission of 1750 g of fossil CO_2 per kg of applied urea-N (only for urea-based fertilizers).

The NH_3 emission from applied mineral fertilizers is calculated by constant emission factors for each group of fertilizer. Instead of the emission factors suggested in Agrammon group (2009) (15% for urea and 2% for all other mineral fertilizer) a set of emission factors was applied that distinguishes a greater number of different fertilizer groups (Asman, 1992). The set of emission for each group of mineral nitrogen fertilizers are reported in Table 10.

In the case of spring-summer crops cultivation, emission into water was not taken into account because nitrates in the soil can be adsorbed by the plants and the evapotranspiration is similar or higher than the precipitation (Spinelli et al., 2013b). In period of heavy rainfall, in autumn and in winter precipitation accelerates uptake by the plants causing leaching of nitrate dissolved in the water. The amount of leaching NO_3-N is calculated by a simple regression model (Nemecek & Schnetzer, 2011).

As reported in Table 11, in accordance with the model to calculate the leaching NO_3-N, the geographical variability of the average annual precipitation should be considered in order to quantify the nitrate in ground water.

Table 10. NH_3-emissions from mineral fertilizers (% N emitted in form of NH_3)

Type of fertilizers	Emission factor for NH_3-N (%)
Ammonium nitrate, calcium ammonium nitrate	2
Ammonium sulphate	8
Urea	15
Multi-nutrient fertilizers (NPK-, NP-, NK-fertilizers)	4
Urea ammonium nitrate	5.7[a]
Ammonia, liquid	3

[a] The weighted average of ammonium nitrate (2/3 of N) and urea (1/3 of N) was taken, since no emission factor is given by Asman (1992).

Table 11. Average annual precipitation for the year 2011 for region

Region	Average precipitation (mm per year) for 2011
Northern America	734
Central America and Caribbean	2,300
Southern America	1,442
Western and Central Europe	768
Eastern Europe	832
Africa	598
South Asia	1,025
East Asia	1,156
West Asia	213
Oceania and Pacific	1,802

Source: data.worldbank.org/indicator/AG.LND.PRCP.MM.

As reported in the IPCC 2007 report, nitrous oxide (N_2O) is one of the major greenhouse gases contributor with 8% to the anthropogenic global warming. Fifty to sixty percent of the anthropogenic induced N_2O emissions comes from agriculture and in particular from direct soil emissions (Mosier et al., 1998). In the IPCC 2006 guidelines the updated default emission factor for nitrogen inputs from mineral fertilizers, organic amendments and crop residues is 1%.

In several cases, the emission factor could be disaggregated based on (1) environmental factors (climate, soil organic carbon content, soil texture, drainage and soil pH) (Table 12) and (2) management-related factors (nitrogen application rate for fertilizer type, type of crop and their residues, with differences between legumes, non-leguminous arable crops, and grass) (Bouwman et al., 2002a,b; Stehfest and Bouwman, 2006). For example, crop residues left on the fields increased the soil organic content (Spinelli et al., 2013b) and also act as important source of N_2O. Harrison et al. (2002) and Novoa and Tajeda (2006) have assumed the following emission factors: 0.2% for crop residues of cereals, 2% for crop residues of vegetables and 1% for crop residues of other arable crops. N_2O emission also increases with higher clay content of the soil, because the possibility of anaerobic conditions increases (Velthof & Oenema, 1995). Lesschen et al. (2011) reported the emission factor for the type of soil: 0.86%, 1.24% and 2.61% for sand, clay and peat soils. Furthermore, there is also interference due to the land use as reported in Table 13.

Table 12. Annual N_2O emissions dependent on nitrogen inputs and their relevant environmental data in agricultural fields

Site	N_2O emission (kg N ha^{-1} yr^{-1})	N input (kg N ha^{-1} yr^{-1})	Precipitation (mm)	Temperature (°C)	STN (g kg^{-1})	pH	SOC (g kg^{-1})	Reference
Cambridgeshire, UK	1.20	190	550	12	-	7.7	29	Dobbie & Smith (2003)
Nottinghamshire, UK	2.40	165	600	12	-	5.7	11.6	Dobbie & Smith (2003)
Potsdam, Germany	0.55-3.89	150	397-569	10.1-11.3	1.4	6.3	9.0	Hellebrand et al. (2003)

Table 12. (Continued)

Site	N_2O emission (kg N ha^{-1} yr^{-1})	N input (kg N ha^{-1} yr^{-1})	Precipitation (mm)	Temperature (°C)	STN (g kg^{-1})	pH	SOC (g kg^{-1})	Reference
Siggen, Germany	2.96	200	792	7.5	4.6	7.0	47.2	Stephan & Karl (2001)
Heino, Netherlands	3.10-13.20	313-743	868-995	9.5-10.5	-	-	-	Velthof and Oenema (1995), Velthof et al. (1996)
Zegveld, Netherlands	11.90-17.30	521-713	820-894	9.5-10.5	-	-	-	Velthof & Oenema (1995), Velthof et al. (1996)
Hokkaido, Japan	3.50-9.90	270-322	642-928	13.7-17.54	2.7	5.5	29.0	Kanako et al. (2002)
Ithaca, USA	1.60-3.80	130	756-926	7.6-7.9	-	-	-	Duxbury et al. (1982)
Madison, USA	3.60-5.20	181-237	584	7.3	-	6.7	12.0	Cates & Keeney (1987)
New Delhi, India	1.09-1.64	120	750	20	0.3	8.1	4.5	Pathak et al. (2002)
Yucheng, China	2.90-3.40	420-480	610	13.1	0.5	7.9	4.5	Dong et al. (2001)
Wuxi, China	3.38-12.99	240-500	1,144-1,150	15.4-18.3	-	6.8	15.0	Zheng et al. (2004)

Table 13. N_2O emission factor for N-input sources (in %) in different land use

Source N Input	Sand		Clay		Peat	
	Grassland	Arable land	Grassland	Arable land	Grassland	Arable land
Nitrate based fertilizer	1.00	0.50	1.50	0.75	2.00	1.00
Ammonium based fertilizer	0.50	0.40	0.75	0.60	1.00	0.80

CONCLUSION

The reduction of the environmental burdens of nitrogen fertilizers in agriculture can be achieved by improving the production phase (by the use of Life Cycle Assessment methodology) and the integrated logistic phase of distribution, and by using the better management practices in the phase of field application. Environmental consequences of field application of N fertilizers represents a key and complex topic, since the environmental damages are affected by several factors: pedo-climatic conditions, crop types, soil and crop

managements, soil moisture, type of fertilizer, timing, and so on. By the way, on the base to the literature evidences exposed before, some guidelines of the better management practices (BMPs) can be identified and summarized.

Snyder et al. (2009) reported four guidelines in order to optimize N fertilizer use in agriculture:

1) when fertilizer is used to increase crop yields, it increases the efficiencies of other energy-consuming inputs used in production. However, since fertilizer use itself involves energy consumption, the importance of applying the optimum rate is underscored.
2) by increasing the net primary productivity of cropland, fertilizers can increase the return of C to the soil as crop residues, mainly as root biomass and rhyzo-deposits.
3) since demand for biofuels increases the need for higher biomass production per unit of land area, it also increases fertilizer use. If the goal is net energy production or fossil fuel offset, this fertilizer use must be efficient.
4) when fertilizer is used to increase crop yields, land for forests and other natural areas can be spared from conversion to cropland.

Several studies explored the possibility to produce fertilizers based on renewable resources. Ahlgren et al. (2008) have studied the use of hydrogen from gasified biomass for the production of ammonia (cereal straw and short rotation willow (*Salix*) coppice) with a reduction of greenhouse gas emissions compared with using natural gas. Indeed, the main energy requirements for fertilizer production are due to fuel and feedstocks used in the manufacture of ammonia (Spinelli et al., 2013b). On the contrary, eutrophication potential was higher in the biomass systems due to nutrient leaching from soil and the acidification was in the same range for both natural gas and biomass (Ahlgren et al., 2008).

Another possibilein obtaining hydrogen is to produce methane by anaerobic digestion of several type of biomass (Collet et al., 2013; Show et al., 2012) and then produce hydrogen from the methane gas. Other aspects that should be considered are the following (IFA, 2009):

- improving energy efficiency in ammonia production because the coal-based ammonia synthesis, especially for the China policy, is expected to increase in coming years and carbon capture and storage could be used to reduce the emission;
- reducing nitrous oxide emissions in nitric acid production by secondary catalytic processes to convert 70-95% of N_2O into dinitrogen (N_2) and water without additional energy use;
- reducing greenhouse gas emissions related to transport and logistics because the future growth is expected where trucking is the primary way for the distribution (Asia, Africa and South America);
- using fertilizer best available management practices to improve agricultural greenhouse gas balances.

The first fundamental choice is the use of the best N source and rates (avoid excessive N applications) for the specific crop and pedo-climatic conditions, in order to improve N crop nitrogen uptake by crops, thereby reducing the N loss via soil microorganisms transformation, as well as in reactive forms. This choice is strictly connected with timing N supply, planned

in order to coincide N applications with crop N uptake demand. Balanced fertilization should be performed by supplying all required nutrients for crop growth, in order to improve crop N use efficiency, and maximize CO_2 fixation. Calibrated fertilizer application can be achieved by using equipment that ensure accurate delivery of prescribed N rates and proper placement. Overlaps application should be avoided. In intensive crop systems of arid and semi-arid lands, an effective irrigation scheduling is basic, not only to improve crops performance but also to keep soil moisture in that value (40-60% WFPS) to reduce the N loss as N_2O. Correct irrigation reduce the also the risk of nitrate leaching. Moreover, in order to avoid NO_3^- leaching, the application of NO_3^- based or any N sources on wet or waterlogged soils and on highly permeable soils should be avoided.

Cover crop can be a successful management to guarantee continuous N uptake, particularly after the application of fertilizers with slow release of N. Soil tillage represents a management that drain soil, reducing in that way anoxic conditions favorable to N_2O emission. Recently, positive results have been obtained by using nitrification and urease inhibitors at reducing N_2O and NH_3 loss, respectively.

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Chapter 2

ENVIRONMENTAL IMPACT OF PHOSPHATE FERTILIZERS AND BY-PRODUCTS ON AGRICULTURAL SOILS

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ABSTRACT

Soil amendment with phosphate fertilizers and by-products such as phosphogypsum becomes of an increasing importance in agriculture. This may lead, however, to soil, plant and groundwater contamination with trace elements inherently present in both products. Monitoring of selected trace elements (Cu, Pb, Zn and Cd) distribution and mobility in a Mediterranean red soil profile has been performed in soil and plant samples collected from various depth intervals at different points in time. Trace elements sequential extraction was conducted on collected samples. Samples were examined under a Scanning Electron Microscopy (SEM) coupled with an Energy Dispersive X-ray spectrometer (EDX). Phosphogypsum increased the solubility of the studied trace elements where they were bound to exchangeable and acid-soluble fractions in higher percentages than reference soil. After the period study of phosphogypsum amendment (16 months), Pb, Zn and Cu were sorbed into mineral soil phases while Cd was mainly found in the exchangeable form. On the contrary, Pb, Zn and Cu were transferred from residual to exchangeable fraction except for Cd in fertilizers application. Cichorium intybus accumulated higher trace elements concentrations than the reference plants, but they remained within normal reported levels except for Cd where it exceeded the recommended tolerable levels in phosphogypsum application, thus signifying potential health threats through contaminated crops. Evidence of fluorine was detected by SEM in the fertilizers amended soil which should be a matter of concern in phosphate fertilizers application.

Keywords: Soil amendment, trace elements, mobility, plant uptake

INTRODUCTION

Large application of phosphate fertilizers and by-products has been practiced on arable lands, to improve crop production, induced soil nutrients deficiency, and increasing the levels of available S and P. However, contamination of agricultural soils with trace elements (TEs) such as Cd, Pb, Zn and Cu, and with fluorine can occur as these elements are transferred during manufacturing from phosphate rock to phosphate fertilizers (PFs) and by products such as phosphogypsum (PG) which is a di-hydrate calcium sulfate. Concentrations of Cd, Pb, Zn and Cu can vary in PFs and PG depending on the origin of phosphate rock and the process used.

Many studies have been conducted on assessing the use of PFs and PG as amendment on agricultural soil (Adriano 1986; Arocena et al. 1995; Conkline 1992; Guttormsen et al. 1995; Jeng and Singh 1995; Lambert et al. 2007; Loganathan et al. 1995; Malavolta 1994; McLaughlin et al. 1996; Morvedt 2005). Cd has been the most concerned element in PFs application since it can accumulate in relatively high amounts in soil and plants which could be harmful to human health (Kirkham 2006). Al-Masri et al. (2004) indicated that Cu, Zn and Cd could be transferred from PG into water and subsequently to the soil horizons, which should be considered when PG is used as amendment to agricultural soils. Furthermore, in studying the cumulative effect of the amendment of PG on the uptake of elements by tomatoes, Abril et al. (2008) showed a possible direct effect of PG amendment in increasing Cd levels in tomato crops, which was less than the permissible limit (50 ppb). On the other hand, earlier studies showed that PFs application in recommended rated on Brazilian soils did not increase TEs concentration to hazardous levels in short and medium terms (De Conceicao and Bonotto 2006; Malavolta 1994). Depending on their different forms and phases in soil or mobility, TEs can be accumulated in plants, migrated into groundwater, or associated with different soil components (e.g., organic matter, clays, Fe and Mn oxides, carbonate minerals). Determination of different forms or phases of a TE, referred to as speciation, is primordial to assess its mobility and thus the potential risk of its bioavailability. Soil properties such as pH play a key role in releasing or holding a TE in a defined phase (Kabala and Singh 2001).

A recent study on the effect of phosphate industry emissions on soil contamination showed that PG had the highest contribution in TEs input into the surface soil (Kassir et al. 2012b). Results suggested that soil contamination with TEs carried with PG and phosphate particulates could be potentially hazardous to plants and groundwater. Nevertheless, further investigations on the behavior, forms and dynamics of these TEs in the soil had been recommended to better assess the risk factor.

It follows that our study has been focusing on the time variation aspect of TEs mobility following PFs and PG application on soil and their transfer factor to plants.

METHODS

Experimental Study Area

For this purpose, an experimental field has been realized in an area located off the Mediterranean east cost in north Lebanon, and extends over 100 km^2 of agricultural lands

(Figure 1). Agriculture is mainly dominated by plantations of olive trees, which is the main crop of the study area. The region has a Mediterranean climate with intensive precipitations between January and May (600-900 mm). According to Darwish et al. (2005), the study area was dominated by a well-drained red soil classified as an association of Gleyic and Vertic Luvisols. The texture of the soil is clay (sand 24%, silt 20%, and clay 56%) with a calcium carbonate reaching 22%. Despite its clay loamy texture, the red Mediterranean soil is distinguished by a strong granular surface structure and porosity promoting intermediate and high soil permeability (Darwish and Zurayk 1997). The organic matter content is low (1.3%). Medium to coarse sub angular and angular blocky aggregates represent the strong structure of the soil. Common fine to medium roots are also found. Common plants growing between olive trees were of *Cichorium intybus* species.

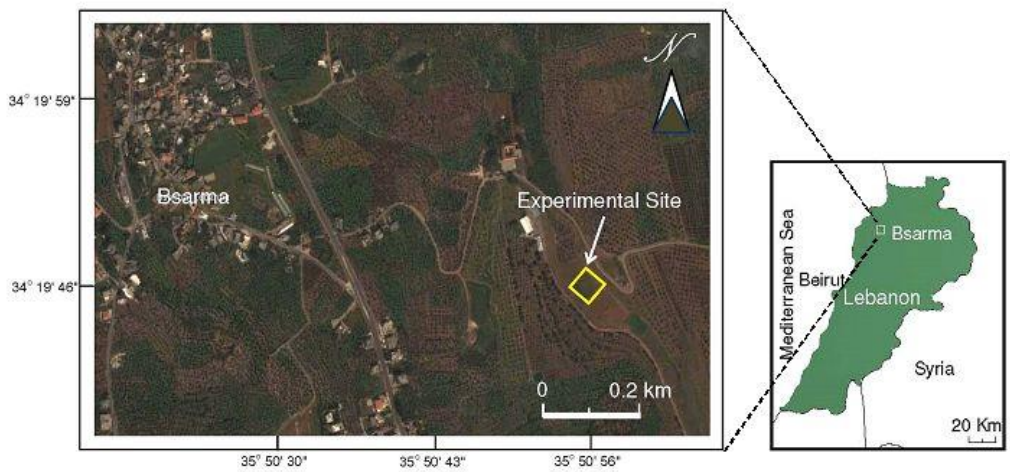

Figure 1. Experimental site localization map.

The field site consists of a land frame of about 256 m^2. The study plot was divided into 24 separate parcels of 1 m^2 each spread over a surface of 49 m^2 and separated by distances of 75 cm (Figure 2). Each parcel was delimited with a wood frame of 1 × 1 × 0.15 m (length × width × height). To simulate the potential source of pollution originating from PF and PG-amended soils, 12 parcels were used for PG application and 12 parcels for PF application as 1 kg and 2 kg of PG and PF respectively were dispersed on the soil surface in every parcel over a surface of 0.5 m × 0.5 m, without homogenization with the soil. A reference parcel was left without amendment.

The PF application quantity (2 kg) is equivalent of an intensity of 8 kg m^{-2} (80,000 kg ha^{-1}) application, about forty times higher than the fertilizer yearly use of 1860 kg ha^{-1} on arable soils in Lebanon (Farajalla et al. 2010). The purpose of this abundant application was to subject the soil to leveraged state of influence induced by PFs application, so as extreme ecological responses to such stimulus could be reached and measured.

Coring was carried out to a depth of 60 cm, using a high power (2,500 W) motor of type Cobra TT. The core shaft used was 100 cm long, 6 cm in diameter. The first soil sample was cored from the reference parcel labeled as R. Then, sample coring had been successively performed in six different parcels labeled as PG1, PG2, PG3, PF1, PF2, and PF3 at six

different subsequent dates: T1=5, T2=12, T3=16 months for PG application and T1=4, T2=11 and T3=15 months for PF application after the initial application and sampling from the reference plot R at T0. Samples were collected from the cored cylindrical soil blocks at 5 cm intervals to a depth of 20 cm, then at 15 cm intervals to a final depth of 55 cm. Plant roots and leaves were also collected simultaneously with soil samplings from each parcel.

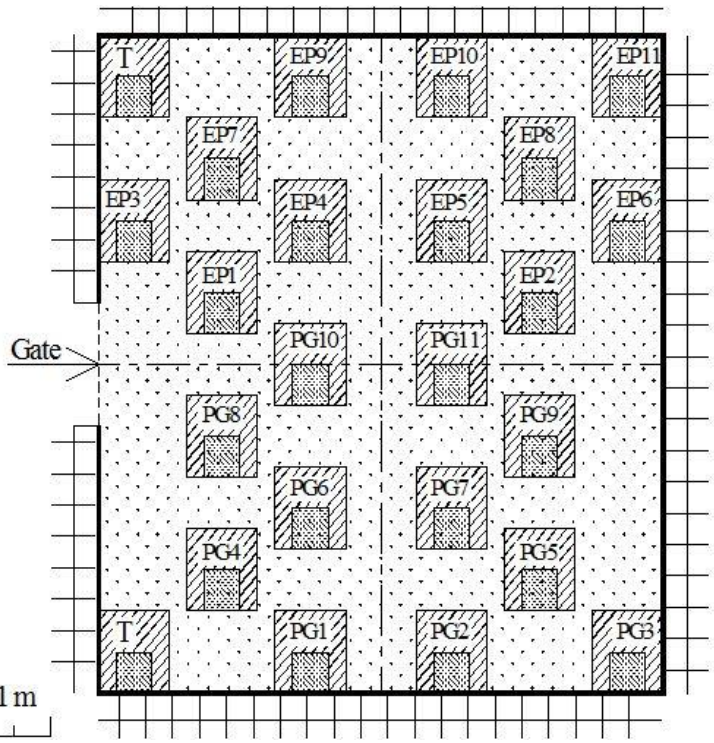

Figure 2. Experimental plot and parcels distribution.

Analytical Methods

Soil samples were cleaned from roots and stones, oven-dried at 50 ºC, sieved to pass a 2 mm sieve and ground to a fine powder using an agate pestle and mortar. Whereas, vegetation samples were washed in ultra-pure water, dried at 60 ºC, and ground to a fine powder using an agate pestle and mortar. The following methods were applied on soil and vegetation samples. Soil pH was determined in a ratio 1:5 soil:ionized water suspension according to AFNOR X 31-103. Total organic carbon (TOC) in soil samples was determined using elemental analyzer of type (Flash EA1112 NC). Cation exchange capacity (CEC) of the soil is measured by displacing the exchangeable cations with a solution of cobalthexamine chloride. The displaced cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) are determined by the flame atomic absorption spectroscopy and the summation is the cation exchange capacity.

Trace elements were measured using an Iris advantage ERS Inductively Coupled Plasma-Optical Emission Spectrometer ICP-OES "ThermoFischer" and trace elements by ICP-MS X7 "ThermoFisher", after melting the samples and phosphogypsum with lithium metaborate,

and dissolution of the melt in dilute nitric acid. Analysis was conducted at SARM (CRPG, Nancy). Ignition loss was determined by heating the samples at 980 ^{o}C during 3 hours.

Vegetation samples and Reference soil samples (IAEA-405) were accurately weighed to approximately 0.25 g directly in microwave PTFE vessels and subjected to 1 mL H_2O_2 (30%) and 9 mL concentrated HNO_3 (65%). The digestion program itself consisted of a 10 min gradual increase in temperature to 200 °C, a 15 min step at 200°C (1,000 W; 106 Pa) and then a stage of ventilation cooling. The digests were filtered through a 0.45 µm and analyzed using an Atomic Absorption Spectroscopy (AAS) "Analytical Gena Zeenit 700" to determine the content of Pb, Zn, Cu, and Cd in roots and leaves plants.

The sequential extractions were carried out progressively on an initial weight of 5 g in polypropylene centrifuge tubes of 50 mL. Five fractions were separated in 5 steps as follows: (1) exchangeable with 40 mL of 1 M $Mg(NO_3)_2$; (2) acid soluble extracted by 40 mL of 1 M CH_3COONa adjusted to pH 5 with CH_3COOH; (3) reducible extracted by 20 mL Na-citrate (78,4 g L^{-1}) and 20 mL $NaHCO_3$ (9.82 g L^{-1}) then adding 1 g of $Na_2S_2O_4$ (80 °C for 4 h); (4) oxidizable extracted by adding 8 mL HNO_3 (0.02M) and 20 mL of H_2O_2 (35%) in small aliquots then 12 mL of 3.2 M CH_3COONH_4 in nitric acid (85 °C for 2 h); (5) residual extracted by 40 mL HNO_3 (65%). Each extraction step was followed by centrifugation the mixtures at 5,000 rpm for 30 minutes and the solutions were separated from the solid residue. Liquors were first filtered on with a membrane filter of ester cellulose of 0.45 µm porosity.

All the solutions were stored in polyethylene vials at 4 °C until analysis and then analyzed by Atomic Absorption Spectroscopy (AAS) to determine the content of Pb, Zn, Cu and Cd in each extracted fraction. Reagent blanks were also analyzed in order to monitor analytical accuracy and precision.

Mineralogical Analysis

Samples were crushed and homogenized before analysis by X-ray diffraction. About 500 mg of homogenized and ground samples were deposited as sub compacted powders within a thin layer of 1mm thickness on a plexiglass disc of 2 cm diameter. Diffractograms were collected and recorded by using a D8-Bruker diffractometer (cobalt radiation source, λ = 1.7889 Å) operating with reflection mode and a 3 s of acquisition time.

Electron microscopy observations were performed with an S-2500 Hitachi SEM (Scan Electron Microscopy) equipped with a Kevex 4850-S EDX (energy dispersive X-ray spectrometer). Trace elements carriers were identified from elemental analysis of individual particles. Stoichiometric ratios were first calculated from atomic percentages given by EDX spectra, and then compared with known mineralogical compositions. For SEM imaging and microanalysis, the powder sample was re-suspended in ethanol under ultra-sonication, and a drop of suspension was evaporated on a carbon-coated copper grid (EuroMEDEX, Mesh200). Samples for SEM-EDX examination were sprinkled onto 2 cm^2 plates and carbon coated. Backscattered Electron Imaging (BEI) was used to identify the particles of interest. In that mode, brightness is related to the average atomic number of materials, and the mineral particles appear as bright spots within the matrix of sediments. The relative abundance of a given heavy metal carrier can then be assessed by conducting systematic microanalysis of bright spots. It should nevertheless be noted that this procedure largely overlooks mineral

phases with low atomic number elements. In order to increase the emission of backscattered electrons, the SEM microscope was generally operated with a beam current of 3 pA and an accelerating voltage of 20 keV.

Statistical Analysis

ANOVA tests were performed on the samples data of trace and major element concentrations and pH values obtained for the soil profile in all parcels. The data was distributed in different levels of two categories; time (T0, T1, T2, and T3, corresponding to parcels R, PG1, PG2, and PG3, PF1, PF2, PF3) and depth (horizons 0-5 cm, 5-10 cm, etc.). When significant differences were found, a multiple comparison of mean values was carried out by the Walker-Duncan test ($P < 0.05$). Normality of variances was examined by the Shapiro-Wilk test, before running ANOVA. Correlation analysis (Pearson r) was carried out between trace and major elements, TE concentrations and soil characteristics. All statistical analyses were performed using SPSS Version 17.

RESULTS AND DISCUSSION

Soil Background Reference

The mineralogical results (XRD) showed that the background soil contained Quartz (SiO_2), Calcite ($CaCO_3$), clay minerals (Montmorillonite, Kaolinite), Anatase (TiO_2) and Hematite (Fe_2O_3). The soil pH showed alkaline range (8.23 ± 0.12), and the cation exchange capacity (CEC) exhibited relatively high values (31.06 ± 0.5 cmol kg^{-1} of dry weight) with a dominance of calcium ions at the exchange sites.

The average background soil contained relatively low Cd concentration (0.30 ± 0.02 mg kg^{-1}) and high Zn concentration (103.35 ± 6.72 mg kg^{-1}). As for Cu and Pb, their average concentrations were found to be 35.84 ± 2.33 mg kg^{-1} and 11.50 ± 0.75 mg kg^{-1}, respectively. No significant differences were found in TEs distribution with depth (till 55cm), except in layer (30-55 cm) where Cu and Pb concentrations decreased with respect to upper layers.

It follows that, the study area presented elevated Zn total concentrations and slightly elevated Cu concentration when compared to the world agriculture soils (20-30 mg kg^{-1} and 50 mg kg^{-1} for Cu and Zn, respectively) (Alloway 1995). Pb and Cd concentrations were within the range of agricultural and normal soils (10-30 mg kg^{-1} and 0.2-1 mg kg^{-1} for Pb and Cd; respectively) (Alloway 1995; Baize 1997). Moreover, the total Zn and Cu concentrations exceeded the background values (12 ± 2 and 64 ± 2 mg kg^{-1} for Cu and Zn, respectively) reported for the arable soils of North Lebanon (Nsouli et al. 2004). In general, the natural occurrence and concentration of the studied elements were homogenous with depth. However, Cu and Pb concentrations decreased in the deeper layer (30-55 cm). Chemical speciation of the studied TEs in the reference soil showed they were mainly associated with Fe and Mn oxides and hydroxides (reducible fraction F3) (64, 45, 43 and 13% for Cd, Pb, Zn and Cu, respectively) and bound to the structure of clays (residual fraction F5) (63, 56, 23 and 11% for Cu, Zn, Pb and Cd, respectively). This partition of the studied elements is due to the

soil nature (Luvisol) with a dominance of swelling-shrinking clays and iron oxides, which agrees with the findings of previous works (Darwish et al. 1988; Lamouroux et al. 1968; Sayegh et al. 1990). In addition, Cu was accumulated in the oxidizable fraction (F4) (20%), in agreement with other findings reported in the literature (Kuo et al. 1983; Zaccone et al. 2007).

Characterization of Amendment Product

PF and PG Amendment

XRD results of PF showed that the main peak recorded in the diffractogram was calcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) and to less extent Anhydrite ($CaSO_4$). The presence of $CaSO_4$ in PF is the result of the acidulation of phosphate rock with H_2O and H_2SO_4. The PF sample showed an acidic pH value of 2.63 ± 0.8. As for PG, XRD results showed that the main peak recorded in the diffractogram was Gypsum ($CaSO_4.2H_2O$) and to a less extent Bassanite ($CaSO_4 \cdot 1/2H_2O$) and Anhydrite ($CaSO_4$). The measured pH value of 6.51 ± 0.5 showed slight acidity of the PG sample.

TEM elemental mapping of PF showed particles of phosphorus, sulfur and calcium. Ca was associated with sulfur (Ca/S=1) and with phosphorus (Ca/P=0.5). Particles of gypsum ($CaSO_4$) and acidulated P-fertilizers ($Ca(H_2PO_4)_2$) were thus identified.

The total concentration of the studied elements (Cu, Zn, Pb and Cd) in the Lebanese manufactured PF showed that Zn was the most abundant TE (199.3 mg kg^{-1}), with the lowest concentration being for Pb (2.66 mg kg^{-1}). Copper and cadmium concentrations were found to be 16.62 and 6.18 mg kg^{-1}, respectively. These results were generally within the average metal concentrations analyzed from a total of 196 European PFs (Cd, 7.4; Zn, 166; Pb, 2.9 mg kg^{-1}) (Nziguheba and Smolders 2008). The total concentration of the studied elements (Cu, Zn, Pb and Cd) in the Lebanese manufactured PG showed that Zn was the most abundant TE (123.60 mg kg^{-1}), with the lowest concentration being for Pb (2.46 mg kg^{-1}). Copper and cadmium concentrations were found to be 5.13 and 3.44 mg kg^{-1}, respectively.

Results of PF speciation showed that the studied metals (Zn, Cd, Cu, and Pb) were mainly present in the exchangeable fraction (F1) (60%, 41.9%, 40.7% and 38%, respectively). They were also found in the acid-soluble fraction (F2) (27.7%, 10.4%, 19% and 34.7%; respectively). Moreover, part of these TEs mass (27% and 23.6% for Pb and Cd; respectively) was bound with reducible fraction (F3). According to the sequential extractions of TEs in PG, however, results of PG speciation showed that the studied metals (Pb, Cu, Zn and Cd) were generally present inside the gypsum lattice (F2) (30%, 47%, 28% and 54%; respectively). Moreover, part of these TEs mass (30, 18, and 32% for Zn, Pb and Cu; respectively) was bound with organic matter (F4) incorporated within the PG. In addition, Pb was also found in the residual fraction (F5) (46%), Cd in the reducible fraction (F3) (20%) and Zn in silica fraction (F5) (36%).

Variation of Total Concentrations in Soil Profile

To analyze the PG or PF originated TEs transfer in soil with time, the concentrations of Cd, Zn, Pb, and Cu, in different soil horizons were plotted against time for the reference parcel R at T0 and incubated parcels PG1, PG2, PG3, PF1, PF2 and PF3 (corresponding to T1=5–, T2=12– and T3=16–month period for PG amendment; T1=4, T2=11 and T3=15 month period for PF amendment). The time axis was started at the date when the PG or PF

was just applied on the study parcels; the corresponding metal concentrations at the different depths represent the soil background concentrations (Figure 3 and Figure 4).

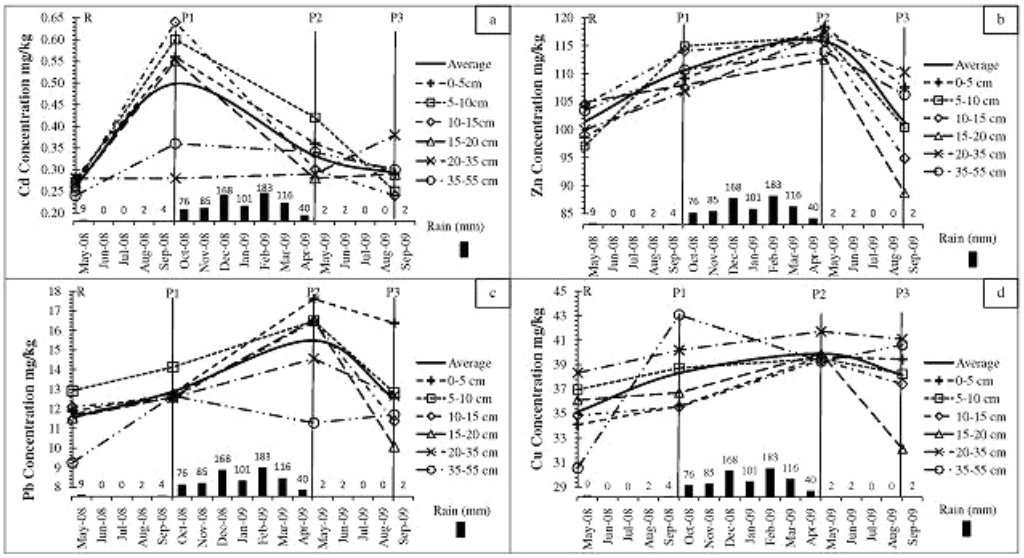

Figure 3. Time-variation of TEs concentrations in the soil profile – Reference parcel R (T=0); PG-amended parcels P1 (T1 = 5 months); P2 (T2 = 12 months); and P3 (T3 = 16 months).

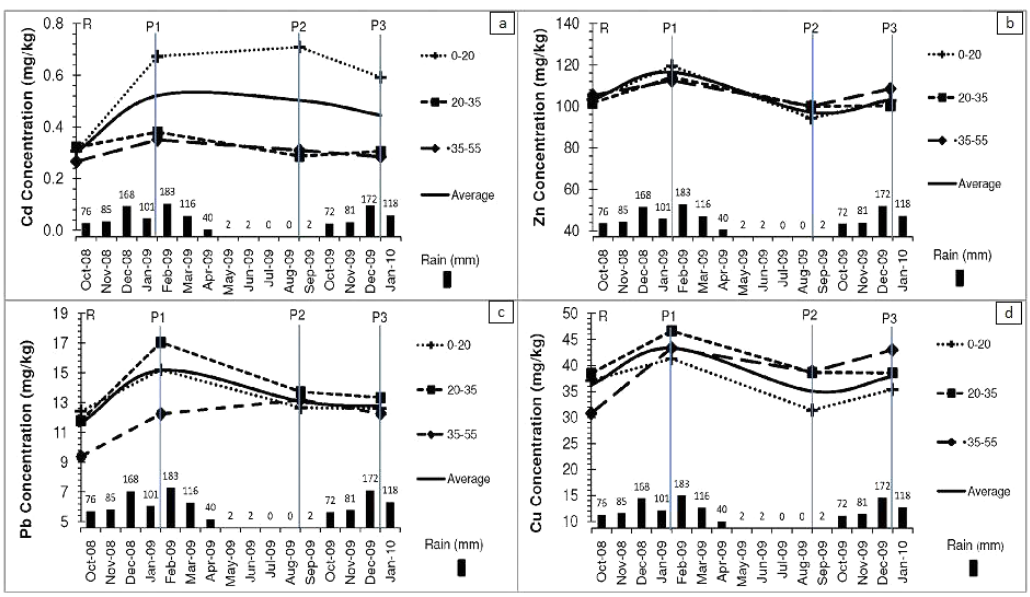

Figure 4. Time-variation of TEs concentrations in the soil profile – Reference parcel R (T=0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months).

It could be seen from PG application Figure 3, for the overall depth, that TEs showed different periods for reaching their peak concentration values. Peaks for TEs occurred in PG1 for Cd (in September) and PG2 for Zn and Pb (in April) before it decreased gradually with time (Spring-Summer) to reach a minimum value in PG3 (in August) within the initial

background range. The time-variation of Cu concentrations over the studied depths exhibited generally a steady function increasing at slight rate with time.

Following PG amendment, a general increase of TEs concentration could be observed. During rainy period (October - April), Cd concentration decreased (0.5 to 0.35 mg kg^{-1}) (Figure 3), whereas Zn, Pb and Cu concentrations were generally increasing at almost all depths (110.61 to 115.76, 12.88 to 15.49, and 38.31 to 39.90 mg kg^{-1} respectively). However, in the deeper layers (35-55 cm), Pb and Cu concentrations decreased ($P< 0.01$) while Zn concentration showed no significant difference between parcels in this particular layer. Cd concentration displayed a significant difference ($P < 0.05$) between parcels (R, PG1, PG2 and PG3) only in the upper layers (0-20 cm). A general remarkable decrease in TEs concentrations was observed over the study period in the layer (10-20 cm) particularly over the spring-summer period. On the other hand concerning PF application, to simplify the tracking of the profiles variations, the eight layers in each parcel were grouped in three depth zones: upper (0-20 cm), intermediate (20-35 cm), and lower zone (35-55 cm); zone TE concentration was taken as the average content of TE in the zone layers. Generally, a similar trend was observed for the studied TEs. For the overall profile, peak concentrations of TEs (Cu: 43.13, Cd: 0.52, Zn: 116.36, and Pb: 14.92 mg kg^{-1}) occurred four months after amendment (in January, parcel P1). Then, TEs average total concentration decreased between parcels P1 and P2 (February-August) to finally reach near background values in parcel P3 (December) (Figure 4). However, Cd average concentration remained relatively constant between parcels P1- P3 (0.44 to 0.52 mg kg^{-1}). Changes in Cd concentration between parcels occurred only in the upper layers (0-20 cm) (0.31 to 0.71 mg kg^{-1}), thus not reaching deeper layers, indicating possible in situ release and recovery. A soil enrichment of about 0.30 mg kg^{-1} (~100% enrichment) in Cd remained in P3 (11 months after PF application) in the upper zone (0-20 cm). Layer 35-55 cm showed an increase in Pb content between P1 and P2 (February-August). Practically, with the exception of Cd, slight variations in TE total concentrations occurred between P2 and P3 (August-December), where the TEs content in the soil approached the background levels, indicating the direct effect of PF on the soil profile took place mainly during around a year following PF application. No evidence of released TEs accumulation in the soil was observed by the end of the sampling period (15 months after PF application)–Except for Cd which was somewhat accumulated in the upper layers (0-20 cm). However, Cd content in this layer was decreasing between P2 and P3, and by extrapolation, it would eventually reach the background level.

A decrease of soil pH following the PF amendment was detected from the surface (0-5 cm) till layer 15-20 cm that showed the lowest value with slightly acidic pH (6.6 ± 0.2) in parcels P1 and P2. Statistically, the fluctuation of TEs concentration in all depths with time proved a significant difference in the average concentration between parcels for Cu, Zn and Pb. The upper layers of the parcels (down to 25 cm) showed a significant difference with time for Cd.

As a result of local and temporary pH decrease after PFs amendment, and heavy rainfall during October-January, TEs average concentrations increased in the soil profile to reach their highest values in parcel P1 (Figure 4). However, despite the relatively massive quantity of PFs amendment, their concentration remained below the permissible limits of TEs in agricultural soils (Cu: 63, Cd: 1.4, Zn: 200, and Pb: 70 mg kg^{-1}) (CCME 1999). TEs were transferred from PF and moved downward through the soil profile with incoming rain water creating temporary leaching conditions. Generally, the continuous rainfall between parcels P1

and P2 (particularly between January and April) caused TEs dilution, and thus a significant decrease in Zn, Cu, and Pb average concentrations was observed in the soil profile, and this trend persisted in the period between April and August, due to TEs uptake by growing plants. However, Cd nearly constant average concentration in the soil profile between parcels P1 and P2 indicated its accumulation in the soil matrix. In the period between September and December (P2-P3), the drying-wetting cycle of the soil caused the re-augmentation of Zn and Cu average concentrations in the soil profile. However, a different behavior was observed for Pb and Cd that showed a slight decrease of their average concentration between P2 and P3 (13.06 and 12.70 mg kg^{-1} for Pb, 0.50 and 0.44 mg kg^{-1} for Cd in P2 and P3, respectively), indicating possible uptake by plants or leaching toward the saturated zone.

Metals Mobility in Soil

In order to assess the chemical form of TEs and thus their mobility and potential risk on the environment, speciation of Cu, Cd, Zn and Pb were performed in all parcels (R, PG1, PG2 PG3, PF1, PF2 and PF3). Five geochemical fractions were determined: the exchangeable fraction (F1), the acid-soluble fraction (F2), the reducible fraction (F3), the oxidizable fraction (F4) and the residual fraction (F5). The time-variations of concentrations of TEs in the chemical fractions (F1, F2, F3, F4 & F5) at the different study depths (0-20 cm, 20-35 cm, and 35-55 cm), were plotted on separate charts for each of the considered metals (Cu, Cd, Zn & Pb) over the study period, as shown in Figure 5 and Figure 6. Different behaviors were shown for the selected metals in the two applications as we explained below:

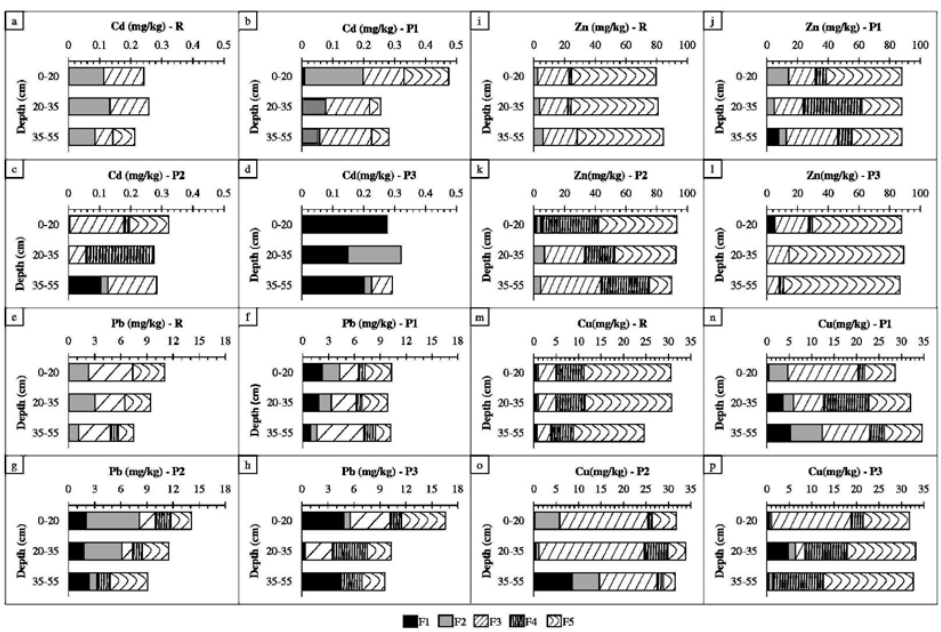

Figure 5. Time-variation of TEs geochemical fractions concentrations in the soil profile – Reference parcel R (T=0); PG-amended parcels P1 (T1 = 5 months); P2 (T2 = 12 months); and P3 (T3 = 16 months). F1–Exchangeable; F2–Acid Soluble; F3–Reducible; F4–Oxidizable; and F5–Residual fraction.

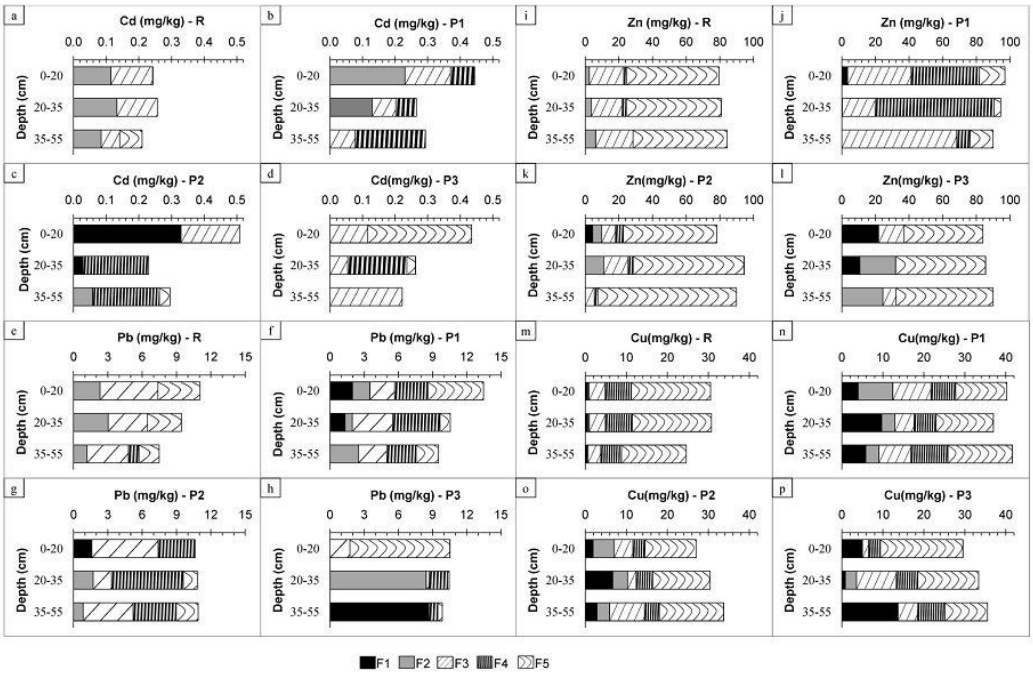

Figure 6. Time-variation of TEs geochemical fractions concentrations in the soil profile – Reference parcel R (T=0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months). F1–Exchangeable; F2–Acid Soluble; F3–Reducible; F4–Oxidizable; and F5–Residual fraction.

Cd Speciation

When PG was amended, Cd solubilization was increased at the depth 0-20 cm (from 46 to 61%, respectively at reference and PG1 parcels) as Cd was found associated to the mobile fractions (exchangeable and acid-soluble fraction F1 and F2) in the parcel PG1, 5 months following PG amendment (Figure 5); this behavior was due to pH decrease (from 8.24 ± 0.13 to 7.73 ± 0.08). This increase was in the detriment of Fe and Mn oxides (F3) (53 to 34% in R and PG1, respectively). In addition, 12 months following PG amendment and after a period of precipitations (winter season), Cd was related to the exchangeable fraction F1 at the depth 35-55 cm. Cd transferred from acid soluble phase of PG (54%), was dissolved in soil profile, and migrated to a depth of 55 cm, which explained the increment of 20% of Cd in the mobile phase when comparing to PG1. The exchangeable fraction was predominated at the end of the study in the PG3 (16 months following PG amendment) in soil profile (87, 90 and 77% in 0-15 cm, 15-35 cm and 35-55 cm, respectively). In soils and sediments polluted with metal wastes, the greatest percentage of the total Cd was associated with the exchangeable fraction (Kuo et al. 1983; Tessier et al. 1980).

On the other hand, a different behavior for Cd was observed in PF application (Figure 6). Cd concentrations increased in the top layer (0-20 cm) in the mobile fractions (F2 and F1 in parcels PF1 and PF2, respectively). Furthermore, Cd related to the oxidizable fraction (F4) appeared in all depths of the soil profile of parcel PF1 (4 months following PF application) and increased at depth 20-55 cm of parcel PF2 (11 months following PF application).

At T3 (fifteen months after PF amendment), the reducible and residual fractions were dominant. The change in Cd speciation over the study period can be attributed to phosphate fertilizer application decreasing soil pH in parcels P1 and P2 at the surface layer (0-20 cm). It is well reported that under acid conditions, Cd was found accumulated in the exchangeable fraction and acid soluble fraction (F1 and F2) (Castillo-Carrion et al. 2007; Li and Thornton 2001). In the end of our study, under alkaline soil conditions, Cd was adsorbed to iron and manganese oxides, organic matter, and clays structure.

Pb Speciation

Concerning PG application, Pb revealed a higher mobility in the amended plot P1 (5 months following PG application) than the reference plot R where Pb appeared in the exchangeable fraction F1 in all depths (2.29 mg kg^{-1} at 0-20 cm; 1.87 mg kg^{-1} at 20-35 cm; 0.91 mg kg^{-1} at 35-55 cm) (Figure 5). At the surface layer (0-20 cm), the amount of Pb in the mobile fractions (F1 and F2) were 20 and 40% in R and P1, respectively. This increase was in the detriment of Fe and Mn oxides (45 and 22 %). This represents a shift of 20% to the mobile fractions. Pb became in a soluble phase and easily translocated down the profile as pH decreased in parcel P1. Pb associated to the mobile fractions (F1 and F2) predominated in parcel P2 (12 months following PG amendment). In parcel P2 (after the wet period), and comparing to P1, this continuous increase of Pb content in the mobile fractions (14, 12 and 20% in 0-20, 20-35, 35-55 cm, respectively), compared to clays and iron oxides fractions, was due to Pb release associated with acid soluble fraction of PG (30%) during rainy periods. At T3 (16 months after PG amendment, summer period), Pb was found related to reducible and clays fractions (F3 and F5) however the amount of exchangeable Pb fraction (F1) in parcel P3 was significant at 0-20 cm and 35-55 cm depth (4.84 and 4.32 mg kg^{-1}, respectively) while Pb was found accumulated (4.02 mg kg^{-1}) in the organic matter and sulfur phase (F4) at 20-35 cm layer. In deed, 18% related to oxidizable fraction was released from PG (in dry condition), due to the high porosity of PG (Rabi and Mohamad 2006) and adsorbed to soil constituents.

On the other hand, when phosphate fertilizers was applied, 81% of Pb was mainly related to the acid soluble fraction (F2) in layer 20-35 cm and 88% to the exchangeable fraction (F1) in layer 35-55 cm at the end of the study (15 months following Pf application) (Figure 6). Hence, Pb solubility increased in depth layers 20-55 cm. However, it is remarkable the appearance of Pb in exchangeable (F1) and oxidizable fractions (F4) in parcels P1 and P2 (4 and 11 months respectively following PF application) comparing to reference plot R. The appearance of Pb in the exchangeable fraction F1 in parcels P1 and P2 (acid pH) is in agreement with different studies (Castillo-Carrion et al. 2007). It can be ascribed to the input of phosphate fertilizer with speciation results showing that 38% of Pb occurred in the exchangeable fraction F1. Pb binding to oxidizable fraction F4 has been reported in the literature (Chaney et al. 1988), generally in the detriment of the residual fraction (F5). The presence of complexing and organic ligands and competing cations, decreasing Pb sorption, could substantially enhance its mobility (Kotuby-Amacher and Gambrell 1988; Puls et al. 1991), which may lead to reinstating, to some extent, the exchangeable fraction F1 (P3 layer 35-55 cm).

Zn Speciation

Phosphogypsum application varied Zn speciation only in parcels P1 and P2 (5 and 12 months following PG application) to finally be restored as reference speciation at the end of the study (16 months following PG application) where the reducible and the residual fractions (F3 and F5) were again dominant in the soil control section under study (Figure 5). However, the mobile fractions (F1 and F2) increased in the soil profile of parcel P1 (5 months following PG application). Indeed, the amount of Zn in the mobile fractions at the layer 0-20 cm is 3 and 16% for parcels R and P1, respectively. This increase was in the detriment of clays fractions (F5). This represents a relative shift of 13% to the mobile fractions. Moreover, Zn was found mostly associated to the oxidizable fraction (F4) in parcel P2 (12 months following PG application). With pH increase due to soil buffering capacity, Zn was finally intercepted by reactive negatively charged soil constituents such as phyllosilicates and iron oxides (Fernandez et al. 2007; Van Oort et al. 2006).

Contrary, Phosphate fertilizer application varied Zn speciation in the soil horizons where the greatest proportion of Zn resided in the oxidizable fraction (F4) in parcel P1 (4 months following PF application, 42 and 75% in layers 0-20 cm and 20-35 cm, respectively), in the detriment of the residual fraction (F5) (Figure 6). This can be related to the soil organic matter's affinity for soluble Zn (Kabata-Pendias and Pendias 1984). Zn was mainly sorbed in clays in parcel P2, 11 months following PF application. At the end of the study (15 months after PF application), Zn mobility was modified, where Zn in the exchangeable and acid soluble fractions (F1 and F2) was in relatively higher concentrations of 21.90 mg kg^{-1}–26%, 31.94 mg kg^{-1}–37%, and 24.40 mg kg^{-1}–27% at 0-20, 20-35 and 35-55 cm, respectively. Zn solubility increased, comparing with parcel P2, in the detriment of residual fraction (at 0-20 and 35-55 cm) and reducible fraction (at 20-35 cm) (net shifts of 14%, 15% and 27% at 0-20, 20-35 and 35-55 cm, respectively, were observed from Fe/Mn oxides and clay fractions to the mobile fractions (F1 and F2).

Cu Speciation

Cu was found in a soluble phase following phosphogypsum application in parcel P1 where Cu was associated to the mobile fractions (exchangeable and acid-soluble) in the soil profile (till depth of 55 cm), due to pH decease (Figure 5). In fact, the presence of Cu in the mobile fractions of parcel P1 increased with depth. This increase continued in parcel P2 only at depth 35-55 cm during winter season to reach 11 %. At the end of the experiment (16 months), Cu was partitioned in the reducible, oxidizable and alumino-silicate fractions of the soil in P3 as reference speciation. However, 19% of Cu content was present in the mobile fraction (F1 and F2) in the layer 20-35 cm. Phosphate fertilizers application induced a mobilization of Cu in the soil profile of parcel P1 (winter period) (3% to 32%, 3% to 35%, and 3% to 23% increase in mobile fractions (F1 and F2) at 0-20, 20-35 and 35-55 cm, respectively, comparing R to P1), with a decrease occurring mainly in the fraction associated with clays (F5) (63% to 32%; 63% to 35%; 64% to 36% at 0-20, 20-35 and 35-55 cm, respectively) (Figure 6). At parcel P2, (summer period), Cu concentrations decreased mainly in the mobile fractions and particularly at layer 0-35cm, this may be due to plants absorption at this period. At the end of the experiment in P3, Cu was partitioned in the reducible, oxidizable and alumino-silicate fractions of the soil. However, 17% and 39% of Cu content was present in the exchangeable fraction (F1) in layers 0-20 and 35- 55 cm.

Zn, Pb, Cu versus Cd

The studied elements were mainly associated to the reducible and residual fractions in reference soil. The application of PG or Phosphate Fertilizers induced a variation in soil speciation. Fe/Mn oxides and clays were the most susceptible to changes that resulted in an increase of Pb, Zn and Cu solubility in soil profile. The presence of soluble phosphates and sulfates in PF amendment (at least 85-90% of the total P in PF is water-soluble) (Chien et al. 2011) may have had a significant impact on Pb, Zn and Cu solubility. Moreover, phosphate fertilizers speciation showed that these elements were associated to the mobile fraction (exchangeable fraction F1) in relative high percent. After fifteen months of amendment (parcel P3), comparing to the reference plot, the mobile fractions and mainly the exchangeable fraction (F1) showed increments in Cu, Pb and Zn amounts relative to the reference (14%, 7.7%, 35.5% for Cu; 23%, 32.9%, 20% for Zn at 0-20, 20-35 and 35-55 cm respectively; and 48%, 72.4% for Pb at 20-35 and 35-55 cm, respectively), which could mobilize these TEs in soil profile, imposing a threat to the ground water. On the contrary, Cd was sorbed in soil particles (clays and Fe/Mn oxides and hydroxides) over the study period. These results were opposite to those of phosphogypsum-amended soil where Cd, after the study period (16 months) was mostly in the exchangeable fraction, whereas Pb, Zn and Cu were sorbed in soil particles (Kassir et al. 2012a). However, phosphogypsum application increased the solublization of the studied elements where they were associated to the exchangeable and acid-soluble fractions in the phosphogypsum which caused their transfer during winter season to soil particles and increased their concentration in soil.

Trace Elements Transfer from Soil to Cichorium Intybus: Phosphate Fertilizers

The TE transfer factor (TF) is a measure of its availability to plant uptake. There are many factors that can influence the TF, including soil properties and TE chemical phases. The exchangeable fraction (F1) is readily available and considered to be the primary nutrient source for plants (Narwal and Singh 1998). The fraction associated with carbonate (F2) is potentially available for plant uptake, depending on soil pH conditions, whereas TE in the other chemical fractions (oxides-F3, organic-F4, and residual-F5) with minimal solubility and very low mobility are not involved with the plants biological activities (Xian 1989). Thus, during the experiment course, the main changing factors affecting TEs bioavailability, hence their TFs, are the soil moisture content (associated with rainfall), soil pH (influenced by PF acidity), and the respective TEs leaching and available fraction concentrations. Cd exhibited the highest TF (0.32 ± 0.04 — 0.52 ± 0.05) among the other TEs in all parcels, followed by Cu (0.16 ± 0.02 — 0.27 ± 0.03). Pb and Zn had comparable TFs (0.08 ± 0.01 — 0.17 ± 0.02) in all parcels, except in P3 where it increased to 0.20 ± 0.03 for Pb, while remained in the same range for Zn (0.11 ± 0.01).

In comparison with the reference parcel, it can be seen from Figure 7 that the TF of Cd, thus its bioavailability, increased in P1 and P2 by about 49% and 78%, respectively, while it dropped back to near its reference value in P3. This variation in Cd bioavailability is in line with its chemical fraction changes depicted in Figure 6. In fact, in the root zone (0-20 cm), the Cd pH-susceptible mobile fraction F2 increased from 0.102 mg kg^{-1} in parcel R to 0.203 mg kg^{-1} in P1 (Figure 6), simultaneously with a drop in the layer average pH from 8.23 ± 0.54 to 6.92 ± 0.45. This was followed by a phase transformation yielding 0.303 mg kg^{-1} of Cd exchangeable fraction (F1) in P2, and the depletion of the mobile fractions in P3 (Figure 6).

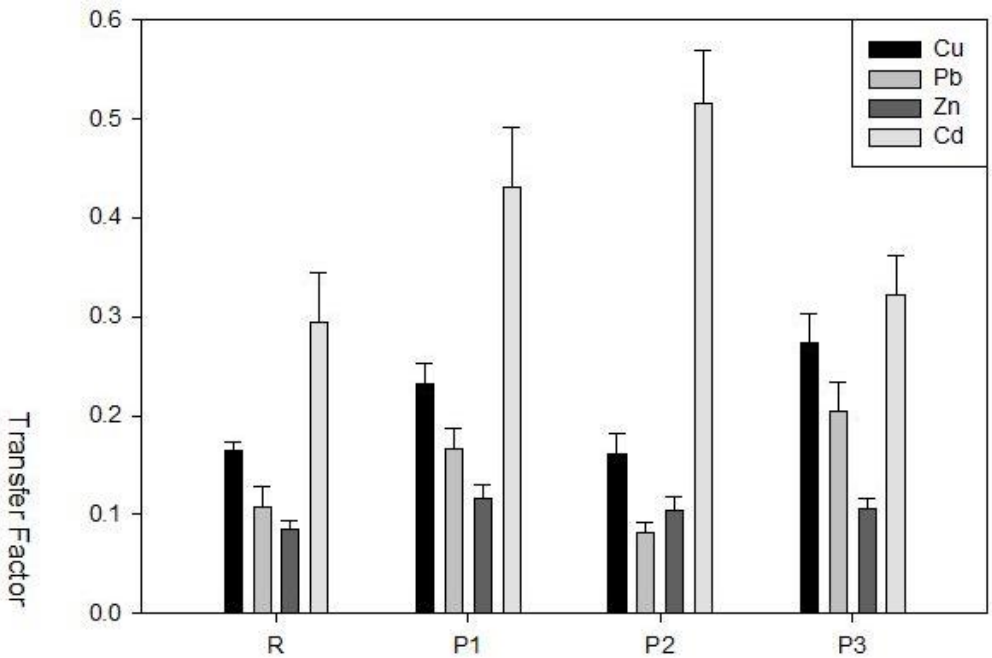

Figure 7. Transfer factor of TEs in the plants – Reference parcel R (T=0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months).

The copper TF went through an increase in P1 by about 44% relative to the reference, declined to the reference value in P2, then increased again by about 71% in P3 (Figure 7). If we examine Figure 6 for Cu fractions in layer 0-20 cm, we find a similar fluctuating pattern in the mobile fractions (F1 & F2) concentrations, with the pH factor favoring the availability of the carbonate fraction (F2) to plant uptake.

Similarly, the Pb TF went through an increase of nearly 52% from R to P1, a drop in P2, followed by an increase of about 86% in P3 (Figure 7). However, this increase in bioavailability of Pb in P3 is contradicted with the prevailing insoluble and residual Pb fractions in layer 0-20 cm (Figure 6). This could be explained by the idea that some plant roots were extended to deeper layer (20-35 cm), where the Pb plant available fraction F2 prevailed in P3 (Figure 6).

As for Zn, plant available fractions (F1 & F2) barely existed in parcels R, P1 and P2, with bio-available fraction F1 appearing in parcel P3 with a concentration of about 20 mg kg^{-1} in layer 0-20 cm (Figure 6). Zn plant availability, however, remained the same in P3 as in the other parcels, with relatively low TF (0.11 ± 0.01) (Figure 7). This could possibly be due to the existence of competing available Cu (László Simon 2000; Tani and Barrington 2005) in P3 layer 0-20 cm (Figure 6).

There was little evidence of significant elevated uptake of TEs in soil extensively amended with PFs. Pb, Zn, Cu and Cd concentrations were within the normal levels reported by Chaney (1989) (2-5 mg kg^{-1} for Pb; 15-150 mg kg^{-1} for Zn; 3-20 mg kg^{-1} for Cu; 0.5-1 mg kg^{-1} for Cd) though they are considerably higher than the reference, particularly for Cd. This phenomenon could be attributed to the formation of TEs phosphate compounds which are non-absorbable. In fact, it is recognized that high levels of phosphate fertilization decrease Zn concentrations in plant tissues (Lindsay 1972; Loneragan and Webb 1993; Moraghan 1984),

which was attributed to the interaction of P with Zn in soil (Grant and Bailey 1997; McLaughlin et al. 1995). Cd availability in plants from PFs depends on many factors such as soil texture, plant species, Cd concentration, and type of fertilizers used (Singh and Myhr 1997).

Trace Elements Transfer from Soil to Cichorium Intybus: Phosphogypsum

Accumulation ratio (element concentration in plant root to element concentration in soil) and transfer coefficient (element concentration in aerial part/element concentration in root) (Baker 1981; Kabata-Pendias and Pendias 2001; Madejon et al. 2002) were determined for the different studied elements (Table 1) in order to provide a better understanding of the relationship between TEs concentrations in soil and plants, and to investigate their potential transfer into the food chain.

Cd showed a high accumulation ratio (3.2 − 3.8) in all parcels with an increment of approximately twenty five times (25x with relation to reference values (0.14). Previous studies displayed that plants could accumulate high amounts of this element even when its concentration in the soil was low (Ciura et al. 2005). However, in a balanced undisturbed soil, Cd in the control plot must have been well retained by the soil particles compared with Cd added with PG which seems to be more readily accessible to plants during its life cycle, and transfer from fertilizer to soil solution, root and soil phase.

According to Ross criteria (Ross 1994), Cd concentration in roots was within the values of contaminated plants (0.03- 3.8 mg kg^{-1}).

Pb exhibited accumulation ratios greater than Zn in all parcels, with increments in parcels P1 and P2 being approximately two times (2x higher than in control soil. This order disagrees with previous studies showing Zn as the most and Pb the least readily accumulated TE in vegetation (Chopin and Alloway 2007; Kabata-Pendias and Pendias 2001). This discrepancy resulted from diversity of factors such as differences in TEs speciation and the consequent variations in mobility and bioavailability, soil conditions, plants age and state of health, and element concentrations (Batista et al. 2007; Kabata-Pendias and Pendias 2001; Ross 1994). However, Pb and Zn concentrations (Figure 8) did not exceed the minimum levels of contamination in plants reported by Ross (1994) (30 - 300 mg kg^{-1} for Pb; 100-400 mg kg^{-1} for Zn).

The accumulation ratio of Cu could be considered constant with time which probably is related to the plants regulation of the uptake of this essential micronutrient. Copper concentration (Figure 8) was not above the minimum values for plants contamination (20-100 mg kg^{-1}) (Ross 1994).

As vegetation can only take up soluble TEs, these were absorbed by plants in mobile forms (i.e., exchangeable, acid soluble). Chicory root exudates (i.e., H^+, acetic acid, organic acids, amino acid) could solubilize or mobilize TEs from the mineral and organic fractions in soil (Carrillo-Gonzalez et al. 2006). Therefore, roots promoted mobilization and uptake of exchangeable, acid soluble and oxidizable TEs (i.e., Pb in exchangeable fraction, Cu and Zn in acid soluble and Zn in complexed form). According to Baker (1981), plants can be classified according to their transfer coefficients as accumulators (TC > 1.5), indicators (TC from 0.5 to 1.5), and excluders (TC < 0.1).

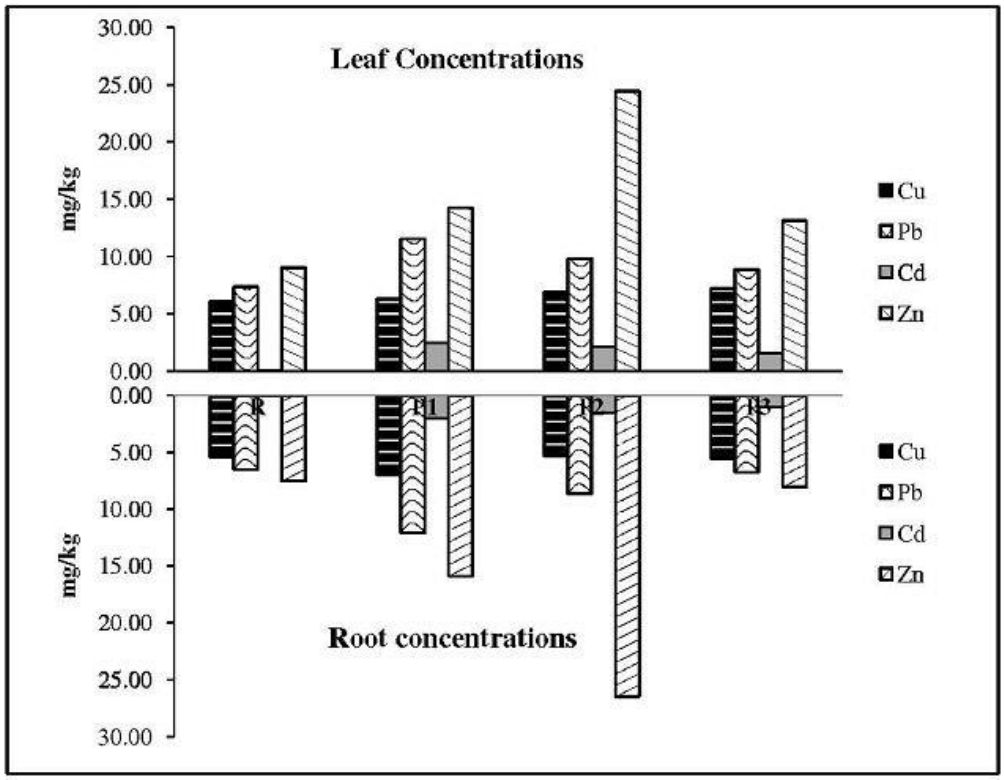

Figure 8. Time-variation of trace element concentrations in plant roots and aerial parts following PG application.

Chicory was demonstrated to be a potential indicator plants for heavy metal contaminated soils (Aksoy 2008; Simon et al. 1996) particularly for Cd. Due to the high rate of Cd transfer, concentration of Cd in leaves was higher than in roots.

According to Simon et al. (1996), Cd concentrations in all chicory plant parts grown in Cd-amended soils, were substantially higher than in controls following the order: leaf > root. Moreover, Chicory leaves growing on soil amended with PG had approximately an average of 2.0 mg kg^{-1} of Cd in all parcels (Figure 8), exceeds the normal levels (0.1-1 mg kg^{-1}), and exceeding the tolerable level (0.5 mg kg^{-1}) recommended for livestock (Chaney 1989).

Transfer coefficient of Cu, Pb and Zn were relatively constant with time. Cu level in leaves was within the normal range (3-20 mg kg^{-1}) (Chaney 1989) and showed no significant difference between parcels and reference. Several studies have reported restricted transport of Cu from contaminated soils to aboveground parts in different species (Ait Ali et al. 2002; Arduini et al. 1996; Dominguez et al. 2008). Pb in leaves was above the normal levels (2-5 mg kg^{-1}) in all parcels and showing significant difference with the reference, but not exceeding the toxic level for livestock (30 mg kg^{-1}) (Chaney 1989).

Though Zn concentration in leaves presented a significant difference between parcels and reference, its concentration remained within the normal values (15-150 mg kg^{-1}) (Chaney 1989). In fact, Zn is a micronutrient whose absorption was closely regulated by plants (Madejon et al. 2007).

These results suggest that Chicory showed a translocation of these elements from roots to leaves particularly for Cd that could present a food chain hazard over the limited study period. In fact, Cd is the most predisposed TE in terms of crop accumulation from soil amendments (Carrillo-Gonzalez et al. 2006). Long term application of PG and derived industrial materials can bring environmental and public health hazards.

CONCLUSION

In summary, absorption of the studied elements by Chicory showed no danger to human health in case of PF application whereas phosphogypsum application increased Cd absorbed in the leaves of Chicory exceeding the norms thus posing a threat to the food chain. However, the risk associated to phosphate fertilizers application was the presence of fluorine detected with Scan Electron Microscopy (SEM) involving further analysis.

SUMMARY

Monitoring of selected trace elements (Cu, Pb, Zn and Cd) distribution and mobility in a Mediterranean amended red soil profile has been performed in soil and plant samples collected from various depth intervals at different points in time. Phosphogypsum amendment increased the solubility of the studied trace elements where they were bound to exchangeable and acid-soluble fractions in higher percentages than reference soil. Pb, Zn and Cu were sorbed into mineral soil phases, while Cd was mainly found in the exchangeable form. On the other hand, Pb, Zn and Cu were transferred from residual to exchangeable fraction, except for Cd, in the case of fertilizers application. *Cichorium intybus* accumulated higher trace elements concentrations than the reference plants, but they remained within normal reported levels, except for Cd where it exceeded the recommended tolerable levels in the case of phosphogypsum amendment.

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SECTION II. ORGANIC FERTILIZERS

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Chapter 3

ACHIEVEMENT OF ZERO EMISSIONS BY THE BIOCONVERSION OF FISHERY WASTES INTO FERTILIZER

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ABSTRACT

Recently, a paradigm for waste policy has been changed, emphasizing resource recycling with zero emissions. In this sense, this chapter provides a brief review of experimental results of the bioconversion of seaweed wastes into fertilizer and discusses recent issues that are involved in zero emissions during fishery waste treatments. Seaweeds or their products are commonly used in agriculture to stimulate plant growth and increase crop productivity. Hence, biodegradation experiments for three different types of seaweeds were executed to determine their reutilization as wastes, and the possibility of the biodegraded culture broths of seaweeds as a liquid fertilizer was examined. The 4-d biodegraded culture broths of all three seaweeds were found to be phytotoxin-free at 1000 dilution ratio and exhibited comparable fertilizing ability with the commercial fertilizer in hydroponic culture with an amino acid content of 1.26 to 8.93 g 100 g protein^{-1}, low concentrations of heavy metals, and an N/P/K level of 1.62-2.63%. As a result, the biodegradation was an eco-friendly means to convert seaweed wastes into liquid fertilizer containing various essential nutrients, such as mono-, di-, and oligosaccharides, amino acids, and mineral elements. This zero-emissions process for treating fishery wastes can lead perfect resource recycling.

Keywords: Zero-emission process, fishery wastes, bioconversion, fertilizer, resource recycling

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INTRODUCTION

Currently, the consumption of fishery products, including seaweeds, to acquire health benefits has steadily increased steadily worldwide. Because of this situation, there has been in increase in the amount of fishery wastes, primarily from industrial processing. Seaweeds are known to be a favorite food in Asian countries (Rouxel et al., 2001). In Korea, approximately one quarter of the total amount of domestically produced seaweeds (approximately 800,000 tons) is discarded annually. However, large quantities of fishery wastes, including seaweeds, have not been efficiently utilized to date. This fishery waste disposal problem greatly affects the local environment. Customarily, the unused fishery wastes are disposed via landfills, incineration, or by dumping into the sea. In addition, the cost for the disposal of the fishery wastes has remarkably increased in Korea since 2012 when dumping wastes into the sea became prohibited according to the London Convention (International Maritime Organization, 2006). Therefore, this situation urgently requires an ecologically acceptable means for the reutilization of fishery wastes.

Conventional methods for the reutilization of fishery wastes are the production of high-protein meals by ensilation (Faid et al., 1997), the recovery of chitin (Cira et al., 2002) and carotenoids (Sachindra et al., 2007) from shrimp waste ensilation, fishmeal production (Hall, 1992; Keller, 1990), the production of protein by fermentation (Hassan & Heath, 1986), composting (Liao et al., 1997), the production of nutrients for lactic acid bacteria by acid hydrolysis (Gao et al., 2006), the production of high-quality fishmeal by fermentation (Yano et al., 2008) and the production of low-salt fish sauce by the fast fermentation of squid processing by-products (Xu et al., 2008). Recently, an eco-friendly means to convert fishmeal wastewater and fish waste into liquid fertilizer has been demonstrated (Kim & Lee, 2009; Kim et al., 2010; Dao and Kim, 2011).

Seaweeds have been consumed by humans and animals since 600 BC, and seaweeds are often used in fertilizer, fungicides, herbicides, and phycocolloids, such as alginate, carrageenan, and agar (Chapman & Chapman, 1980). Seaweeds or their products (extracts, composts, soil conditioners) have been commonly used in agriculture to enhance plant growth and crop productivity. These compounds have been reported to have beneficial effects on seed germination, plant yield, root growth, tolerance to plant stress and plant resistance to infections or insect attack (Abetz, 1980; Blunden, 1991; Sivasankari et al., 2006). These beneficial effects are because these products can contain growth-promoting hormones, trace elements, organic compounds, such as carbohydrates, amino acids and vitamins, and stimulatory and antibiotic substrates (Crouch & van Staden, 1993). Seaweeds also have been used for the preparation of composts, and seaweed compost has been proposed as an amendment for horticultural soil (Eyras et al., 2008). Therefore, the use of seaweeds or their products can minimize the use of chemical fertilizers, maintain fertility and overcome nutrient deficiency (Papenfus et al., 2013). Recently, the co-composting of seaweed and fish waste has been reported to produce a fertilizer for use in organic agriculture (López-Mosquera et al., 2011). Seaweed compost with a good fertilizer quality has also been demonstrated using halotolerant and alginate-degrading bacteria (Tang et al., 2011).

The disposal and utilization of seaweed wastes have become a worldwide issue to preserve the marine environment and to recycle organic substances. In the same manner, the Korean government recognized this issue, and accordingly, the Ministry of the Environment

changed the paradigm for the waste policy, emphasizing resource recycling with zero emissions (Mathews, 2012). Therefore, this policy urges ecologically acceptable methods for the reutilization of fishery wastes. Recently, the conversion of fish waste into liquid fertilizer has been demonstrated using proteolytic bacteria, indicating that the fermented broth of fish waste could be a valuable resource for agriculture (Dao & Kim, 2011; Kim & Lee, 2009; Kim et al., 2010). This eco-friendly means is considered the most suitable for the ideal resource recycling of seaweed wastes with no additional emissions of waste. Therefore, this chapter presents the results of a study that aimed to develop biodegradation products from three different types of seaweeds (green, red and brown seaweeds) and to determine their suitability as fertilizers. For this purpose, phytotoxicity tests, analyses of amino acid and mineral element compositions, and hydroponic cultures were accomplished to examine the fertilizing values of the biodegraded culture broths as fertilizers, and their fertilizing values were compared with those values of biodegraded culture broths of fish wastes.

EXPERIMENTAL AND METHODS

Microorganism

Green Seaweed

The bacterial strain that was used for the biodegradation of green seaweed polysaccharides was *Bacillus licheniformis* TK3-Y (GenBank Accession No.: AB374901.1), which was isolated from silt and sandbar locations in a coastal area near Busan (Korea), where green seaweed often drifts and accumulates. This strain formed a transparent ring on carboxymethylcellulose (CMC) and skim milk agar around each colony according to the plate assay (Kasana et al., 2008). Additionally, its colony had a tinge of blue on the spirit blue agar, indicating that this strain possesses high CMCase, protease and lipase activities. Based on microscopic observations, *B. licheniformis* TK3-Y was motile in its vegetative state. This strain possessed gram-positive rods, which measured 0.5-1 μm in width and 2-4 μm in length, and formed endospores. Because *B. licheniformis* TK3-Y is a novel strain that exhibits the distinct ability to degrade CMCs, proteins and lipids, we have applied for a Korean patent (No.: 10-2012-0040487) after depositing this strain into the Korean Agricultural Culture Collection (KACC) as KACC91709P. The pure culture was maintained on a 1.5% nutrient agar plate at 4 °C until use and transferred to a fresh agar plate every month. Periodically, the potential degrading ability of this strain was checked on CMC, skim milk and spirit blue agar by the plate assay.

Red Seaweed

The bacterial strain that was used for the biodegradation of red seaweed polysaccharides was *Bacillus alcalophilus* (GenBank Accession No.: EU231621.1), which was isolated from silt and sandbar locations in a coastal area near Busan (Korea), where red seaweed often drifts and accumulates. This strain formed a yellow-colored ring on agar and carrageenan agar around each colony according to the plate assay (Saraswathi et al., 2011), indicating that this strain possesses high agarase and carrageenanase activities. Based on microscopic observations, *B. alcalophilus* was motile in the vegetative state. This strain possessed gram-

positive rods, which measured 0.5-1.2 μm in width and 2-4 μm in length, and formed endospores. The colors of the colonies that formed on *Porphyra* powder, agar and carrageenan media were orange, pink and white, respectively. Because this strain demonstrated distinct degradation abilities for both agar and carrageenan, we have applied for a Korean patent (No.: 10-2012-0024992) after depositing this strain into KACC as KACC91705P. The pure culture was maintained on a 1.5% nutrient agar plate at 4 ℃ until use and transferred to a fresh agar plate every month. Periodically, the potential degrading ability of this strain was checked on both agar and carrageenan agar by the plate assay.

Brown Seaweed

The bacterial strain that was used for the biodegradation of brown seaweed polysaccharides was *Microbacterium oxydans* (GenBank Accession No.: HQ113206.1), which were isolated from silt and sandbar locations in a coastal area near Busan (Korea), where brown seaweed often drifts and accumulates. This strain formed a transparent ring (on alginate agar) or a yellow-colored ring (on laminarin agar) around each colony according to the plate assay (Gacesa & Wusteman, 1990; Lee & Chang, 1995), indicating that this strain possesses high alginate lyase and laminarinase activities. Based on microscopic observations, *M. oxydans* was extremely motile in the vegetative state and displayed Gram-positive rods, which measured 0.4-0.8 μm in width and 1.0-2.0 μm in length. The strain occurred both singly or in random groups, was catalase-positive and did not form endospores. Because this novel strain exhibits the distinct ability to degrade both alginate and laminarin, we have applied for a Korean patent (No.: 10-2011-0097411) after depositing this strain into KACC as KACC 91657P. The pure culture was maintained on a 1.5% nutrient agar plate at 4 ℃ until use and transferred to a fresh agar plate every month. Periodically, the potential degrading ability of this strain was checked on both alginate and laminarin agar by the plate assay.

Culture medium

Green Seaweed

A fresh colony of *B. licheniformis* TK3-Y from a nutrient agar plate was transferred with a platinum loop to a 10-ml tube containing 5 ml *Ulva* culture medium (10 g l^{-1} *Ulva* powder, 5 g l^{-1} yeast extract, 2.4 g l^{-1} NH_4Cl, 2 g l^{-1} KH_2PO_4, and 0.5 g l^{-1} NaCl). The *Ulva* powder (mixture of *U. pertusa* and *U. lactuca*) was purchased from a local market and ground using a porcelain mortar and pestle. The final samples were sieved to achieve particle size homogeneity using a 100-mesh sieve. The ground powder was sonicated for 1 h after treatment with 1% (v v^{-1}) H_2SO_4 at 121 ℃ for 15 min to increase its solubility. The pH of the culture medium was adjusted to 6 before autoclaving, and the culture medium was sterilized at 121℃ for 15 min. After inoculation, the tube was incubated in a rotary shaking incubator at 50 ℃ and 180 rpm for 3 d until cells reached a late-log phase. A 5% (v v^{-1}) inoculum from this tube was then used to inoculate 100 ml of the *Ulva* culture medium in a 250 ml Erlenmeyer flask, and the flask was incubated for 2 d. Then, wet cells from the flask were collected by centrifugation at 8000 rpm, and the harvested cells were used as an inoculum for the biodegradation experiment.

Red Seaweed

A fresh colony of *B. alcalophilus* from a nutrient agar plate was transferred with a platinum loop to a 10-ml tube containing 5-ml autoclaved *Porphyra* culture medium (10 g l^{-1} *P. yezoensis* powder and 1 g l^{-1} of NH_4Cl, pH 7.5). The *Porphyra* powder was purchased from a local market and ground using a porcelain mortar and pestle. The final samples were sieved to achieve particle size homogeneity using a 100-mesh sieve. The ground powder was boiled for 40 s after treatment with 0.1% (v v^{-1}) H_2SO_4 at 121 °C for 15 min to increase its solubility. After inoculation, the tube was incubated in a rotary shaking incubator at 30 °C and 180 rpm for 3 d until cells reached a late-log phase. A 5% (v v^{-1}) inoculum from this tube was then used to inoculate 100 ml of the *Porphyra* culture medium in an 250 ml Erlenmyer flask, and the flask was incubated for 2 d. Then, wet cells from the flask were collected by centrifugation at 8000 rpm, and the harvested cells were used as an inoculum for the biodegradation experiment.

Brown Seaweed

A fresh colony of *M. oxydans* from a nutrient agar plate was transferred with a platinum loop to a 10-ml tube containing 5-ml autoclaved *Laminaria* culture medium (10 g l^{-1} *L. japonica* powder, 0.1 g l^{-1} of $MgSO_4$, 0.1 g l^{-1} of NaCl, 0.1 g l^{-1} of $CaCl_2$, 2 g l^{-1} of $(NH_4)_2SO_4$, and 0.5 g l^{-1} of KH_2PO_4, pH 6.8). The *Laminaria* powder was purchased from a local market and ground using a porcelain mortar and pestle. The final samples were sieved to achieve particle size homogeneity using a 100-mesh sieve. The ground powder was boiled for 40 s after treatment with 0.1% (v v^{-1}) H_2SO_4 at 121 °C for 15 min to increase its solubility. After inoculation, the tube was incubated in a rotary shaking incubator at 30 °C and 180 rpm for 3 d until cells reached a late-log phase. A 5% (v v^{-1}) inoculum from this tube was then used to inoculate 100 ml of the *Laminaria* culture medium in a 250 ml Erlenmyer flask, and the flask was incubated for 2 d. Then, wet cells from the flask were collected by centrifugation at 8000 rpm, and the harvested cells were used as an inoculum for the biodegradation experiment.

Biodegradation of Seaweed

To characterize the degradation of green seaweed polysaccharides by *B. licheniformis* TK3-Y, experiments were performed in a 1000 ml flask (with a 250 ml working volume). The culture medium that was used for the degradation experiment was the same medium that was used for the culture in tubes, as described above. The previously harvested cells were proliferated for 12 h under the same culture conditions and used as an inoculum (0.5%, w v^{-1}) for this experiment. After inoculation, the flask was incubated in a rotary shaking incubator at 50 °C and 180 rpm for 5 d.

To characterize the degradation of red seaweed polysaccharides by *B. alcalophilus*, experiments were performed in a 1000 ml flask (with a 250 ml working volume). The culture medium that was used for the degradation experiment was the same medium that was used for the culture in tubes, as described above. The previously harvested cells were proliferated for 12 h under the same culture conditions and used as an inoculum (0.5%, w v^{-1}) for this experiment. After inoculation, the flask was incubated in a rotary shaking incubator at 30 °C and 180 rpm for 5 d.

To characterize the degradation of brown seaweed polysaccharides by *M. oxydans*, experiments were performed in a 1000 ml flask (with a 250 ml working volume). The culture medium that was used for the degradation experiment was the same medium that was used for the culture in tubes, as described above. The previously harvested cells were proliferated for 9 h under the same culture conditions and used as an inoculum (0.5%, w v^{-1}) for this experiment. After inoculation, the flask was incubated in a rotary shaking incubator at 30 °C and 180 rpm for 5 d.

In each biodegradation experiment, samples were periodically taken from each flask to measure the changes in pH, cell density and in the concentration of reducing sugars. The samples were also analyzed to examine the concentrations of COD_{Cr}, TN, cations and anions.

Seed Germination Test

The phytotoxicity of the biodegraded culture broths of seaweeds was evaluated by a seed germination test according to the method of Wong et al. (2001). Ten milliliters of each culture broth was filtered through a 0.45-μm membrane filter after centrifugation at 8000 rpm for 10 min. In parallel, 10 ml of the culture broth containing cells was also tested. For measurements of seed germination and root length, 5 ml of the filtrate or of the original culture broth at various dilution ratios was pipetted into a sterile Petri dish that was lined with Whatman #1 filter paper. Ten cress (*Lepidium sativum*) seeds were placed evenly in each Petri dish and incubated at 25 ºC in the dark at 75% humidity. After 72-h incubation, the seed germination and root length in each Petri dish were measured against the control using distilled water. The percentages of relative seed germination (RSG), relative root growth (RRG), and germination index (GI) were estimated according to the following formula (Hoekstra et al., 2002):

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the culture broth}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\text{RRG (\%)} = \frac{\text{Mean root length in the culture broth}}{\text{Mean root length in the control}} \times 100$$

$$\text{GI (\%)} = \frac{\text{RSG} \times \text{RRG}}{100}$$

Hydroponic Culture

Kidney bean and barley were selected as reference plants and cultivated in a mini-hydroponic culture pot ($5 \times 12 \times 8$ cm^3) against the control to evaluate the fertilizing ability of the biodegraded culture broths of seaweeds. For the hydroponic cultures, each culture broth at maximal degradation and its mixture were used, and a commercial seaweed fertilizer, which sold at a moderate price in Korea, was tested for relative evaluation by comparison. The mixed culture broths of seaweeds and fish (mackerel) wastes were also tested. The hydroponic culture pot was composed of a glass vessel and a plastic screen inside. In each pot, 10 seeds of kidney bean or 20 seeds of barley were placed on top of the plastic screen.

Each 320-ml solution of 1000-fold-diluted seaweed culture broth or of the commercial liquid fertilizer was added underneath the plastic screen to fully soak the seeds. Each prepared pot was covered with aluminum foil to keep the seeds in the dark. After the seeds germinated, all the pots were placed by the window all day to provide enough sunlight for plant growth. Light from two light bulbs (100 W) that were placed 50 cm from the pots was provided instead on cloudy days. The air temperatures at day and night were maintained at approximately 24 and 18 °C, respectively, by natural ventilation and heating. The water temperature was approximately 17 °C, and the relative humidity in the culture room was approximately 65% on average**.** Each solution of the culture broths or commercial liquid fertilizers was refreshed every 3 d. Measurements of plant growth (stem height, stem thickness, leaf number and leaf length) were periodically performed for both kidney bean and barley.

Analyses

The cell density, which indicates the number of viable cells, was measured after the samples were diluted and plated on nutrient agar. Cell density is expressed as colony forming units (CFU) per ml of sample. The concentrations of COD_{Cr} and TN were analyzed by a water-quality analyzer. The concentrations of Na^+, NH_4^+, Cl^-, NO_2^-, NO_3^-, and PO_4^{3-} were estimated by ion chromatography (Metrohm 792 Basic IC, Switzerland). The columns that were used in these analyses were Metrosep C2-150 (150×4.0 mm) and Metrosep Supp 5-150 (150×4.0 mm) for cations and anions, respectively.

All measurements were performed in triplicate. The composition of amino acids and heavy metals in the culture broth of each seaweed was analyzed at the Korea Basic Science Institute (Seoul, Korea).

The characterization of the microorganisms' ability to degrade each seaweed was deduced from the quantity of reducing sugars. Thin-layer chromatography (TLC) was also used to differentiate and identify various seaweed-decomposition products. The reducing sugars were quantified by the 3,5-dinitrosalicylic acid (DNS) method (Chaplin & Kennedy, 1986), using glucose as the standard substrate for green seaweed and brown seaweed polysaccharides and using galactose as the standard substrate for red seaweed polysaccharides. TLC was performed by the ascending method, using Silica Gel-60 TLC plates (E. Merck, Darmstadt, Germany). n-butanol:acetic acid:water (2:1:1), n-butanol:ethanol:water (3:2:2) and n-butanol:isopropanol:ethanol:water (2:3:3:2) solutions were used to develop the green seaweed, red seaweed and brown seaweed decomposition products on the TLC plates, respectively.

The products that were depolymerized from green seaweed polysaccharides were visualized by heating at 120 °C for 15 min, after spraying with 10% (v v^{-1}) H_2SO_4 in ethanol. The products that were depolymerized from red seaweed polysaccharides were visualized by heating at 110 °C for 10 min, after spraying with 0.2% naphthoresorcinol solution and 10% (v v^{-1}) H_2SO_4 in ethanol. The products that were depolymerized from brown seaweed polysaccharides were visualized by heating at 110 °C for 20 min, after spraying with 10% (v v^{-1}) H_2SO_4 in ethanol.

RESULTS AND DISCUSSION

Seaweed fertilizer has been found superior to chemical fertilizer due to the high level of organic matter aids in retaining moisture and minerals in the upper soil level that are available to the roots (Blunden, 1991). This finding is because seaweed contains not only nitrogen, phosphorus and potash content but also trace elements and metabolites, which increase its fertilizing value (Booth, 1969). In this chapter, therefore, biodegradation characteristics for various types of seaweeds were determined, and the fertilizing values of the biodegraded culture broths of the seaweeds were evaluated.

Biodegradation of Seaweed Polysaccharides

Compared with terrestrial plants, seaweeds possess a high water content of approximately 70-90%, a relatively high protein content of approximately 10% and varying levels of carbohydrates (Park et al., 2008). The carbohydrate contents of green, red and brown seaweeds are 25-50, 30-60 and 30-50%, respectively, depending on species, habitat, season, part of the seaweed body, and maturity. The different chemical compositions of seaweeds are shown in Table 1. Major polysaccharides that are contained in seaweeds vary and thus, are composed of different chemical bonds. To reutilize the seaweed waste, the complicated molecular structure must be hydrolyzed. Recently, aerobic biodegradation was offered as an ecologically acceptable alternative for the treatment of fishery wastes (Kim & Lee, 2012).

Table 1. Chemical composition of seaweeds

Seaweed	Water[a]	Carbohydrate[b]	Protein[b]	Lipid[b]	Ash[b]
Green[c]	88.0-92.0	38.0-55.4	8.5-26.0	1.4-7.9	19.6-49.8
Red[d]	70.0-90.0	43.8-68.3	27.0-38.6	0.3-6.2	8.1-38.5
Brown[e]	86.0-94.0	30.3-68.5	5.6-20.0	0.2-4.2	5.1-46.0

[a] Fresh weight %

[b] Dry weight %

[c] Data that were obtained from Aguilera-Morales et al. (2005), Lahaye and Robic (2007), Pena-Rodriguez et al. (2011), Wong and Cheung (2000), and Yaich et al. (2011).

[d] Data that were obtained from Dawczynski et al. (2007), Gressler et al. (2010), Marinho-Soriano et al. (2006), and Matanjun et al. (2009).

[e] Data that were obtained from Dawczynski et al. (2007), Kim et al. (2011), McDermid and Stuercke (2003), and Marinho-Soriano et al. (2006).

Green Seaweed

A time course monitoring the degradation of green seaweed polysaccharides by *B. licheniformis* TK3-Y is shown in Figure 1. As shown in Figure 1A, the pH steadily increased from 6.05 to 8.24 after 5 d as the biodegradation proceeded. The cell number increased from 4.7×10^5 CFU ml^{-1} to 8.8×10^7 CFU ml^{-1} after 3 d, and then decreased somewhat to 6.5×10^7 CFU ml^{-1} near the end. The concentration of reducing sugar was 2.94 g l^{-1} and decreased until 3 d. Then, the concentration of reducing sugar slightly increased to 0.52 g l^{-1} after 5 d. Reducing sugars that were present at the beginning of biodegradation were due to the pretreatment of *Ulva* powder to improve its solubility.

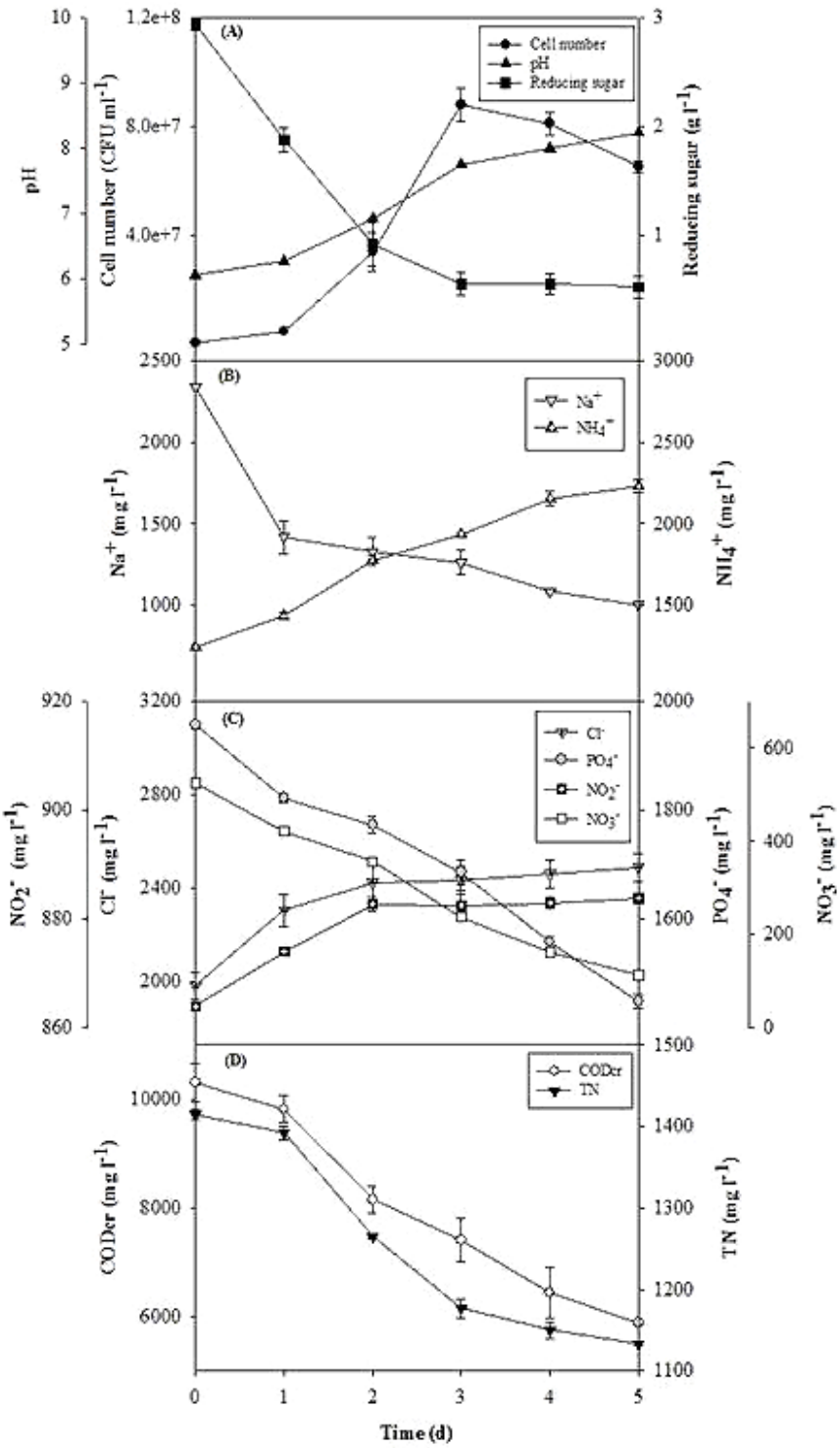

Figure 1. Results of biodegradation of green seaweed. Changes in the cell number, pH and in the concentration of reducing sugars (A), the concentrations of cations (B), the concentrations of anions (C), and the concentrations of COD_{Cr} and TN (D). Error bar: the mean±S.D. of three replicates.

The drop in the concentration of reducing sugars was due to consumption by the cells. It was reported that glucose could significantly induce cellulase (Dhillon et al., 1985). After the *B. licheniformis* TK3-Y strain utilized the monosaccharide, the active biodegradation of green seaweed polysaccharides could occur after 3 d.

Based on the changes in pH, cell number, and in the concentration of reducing sugars, the 4-d culture broth was selected, and its fertilizing ability was later tested.

Figure 1B and Figure 1C present the changes in the concentrations of cations and anions during the biodegradation of green seaweed polysaccharides. As shown in Figure 1B, the concentration of Na^+ steadily decreased from 2342.1 to 1002.2 mg l^{-1}, whereas the concentration of NH_4^+ increased from 1243.6 to 2231.7 mg l^{-1}. During biodegradation, the concentrations of Cl^- and NO_2^- increased, whereas the concentrations of PO_4^{3-} and NO_3^- decreased due to their partial utilization by cells (Yamamoto et al., 2005). The convergence of these changes was accomplished at approximately 3 d of culturing. The increase in the concentrations of Cl^- and NO_2^- was most likely due to the decrease in Na^+ and to the conversion of NH_4^+, respectively. The high content of NaCl in the seaweed digestate causes its ineligibility for use as fertilizer (Cecchi et al., 1996). It is recommended that a low level of NaCl be contained in the biodegraded culture broth of seaweed for commercial use.

The average removal percentages of COD_{Cr} and TN were 42.9 and 19.9%, respectively (Figure 1D). The COD_{Cr}/TN ratio decreased from 7.3 to 5.2 in the end such that the microbial degradation occurred under a relatively stable C/N ratio. The C/N ratio was low compared with that (10.5-13.1) achieved in seaweed composting (Tang et al., 2011).

As green seaweed polysaccharides were degraded by *B. licheniformis* TK3-Y over time, TLC revealed the migration of degraded oligosaccharides with different degrees of polymerization (DP) (Figure 2). Although the bands of DP 1-3 that were produced by the *Ulva* powder pretreatment were observed at the beginning of the experiments, these bands weakened over time in the culture.

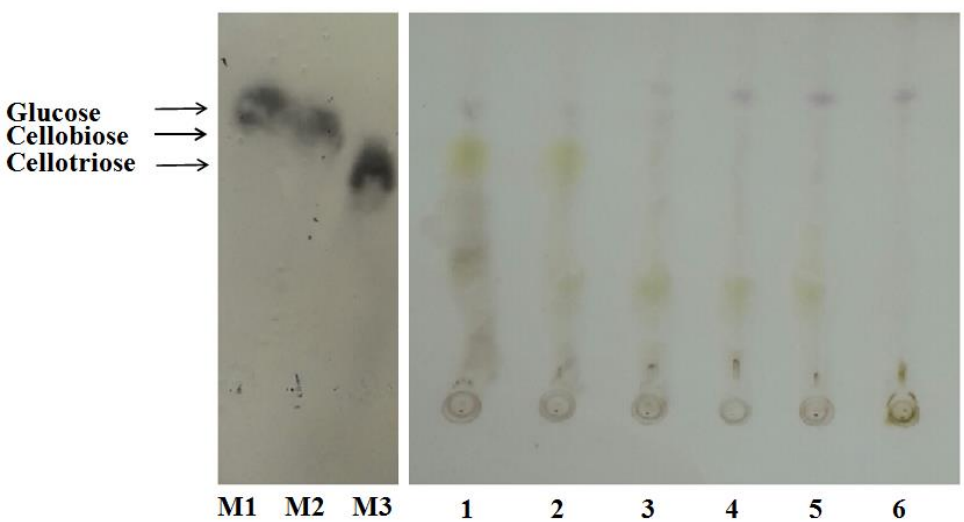

Figure 2. TLC of the degradation products in the biodegraded culture broth of green seaweed. M1-M3 indicates standard markers. Lane 1, day 0; lane 2, day 1; lane 3, day 2; lane 4, day 3; lane 5, day 4; and lane 6, day 5 of cultivation.

It was clearly observed that a glucose band appeared again after 3 d of culturing, which supported the idea that the *B. licheniformis* TK3-Y strain degraded green seaweed polysaccharides in an exolytic manner. These depolymerized products in the culture broth of the green seaweed may contribute to plant growth because seaweed oligosaccharides have been known to stimulate plant growth by enhancing carbon and nitrogen assimilation, basal metabolism and cell division (Gonzalez et al., 2013). In addition, these decomposed products might affect soil aggregation (Haslam & Hopkins, 1996).

Red Seaweed

A time course monitoring the degradation of red seaweed polysaccharides by *B. alcalophilus* is shown in Figure 3. As shown in Figure 3A, the pH steadily increased from 7.62 to 8.40 after 5 d as the biodegradation proceeded.

The cell number increased from 6.0×10^5 CFU ml^{-1} to 3.1×10^7 CFU ml^{-1} after 2 d, and then was almost maintained until the end. The concentration of reducing sugar was 0.96 g l^{-1} and decreased to 0.74 g l^{-1} after 1 d. Then, the concentration of reducing sugar slightly increased to 0.77 g l^{-1} after 5 d. Reducing sugars that were present at the beginning of biodegradation were due to the *Porphyra* powder pretreatment to improve its solubility. The drop in the concentration of reducing sugars was due to their consumption by the cells. Oligosaccharides are a better inducer for agarase production (van der Meulen & Harder, 1976). After *B. alcalophilus* utilized the oligosaccharides, the active biodegradation of red seaweed polysaccharides could occur after 3 d. Based on the changes in pH, cell number, and in the concentration of reducing sugars, the 4-d culture broth was selected, and its fertilizing ability was later tested.

Figure 3B and Figure 3C present the changes in the concentrations of cations and anions during the degradation of red seaweed polysaccharides. As shown in Figure 3B, the concentration of Na^+ slightly decreased from 989.0 to 955.2 mg l^{-1}, whereas the concentration of NH_4^+ increased from 59.2 to 106.3 mg l^{-1}. During biodegradation, the concentrations of anions did not change much; however, the concentrations of Cl^- and PO_4^{3-} increased, whereas the concentrations of NO_2^- and NO_3^- decreased due to their partial utilization by cells. The content of NaCl in this culture broth was not as high as that of the green seaweed culture broth. This observation was because the initial content of NaCl in each culture medium was quite different.

The average removal percentages of COD_{Cr} and TN were 25.9 and 12.1%, respectively (Figure 3D). The COD_{Cr}/TN ratio decreased from 13.5 to 11.4 in the end such that the microbial degradation occurred under a relatively stable C/N ratio. The C/N ratio was similar to that (10.5-13.1) achieved in seaweed composting (Tang et al., 2011).

As red seaweed polysaccharides were degraded by *B. alcalophilus* over time, TLC revealed the migration of degraded oligosaccharides with different DP (Figure 4). Although the bands of DP 1-3 that were produced by the *Porphyra* powder pretreatment were observed at the beginning of the experiments, these bands weakened over time in culture.

It was clearly observed that galactose and oligosaccharide bands appeared again after the 4 d culture, supporting the idea that the *B. alcalophilus* strain degraded red seaweed polysaccharides. Red seaweed extract has been demonstrated to affect the growth, yield and nutrient uptake of soybeans (Rathore et al., 2009). Accordingly, these depolymerized products in the culture broth of the red seaweed may be good candidate compounds for plant growth.

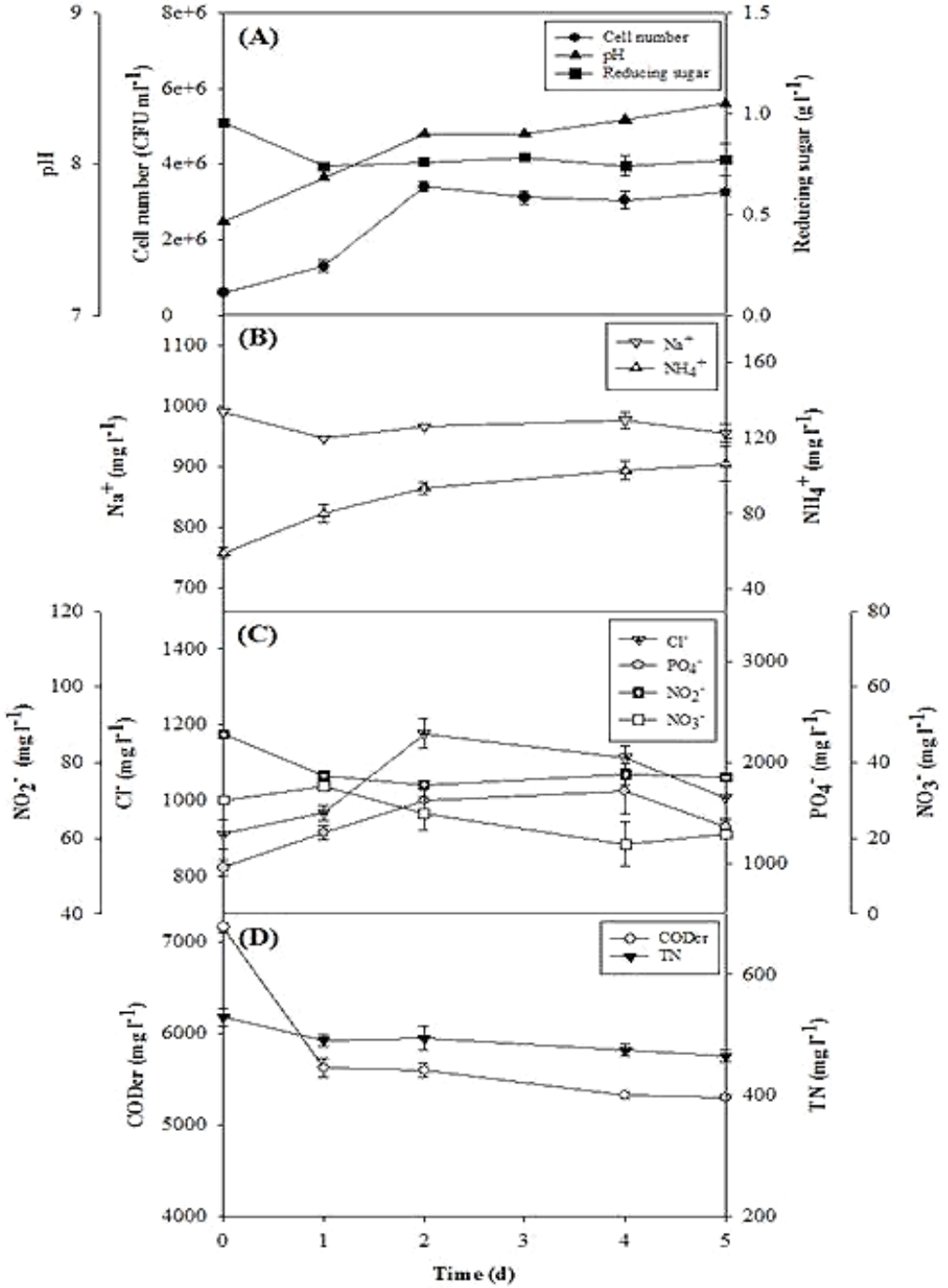

Figure 3. Results of biodegradation of red seaweed. Changes in the cell number, pH and in the concentration of reducing sugars (A), the concentrations of cations (B), the concentrations of anions (C), and the concentrations of COD_{Cr} and TN (D). Error bar: the mean±S.D. of three replicates.

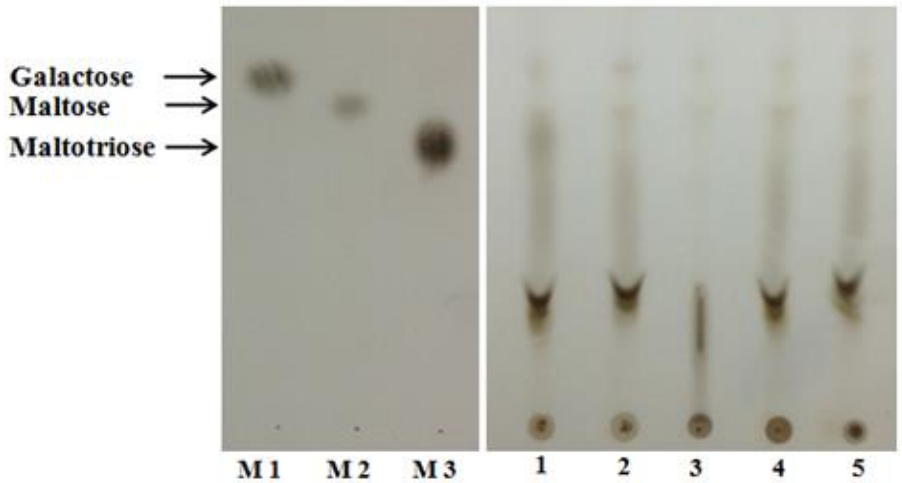

Figure 4. TLC of the degradation products in the biodegraded culture broth of red seaweed. M1-M3 indicates standard markers. Lane 1, day 0; lane 2, day 1; lane 3, day 2; lane 4, day 4; and lane 5, day 5 of cultivation.

Brown Seaweed

A time course monitoring the degradation of brown seaweed polysaccharides by *M. oxydans* is shown in Figure 5. As shown in Figure 5A, the pH steadily decreased from 6.09 to 4.20 after 5 d as the biodegradation proceeded. The cell number increased from 4.5×10^6 CFU ml^{-1} to 1.6×10^7 CFU ml^{-1} after 2 d, and then was almost maintained until the end. The concentration of reducing sugar was 0.18 g l^{-1} and steadily increased to 0.35 g l^{-1} after 5 d. The small amount of reducing sugars that were present at the beginning of biodegradation was due to the *Laminaria* powder pretreatment to improve its solubility. The production of alginolytic enzymes has been shown to improve in the presence of glucose and peptone (Alekseeva et al., 2004). Most likely, *M. oxydans* utilized this reducing sugar, resulting in the active biodegradation of brown seaweed polysaccharides at approximately 3 d. Based on the changes in pH, cell number, and in the concentration of reducing sugars, the 4-d culture broth was selected, and its fertilizing ability was later tested.

Figure 5B and Figure 5C present the changes in the concentrations of cations and anions during the degradation of brown seaweed polysaccharides. As shown in Figure 5B, the concentration of Na^+ slightly decreased from 1322.0 to 1301.0 mg l^{-1}, whereas the concentration of NH_4^+ slightly decreased from 109.0 to 98.5 mg l^{-1}. During biodegradation, the concentrations of Cl^-, NO_3^- and PO_4^{3-} slightly decreased, whereas the concentration of NO_2^- was almost maintained.

The average removal percentages of COD_{Cr} and TN were 21.4 and 5.0%, respectively (Figure 5D). The COD_{Cr}/TN ratio decreased from 28.8 to 23.8 in the end under a relatively stable C/N ratio. This high C/N ratio was dependent on the microbial characteristics.

As brown seaweed polysaccharides were degraded by *M. oxydans* over time, TLC revealed the migration of degraded oligosaccharides with different DP (Figure 6). It was clearly observed that a glucose band appeared after 3 d culture, supporting the idea that the *M. oxydans* strain degraded brown seaweed polysaccharides in an exolytic manner. Alginate oligosaccharides have been reported to promote the germination and shoot elongation of

certain plants (Yonemoto et al., 1993). Accordingly, these depolymerized products in the culture broth of the brown seaweed may contribute to plant growth.

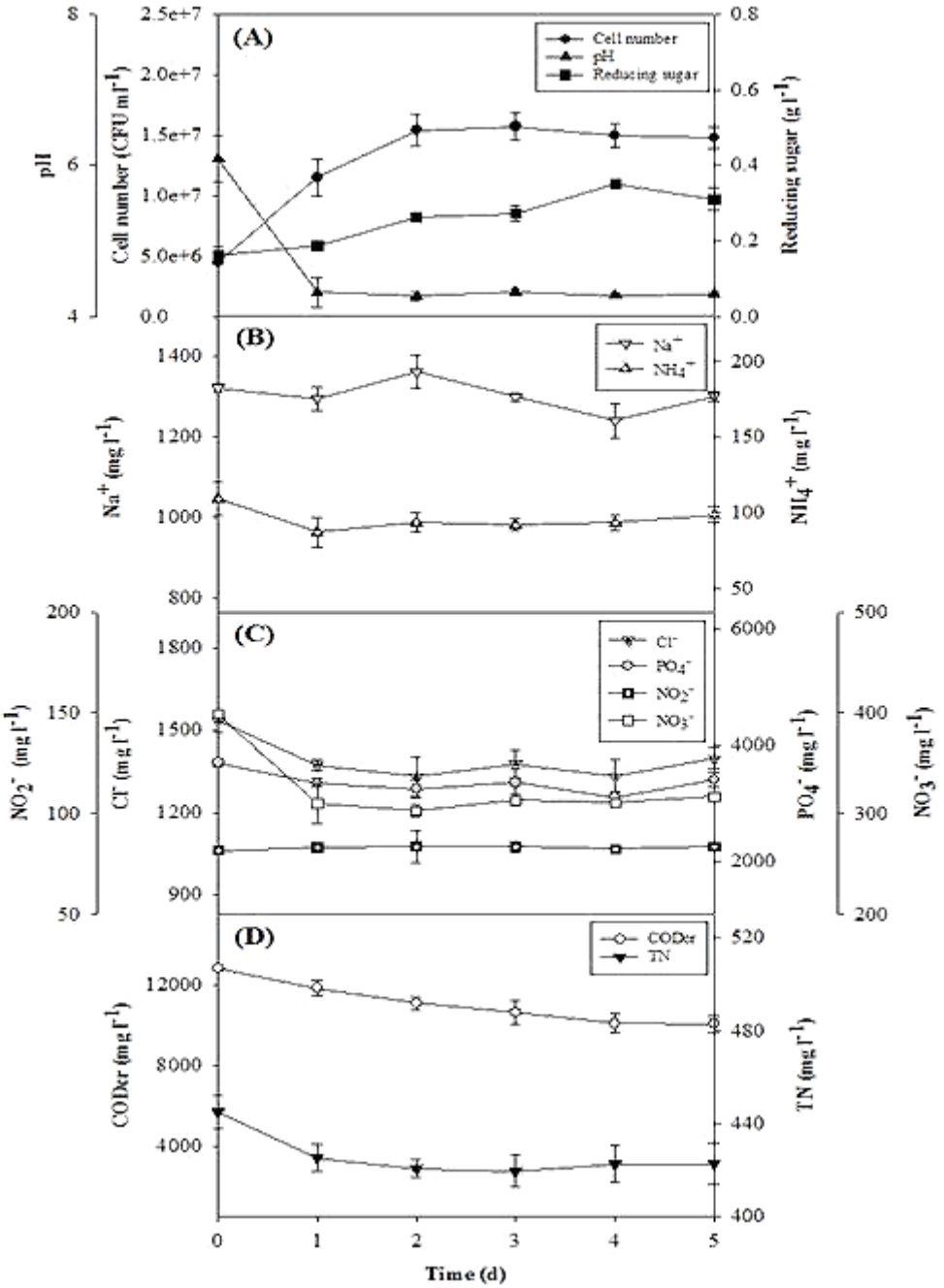

Figure 5. Results of biodegradation of brown seaweed. Changes in the cell number, pH and in the concentration of reducing sugars (A), the concentrations of cations (B), the concentrations of anions (C), and the concentrations of COD_{Cr} and TN (D). Error bar: the mean±S.D. of three replicates.

Properties of the culture broths

The biodegraded culture broth of seaweed could be used as a fertilizer because this broth contained compounds that are potentially useful for plant growth. However, the application of the culture broth depends on the absence of any toxicity and on the abundance of organic and inorganic sources of nutrients. To assess the feasibility of liquid fertilizers, hydroponics was used in this chapter because this method could provide a convenient means of studying the nutrient uptake by plants. In addition, this method is free of confounding or uncontrollable variations in the soil nutrient supply (Nhut et al., 2006).

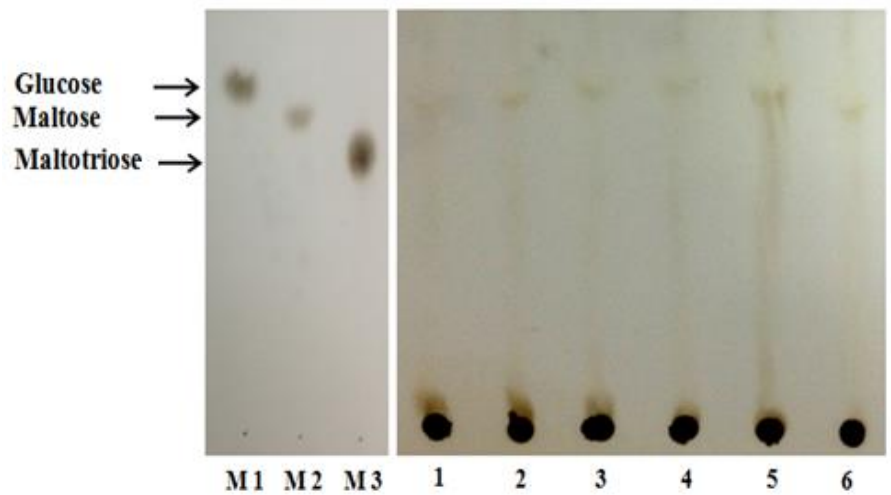

Figure 6. TLC of the degradation products in the biodegraded culture broth of brown seaweed. M1-M3 indicates standard markers. Lane 1, day 0; lane 2, day 1; lane 3, day 2; lane 4, day 3; lane 5, day 4; and lane 6, day 5 of cultivation.

Phytotoxicity

In aerobic biodegradation, sufficient aeration promotes the conversion of organic matters into nonobjectionable stable products, such as CO_2, SO_4^{2-}, NO_3^-, etc. However, in incomplete aeration, organic acids can accumulate, which have harmful effects on plant growth if the fertilizer is incorporated into the soil (Jakobsen, 1995). To examine the fertilizing value of the biodegraded culture broths of seaweeds, their GI tests were accomplished at various dilution ratios, with the criterion of phytotoxin-free at GIs that were higher than 50% (Zucconi et al., 1985). As shown in Figure 7, the filtered culture broth of red seaweed showed the lowest phototoxicity even at low dilutions, and all filtered culture broths were phytotoxin-free at the dilution ratios of more than 500-fold. Low values of GI indicate that some characteristics that existed had an adverse effect on the root growth of cress seeds. This adverse effect may be attributed to the release of high concentrations of ammonia and low molecular weight organic acids (Wong, 1985; Fang & Wong, 1999) because cress is known to be sensitive to the toxic effect of these compounds (Fuentes et al., 2004). At the dilution ratio of 1000, GI values of the filtered culture broths of green, red and brown seaweeds were 94.5, 89.9 and 90.5%, respectively, which indicates almost no existence of any compounds that adversely affecting root growth after seed germination.

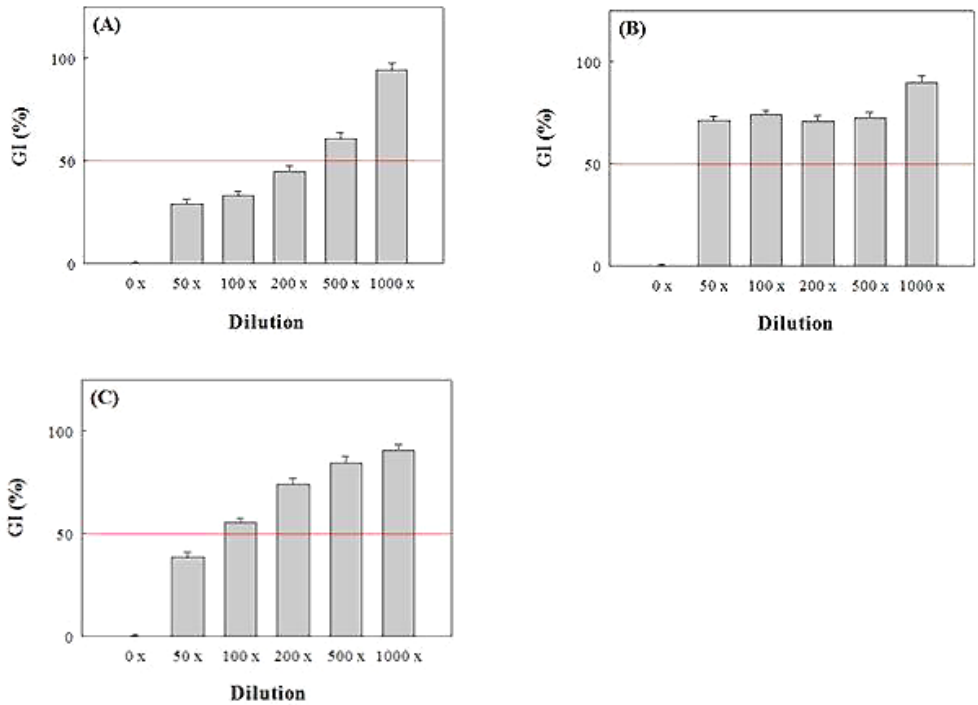

Figure 7. Phxtotoxicity-test results of filtered 4-d culture broths of seaweeds at various dilutions. (A) green seaweed; (B) Red seaweed; and (C) Brown seaweed.

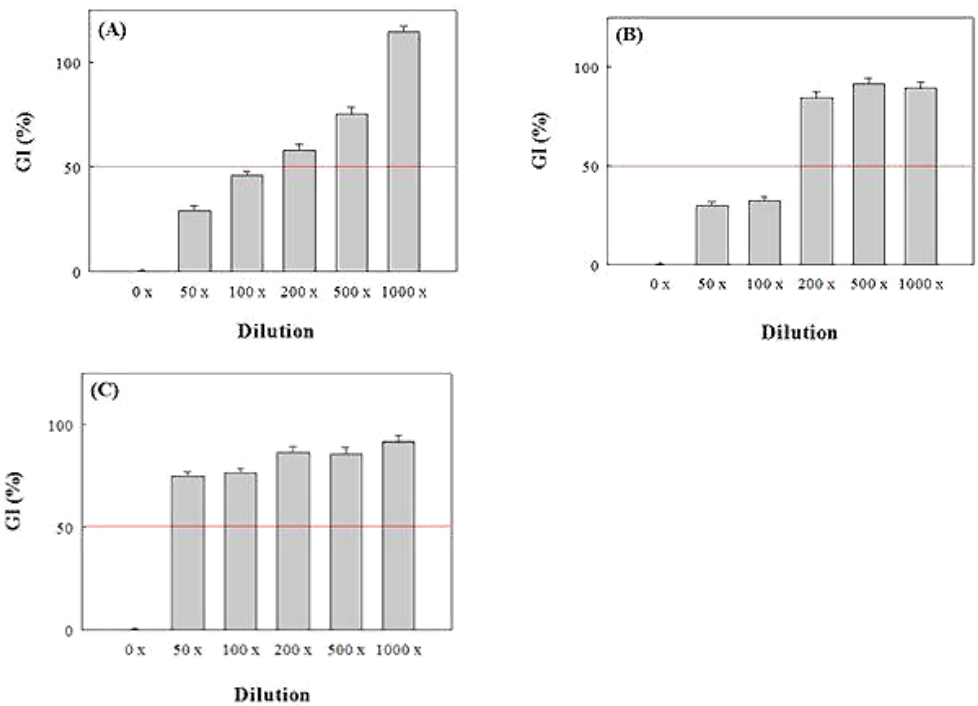

Figure 8. Phxtotoxicity-test results of 4-d culture broths of seaweeds including cells at various dilutions. (A) Green seaweed; (B) Red seaweed; and (C) Brown seaweed.

This result suggested the possibility of seaweed culture broth as liquid fertilizer because the1000-fold diluted liquid fertilizer could be used more often in horticulture for general purposes. Similar to the culture broth of fish waste (Kim et al., 2010), there was no phytotoxicity present in 1,000-fold-diluted culture broths of seaweeds.

The GI tests were also executed for the original culture broths of seaweeds containing cells, and the results are presented in Figure 8. The GI values of all the seaweed culture broths tended to increase with the presence of cells. It has been reported that phytotoxicity caused by organic compounds can be remedied by aerobic decomposition (Wong et al., 2001), and in particular, biofertilizers containing microorganisms can promote plant growth (Rokhzadi *et al*., 2008). For this reason, relatively better results may be observed with the culture broths of seaweeds that contain polysaccharide-degrading bacteria.

Amino Acid Composition

The amino acid composition of the biodegraded culture broths of seaweeds was analyzed because amino acids are an essential part of the active fraction of organic matter in a fertilizer. The amino acid levels of the biodegraded culture broths are presented in Table 2.

Table 2. Amino acid composition of the biodegraded culture broths (unit: g 100 g protein^{-1})

Amino acid	Green seaweed	Red seaweed	Brown seaweed
Lysine	3.22	0.70	0.06
Histidine	0.22	n.d.[a]	n.d.
Arginine	n.d.	n.d.	n.d.
Aspartic acid	0.05	0.06	0.06
Threonine	n.d.	n.d.	n.d.
Serine	n.d.	n.d.	n.d.
Glutamic acid	n.d.	n.d.	0.24
Proline	n.d.	n.d.	0.04
Glycine	0.03	0.07	0.06
Alanine	n.d.	n.d.	0.23
Valine	0.01	0.06	0.05
Cysteine	n.d.	0.48	n.d.
Tryptophane	n.d.	n.d.	n.d.
Methionine	0.78	0.46	n.d.
Leucine	0.12	n.d.	0.07
Tyrosine	1.49	n.d.	0.03
Isoleucine	n.d.	n.d.	0.03
Phenylalanine	2.6	n.d.	0.39
Glutamine	0.41	0.64	n.d.
Asparagine	n.d.	n.d.	n.d.
Total	8.93	2.47	1.26

[a] n.d.: not detected.

Each culture broth contained essential amino acids in different proportions, and the levels of arginine, threonine, serine, tryptophan and asparagines were not detected in all cases. The levels of total amino acids in the culture broths of green, red and brown seaweeds were 8.93, 2.47 and 1.26 g 100 g protein^{-1}, respectively. The level of total amino acids in the culture

broth of green seaweed was superior to those levels in red and brown seaweeds. Particularly, the levels of lysine, methionine, tyrosine and phenylalanine constituted a substantial amount of the total amino acids of green seaweed compared with those levels in both red and brown seaweeds. The sulfur-containing amino acid, methionine, was known to be a nutritionally important essential amino acid and to be the precursor of several metabolites that regulate plant growth (Amir et al., 2002). Relatively high levels of methionine that were in the culture broths of green and red seaweeds could increase their fertilizing value.

As shown in Table 2, there were some pronounced differences among the amino acid compositions of green, red and brown seaweeds. These differences were most likely due to the seaweed species that were used and to the degree of microbial degradation. These levels of total amino acids that were in the culture broth of seaweeds were less than half of that in fermented fish wastes (Kim et al., 2010).

The reported amino acid compositions of different seaweed extracts are presented in Table 3. The levels of each essential amino acid that were in seaweed extracts were almost higher than that of biodegraded culture broths of seaweeds that were used in this study, which resulted in much higher levels of total amino acids. This finding indicates that some amino acids were consumed by microorganisms during the culturing of the seaweeds. It was reported that high levels of aspartic acid and glutamic acid contribute to the special flavor and taste of the seaweeds (Mabeau et al., 1992).

Table 3. The reported amino acid composition of seaweeds (unit: g 100 g protein^{-1})

Amino acid	*U. lactuca*[a] (Green)	*P. tenra*[b] (Red)	*L.digitata*[c] (Brown)	FAO/WHO requirement[d]
Lysine	6.5	4.5	4.8	1.6
Histidine	1.4	1.4	2.4	1.6
Arginine	6.2	16.4	3.0	
Aspartic acid	12.9	7.0	4.7	
Threonine	6.3	4.0	3.4	0.9
Serine	6.9	2.9	2.5	
Glutamic acid	12.9	7.2	3.9	
Proline	4.1	6.4	1.9	
Glycine	6.7	7.2	3.3	
Alanine	9.2	7.4	4.5	
Valine	9.2	6.4	6.0	1.3
Cysteine	1.9	-	2.0	1.7[e]
Tryptophan	-	1.3	0.2	0.5
Methionine	2.4	1.1	1.5	
Leucine	8.3	8.7	4.5	1.9
Tyrosine	6.0	2.4	1.7	1.9[f]
Isoleucine	4.8	4.0	2.6	1.3
Phenylalanine	2.7	3.9	2.8	

[a] Data that were obtained from Yaich et al. (2011).
[b] Data that were obtained from Fleurence (1999).
[c] Data that were obtained from Kolb et al. (2004).
[d] Requirement pattern for adults.
[e] Cysteine + Methionine.
[f] Tyrosine + Phenylalanine.

The levels of those amino acids were high in the seaweed extracts, whereas these levels were extremely low in the biodegraded culture broths of seaweeds, which implied the disappearance of the seaweed flavor by microbial degradation. Moreover, the levels of almost all their essential amino acids in the seaweed extracts were comparable with those amino acid levels of the FAO/WHO requirement, whereas those amino acid levels of the biodegraded culture broths were not comparable. Leucine, tyrosine and phenylalanine were known to be the limiting amino acids of some species of seaweeds (Wong & Cheung, 2000); however, these amino acids were not applicable to the seaweed extracts that were examined. Accordingly, amino acid compositions would somewhat vary among seaweed species.

Major and Noxious Components

It is important to improve the utilization of fertilizer nutrients because fertilizer is responsible for the growth of plants. Although the organic matter of the fertilizer directly affects crop growth and yield, the combined use of organic and inorganic sources is essential to augment the efficiency of nutrients (Lian, 1994). Three major soil nutrients are nitrogen (N), phosphorus (P) and potassium (K), and their deficiency can adversely affect crop yield. In this sense, the concentrations of the three primary nutrients (N, P, and K) with heavy metals in the 4-d culture broths of seaweeds were analyzed and compared with those concentrations in the fermented broth of fish waste (Table 4).

Table 4. Comparison of concentrations of major and noxious components between the biodegraded culture broths of seaweeds and fermented broth of fish wastes

Measurement		Seaweed[a]			Fish wastes[b]
		Green	Red	Brown	
N, P, K (%)	N	2.19	1.94	1.35	1.57
	P	0.31	0.06	0.15	0.31
	K	0.13	0.03	0.12	0.45
Noxious compounds (mg kg^{-1})	Pb	0.016	0.001	0.013	n.d.[c]
	As	0.078	0.176	0.47	n.d.
	Cd	0.001	0.001	0.002	n.d.
	Hg	n.d.	n.d.	0.054	0.02
	Cr	0.083	0.034	0.029	0.26
	Cu	0.078	0.061	0.029	n.d.
	Ni	0.054	0.01	0.046	n.d.
	Zn	0.247	0.063	0.162	1.72

[a] Data that were obtained from this study.

[b] Data that were obtained from the study of Kim et al. (2010).

[c] n.d.: not detected.

The concentrations of N, P and K were 2.19, 0.32 and 0.13% for green seaweed culture broth, 1.94, 0.06 and 0.03% for red seaweed culture broth, and 1.35, 0.15 and 0.12% for brown seaweed culture broth, respectively. There were some pronounced differences among seaweeds, and these quantities of N, P and K are small, similar to the fermented broth of fish waste, compared with those quantities in commercial fertilizers. In contrast, the

concentrations of noxious compounds in the biodegraded culture broths of seaweeds were approximately similar to those concentrations in the fermented broth of fish waste. These values were known to be lower than those values for commercial fertilizers. Seaweeds were known to contain significant amounts of essential mineral elements (Yaich et al., 2011), and, among these elements, heavy metals were regarded as toxic.

Therefore, high concentrations of heavy metals in untreated seaweed leachate and in the digestate, which was obtained after anaerobic digestion (one of the disposal methods of seaweed), were of concern (Cecchi et al., 1996; Nkemka & Murto, 2012). Moreover, seaweed is classified as a toxic waste due to its high Cd content, and the usage of the digestate as a bio-fertilizer is restricted in Sweden (Nkemka & Murto, 2012). However, the contents of Cd in the biodegraded culture broths of seaweeds were extremely low. Microbial degradation may significantly reduce the Cd level.

Hydroponic Culture

The use of the biodegraded culture broths of seaweeds is aimed at elongating the root and at overall plant growth by improving the uptake of available nutrients in the soil. In this sense, the fertilizing ability of the culture broths of seaweeds was tested by hydroponic cultures, which contained kidney bean and barley. In parallel, the fertilizing ability of the mixed broths (mixture of the culture broths of the three seaweeds, and the mixture culture broths of the three seaweeds and fish waste) was also tested together with a commercial seaweed fertilizer.

The results of their comparison in a 15-d hydroponic culture are presented in Figure 9 for kidney bean and in Figure 10 for barley, respectively.

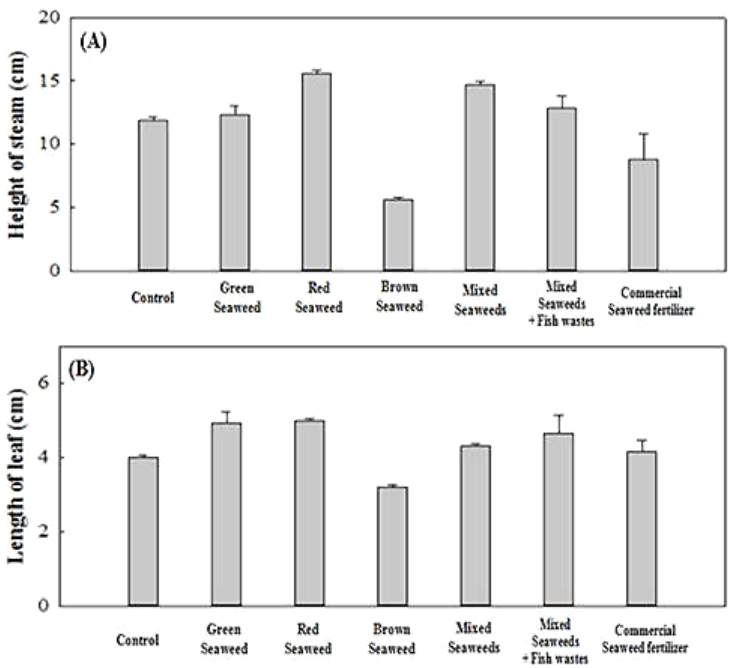

Figure 9. Results of 15-d hydroponic cultures of kidney bean using 4-d culture broths of seaweeds, mixture of seaweeds, mixture of seaweeds and fish wastes, and commercial seaweed fertilizer.

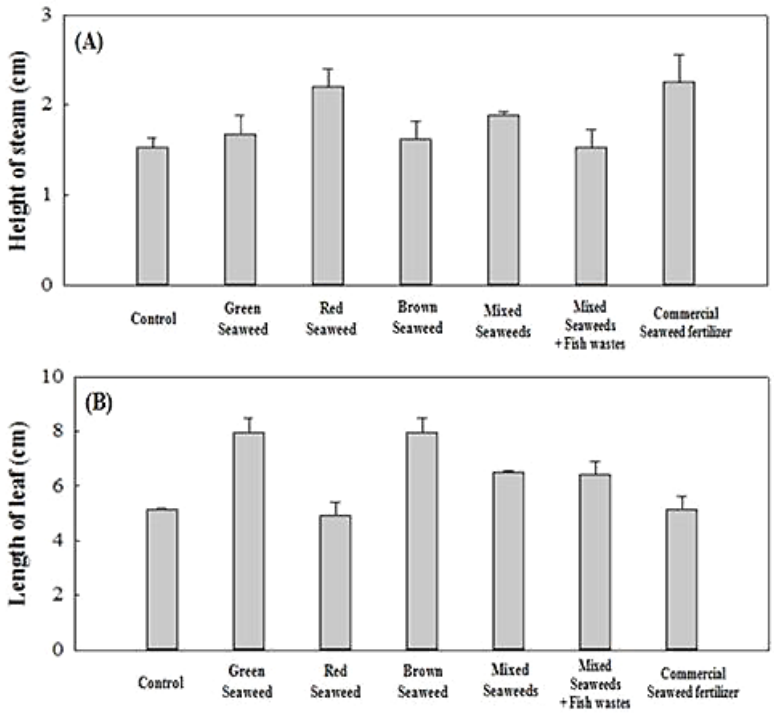

Figure 10. Results of 15-d hydroponic cultures of barley using 4-d culture broths of seaweeds, mixture of seaweeds, mixture of seaweeds and fish wastes, and commercial seaweed fertilizer.

During the 15-d hydroponic cultures, the stems and leaves of both plants grew as the roots elongated. The 4-d culture broths of seaweeds, except for the brown seaweed, exhibited better growth (improved stem height and leaf length in the kidney bean hydroponic culture) than commercial seaweed fertilizer (Figure 9).

The mixture of the culture broths of the three seaweeds and its mixture with fish waste also exhibited comparable results with those results of the commercial seaweed fertilizer. Except for the brown seaweed culture broth, this trend was also observed in the result of the barley hydroponic culture within the significant difference (Figure 10). The brown seaweed culture broth exhibited better growth for barley than for kidney bean. Consequently, the fertilizing ability of all the 4-d culture broths of seaweeds was comparable with the commercial seaweed fertilizer. This result was not surprising not only because seaweed extracts are non-toxic, non-polluting and non-hazardous to human, animals and birds, unlike chemical fertilizers (Dhargalkar & Pereira, 2005) but also because seaweeds contain naturally occurring plant-growth regulators (Gupta et al., 2011).

Zero Emissions

In general, some wastewater is unavoidably generated during the process of fishery waste reutilization (Yano et al., 2008). As observed in this chapter, however, there were no additional emissions of waste during the bioconversion for the production of liquid fertilizer from seaweed waste. Therefore, this process is zero-emissions with perfect resource recycling. This method can provide the acquisition of a novel resource as the liquid fertilizer from seaweed wastes and can prevent coastal area pollution or the disruption of the climate.

This method could also lead to the achievement of green growth via the promotion of resource recycling, which is a current goal in Korea (Mathews, 2012). In addition, this process could address impending problems concerning the release of fishery wastes into the sea.

CONCLUSION

This chapter examined the possibility of using biodegraded culture broths of seaweed wastes as a liquid fertilizer. The profiles of the major reaction parameters during the biodegradation indicated that the stable metabolic reactions of seaweed polysaccharides took place and that the 4-d biodegraded culture broths of three different types of seaweeds exhibited comparable fertilizing ability with the commercial fertilizer with non-phytoxocity. This finding implies that the culture broths of seaweeds adequately contain plant-growth-enhancing compounds, including oligosaccharides, amino acids and mineral elements. Together with fishery wastes, therefore, this successful reutilization of seaweed wastes accomplished perfect resource recycling, exhibiting a zero-emissions process. In addition, the reutilization of fishery wastes, including seaweeds and fish, as a liquid fertilizer can save money and reduce environmental pollution by the replacement of chemical fertilizers. Furthermore, this result addresses the impending problems that are related to the release of fishery wastes into sea and to loss recovery.

ACKNOWLEDGMENTS

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Chapter 4

BIOCHAR

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ABSTRACT

Biochar, a carbonaceous material produced by pyrolysis, can be used as soil amendment to improve soil properties. As some of the carbon is converted into a recalcitrant form rendering it more resistant to biodegradation, land application of biochar is promoted as a beneficial mean for carbon sequestration and as an offsetting mechanism for carbon emission. The agronomic efficacy of biochar and its effects on improving soil properties is highly process- and feedstock-dependent. Feedstock nutrient recovery in biochar tends to decrease with temperature while remaining nutrients redistribute into more recalcitrant and less readily available forms for plant uptake. Pyrolysis of biomass at higher temperature increases biochar liming capacity which contributes to increase in pH of dystrophic, acidic, and highly weathered soils where biochar improvement of soil fertility has shown to be most pronounced and consistence. Biochar showed to improve several soil quality indicators including cation exchange capacity, bulk density, and carbon content. When produced as the main product under slow pyrolysis conditions, biochar can be engineered to achieve desired characteristics; conversely, when produced as a byproduct in fast-pyrolysis, post-production augmentation procedures to improve desired biochar characteristics need to be considered in order to maximize biochar impact on soil fertility. Effect of pyrolysis conditions, feedstock source, and composition on biochar characteristics and effect of biochar application on soil fertility are discussed.

Keywords: pyrolysis, biochar, soil pH, carbon black, aromatic carbon, nutrients

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INTRODUCTION

Biochar is a carbonaceous solid material composed of charred and partially carbonized biomass (charcoal) used as soil amendment. Use of material from the continuum of fresh organic residue and/or raw organic waste to their post-combustion ash as soil amendment is known to be practiced for thousands of years. The historic use of combustible and/or partially combustible organic residue and their long lasting effect of improving soil organic carbon content and soil fertility are documented on lowland humid tropical soils in the Amazonian basin (Glaser et al., 2001) and in similar climatic zones in West Africa (Fairhead and Leach, 2009). Moreover, the high productivity of USA Midwest prairie soils has been attributed in part to the soils high black carbon content - a buildup of charred material over centuries of spontaneous prairie fires (Laird et al., 2009). Charred material contains recalcitrant organic matter and carbonized moieties of slower decomposition rates than the original feedstock material. Amid the rapid increase of CO_2 in the atmosphere and the opportunity of building soil organic matter, sustaining and improving soil fertility, and increasing carbon sequestration by incorporating rather recalcitrant organic residue in soil sprung the promotion of the use of this material as soil amendment (Lehmann et al., 2006). The product is produced from similar feedstock and under the same process as charcoal; yet, promoting its use needed a sharp distinction from charcoal in order to distance it from the impact of fossil fuels on carbon emission, which is associated with 'coal'. Thus, the term 'biochar' was devised and is currently used to indicate of a charred material that is associated with sustainable use of biomass - with cyclical, if not reverse, impact on carbon emission and with a target use as soil amendment (Lehmann and Joseph, 2009).

Similar to charcoal, biochar is produced by thermal decomposition of organic matter in oxygen-limited environment. Biochar can be produced in a very low-cost and crude fabricated devise or in more sophisticated and regulated pyrolysis chamber. Depending on production objectives and target product, pyrolysis is conducted at a wide range of temperatures, bordering torrefaction at low temperatures (250 to 350 °C) and gasification at high temperatures (> 750 °C). During pyrolysis, cellulose and hemicellulose are decomposed at lower temperatures producing volatile compounds, while lignin decomposes at much higher temperature. The three main products of pyrolysis include char, organic vapor and steam, and gasses (mainly CO_2, CO, CH_4, and H_2). High heating rates (>500 °C s^{-1}) and short vapor residence time (<4 s) under moderate temperatures (350 to 450 °C) favor yield of liquid (bio-oil), while longer vapor residence time and lower temperatures (300 to 400 °C) favor char formation. High temperature (800 °C) and long vapor residence time favor gas formation. There are two main biochar production methodologies, (1) 'fast' pyrolysis, with reaction times of millisecond to few seconds and (2) 'slow' pyrolysis, with reaction times greater than 30 minutes. In the former, pyrolysis is used for thermal conversion of cellulosic biomass to produce bio-oil with biochar being generated as a byproduct where pyrolysis conditions are optimized to maximize bio-oil yield and quality. In the slow pyrolysis, the main product is biochar and hence conditions are optimized to maximize biochar production and quality. Both pyrolysis conditions and feedstock origin have a profound impact on biochar properties. Hence, biochar characteristics and its effects on soil properties and fertility are both process- and feedstock-dependent.

Similar to compost, biochar is a process-defined material, i.e. biochar is defined by the process in which it is developed and produced rather than by a specific property, e.g. chemical composition, molecular formulation, or mineral structure. Hence, biochar properties encompass a wide range of characteristics, depending on feedstock material and pyrolysis conditions.

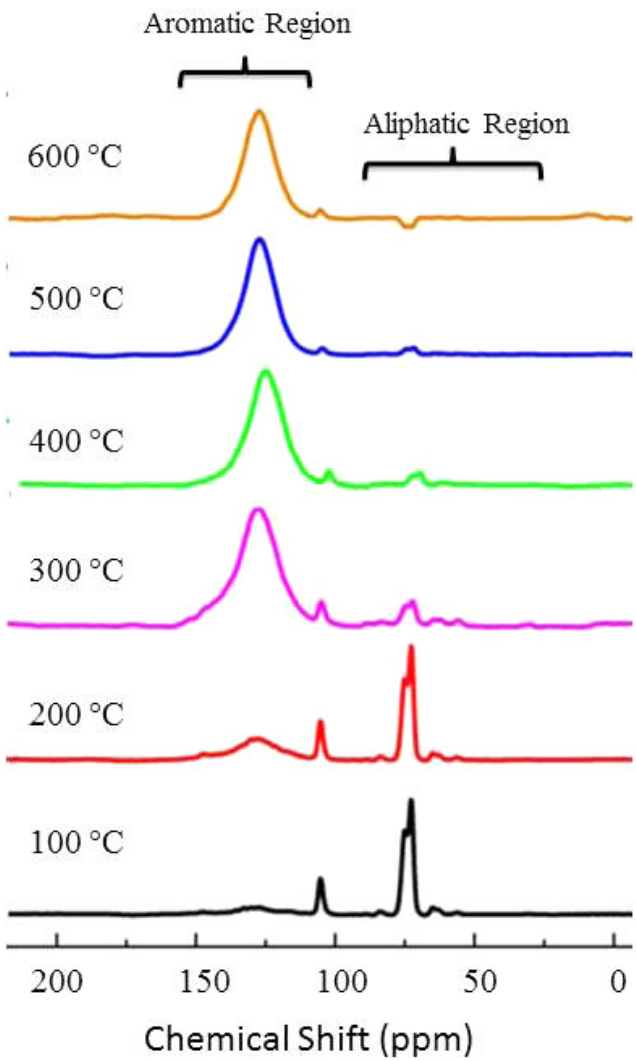
Analogous to compost characterization specifications and certification, guidelines and specifications to meet a set of minimal criteria were developed to serve as guidelines for standardization of biochar product and use as soil amendment (IBI, 2012). The objective of this chapter is to provide the framework for understanding the effect of pyrolysis conditions, feedstock source, and composition on biochar characteristics, and to synthesize existing knowledge and discuss the effect of biochar application on soil fertility.

EFFECT OF FEEDSTOCK AND PYROLYSIS CONDITIONS ON BIOCHAR PROPERTIES

Common feedstock to produce biochar include organic waste (e.g. green and animal waste), crop and timber residues (e.g. corn *[Zea mays L.]* and sorghum *[Sorghum bicolor]* stover, sawdust), as well as dedicated cellulosic energy crops (e.g. switchgrass *[Panicum virgatum]* and miscanthus *[Miscanthus giganteus]*). Biochar is commonly produced at temperatures ranging from 350 to 700 °C and its yield increases with (1) decrease in pyrolysis temperature and (2) increase in pressure, feedstock density and particle size, ash, lignin, and alkali and alkaline earth metal content (Antal et al., 1990; Richards & Zhang, 1991; Wornat et al., 1992; Antal & Gronli, 2003; Demirbas, 2004; Yang et al., 2007; Mayer et al., 2012; Lee et al., 2013).

Biochar porosity and surface properties have a marked impact on its ability to interact with and retain water and nutrients in soil. Biochar is an amphoteric material with pH usually above neutral; and as pyrolysis temperature and duration increase, surface area, pH, Lewis base and ash, and C content also increase (Ramon et al., 1999; Rutherford et al., 2004; Yang et al., 2007; Kwapinski et al., 2010; Tsai et al., 2012). As pyrolysis temperature increases and cellulose and hemicellulose decompose, the aliphatic carbon content in the biochar decreases while aromatic carbon content increases (Figure 1), leading to an increase in hydrophobicity associated with basic groups in the aromatic structures (Chun et al., 2004; Demirbas, 2004; Rutherford et al., 2004; Kwapinski et al., 2010).

Evaluating the behavior of different organic material during pyrolysis, Yang et al. (2007) found that losses of hemicellulose occurred at low temperatures (220 to 300 °C) and was associated with high CO_2 losses (attributed to losses of carboxyl groups), cellulose rapid losses occurred at temperature between 300 to 400 °C with high CO release (attributed to losses of carbonyl groups). Lignin losses occurred throughout the temperature range (160 to 900 °C) and were associated with high H_2 and CH_4 release from the thermal cracking of methoxyl and aromatic C and H of the highly aromatic lignin structure. Fused aromatic ring structures developed with increasing pyrolysis reaction times and/or peak temperature, providing the matrix in which microporosity develops, with pores <2 nm in diameter (Rutherford et al., 2004; Wu et al., 2012).



(modified from Li et al., 2013).

Figure 1. Solid-state ^{13}C NMR spectra of rice straw and rice bran at various charring temperatures.

Unlike graphite sheets which orient themselves into compact sheet structures at high temperatures, heterogeneous high-oxygen rich materials (>5% oxygen by mass) such as biochar produced at lower temperatures (ca. <700 °C) tend to develop cross-link structures that maintain the random orientation of the developed graphene/graphite like short-order crystalline, withholding the voids that contribute to its porosity. Yet, development of porosity in biochar follows a bell-shape pattern, increasing as temperature increases up to a critical temperature range from which porosity declines, at values which are feedstock dependent (Rutherford et al., 2004). Rutherford et al. (2004) noted that microporosity development coincided with loss in total mass of aromatic carbon and suggested that microporosity

develops within the fused-ring matrix. Using cellulose, lignin, and woody material, these researchers observed that total and microporosity developed at temperatures above 300 °C and increased with temperature and heating time (Rutherford et al., 2004). The extent of porosity development depends on material composition, with lignin developing porosity at higher temperatures than cellulose. The increase in biochar surface area was correlated with increase in microporosity (Rutherford et al., 2004), with cellulose surface area increasing from 2.1 $m^2 g^{-1}$ in the untreated cellulose to 147 $m^2 g^{-1}$ at 400 °C, and lignin from <1 $m^2 g^{-1}$ in the untreated material and with no significant increase below 350 °C to only 2.0 $m^2 g^{-1}$ at 400 °C and up to 162 $m^2 g^{-1}$ during pyrolysis at 500 °C for 1h. Similar trends of increase in porosity and surface area were reported for poplar (exact specie unknown) and ponderosa pine (*Pinus ponderosa*) wood when pyrolyzed at 500 °C for 1h; surface area increased from <2.0 $m^2 g^{-1}$ for both woody materials to 354 and 501 $m^2 g^{-1}$, respectively. While lignin surface area and porosity increased with increase in exposure time at each temperature level, surface area and porosity of cellulose, poplar and pine wood, and pine bark decreased at exposure times longer than 24 h (Rutherford et al., 2004).

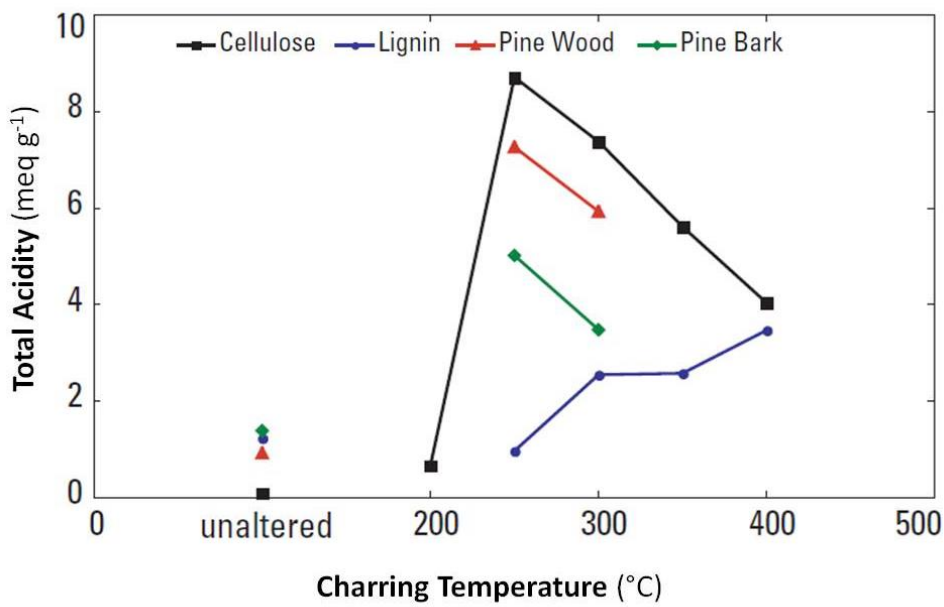
Similarly, steam activation, i.e. physical activation of biochar using hot stream of water vapor, also showed to markedly increase biochar surface area, attributed almost entirely to the increase in inner sphere pores (Lima et al., 2010). Porosity and surface area of biochar of different feedstock produced at 500 °C in a fast pyrolysis fluidized reactor with residence time of 0.1-1.0 s was measured before and after steam activation at 800 °C for 45 min. While all biochar had negligible surface area (<4.0 $m^2 g^{-1}$) and non-detected porosity, steam activation resulted in dramatic increases in biochar surface area (Lima et al., 2010). For example, surface area of biochars derived from alfalfa (*Medicago sativa L.*) stems, switchgrass, and corn stover increased from 2.3 to 204, 0.3 to 293, and 3.1 to 455 $m^2 g^{-1}$, respectively; with microporosity constituting 79, 85, and 75% of total surface area, respectively (Lima et al., 2010).

Inasmuch as pyrolysis temperature affects biochar surface area and porosity, it also affects its surface chemical properties (Boehm, 1994; Ramon et al., 1999; Brennan et al., 2001; Chun et al., 2004). Aromatic regions with π electron-rich areas (Lewis bases) at the carbon plane contribute to biochar basicity while oxygen surface oxides tend to reduces carbon plane electronic density, reducing its basicity and providing hydrophilic sites to the otherwise hydrophobic surface associated with the aromatic π-electrons (Boehm, 1994; Ramon et al., 1999; Brennan et al., 2001). Increase in pyrolysis temperature increases the content of aromatic structures in the biochar and the orientation and condensation of the aromatic groups into amorphous graphene/graphite-like structure at higher temperatures. Furthermore, increase in production temperature reduces biochar oxygen content while shifting the proportion of remaining oxygen from strong acid functional groups, such as carboxyl into weaker phenolic groups of low pK_a or oxygen-containing groups, such as pyrone-type structures, as well as from other impurities, such as amine groups that further contribute to char basicity (Boehm, 1994). Increasing temperature from 300 to 700 °C of biochar produced from wheat residue led to reduction in (1) total acidic surface functional groups, from 2.83 to 0.30 mmol g^{-1}; (2) functional groups surface density, from 15 to 1 groups nm^{-2}; (3) carboxyl groups, from 0.74 to 0.17 mmol g^{-1}; and (4) O/C ratio and biochar hydrophilicity; while increasing (1) basic functional groups content, from 0.04 to 0.29 mmol g^{-1} and (2) surface area, from 116 to 363 $m^2 g^{-1}$, all respectively (Chun et al., 2004). Indeed, the point of zero charge (PZC), i.e. pH where the net surface charge is zero, of carbonized

material was inversely related to its oxygen content, i.e. PZC increases with decrease in oxygen content (Boehm, 1994; Ramon et al., 1999).

The pattern described above, i.e. increase in aromatic structures and reduction in biochar oxygen content and strongly acidic functional groups with increase in pyrolysis temperatures, leads to an increase in biochar pH and hydrophobicity, resulting in decrease of biochar cation exchange capacity (CEC). This decrease, however, leads to increase in biochar's (1) anion exchange capacity, (2) affinity to polar organic molecules, and (3) specific sorption ability and affinity towards heavy metals (Chun et al., 2004; Lima & Marshall, 2005; Lima et al., 2010; Uchimiya et al., 2010) as the π electron-rich areas (Lewis bases) act as ligands forming coordinated covalent bonding with transition metals. Moreover, the increase in temperature increases biochar surface area, which compensates to some extent for the loss of strong acid functional groups density.

Using Boehm titration method to assess biochar acidic functional groups, Rutherford et al. (2008) evaluated the effect of feedstock type, pyrolysis peak temperature, and reaction time on development of biochar surface acidic functional groups. Pyrolysis resulted in initial increase in biochar total acidity compared to the original feedstock (cellulose, lignin, pine wood, and pine bark). While marked increase in total acidity with a sharp peak at 250 °C was noted in cellulose derived biochar, a much moderated development of total acidity was noted in lignin derived biochar (Figure 2). Furthermore, total acidity of lignin increased throughout the temperature range (200 to 400 °C), while that of cellulose and pine bark and wood decreased at pyrolysis temperatures above 250 °C (Figure 2).



(U.S. Geological Survey/ figure by Rutherford et al., 2008).

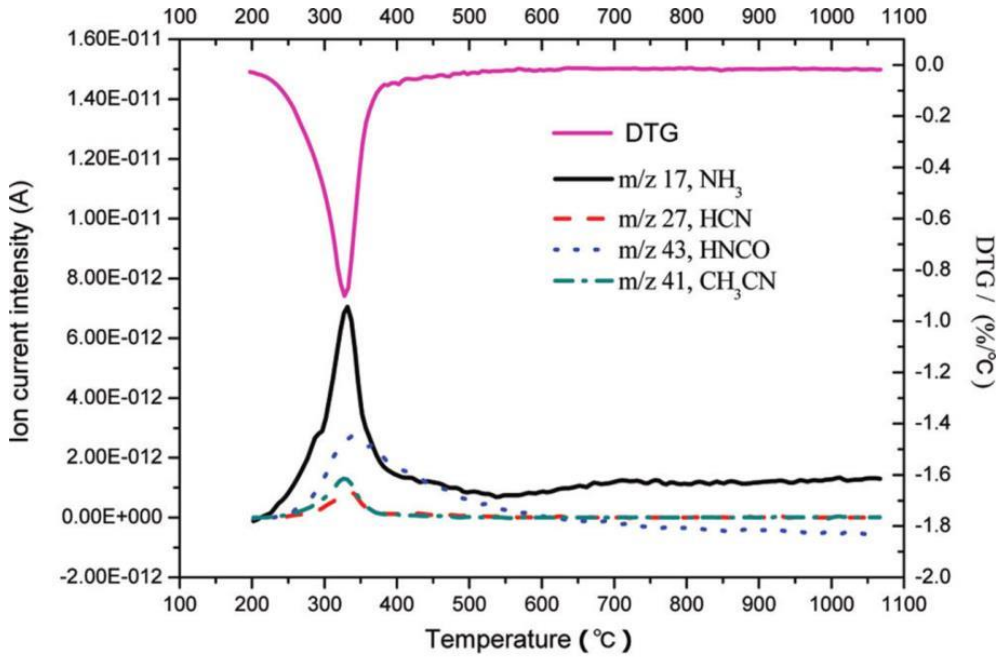
Figure 2. Total acidity of cellulose, lignin, pine wood, and pine bark after 72 hours of charring at various temperatures.

While much of the surface acidity in the cellulose and pine materials were attributed to strong acidic groups (e.g. carboxylic groups), moderately acid groups (i.e. phenols of low pK_a values) and lactones had a substantial contribution to total acidity of lignin derived biochar (Rutherford et al., 2008).

Inasmuch as biochar CEC is affected by feedstock source and composition, CEC decreases with increase in production temperature due to the effect of production temperature on oxygen functional group and total acidity. Gaskin et al. (2008) showed significant decrease in CEC of biochars made from chicken manure (61.1 to 38.3 cmol kg^{-1}), peanut (*Arachis hypogaea L.*) hull (14.2 to 4.63 cmol kg^{-1}), and pine chips (7.27 to 5.03 cmol kg^{-1}) as production temperature increased from 400 to 500 °C, all respectively. Similar findings, i.e., reduction of biochar CEC and increase in biochar pH, surface area, and porosity were reported for chicken manure biochar produced between 300 to 600 °C (Song & Guo, 2012) and sewage sludge biochar produced at 400 and 600 °C (Mendez et al. 2013). During pyrolysis of rice straw at different temperatures (300 to 700 °C) and duration (1 to 5 h), Wu et al. (2012) found a decrease from 184 to 46 cmol kg^{-1} in biochar surface acidity and an increase from 84 to 147 cmol kg^{-1} in surface alkalinity as pyrolysis temperature increased from 300 and 700 °C, all respectively. A slight increase in biochar CEC produced at 300 and 400 °C (57 to 62 cmol kg^{-1}, respectively) was followed by a sharp decrease to 32 and 23 cmol kg^{-1} at 500 and 700 °C, respectively; with pyrolysis duration (1 to 5 h) with no significant or consistent effect (Wu et a., 2012). Overall, CEC, Olsen extractable P, extractable Ca, K, and Mg, and total N content increased with temperature, reaching a maximum at 400 °C (Wu et al., 2012).While steam activation is known to have marked effect on biochar surface area (Lima et al., 2010), conducted at the same temperature as biochar production temperature, steam activation reduced or had no significant effect on CEC of the pine chip derived biochar (Gaskin et al., 2008).

Biochar CEC increases once biochar is applied to soil because of both biotic and abiotic oxygenation processes that occur under natural soil conditions and are positively correlated with annual mean temperature (Cheng et al., 2006). However, the oxygenation process is slow and affects mostly the external biochar surface (Nguyen et al., 2009). As such, this effect may be limited as the high surface area of biochar is attributed to the increase in inner sphere porosity, which occurs with increase in pyrolysis temperature and/or upon (steam) activation processes (Lima et al., 2010). In all, low production temperature (ca. 250 to 350 °C) promotes higher total acidity, and as a result a higher CEC, while high temperatures (ca. >500 °C) tend to promote higher total surface area, with much of the increase resulting in inner surface area of developed pores. Such increase in porosity, and subsequent increase in surface area will be diminished at elevated temperatures (ca. >700 °C).

Feedstock nutrient recovery and availability for plant uptake tend to decrease with increase in pyrolysis temperature. Much of the loss of nutrients as volatile compound (e.g. N and S) occur at temperatures above 350 - 400 °C and is process- and feedstock-dependent (Gaskin et al., 2008; Wang et al., 2010; Wu et al., 2012). The remaining nutrients are redistributed into chemical forms less available for plant uptake. Wang et al. (2010) showed N loss as ammonia (NH_3), hydrogen cyanide (HCN), isocyanic acid (HNCO), and acetonitrile (CH_3CN) from pyrolysis of wheat (*Triticum spp.*) straw starting at 250 °C, to sharply peaked at 350 °C, and to tapered off with minimal changes at temperatures above 550 °C (Figure 3). Increase in pyrolysis temperatures also results in decrease in hydrolysable organic N content and increase in aromatic and condensed heterocyclic N structures in the biochar.



(Wang et al., 2010; Reprinted with permission from Energy and Fuels. Copyright 2013, American Chemical Society).

Figure 3. Emission of nitrogen species during pyrolysis of wheat straw.

These changes in N dynamics occur as consequence of both (1) the relative increases in their proportion as decomposition of hydrolysable N progress with temperature and (2) the synthesis of aromatic and heterocyclic N from aliphatic or low-molecular weight heterocyclic structures during the pyrolysis process (Almendros et al., 2003). Overall, increase in pyrolysis temperature decreases feedstock N recovery in biochar and reduces the availability of the remaining nitrogen. Noteworthy, the content of N and other elements in biochar increases in the process at lower temperature due to preferential loss of O and C. Overall, decrease in N due to volatilization occurs at higher temperatures (ca. above 500 °C) as reported by others (Tsai et al 2012; Wu et al., 2012). Inasmuch as heterocyclic N is suggested to be plant available, being trapped in a not-readily available carbonaceous structure is likely to limit its availability for plant uptake (Wang et al., 2012).

Sulfur is released as carbonyl sulfide (COS) and hydrogen sulfide (H_2S) at 200 - 400 °C, which is attributed to the decomposition of organically-bound sulfur, while release of SO_2 at temperatures above 950 °C is attributed to evaporation and/or transformation of inorganic sulfate (Wang et al., 2010). Negligible potassium loss at temperatures below 600 °C was reported in pyrolysis of wheat straw (Wang et al., 2010). Yu et al. (2005; in Chan & Xu, 2009) reported a sharp loss of nearly 50% in K during pyrolysis of rice (*Oryza sativa*) straw at temperature between 473 to 673 °C, attributed mostly to the loss of the water soluble K fraction. Inasmuch as other nutrients such as P tend to concentrate in the biochar with increase in peak pyrolysis temperature, they also tend to shift into chemical forms that are less available for plant uptake, e.g. apatite-type minerals (Chan & Xu, 2009; Gaskin et al., 2008). Using giant reed (*Arundo donax L.*), Zheng et al. (2013) also showed a decrease in water

soluble P and NH_4^+ with increase in pyrolysis temperature from 300 to 600 °C; yet the authors reported increase in soluble K with increase in peak pyrolysis temperature.

The fact that nutrient availability decreased with increase in peak pyrolysis temperature implies that pyrolysis can favorably be used to mitigate elevated excess nutrient content in organic waste (e.g. chicken manure). Waste volume and pathogen reduction accompanied by entrapment of the manure-borne excess nutrients into less mobile phases should be viewed favorably as it lessen the adverse environmental impact associated with manure application while still maintaining high levels of available nutrients (Lima & Marshall, 2005; Gaskin et al., 2008; Lima et al., 2009; Hass et al., 2012). Gaskin et al. (2008) evaluated nutrient content and availability in biochar produced from chicken litter, peanut hulls, and pine chips feedstock at two different temperatures, 400 and 500 °C. The increase in temperature increased the nutrient content; however, it decreased their availability for plant uptake, as measured by the Mehlich-1 extraction. Nevertheless, while Mehlich-1 available P and K decreased with temperature in all biochars, their concentration in poultry litter biochars (5.33 and 38.1 g kg^{-1}, respectively) were much higher than biochar from peanut hulls (0.57 and 5.91 g kg^{-1}, respectively) or pine chips (0.04 and 0.41 g kg^{-1}, respectively). Hass et al. (2012) showed a reduction in soil Mehlich-3 available K, P, and, S and increase in Cu and Zn with increase in production temperature of chicken manure biochar (350 vs. 700 °C) when incubated for eight weeks in a Typic Hapludult soil (pH 4.8). Yet, nutrient availability increased with biochar application, and to alarming levels at high application rates (equivalent to > 20 Mg ha^{-1}), as high levels of total P and PO_4^{2-} appeared in the leachate (above 4 and 2 mg L^{-1}, respectively). Moreover, Hass et al. (2012) showed that at application rate of 10 Mg biochar ha^{-1}, Mehlich-3 extractable P reached levels (>200 mg kg^{-1}) considered critical threshold value above which excess P can be expected in runoff (>1 mg L^{-1}; Sharpley et al., 1996).

Biochar liming capacity is an influential property affecting soil fertility (Verheijen et al., 2009). While most biochar have high pH (7 – 11), their pH value do not reflect their acid neutralization capacity or 'liming capacity' – the ability to increase soil pH, usually expressed as percent calcium carbonate equivalent (CCE). Biochar CCE is limited mostly to the mineral or ash content and composition. For a given feedstock, biochar CCE increases with pyrolysis temperature due to the increase in ash and metal content and due to transformation of alkali (K and Na) and alkaline earth (Ca and Mg) metals into oxide and carbonate minerals such as K_2O, CaO, $Ca/MgCO_3$ and /or MgO. Yuan et al. (2011a) showed an increase in biochar pH and alkalinity with pyrolysis temperature (300 to 700 °C) in biochars from canola (*Brassica campestris L.*), corn, soybean (*Glycine max L.*), and peanut straws. The authors suggested that the $CaCO_3$ generated at the higher temperatures (500 and 700 °C) is the dominant component contributing to biochar alkalinity at these temperatures, while biochar acidic functional groups, such as hydroxyl and carboxyl, contribute to biochar alkalinity at lower temperature (300 °C). Conversely, Wu et al. (2012) showed an increase in $CaCO_3$ content in rice straw biochar produced at low temperatures (300 to 500 °C) and a decrease at higher pyrolysis temperatures (600 to 700 °C). However, biochar ash content and alkalinity tend to increase with pyrolysis temperature (Yuan et al., 2011a; Yuan et al., 2011b; Wu et al., 2012).

While ash content might be an indicator of biochar CCE, the actual contribution of the ash depend on its elemental and mineral composition, as different plants result in different nutrient composition. For example, woody biomass generally has very low Si content (0.05 to 0.11); oak (*Quercus spp*) accumulates more Ca and K than pine (*Pinus spp*) (Ragland et al.,

1991). While most cereal crops tend to accumulate higher Si compared to legumes, 1 to 3% vs. <0.5%, respectively (Marschner, 1995), biochars from cereal crops are likely to result in high ash content but of lower CCE value as relative contribution of SiO_2 to biochar CCE is much lower than that of alkali or alkaline carbonate and/or oxides. Indeed, plants can differ markedly in their basic cations content and ash alkalinity (Tang & Rengel, 2003). Legumes (and those of temperate zones more than those of tropical ones) tend to accumulate excess basic cations, resulting in much higher alkaline ash content than non-legume plants (Bolan et al., 1991).

While base accumulation of cereal crops ranges from 25 to 75 cmol kg^{-1}, that in pasture legumes plants ranges from 61 to 255 cmol kg^{-1} (Tang & Rengel, 2003). Producing biochar from straw of different plant materials at 350 °C for 4 hours, Yuan et al. (2011a, 2011b) found that biochar from legume feedstock resulted in higher biochar alkalinity, ranging from 217 to 326 cmol kg^{-1} (mung bean [*Vigna radiata*] > peanut > soybean > pea [*Pisum sativum*]> faba bean [*Vicia faba*]) compared to biochar from non-legume feedstock which ranged from 120 to 191 cmol kg^{-1} (canola > corn > rice > wheat). Incubated in an Ultisol (pH 4.2) at a rate of 10 g kg^{-1} for 50 days, biochar from legume straw increased the soil pH by 0.5 to 0.7, while the biochar from non-legume straw increased the soil pH by only 0.1 to 0.4 (Yuan et al., 2011b). At the end of the 50 d incubation, only pea and soybean straw biochars increased soil pH above the pH achieved by their respective raw material applied to soil at 20 g kg^{-1} (Yuan et al., 2011b).

Organic wastes of initially high ash content, e.g. chicken, dairy and hog manures, likely result in biochar of relatively high CCE. Yet, incubating an Ultisol (pH 4.8) for eight weeks with chicken manure biochar produced at 350 and 700 °C, at similar application rate as in Yuan et al. (2011b), i.e. 10 g kg^{-1}, resulted in increases in soil pH by only 0.3 and 0.5 pH units, respectively (Hass et al., 2012). Soil pH increased to 5.8 and 5.9 by the end of the incubation at application rates of 20 and 40 g kg^{-1} of biochar produced at 700 and 350 °C, respectively. Based on linear regression between biochar application and soil pH, the authors calculated that 36 and 56 g kg^{-1} (i.e. equivalent to 73 and 112 Mg ha^{-1}) of chicken manure biochar produced at 700 and 350 °C, respectively, were needed to increase the soil pH to 6.4, a level achieved with 3 g kg^{-1} (6 Mg ha^{-1}) of dolomitic lime (Hass et al., 2012). Such high biochar application rates may not be attractive replacement option for lime. Moreover, calculated on a volume basis the spread between biochar and dolomitic lime application rate more than double as bulk density of biochar is less than half that of lime. However, as additional benefits associated with biochar application emerge such an investment might be justified.

Figure 4 provides a conceptual illustrative summary for the effect for pyrolysis peak temperature on selected biochar properties that discussed above. Initial mineral and organic feedstock composition will impact biochar composition at each peak temperature, with feedstock of high lignin to (hemi) cellulose ratio (i.e. woody biomass) likely to result in lower carbon loss during pyrolysis, higher total carbon recovery in biochar, higher stability of the recovered carbon, and higher surface area than that produced from feedstock of lower lignin:(hemi)cellulose ratio (e.g. grass). Yet, such biochar likely will result in lower CEC, lower mineral (nutrients, liming) content, and to exhibit a lower concentration of mineral content during pyrolysis due to lower losses of initial feedstock carbon matrix.

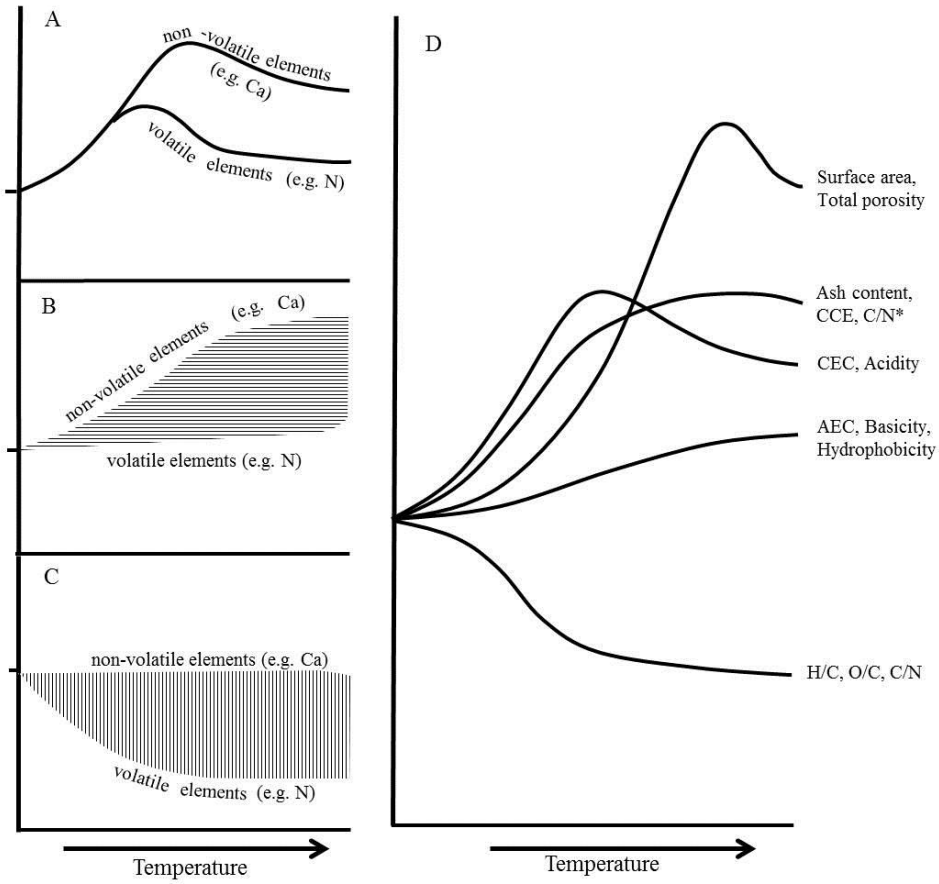


Figure 4. Conceptual sketch of (A) biochar nutrient availability, (B) nutrient content, (C) feedstock nutrient recovery, and (D) selected properties changes with increase in pyrolysis temperature relative to feedstock state. Rates and peaks varies and are process, feedstock and element dependent (CCE, calcium carbonate equivalent; CEC cation exchange capacity; AEC, anion exchange capacity); * C/N ratio increase at higher rate and do not decline at elevated temperature (a dedicated line was avoided for the purpose of simplicity).

EFFECT OF BIOCHAR APPLICATION ON SOIL QUALITY INDICATORS

Biochar used as soil amendment affects chemical, physical, and biological soil quality indicators (Laird et al., 2010a, 2010b; Jeffery et al., 2011; Biederman & Harpole, 2012; Chintala et al. 2013). Using over 100 studies in a meta-analysis, Biederman & Harpole (2012) found that biochar increased aboveground biomass, crop yield, soil microbial biomass, rhizobia nodulation, plant K tissue concentration, soil NPK, and total soil carbon (C) compared to control conditions. Yet, biochar applications resulted in no significant response in belowground biomass, ratio between above and below ground biomass, mycorrhizal root colonization, soil inorganic N, as well as plant tissue N content (Biederman & Harpole, 2012). Incubated with a fine-loam US Midwest Hapludolls topsoil (pH 6.4), slow pyrolysis biochar derived from mixed hardwood (oak [*Quercus spp.*] and hickory [*Carya spp.*]) material was shown to decreased soil bulk density and leaching of nutrient while increasing

soil effective CEC, %C, C/N ratio, water retention, and plant available water (Laird et al., 2010a, 2010b). At high application rates (>5 g kg^{-1} soil), biochar also increased soil surface area and Mehlich-3 extractable P (Laird et al., 2010a). Similarly, positive effects of biochar application on soil quality indicators were reported by Novak et al. (2009b) for a Typic Kandiudults loamy sand coastal plain soil from Eastern US. Application of pecan [*Carya illinoinensis* (Wangenh.) K. Koch] shell biochar (up to 20 g kg^{-1}) increased soil pH and reduced exchangeable acidity, but had no effect on soil CEC. In addition, the biochar application reduced S and Zn availability, while increasing availability of other plant essential nutrients, such as Ca, K, Mn, and P as determined by the Mehlich-1 extraction. The reduction in soil exchangeable acidity was attributed to dissolution of alkaline earth metal oxides in the biochar ash (e.g. CaO), leading to speciation of soluble Al into nontoxic species for plants or precipitation as aluminum oxide (Novak et al., 2009a, 2009b).

Biochar affects soil nitrogen dynamics through sorption and chemical transformations of both inorganic and organic forms of N; these processes are biochar- and N species-dependent. Sorption of the plant nutrients ammonium (cationic form) and nitrate (anionic form) will greatly depend on the biochar chemical and physical properties, e.g. cation exchange capacity, anion exchange capacity, surface area, and pH, which all depend on the pyrolysis temperature (Figure 4). Ammonium likely sorbs on biochars by electrostatic interactions since most biochars possess negative charges as suggested by the biochar cation exchange capacity. In general, ammonium is sorbed in biochars produced at temperatures ≥300 ºC, as demonstrated by sorption isotherms of ammonium on biochars from cacao (*Theobroma cacao*) shell and corn cobs at 300-350 ºC (Hale, et al., 2013), pine wood at 450 ºC (Sika & Hardie, 2013), Brazilian pepperwood (*Schinus terebinthifolius*) at 300, 450, and 600 ºC, sugarcane bagasse (*Saccharum officinarum*), peanut hull, and bamboo (*Bambuseae spp*) produced at 300 and 600 ºC (Yao, et al., 2012). Oddly, biochars produced at 450 ºC from sugarcane bagasse, peanut hull, and bamboo did not sorb ammonium (Yao, et al., 2012). As biochar properties change with time due to biogeochemical processes, e.g. redox reactions, and microbial colonization; sorption of ammonium is also affected, e.g. Singh et al. (2010) observed that 4 months after applying biochar to soils, the leaching of ammonium decreased, attributed to the increase of biochar sorptivity as biochar surface oxidizes with aging. Another factor affecting ammonium sorption on biochar is pH since aqueous NH_4^+ and gas NH_3 exist in equilibrium (pK_a of NH_3 is 9.2 at 20 ºC); as pH increases from neutral to alkaline, gas NH_3 starts forming while aqueous NH_4^+ concentration decreases (Bates & Pinching, 1950). Zhang et al. (2013) demonstrated that the efficacy of corn biochar (produced at 600 ºC) for ammonium sorption decreased about 35% as the pH solution increased from 4 to 10; perhaps due to the conversion of aqueous NH_4^+ to the gas NH_3. Taghizadeh-Toosi et al. (2012) observed that ^{15}N labeled NH_3 sorbed on Monterey pine (*Pinus radiata*) biochars (300 to 500 ºC, pH_{H2O} 5.2 to 7.8) and that the sorbed NH_3 was available for plant uptake as demonstrated when perennial ryegrass was grown in a Temuka silt loam soil (Endoaquept) amended with enriched ^{15}N labeled NH_3 biochars; NH_3 sorption is attributed to the carboxyl acid functional groups (Asada, et al., 2002; Kastner, et al., 2009) and low pH of biochars (Kastner, et al., 2009). Acidified biochar from pine chips (slow pyrolysis at 400 ºC), when applied to an amended soil with poultry litter, reduced NH_3 losses by 59% (Doydora, et al., 2011). However, Sika and Hardie (2013) observed that the sorbed ammonium on pine wood biochar was not readily available for plant uptake. The authors found only small amounts of

exchangeable ammonium (using 2 M KCl extractant) when a sandy soil was incubated with biochar and fertilizer for 6 weeks.

Sorption of nitrate is expected to be of lesser extent than ammonium since the biochar's anion exchange capacity is smaller than the cation exchange capacity (Figure 4). Studies had demonstrated that nitrate sorption occurs on biochars produced at ≥450 ºC, e.g. (1) pine wood produced at 450 ºC (Sika & Hardie, 2013), (2) Brazilian pepperwood, (3) peanut hull produced at 600 ºC (Yao, et al., 2012), and (4) bagasse sugarcane produced at ≥700 ºC (Kameyama, et al., 2012). However, nitrate did not sorb on biochars from bagasse sugarcane and bamboo produced at 600 ºC (Yao, et al., 2012), cacao shell and corn cobs produced from 300 to 550 ºC (Hale, et al., 2013). The above discrepancy on nitrate sorption by biochar bagasse sugarcane might be due to the different conditions that the sorption isotherms were conducted. Biochars were rinsed with DI water before use to remove impurities and the sorption equilibrium was set for 20 h (Yao, et al., 2012) or biochars used without rinsing and the sorption equilibrium was set at 120 h (Kameyama, et al., 2012). It is hypothesized that base functional groups of biochar produced at high temperatures play an important role on nitrate sorption on biochars (Kameyama, et al., 2012). Although nitrate is weakly sorbed on biochar, nitrate residence's time in soils might increase in biochar amended soils as compared to soils without biochar; thus, nitrate might be available for plant uptake (Kameyama, et al., 2012; Sika & Hardie, 2013) while reducing nitrate leaching (Kameyama, et al., 2012; Sika & Hardie, 2013; Singh, et al., 2010; Yao, et al., 2012).

Soil organic N transformations and microbial community are influenced by biochar additions to soils. Biochar produced at lower temperatures contains more labile C (Figures 2 and 4) and N than one produced in higher temperatures, hence, acting in part as substrate. Similarly, biochar C/N ratio and liming capacity (Figure 4) are expected to increase with increase in biochar peak production temperature and to affect soil microbial community structure and function. Ameloot et al. (2013) observed that the addition of poultry litter or pine chips biochars produced at 400 and 500 ºC to acid soils (pH_{KCl} 4.6, 5.3) increased the soil bacteria:fungi ratio (B:F ratio), with biochars produced at 500 ºC shifting soil B:F to higher values than those produced at 400 ºC. The additions of biochar from silage corn induced mineralization of the soil recalcitrant N to ammonium (350 ºC biochar >550 ºC biochar >control), but no differences were observed on the mineralization of the labile organic N, suggesting that biochar additions promote SOM turnover, a priming effect induced by biochar (Nelissen et al., 2012). Furthermore, the addition of biochar of lower C/N ratio (poultry litter) resulted in net N mineralization while addition of the higher C/N ratio pine chips biochar resulted in net N immobilization (Ameloot et al., 2013). While filamentous fungi dominate aerobic mesophilic flora in soils of pH below 5.5, bacterial population increases with increase in soil pH and available N, with optimal activity of decomposer community occurring at natural pH, i.e. from 6.5 to 7.2 values (Alexander, 1964). The effects of pH, substrate and N availability on microbial community and B:F ratio are also well documented in non-biochar related studies conducted at different ecosystems and land uses (Bardgett et al., 1996; Blagodatskaya & Anderson, 1998; Baath & Anderson, 2003; De Vries et al., 2006; Rousk et al., 2009). Hence, reduction in fungi and increase in bacteria and an increase in overall soil microbial activity with increase in N availability as shown by Ameloot et al. (2013) is an expected outcome (Alexander, 1964), especially in acid soils where biochar application results in increase in soil pH towards, but not exceeding, natural pH values.

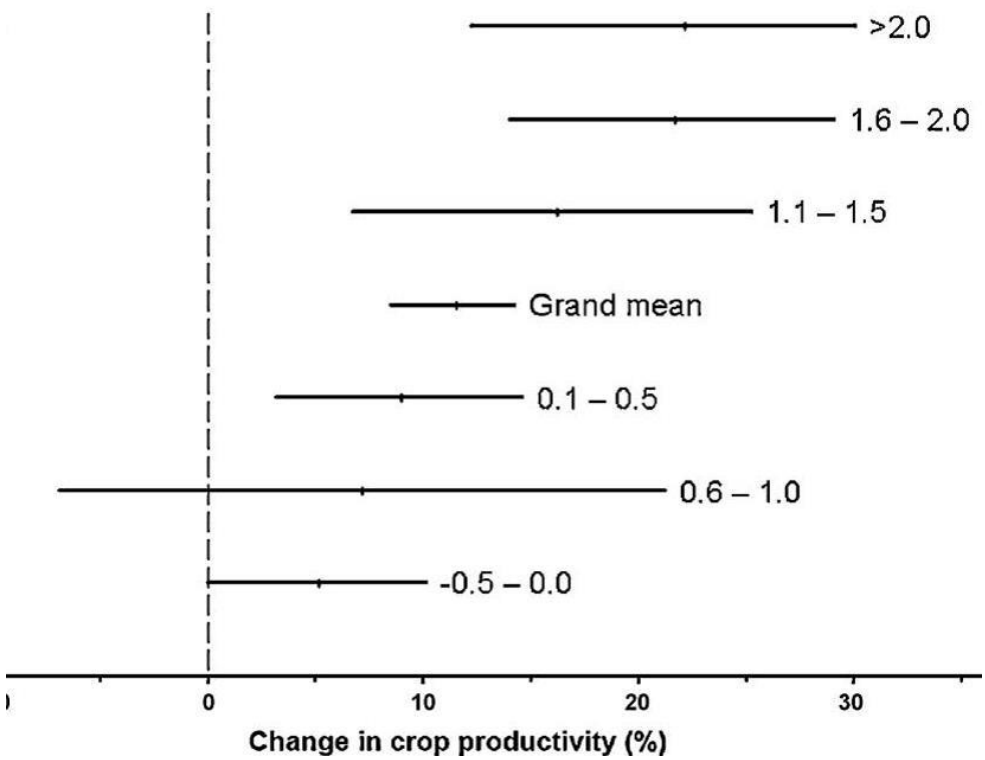
Biochar spatial structure and inner void volume is suggested as habitat for fungi and bacteria, providing physical protection from soil predators (Lehmann et al., 2011; Warnock et al., 2007). Yet, examining 3 year old field-aged biochar particles Quilliam et al. (2013a) found microbial colonization to be evenly distributed along biochar particles transects with no evidence for increase in microbial presence at the internal areas of biochar compared to biochar-soil interface. Moreover, CO_2 evolution rates from the soil brushed from biochar surface were similar to that of the bulk soil, both of which were higher than that of internal or external surfaces of field-aged biochar, which were not significantly different from each other (Quilliam et al., 2013a). Biochar also suppresses intercellular signaling and disrupts communication within a growing multicellular microbial system, the degree of which was positively correlated to biochar surface area and peak production temperature; this phenomenon is attributed to the affinity of biochar for acyl-homoserine lactone, the intercellular signaling molecule used in this experiment (Masiello et al., 2013). Several studies had demonstrated the suppressive effect of biochar on development of powdery mildew (*Leveillula taurica*) on tomato (Elad et al., 2010), and reductions in the percentage of root lesions caused by *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum*, where biochar also showed to improve AM fungi root colonization and increase in root growth and mass (Elmer & Pignatello, 2011). Biochar also increased abundance of genes encoding for denitrification-associated enzymes and of denitrifying bacteria, resulting in lower N_2O emission during composting of organic waste (Wang et al., 2013). Soil pH is known to significantly affect N_2O emission (Firestone, 1982) and to affect abundance of genes encoding for denitrification associated enzymes and of denitrifying bacteria (Cuhel et al., 2010), similar to the above finding by Wang et al. (2013). Noteworthy, the observed reduction in the proportion of N_2O in N gas emission (i.e. $N_2O/[N_2O + N_2]$) was due to increase in N_2 emission, amid a rather constant emission of N_2O throughout the pH range tested, 5.5 to 7.7 (Cuhel et al., 2010). Conversely, a sharp increase in soil N_2O emission at soil pH below 5.0-5.5 was reported by others where both biotic and abiotic denitrification mechanisms, with nitrite playing a significant role in the abiotic pathway (Van Cleemput & Samater, 1995; Morkved et al., 2007). Regardless of the mechanisms involved, their proportion and/or relative significance, N_2O emission from soil is tightly and inversely correlated with soil pH. Inasmuch as biochar effect on soil pH may improve activity of N_2O-reducing organism (Yanai et al., 2007), biochar, because of its aromaticity (Figure 1), can act as electron donor and acceptor (Joseph et al., 2010), facilitating the transfer of electrons ("electron shuttle"), and promoting the reduction of N_2O to N_2 (Cayuela et al., 2013).

Biochar application also showed to affect positively soil physical properties, including soil bulk density, aggregation, available water content, and soil hydraulic conductivity (Novak et al., 2009a; Herath et al., 2013). Biochar application improved soil water holding capacity in coarse texture soils but not in soils of high organic matter content or fine texture (Novak et al., 2009a; Karhu et al., 2011; Uzoma et al., 2011a; Abel et al., 2013; Masto et al., 2013). Biochar properties including polarity, surface area, total and micro porosity improve some soil physical properties; soil available water content was improved with biochar of high pyrolysis temperature (Mendez et al., 2013), whereas soil aggregation was improved with increase in biochar hydrophilicity which improved over time (Novak et al., 2009a; Herath et al., 2013). Yet, effect of biochar on soil nutrients, microbial growth, and mycorrhizal colonization was found to be temporary as no significant effect was noted in these soil quality

indicators three years after chipped wood trunk biochar was applied to a sandy clay loam Eutric Cambisol at rates of up to 50 Mg ha^{-1} (Quilliam et al., 2012; Quilliam et al., 2013a).

EFFECT OF BIOCHAR APPLICATION ON CROP YIELD

The main positive effect of biochar on plant performances is through its liming potential and its effect on soil pH. As assessed in meta-analysis studies, biochar application resulted in moderate (10-15%), yet significant increase in crop productivity (Jeffery et al., 2011). Positive response to biochar application occurred (1) on coarse texture soils more than on soils of finer texture, (2) with increase in application rate, (3) when applied in combination with fertilizers, (4) with increase in biochar alkalinity, and (5) when larger increase in soil pH occurred in response to biochar application (Verheijen et al., 2009; Jeffery et al., 2011; Biederman & Harpole, 2012). When biochar applications did not increase the native soil pH, it resulted in no significant effect on crop yield; conversely, when biochar applications increased the soil pH, it resulted in an increase in crop yield (Figure 5). Inasmuch as increasing soil pH leads to decrease in nutrient availability and subsequent deficiencies at high soil pHs (ca. pH>8), raising pH of acid soils ameliorates acid soils growth limiting factors (Parker et al., 1988; Fox et al., 1991; He et al., 1999; Ritchey & Snuffer, 2002).



(Jeffery et al., 2011, Elsevier, with permission).

Figure 5. Percent changes in crop productivity as influenced by changes in soil pH upon addition of biochar. Points show means of treatments, horizontal bars show 95% confidence intervals.

Increase in pH of an acid soil leads to increase soil CEC as soil pH rises above soil mineral PZC, or as it leads to dissociation of functional groups of amphoteric material (e.g. organic matter) or variable charge minerals such as free oxides of Fe, Al, and Mn. Increasing pH also increases soil base saturation, while reducing the activity and subsequent toxicity of Al and Mn. Consistent and beneficial effects of biochar application on crop yield in acid soils is well documented (Chan et al., 2008; Kimetu et al., 2008; Hossain et al., 2010; Major et al., 2010; Van Zwieten et al., 2010; Peng et al., 2011; Uzoma et al., 2011a, 2011b). Van Zwieten et al. (2010) reported positive effect on yield of wheat, soybean, and radish (*Raphanus sativus*) grown in a ferrosol (pH_{CaCl_2} 4.2), but not in a calcareous soil (pH_{CaCl_2}7.7), when amended with biochar produced at 550 °C from paper mill sludge mixed with waste wood chips and applied at rate of 10 Mg ha^{-1}. Aluminum and Mn toxicity are known to be growth limiting factors in acid soils (Fay et al., 1978; Fox et al., 1991; He et al., 1999) and overcoming such limitation was likely achieved as biochar application resulted in increase in soil pH (from 4.2 to >5.0), increase in CEC and exchangeable Ca, and decrease in soil exchangeable Al (Van Zwieten et al., 2010). Inasmuch as Al^{3+} is thought to be the predominant species responsible for plant growth reduction, Kinraide and Parker (1990) suggested that plant growth was more sensitive to $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ than Al^{3+} since the presence of H^{+} (at the low pH where Al^{3+} predominates) alleviates Al^{3+} toxicity through competition with Al^{3+} at the root cell plasma membrane (Kinraide & Parker, 1990; Kinraide, 1993). Aluminum uptake inhibition by base cations was found to decrease in the order of $Ca^{2+} > Mg^{2+} \approx Sr^{2+} >> K^{+} \approx Na^{+}$ (Kinraide & Parker, 1987). Traditional management practices used to ameliorate Al toxicity and increase pH of acid soils include use of limestone, dolomite, by-products such as fly ash, fluidized-bed combustion by-products, wood ash, as well as organic amendments such as mulch and green manures (Duong & Diep, 1986; Hue & Amien, 1989; Wang et al., 1999; Ritchey & Snuffer, 2002; Ritchey et al., 2004; Qin & Chen, 2005).

In acid soils where Al and/or Mn toxicity have lesser effect on plant growth (e.g. pH ≥ 5.5, high organic matter, or the use of tolerant plants to acid conditions), biochar application may have limited effect on plant response. No effect of biochar (pH 7.8) from Monterey pine (*Pinus radiate*) on perennial ryegrass (*Lolium perenne* L.) herbage biomass yield and herbage N content was observed when biochar was applied to pasture land with an Andic Dystrudepts soil (pH 5.5) at application rates of up to 30 Mg ha^{-1} (Taghizadeh-Toosi et al., 2011). In another study, biochar and organic amendments (sawdust, manure, and tithonia [*Tithonia spp.*] green biomass), improved maize grain yield in two consecutive seasons along a chronosequence of a degraded soil previously treated with mineral fertilizers (Kimetu et al., 2008). Yet, no significant differences were found between biochar and sawdust treatments in all but the first year at the most drastically disturbed soil (Kimetu et al., 2008). The above effect is likely in part due to the role of organic matter in ameliorating aluminum toxicity by binding Al poly/monomeric species (Marschner, 1995, Haynes & Mokolobate, 2001; Ma et al., 2001). Green manure and mulch showed to be an effective agents in alleviating Al toxicity (Hue & Amien, 1989; Duong & Diep, 1986; Qin & Chen, 2005); where leguminous residues (*Calopogonium*, soybean, and *Leucaena*) were more effective in reducing monomeric Al in solution of an Indonesian red-yellow podzolic soil than non-leguminous residues (*Imperata*, sugarcane, and rice) (Berek et al., 1995).

Biochar also showed to have different effect on yield in subsequent years when applied to different soils. While wood biochar application (8 and 20 Mg ha^{-1}) to a Typic Haplustox

(pH_{KCl} 3.9) had no effect on first season maize growth, a significant grain yield increase during the subsequent three seasons was attributed to greater plant available Ca and Mg in the biochar treated soil as compared to the control (Major et al., 2010). However, a nil effect of dairy manure biochar application (22.4 Mg ha^{-1}) to a Xeric Haplocalcids soil (pH 7.6) on silage corn yield in the first season followed by a significant yield reduction in the following season was attributed to development of N deficiency (Lentz & Ippolito, 2012).

In addition to acidity, nutrient deficiency is another limiting factor for plant growth in acid soils, caused by the weathering processes during the pedogenic development of acid soils. Van Zwieten et al. (2010) showed that only upon addition of fertilizer concurrently with biochar derived from the slow pyrolysis of paper mill did significant increase in wheat yield occurred in an acid soil (2.5-fold). Similarly, Hossain et al. (2010) found limited improvement in cherry tomato (*Lycopersicon esculentum*) yield grown in an acid soil (pH 4.6) amended with biochar from wastewater biosolids, which was lower than that achieved by fertilizers alone. Plant productivity nearly doubled upon using biochar in combination with fertilizer (Hossain et al., 2010). The marked response in plant growth to fertilizer application in biochar-amended soils suggests that inasmuch as biochar likely ameliorated soil acidity, nutrient availability limits plant growth.

A combined effect of increasing soil pH and supplementing crop nutrient requirement is likely to be achieved by using biochar from animal waste feedstock such as chicken and dairy manure. A marked positive effect of chicken manure biochar on crop productivity was found (Jeffery et al., 2011), likely due to the high nutrient load in the chicken manure biochar. As shown by Hass et al. (2012), adequate nutrient levels and excess available P can be expected in chicken manure biochar amended soil. Applying cow manure biochar to a sandy soil (pH 6.4) at rate of up to 20 Mg ha^{-1} resulted in increase in maize grain yield, dry matter, water use efficiency, and grain nutrient content at biochar application rates of 15 and 20 Mg ha^{-1}, but not at 10 Mg ha^{-1} (Uzoman et al., 2011b). Applied to an acidic ferralsol (pH 4.7), Slavich et al. (2013) showed contrasting effects of feedlot manure and green waste biochars on productivity of annual ryegrass (*Lolium multiflorum*) and peanut. While the manure biochar increased both productivity and N use efficiency of annual ryegrass and dry matter yield of peanut, no improvement was noticed by green waste biochar. The authors attributed the differences to higher total (6900 and 190 mg kg^{-1}) and available P (73 and 6 mg kg^{-1}, respectively) in the manure biochar compared to the green waste biochar, which were still maintained at higher levels in the soil three years after application (Slavich et al., 2013). In a similar soil type, Van Zwieten et al. (2013) showed increase in fresh corn cob yield in a field treated with poultry manure biochar applied at the same N-level as urea treated corn. While resulting in similar yield and corn N content as in raw poultry manure treatment, biochar treatment resulted in lower levels of N_2O emission and carbon mineralization rate (Van Zwieten et al., 2013).

Crop productivity response to biochar application was found to also be plant-type dependent, where annuals did better than perennials and legumes better than some grasses (Jeffry et al., 2011; Biederman & Harpole, 2012). As the spread between the native soil pH and the pH at which optimal growth condition of a given plant increases, a reduction in performances is expected; and narrowing the pH gap will improve plant performances. Nil to adverse effect of biochar on ryegrass productivity in acid soils (Jeffery et al., 2011) is likely to the fact that ryegrass is an acid tolerant plant and hence may not benefit much from an increase in soil pH, which may not have been the limiting factor for ryegrass yield, and in fact

could further lead to reduction in plant available nutrients. Hart and Mellbye (2010) for example, showed that annual ryegrass growth and yield indicators did not improve by raising soil pH above 5.3. Conversely, increase in productivity of legumes when biochar is applied in acid soils (Jeffery et al., 2011) is likely to the fact that nodulation and N fixation by legumes are very pH-sensitive (Zahran, 1999).

Evans et al. (1980) showed that N fixation does not occur at pH <4.8 and that nodulation was about 10 times more sensitive to acidity than did rhizobia or root growth. As available Al^{3+} in acid soils inhibits both nod gene expression and rhizobia activity (Zahran, 1999 and references therein), a marked increase in legume crop productivity should be expected in response to biochar application due to its liming effect. This can likely explain the lack of adequate response of peanut to NPK, lime and/or biochars as soil pH was below 5.5 throughout the study reported by Slovich et al. (2013). The positive relationship between aboveground growth and pyrolysis temperature were hypothesized to relate to reduction in volatile and other organic compounds at high pyrolysis temperatures that can otherwise limit plant growth (Biederman & Harpole, 2012). Yet, the positive effect of biochar pH and pyrolysis temperature on plant growth is likely to occur due to the positive trajectory and correlation of these characteristics with biochar ash, alkalinity, and liming content (neither of which was included in the meta-analysis), especially as increase in pyrolysis temperature adversely affect biochar nutrient availability.

The effect of biochar on yield in soils of near or above natural pH is minor and inconsistent (Van Zwieten et al., 2010; Lentz & Ippolito, 2012; Schnell et al., 2012; Quilliam et al., 2012; Alburquerque et al., 2013; O'Toole et al., 2013). While improving wheat grain yield and aboveground biomass, use of wheat straw or olive-tree pruning biochar at rates of up to 2.5% (wt/wt) to a loamy sand soil (pH 6.5) did not improved wheat yield parameters as compared to the addition of mineral fertilizer alone (Alburquerque et al., 2013). Moreover, while improving soil NH_4^+ and resin PO_4^{3-}, and plant P content, biochar application negatively affected plant N and micronutrient (Fe, Mn, Cu) content, likely due to a low N mineralization rate and reduction in nutrient availability as soil pH increased to 8.2 and 7.6 in the high application levels of olive-tree pruning and wheat straw biochar, respectively (Alburquerque et al., 2013). Similarly, wheat straw biochar applied to a sandy loam soil (pH 6.8) resulted in no or negative effect on perennial ryegrass biomass and led to reduction in foliar N, Ca, and Mg content (O'Toole et al., 2013).While increasing nitrogenase activity, wood biochar (450 °C for 48 h) applied at rates of up to 50 Mg ha^{-1} to a soil with neutral pH showed no significant positive effect on white clover (*Trifolium repens*) root or shoot biomass and nodulation (Quilliam et al., 2013b). In the above study by Van Zwieten et al. (2010), application of biochar in combination with fertilizer had mixed effects, negatively effecting wheat and radish yield grown in calcareous soil (pH 7.7). In absence of fertilizer application, biochar had no effect on soybean and wheat yield, while a positive effect on radish yield was noted in response to application of biochar with a higher K content (0.2 vs 1.0 cmol K kg^{-1}) and slightly lower pH and CCE (9.4 vs. 8.2, and 33 vs. 29; Van Zwieten et al., 2010).

It is apparent that different feedstock and pyrolysis conditions result in biochar of different properties and characteristics. Consistent and detailed information about the product, its feedstock, and production process will benefit the end users in their decision making process of the proper biochar for amending soils deficiencies and meeting crop requirements.

BIOCHAR PRODUCT AND TESTING STANDARDIZATION

Biochar is produced from feedstock of different origin and quality, mixing thereof, and by pyrolysis conducted under different set of conditions and systems. As such, biochars encompass wide variety of characteristics and may differ markedly in their properties and impact. The International Biochar Initiative (IBI) organization recently published guidelines to establish common definition for feedstock, biochar, as well as to standardize biochar testing methods, provide minimal characterization recording and reporting requirements, and labeling standard for the biochar products (IBI, 2012). IBI distinguishes between 'processed' and 'unprocessed' feedstock, where the former includes any chemical and/or biological process the feedstock was exposed to prior to pyrolysis, e.g. paper pulp sludge, manures, distillers grains, and biomass portion of municipal solid waste. In addition, animal parts and any biomass grown on contaminated soils are considered processed feedstock and as such are subject to a more rigorous and frequent testing requirements. IBI proposed three test categories to provide uniform presentation format for (a) basic biochar properties, (b) toxicology, and (c) characterization of properties relevant to soil fertility. Much of the categories, tests, and limits are adopted from existing standards and limits previously established for toxicants in soil and soil amendments such as land application of biosolids, compost, and wood ash. The basic category (A) include essential properties of biochar used in soil analysis: moisture content, elemental analysis (C, H, N), organic C, pH, electrical conductivity, liming capacity, particle size distribution, ash content, and $H:C_{org}$ molar ratio. Organic carbon is divided into three categories: Category 1: carbon content >60, Category 2: between 30 to 60, and Category 3: between 10 to 30% organic C by weight (dry basis). While information about all other properties in the basic category needs to be reported only, biochar is required to have a $H:C_{org}$ molar ratio of <0.7. The $H:C_{org}$ ratio is considered an intrinsic indicator for the degree of organic carbon stability that is correlated with the production of fused aromatic ring structures from the thermochemical alteration of the feedstock (IBI, 2012). A ratio of 0.7 is used to distinguish between biochar and biomass that has not been thermochemically altered, or material that was altered to an insufficient lesser degree (IBI, 2012). As several studies had shown, biomass pyrolyzed at 300 or 350 °C results in $H:C_{org}$ ratios higher than 0.7; such ratio may lead to the biochar production at high temperatures, that will positively affect biochar carbon stability; however, it is likely to adversely affect biochar properties of agronomic benefits, such as nutrient availability and CEC (Wu et al., 2012; Rutherford et al., 2004; Rutherford et al., 2008). The basic category is followed by the biochar toxicity assessment test category (B), which include earthworm avoidance test, germination inhibition test, testing for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and furans, and content of metals and non-metal elements of environmental concern (e.g. Hg, As, Se, Cd, and Pb). While values of B, Cl, and Na only need to be declared, numerical values of Maximum Allowable Thresholds (MAT) for selected chemical compounds or elements are given as a range based on established thresholds for such toxicant in soil (Table 1). The ranges published by IBI are based on MAT values from Australia, Canada, EU, UK, and USA where long established regulation for such toxicants in soils and or other substrates exist. Category C is optional and provides information about volatile matter, mineral N, total K, total and available P, and total and external surface area (no threshold values exist).

Table 1. Numerical values established by IBI for biochar toxicity assessment reporting

Element/compound	Maximum Allowable	Threshold range[†]
PAHs	6 – 20	μg g^{-1} TM[‡]
Dioxin/Furan (PCCD/F)	9	ng kg^{-1} I-TEQ[§]
PCBs	0.2 – 0.5	μg g^{-1} I-TEQ
Arsenic	12 – 100	μg g^{-1} DW[¶]
Cadmium	1.4 – 39	μg g^{-1} DW
Chromium	64 – 100	μg g^{-1} DW
Cobalt	100 – 150	μg g^{-1} DW
Copper	63 – 1500	μg g^{-1} DW
Lead	70 – 500	μg g^{-1} DW
Molybdenum	5 – 75	μg g^{-1} DW
Mercury	1 – 17	μg g^{-1} DW
Nickel	47 – 600	μg g^{-1} DW
Selenium	1 – 100	μg g^{-1} DW
Zinc	200 – 2800	μg g^{-1} DW

[†] See text for clarification; [‡] Total mass; [§] International Toxicity Equivalent; [¶] Dry weight basis.

CONCLUSION

Biochar is likely to have a positive and prolonged effect on soil properties, the effects of which are yet to be fully appreciated given the relatively short-term studies being reported so far. The agronomic value of biochar in improving soil fertility is highly process- and feedstock-dependent. Biochar CEC decreases with increase in pyrolysis peak temperature above ca. 350 – 400 °C, whereas porosity, anion exchange capacity, hydrophobicity, surface area, and carbon stability increase. The increase in surface area and porosity may compensate for the reduction in CEC while also providing habitat promoting microbial activity. In addition, pyrolysis at higher temperature increases biochar liming capacity. Yet, feedstock nutrient recovery and biochar hydrophility as well as plant available nutrient all decrease with increase in pyrolysis temperature. Optimization is needed between increasing pyrolysis temperature to enhance carbon stability on the one hand and producing biochar of high CEC and nutrient content and availability on the other hand.

Biochar improves several soil quality indicators including CEC, bulk density, and carbon content. The most profound effect of biochar on soil fertility and plant growth was shown to be due to its CCE and the ability to increase the pH of dystrophic, acidic, and highly weathered soils where biochar improvement of soil fertility has shown to be most significant and consistent. Positive plant response to fertilizers application in biochar-amended soils suggests that while improving growing conditions and ameliorating acid soils growth limiting factors such as aluminum toxicity, nutrient content and availability become the main growth limiting factor. Biochar from animal manure feedstock showed to maintain higher nutrient content than biochar form herbaceous and woody feedstock and to, when applied at sufficient application rate, provide for crop nutrient requirements. When produced as the main product under slow pyrolysis conditions, biochar can be engineered to achieve desired characteristics; conversely, when produced as a byproduct, post-production augmentation procedures to

improve desired biochar characteristics need to be considered and included in order to maximize biochar impact on soil fertility.

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SECTION III. BIOFERTILIZERS

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Chapter 5

COMPARATIVE STUDY OF BIO- AND CHEMICAL FERTILIZATION IN STRAWBERRY PRODUCTION

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ABSTRACT

The present study illustrates the effect of biofertilizer and chemical fertilizer applications on the microbiological properties of the soil, the yield-related characteristics, phenolic fruit composition and antioxidant capacity of 'Clery' 'Joly' and 'Dely' strawberries under greenhouse conditions. Plants were treated with biofertilizer (liquid inoculum combined of *Azotobacter*, *Derxia* and *Bacillus* genera) and chemical fertilizer (Polyfeed NPK Water Soluble Fertilizer). The microbiological properties of soil were monitored by determining the total microbial count, numbers of soil fungi, actinomycetes, oligonitrophilic bacteria and *Azotobacter* using indirect counting methods involving plating out a soil suspension onto appropriate selective culture media. The yield-related characteristics of the tested plants were evaluated for generative potential parameters and morphometric traits. Since strawberry fruits are important sources of health-related compounds, special attention was given to the total phenolics content as well as to identified individual polyphenolic compounds, and consequently to the total antioxidant capacity. The results obtained suggested that application of the biofertilizer induced the highest increase in the numbers of oligonitrophilic bacteria and *Azotobacter*, whereas soil fungal and actinomycetes count significantly decreased. Significantly higher values of fruit weight, length and width were obtained in the chemical fertilizer treatment. Application of biofertilizer has given the best results in terms of anthocyanins and kaempferol content, as well as the content of ellagic, gallic, and protocatechuic acids. The positive influence of biofertilizer on the level of total phenolics and consequently on the total antioxidant capacity was clearly expressed. Considering the biological properties of the examined soil and fruit quality characteristics, certain amounts of mineral nitrogen

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can be replaced with biological nitrogen in order to obtain healthy and environmentaly-safe products.

Keywords: Biofertilizer, chemical fertilizer, microorganisms, strawberry

INTRODUCTION

Plant nutrients are essential for the production of crops and healthy food for the world's expanding population (Chen, 2006). The world population is expected to reach over 10 billion in the year 2050 while agricultural production is growing at a slower rate of about 1.8% annually (Sheikh, 2009). According to generally accepted calculations, between now and 2050, the world-wide demand for food will increase by 70%. With the conventional methods of agriculture, agricultural production will not be able to grow fast enough to meet the needs of the rapidly growing population. In order to overcome this problem, there is a pressing need to intensify agricultural production in an ecologically responsible manner. Therefore, the agricultural production must be focused on satisfying basic criteria of the sustainable production concept where the fertilization strategy, among other practices, plays an important role.

Worldwide experience in agricultural development has provided much evidence that fertilizer application is the most efficient measure for the sustainable and increasing crop production (Wang et al., 2013). However, research in this area is mostly focused on increasing crop yields, whereas their cumulative effects (changes in soil biological and chemical properties) are often neglected (Mandić et al., 2012). Numerous studies have shown that increasing utilization of synthetic nitrogen fertilizers significantly contributes to a series of undesirable effects and results in excessive environmental pollution. In particular, investigations indicate that nitrogenous fertilizers used in agriculture contaminate surface and underground waters (Beman et al., 2005), enhance N_2O emissions into the atmosphere (Galloway et al., 2003) and result in possible nitrate accumulation in plants. Accordingly, attention has been focused on the use of different organic materials (e.g., animal manures, crop residues, green manures, etc.) and biofertilizers (microbial inoculants) as an alternative and/or a supplement to costly mineral fertilizers (Alfonso et al., 2005; Rolli, 2007; Mandic et al., 2011; Pešaković et al., 2011; Pešaković et al., 2012; Djukic et. al., 2012a,b; Pešaković et al., 2013). The main scope of these methods is to provide greater efficiencies, increase the quality of agricultural products, reduce costs and *improve* the *quality* of life for growing world population (Carvajal-Muñoz and Carmona-Garcia, 2012). Numerous studies (Higa and Parr, 1994; Reddy, 2005; Tognetti et al., 2005) showed that rational use of microbial inoculants, can provide certain physical (improving structure and aggregation of soil particles, reducing compaction, and increasing the pore spaces and water infiltration), chemical (improving nutrients availability in the soil, leaving free elements to facilitate their absorption by the root system), and microbiological properties (suppression or control through competition of pathogenic populations of microorganisms present in the soil and increasing microbial biodiversity creating suitable conditions for the development of beneficial microorganisms) of soil. Saharan and Nehra (2011), also reported that utilization of Plant Growth Promoting Rhizobacteria (PGPR) as microbial inoculants can contribute to plant growth promotion. In the last few years, the number of PGPR that have been identified has

considerably increased. Various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes* and *Bacillus* have been reported to enhance plant growth (López-Valdez et al., 2011; Luković et al., 2012; Pešaković et al., 2013).

Taking into account the significant increase in consumer interest in strawberry products, there is a need to determine sustainable agricultural practices that should be performed in fruit plantings. Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is a highly valued fruit crop, giving the quickest return in shortest time and receiving a lot of attention in relation to yield and fruit quality (Milivojević et al., 2012a; Pešaković et al., 2013). The permanent trend of increased strawberry consumption, both as fresh and processed fruits, indicates possibilities for expanding the production. To achieve this goal, it is of vital importance to select cultivars, which are both high-yielding and produce consistently flavorful fruits, thus making them more desirable to consumers (Nikolić et al., 2009; Paydas Kargi et al., 2012). In this regard, the breeding programs of strawberry fruit have aimed to improve yield and fruit quality characteristics (sensorial and nutritional), the adaptation to different growing systems and recently, research has been focused on developing ecologically cultivated strawberries (Capocasa et al., 2008; Magnani et al., 2009; Luković et al., 2012; Pešaković et al., 2013). Keeping the focus on the three postulates: quality, economic sustainability and attention to the environment, the breeding programs were improved in efficiency and achieved certain results that are currently very profitable and have also opened the door for important future developments. Consequently, permanent introduction of new strawberry selections and their testing in different environments, with various cultivation techniques and nutrition protocols plays an essential role in obtaining viable information on what cultivars perform well and possess more beneficial traits in certain conditions (Tulipani et al., 2008; Kiprijanovski et al., 2012). In addition to assortment innovation, growing techniques and applied agricultural practices are also important factors in determining yield and fruit nutritional quality (Milivojević et al., 2009).

Many greenhouse and container-production operations can be classified as intensive agriculture because they use different substrates, combination of fertilizers, irrigation methods, insecticides, and fungicides to achieve high-volume production on small acreages (Lea-Cox et al., 2001). Gao et al. (2002) emphasize general advantages of hydroponics growing systems that are also capable of controlling water and nutrient management as well as microclimate conditions inside the greenhouse. It is especially important to note that a specification of agricultural practices should be ideally adjusted to the needs of a single cultivar or a group of cultivars with similar requirements.

Intensive farming practices that warrant high yields and fruit quality often require extensive use of chemical fertilizers, which are costly and create environmental problems. McArthur and Eaton (1988) reported that high rates of NPK fertilizers increased the numbers of leaves and runners of strawberry, delayed fruit ripening and enhanced the number of achenes per fruit.

Variation in strawberry yields associated with application of different chemical fertilizers may be explained by their physiological functions, rates and time of application, production system, cultivar specificities, weather conditions and cultural practices (Tagliavini et al., 2005; Hargreaves et al., 2008; Parmar and Sindhu, 2013). An excessive fertilization of strawberry plants with nitrogen (N) in autumn significantly increased biomass, but did not affect either fruit yields or quality (Tagliavini et al., 2005). But in spring time, strawberry

plants show an increased demand mainly for nitrogen and potassium, which continues to rise during the blossom and fruit-setting phases (Chepiñski et al., 2010). Potassium (K) is the second essential macronutrient and the most abundantly absorbed cation that plays an important role in the growth, metabolism and development of strawberry plants. However, due to an imbalanced fertilizer application, potassium deficiency is becoming one of the major constraints in plant production (Parmar and Sindhu, 2013). To overcome the problems related to increasing utilization of synthetic nitrogen fertilizers, modern strawberry production is based on the possibility of using some alternative methods, such as biofertilizations that may enhance crop yields without adverse effects on soil properties and ensure basic criteria of sustainable fruit production (Umar et al., 2009; Singh and Singh, 2009; Lütfi and Murat, 2009; Güneş et al., 2009; Pešaković et al., 2011; Pešaković et al., 2012; Pešaković et al., 2013).

It is a well known fact that nutrient needs could be defined as those amounts necessary to be absorbed to maximize plant performance. Within sustainable strawberry production this performance cannot be identified only by vegetative growth, but also has to include yield and nutritional fruit quality, as well as minimum or no risk of environmental pollution (Agulbeiro-Santos, 2009). Considerations of the biofertilizer impact on nutrient bioavailability in strawberry plants and particularly its contribution to yield-related components have also received much attention and are important directions for future research (Lütfi and Murat, 2009; Singh and Singh, 2009). In accordance with their findings, yield increase due to biofertilizer application might be caused by the microorganisms' production of plant hormone-like substances, such as indoleacetic acid, gibberellic acid, cytokinins and ethylene.

Numerous studies have already shown that strawberries are a good source of natural antioxidants (Anttonen et al., 2006; Capocasa et al., 2008; Milivojević et al., 2011). An important question arising from the preliminary screening of antioxidant activity in strawberry fruit concerns the polyphenolic profiles, i.e., which components within these profiles contribute the most to differences in bioactive potential. These components are mainly represented by flavonoids, phenolic acids and tannins, which play an important role in controlling oxidative reactions in the human body and exhibit anticarcinogenic activities (Määttä Riihinen et al., 2004; Milivojević et al., 2012b). Flavonoids are polyphenolic phytochemicals that constitute a large group of secondary plant metabolites. Among them, flavonols such as quercetin, kaempferol and myricetin, and their derivatives (primarily glycosides) are considered the dominant flavonoids in strawberry fruit (Milivojević et al., 2011).

Anthocyanins, the pigments responsible for the red color, also make an important contribution to the total antioxidant activity of strawberry fruit (Cordenunsi et al., 2005). Additionally, strawberries are known for a phenolic acid content (ellagic, gallic, *p*-coumaric etc.) that constitutes about one-third of the dietary phenols. Ellagic acid, present in relatively high levels in strawberries, has been suggested as a major phenolic acid providing anticarcinogenic effects (Cordenunsi et al., 2005). Milivojević et al. (2011) noticed large differences in the ellagic acid content between wild strawberry *F. vesca* and cultivated varieties confirming that the content of each antioxidant may vary with genotype/cultivar. Nutritional fruit quality is also affected by environmental conditions, cultivation techniques,

ripening season, pre-harvest and post-harvest conditions, shelf-life and processing (Capocasa et al., 2008).

Furthermore, only limited knowledge is available regarding the possibility of improving strawberry nutritional traits by applying different types and levels of fertilizers. Anttonen et al. (2006) pointed out the lowest fertilization level increased the contents of flavonols and ellagic acid from 19 to 57%, whereas up to 4-fold differences were found in the flavonol content between the tested cultivars. Therefore, the intention here is to narrow the information to results that suggest a direct connection between nutrient uptake and fruit quality. In order to get a complete understanding of this subject, a future review should embrace a broader access to information including the effect on nutritional fruit quality of both biofertilizers and chemical fertilizers.

The research reported in this chapter was designed to evaluate and discuss the effect of the biofertilizer (various bacteria which act as PGPR) and chemical fertilizer (Polyfeed NPK Water Soluble Fertilizer) on the microbiological soil properties and the desirable properties in terms of yield and fruit quality of the three strawberry cultivars under greenhouse conditions. In addition to this, the aim of this comparative study was to establish whether the application of biofertilizers can be an appropriate method in commercial strawberry production, replacing the utilization of chemical fertilizers.

METHODS

Plant Material and Experimental Design

The study was conducted on strawberry plants in the greenhouse at the Fruit Research Institute Čačak (43^{o} 53' N latitude, 20^{o} 20' E longitude, 225 m altitude), during a 2-year period (2012-2013). The experimental design was a split-plot arrangement based on a randomized complete block design with three replications. Frigo plants were established at the beginning of March 2012 using 3 dm^{3} plastic pots filled with Klasmann substrate (TS1, 0.7 g l^{-1} nutrient content). It is a mixture of fine white sphagnum peat (0–10 mm) and perlite (25%). The substrate is slightly acidic (pH=6), enriched with water-soluble nutrients, microelements and wetting agents. The treatments contained two fertilizer types i.e., the biofertilizer (liquid inoculum combined of *Azotobacter*, *Derxia* and *Bacillus* genera), and the chemical fertilizer (Polyfeed NPK Water Soluble Fertilizer). The application was performed on total 60 plants of each investigated cultivar ('Clery', 'Joly' and 'Dely') in three replications per 20 plants. The tested cultivars had been released by the CIV breeding program (Consorzio Italiano Vivaisti, Ferrara).

The biofertilizer formulation is a combination of nitrogen-fixing and phospho-mineralizing bacteria (*Azotobacter chroococcum, Azotobacter vinelandi, Bacillus megatherium, Bacillus lichenformis and Bacillus subtilis*). Polyfeed NPK is a water-soluble chemical fertilizer (production line of 'Haifa Chemicals Ltd'- Israel). The bifertilizer was applied during plant establishment by dipping the roots in a liquid inoculum of bacteria for 30 minutes.

Bacteria titer in the inoculum ranged within 20–40 x 10^{6} CFU cm^{-3}. The plants were watered every 7 days with 100 cm^{3} inoculum per growing pot during the vegetative period.

The application of the chemical fertilizer was performed based on the phenological growth stages: Poly-Feed 11:44:11 + Me (initial fertilization immediately following the completion of planting); Poly-Feed 20:20:20 + Me (supplementary nutrition during the intensive vegetative development and flower buds appearance, 2 times within a 7 to 10 days' interval); Poly-Feed 16:8:32 + 2MgO + Me in conjunction with the Multi-Cal 15.5:0:0 + 26.5 CaO and Multi-K Mg 12:0:43 + 2MgO (supplementary nutrition during the flowering, pollination and fruit set, every 10 days). All the above mentioned combinations of the chemical fertilizer were applied in concentration of 1-1.5 g plant^{-1}.

Soil Microbiological Analysis

Soil samples for microbiological analysis were taken twice during the growing seasons and calculated as an average number of colony forming units (CFUs) in the plate. Total numbers of microorganisms, the number of fungi, actinomycetes, *Azotobacter* and oligonitrophils were determined on agar plates by the Serial Dilution Plate method. The medium used for enumeration of total numbers of microorganisms, fungi, actinomycetes and oligonitrophils was soil agar, Czapek's medium, Krasilnikov medium and Fyodorov medium, respectively. *Azotobacter* number was determined on Fyodorov medium by the fertile drops method (Pochon and Tardideux, 1962). The incubation for the total number of microorganisms and actinomycetes took 7 days, and 5, 4 and 2 days for fungi, oligonitrophils and *Azotobacters*, respectively, at the temperature of 28°C.

Generative Potential and Fruit Quality Traits

During the two-year research period, fruit number per plant, yield per plant, morphometric and chemical fruit properties were monitored. Fruit were counted from each plant in order to determine the total fruit number per plant. Yield per plant was obtained by collecting and weighing fruit from each harvest date during the season from April 25th to May 20th in both years of investigation.

To assess the fruit quality, a sample of 25 fruits per replication were randomly selected to determine the average fruit weight using the METTLER balance (± 0.01 g accuracy) and data were expressed in g per fruit. Fruit dimensions (length, width and fruit shape index) were also determined in the samples by the 'Inox' Vernier scale (± 0.05 mm accuracy) and data were expressed in mm.

Chemical Analysis

Fruit Sample Preparation

The fruits of 'Clery', 'Joly' and 'Dely' strawberries were sampled in triplicate at optimal ripening stage and 100 g fruits per sampling were taken for biochemical analysis and stored at -20 °C for subsequent extraction. Samples were frozen by pouring into liquid nitrogen and homogenized using a stainless steel blender. Grinded sample (4.0 g) was vigorously stirred

with 40 mL of extraction solution consisting of methanol and distilled water (80% v/v) for 2 h in the dark, at room temperature. The mixture was centrifuged in two sequential times for 15 min at 3500 rpm, and supernatant was filtered through a 0.45 µm Minisart filter prior to analysis.

Determination of Individual Phenolic Compounds

Samples were prepared according to the method of Hertog et al. (1992) and analyzed using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 x 150 mm, 3.5 µm particles).

The injection volume was 5 µL and the temperature was set at 30 ºC. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0─10 min, 10% of B in A; 10─25 min, 15─50% of B in A; 25─30 min, 50-80% of B in A; 30─32 min, 10% of B in A. By using this gradient (flow rate 0.5 ml/min), a good level of purity and separation was achieved in the fruit samples. The HPLC equipment was used with a diode array detector (DAD).

Phenolic compounds were detected at 260 nm (protocatechuic acid, ellagic acid), 280 nm (gallic acid, *p*-coumaric acid), 360 nm (kaempferol), and 520 nm (cyanidin-3-glucoside, pelargonidin-3-glucoside). Phenolic compounds were identified according to the peak retention time (RT) and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g FW.

Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Liu et al., 2002), with results expressed as milligrams of gallic acid equivalents (GAE) /100 g fresh weight. Shortly, 40 µL of fruit extracts or gallic acid standard solution was mixed with 3.16 mL of distilled water. In the next phase, 200 µL of Folin-Ciocalteu reagent was added and allowed to stand for 8 minutes before adding 600 µL of 20% Na_2CO_3 solution. The solution was well mixed and absorbance at 765 nm against an appropriate blank was determined after 2 hours.

Determination of the Total Antioxidant Capacity (TAC)

Antioxidant capacity was determined using the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sanchez-Moreno et al., 1998). An aliquot of 0.1 mL of the fruit phenolic extraction was added to 3.9 mL of DPPH solution in methanol (0.060 mM) and vortexed.

A control sample, containing the same volume of solvent in the place of the extraction, was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 30 minutes, the absorbance at 515 nm was recorded to determine the

concentration of the remaining DPPH. The results were expressed as the Trolox equivalent antioxidant capacity (μmol TE /100 g FW). Trolox standard solutions were prepared at a concentration ranging from 50 to 300 μM.

Statistical Analysis

The data from the 2-year investigation were subjected to the analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The Least Significance Difference (LSD) was used to compare the treatment means and treatments declared different at $p = 0.05$ level of significance.

RESULTS

Soil Microbial Counts

The total number of microorganisms and the numbers of fungi, actinomycetes, *Azotobacters* and oligonitrophils in the soil are presented in Table 1.

The analysis of variance showed significant effect of fertilizer on the number of fungi, actinomycetes, *Azotobacters* and oligonitrophils. A significant effect of the cultivar on the number of *Azotobacter* and oligonitrophils was also observed. Compared to different fertilizers, the highest population of the total number of microorganisms, *Azotobacters* and oligonitrophils was recorded in the biofertilizer treatment (125.22 CFU·10^6/g d.m. soil, 39.06 CFU·10^2/g d.m. soil, 26.78 CFU·10^5/g d.m. soil, respectively), whereas the highest fungal and actinomycetes number was recorded in the chemical application treatment (79.17 and 33.50 CFU·10^5/g d.m. soil, respectively). Considering the cultivar influence, the total number of microorganisms, as well as the number of *Azotobacter* (129.92 CFU·10^6/g d.m. soil and 33.08 CFU·10^2/g d.m. soil, respectively) was observed to be the highest in cv 'Dely'. Conversely, a very high number of fungi, actinomycetes and oligonitrophils was recorded in cv ‘Joly’ (67.92, 28.42 and 29.00 CFU·10^5/g d.m. soil, respectively).

Generative Potential and Fruit Quality Traits

Generative Potential

The influence of the fertilizer and cultivar and the effect of their interactions on the generative potential of 'Clery', 'Joly' and 'Dely' strawberries are presented in Table 2.

The number of fruits and yield per plant were significantly different depending on the cultivar tested. It was observed that cv 'Clery' ranked the highest regarding the fruit number and yield per plant (36.2 and 0.78 kg, respectively). In the various fertilizer treatments, biofertilizer expressed a positive influence on the fruit number per plant (29.8) and consequently the yield per plant (0.71 kg). Nevertheless, no significant differences between the two fertilizer types were observed in the obtained results concerning the generative potential.

Table 1. The influence of the fertilizer type and cultivar on the soil microorganisms number

Factor			Total number of microorganisms (CFU·106/g d.m. soil)	Number of			
				Fungi (CFU·105/g d.m. soil)	Actinomycetes (CFU·105/g d.m. soil)	Azotobakter (CFU·102/g d.m. soil)	Oligonitrophils (CFU·105/g d.m. soil)
Fertilizer (A)		Biofertilizer	125.22±3.48a	57.94±2.55b	23.22±0.79b	39.06±1.10a	26.78±1.28a
		Chemical fertilizer	121.50±3.32a	79.17±1.33a	33.50±1.71a	21.44±1.39b	22.94±0.97b
Cultivar (B)		'Clery'	122.00±4.13a	66.00±3.85a	26.50±2.15a	30.75±3.11ab	22.08±0.87b
		'Joly'	118.17±4.60a	67.92±4.37a	28.42±2.14a	26.92±2.79b	29.00±1.67a
		'Dely'	129.92±3.13a	71.75±3.78a	30.17±2.36a	33.08±3.04a	23.50±0.99b
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	124.17±6.33a	55.00±3.07a	21.83±0.70a	40.00±1.77a	23.17±1.19a
		'Joly'	121.00±8.27a	56.67±5.35a	22.67±1.76a	35.00±1.29a	31.83±2.24a
		'Dely'	130.50±2.32a	62.17±4.74a	25.17±1.22a	42.17±1.45a	25.33±1.43a
	Chemical fertilizer	'Clery'	119.83±5.75a	77.00±2.76a	31.17±3.34a	21.50±2.28a	21.00±1.21a
		'Joly'	115.33±4.64a	79.17±2.23a	34.17±1.96a	18.83±2.55a	26.17±2.01a
		'Dely'	129.33±6.13a	81.33±1.89a	35.17±3.60a	24.00±2.34a	21.67±0.95a
ANOVA							
A			ns	*	*	*	*
B			ns	ns	ns	*	*
A x B			ns	ns	ns	ns	ns

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test; ns - non significant differences.

Fruit Quality Traits

Morphometric Fruit Traits

The results obtained concerning the fruit weight, length and width were significantly influenced by the fertilizer and cultivar (Table 3).

Table 2. The influence of the fertilizer type and cultivar on the generative potential of strawberry fruits

Factor			Number of fruits/plant	Yield/plant (kg)
Fertilizer (A)		Biofertilizer	29.78±1.62a	0.71±0.03a
		Chemical fertilizer	28.61±1.32a	0.67±0.03a
Cultivar (B)		'Clery'	36.25±1.16a	0.78±0.02a
		'Joly'	26.08±1.05b	0.76±0.03a
		'Dely'	25.25±0.97b	0.53±0.01b
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	37.67±1.86a	0.79±0.02a
		'Joly'	26.17±1.62a	0.79±0.04a
		'Dely'	25.50±1.43a	0.54±0.02a
	Chemical fertilizer	'Clery'	34.83±1.30a	0.77±0.03a
		'Joly'	26.00±1.48a	0.72±0.04a
		'Dely'	25.00±1.44a	0.52±0.02a
ANOVA				
A			ns	ns
B			*	*
A x B			ns	ns

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

Table 3. The influence of the fertilizer type and cultivar on morphometric traits of strawberry fruits

Factor		Weight (g)	Length (mm)	Width (mm)	Fruit shape index
Fertilizer (A)	Biofertilizer	23.33±0.91b	42.76±1.41b	38.34±0.92b	1.11±0.02a
	Chemical fertilizer	25.04±1.02a	43.53±1.41a	39.00±0.88a	1.11±0.02a

Factor			Weight (g)	Length (mm)	Width (mm)	Fruit shape index
Cultivar (B)		'Clery'	21.60±0.48b	40.15±0.27b	34.79±0.20c	1.15±0.01a
		'Joly'	29.46±0.61a	51.19±0.35a	43.62±0.15 a	1.17±0.01a
		'Dely'	21.51±0.38b	38.09±0.22c	37.58±0.17b	1.01±0.00b
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	20.37±0.18a	39.50±0.27a	34.34±0.25a	1.15±0.01a
		'Joly'	28.27±0.88a	50.85±0.41a	43.40±0.14a	1.17±0.01a
		'Dely'	21.37±0.56a	37.93±0.34a	37.26±0.14a	1.02±0.01a
	Chemical fertilizer	'Clery'	22.83±0.61a	40.80±0.28a	35.25±0.16a	1.16±0.01a
		'Joly'	30.65±0.55a	51.53±0.56 a	43.85±0.24a	1.18±0.02a
		'Dely'	21.65±0.57a	38.25±0.28a	37.90±0.26a	1.01±0.00a
ANOVA						
A			*	*	*	ns
B			*	*	*	*
A x B			ns	ns	ns	ns

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

A significant effect of the cultivar on the index of fruit shape was also evident. In general, morphometric traits were found to be better in the chemical fertilizer treatment (25.04 g, 43.53 mm and 39.00 mm, respectively) except for the index of fruit shape whose values were identical in both fertilizer treatments (1.11). Among the cultivars tested, 'Joly' exhibited the highest values of fruit weight, length and width (29.5 g, 51.19 mm and 43.62 mm, respectively), whereas index of fruit shape was similar to that of cv. 'Clery' corresponding to long conical shape with values above 1.15.

Chemical Fruit Traits

Individual Phenolic Compounds

Kaempferol was identified from the group of flavonols in all cultivars tested. Its content was highly affected by the fertilizer and the interaction effects of the fertilizer and cultivar (Table 4 and Figure 1).

In the different fertilizer treatments, biofertilizer expressed a more positive influence on the kaempferol content (1.09 mg/100 g FW) than the chemical fertilizer. All cultivars tested were characterized by a similar kaempferol content, so no statistically significant differences were observed among cultivars related this compound. Considering the interaction effect of the fertilizer type and cultivar, the only significant differences observed were those in cv. 'Joly' (Figure 1).

Table 4. The influence of the fertilizer type and cultivar on kaempferol content in strawberry fruits

Factor			Kaempferol content (mg/100 g FW)
Fertilizer (A)		Biofertilizer	1.09±0.08a
		Chemical fertilizer	0.76±0.05b
Cultivar (B)		'Clery'	0.85±0.06a
		'Joly'	1.04±0.14a
		'Dely'	0.87±0.04a
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	0.95±0.09b
		'Joly'	1.45±0.13a
		'Dely'	0.87±0.05bc
	Chemical fertilizer	'Clery'	0.76±0.09bc
		'Joly'	0.63±0.07c
		'Dely'	0.87±0.06bc
ANOVA			
A			*
B			ns
A x B			*

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

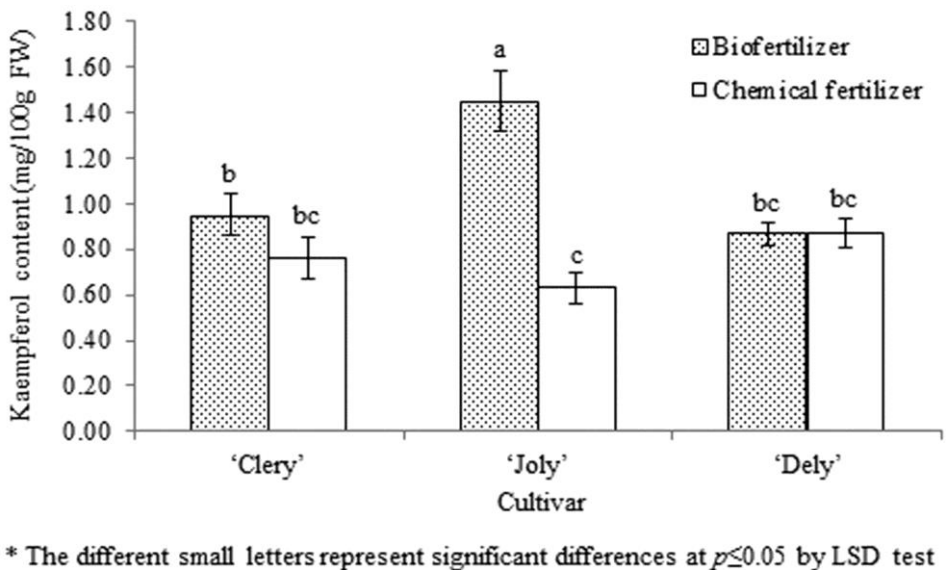

Figure 1. Interaction effect of the fertilizer type and cultivar (A x B) on kaempherol content in strawberry fruits.

A significant impact of the fertilizer type on the content of identified phenolic acids (ellagic, gallic, protocatechuic) in this study, except *p*-coumaric, is reported in Table 5. The highest levels of ellagic, gallic and protocatechuic acids were registered in the biofertilizer treatment (14.43, 2.19, and 0.15 mg/100 g FW, respectively).

As far as the effect of cultivar is concerned, it was observed that the ellagic acid content was not the only trait that was significantly influenced by this factor, since it was established that the 'Dely' cultivar also contained higher quantities of ellagic, gallic, *p*-coumaric and protocatechuic acids (14.19, 2.35, 0.95 and 0.18 mg/100 g FW, respectively).

In terms of the interaction effect of the fertilizer type and cultivar, the only significant differences observed were those in the content of *p*-coumaric acid (Figure 2).

The anthocyanins content (cyanidin-3-glucoside and pelargonidin-3-glucoside) in the fruits of cvs 'Clery', 'Joly' and 'Dely' subjected to analysis of variance showed a significant effect of the fertilizer and the cultivar, as well as their interactions (Table 6 and Figures 3, 4). The results indicate that the biofertilizer treatment enhanced levels of cyanidin-3-glucoside and pelargonidin-3-glucoside in strawberry fruits achieving approximately 2-fold higher values than those obtained in the chemical fertilizer treatment.

Table 5. The influence of the fertilizer type and cultivar on the phenolic acids content in strawberry fruits

Factor			Fenollic acids content (mg/100 g fw)			
			Ellagic	Gallic	*p*-Coumaric	Protocatechuic
Fertilizer (A)		Biofertilizer	14.43±1.02a	2.19±0.21a	0.73±0.12a	0.15±0.01a
		Chemical fertilizer	9.77±0.67b	1.21±0.19b	0.71±0.15a	0.12±0.01b
Cultivar (B)		'Clery'	11.05±1.37a	1.69±0.27b	0.42±0.08b	0.12±0.01b
		'Joly'	11.06±1.23a	1.05±0.25c	0.78±0.22ab	0.12±0.01b
		'Dely'	14.19±0.98a	2.35±0.19b	0.95±0.14a	0.18±0.00a
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	14.08±2.03a	2.24±0.43a	0.36±0.01b	0.14±0.01a
		'Joly'	12.96±2.23a	1.67±0.34a	0.46±0.06b	0.13±0.02a
		'Dely'	16.26±0.61a	2.66±0.20a	1.38±0.10a	0.19±0.01a
	Chemical fertilizer	'Clery'	8.03±0.65a	1.14±0.15a	0.49±0.15b	0.09±0.01a
		'Joly'	9.16±0.48a	0.44±0.04a	1.11±0.41a	0.10±0.01a
		'Dely'	12.11±1.46a	2.05±0.28a	0.53±0.01b	0.18±0.00a
ANOVA						
A			*	*	ns	*
B			ns	*	*	*
A x B			ns	ns	*	ns

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

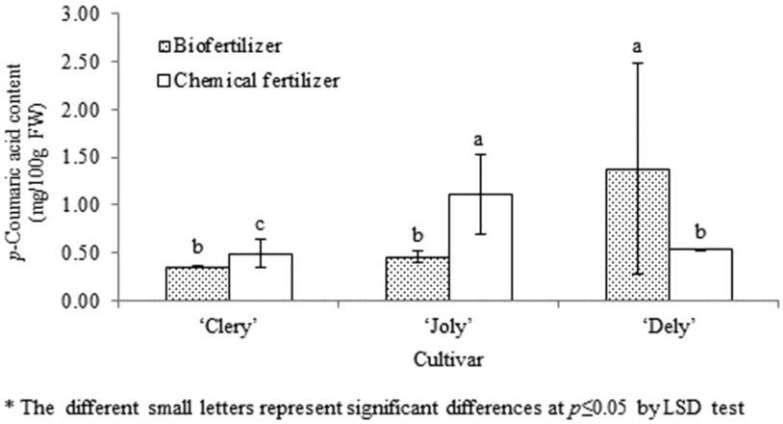

Figure 2. Interaction effect of the fertilizer type and cultivar (A x B) on *p*-coumaric acid content in strawberry fruits.

Table 6. The influence of the fertilizer type and cultivar on the anthocyanins content in strawberry fruits

Factor			Anthocyanins content (mg/100 g FW)	
			Cyanidin-3-glucoside	Pelargonidin-3-glucoside
Fertilizer (A)		Biofertilizer	6.30±0.98a	15.97±0.78a
		Chemical fertilizer	3.93±0.13b	8.60±0.97b
Cultivar (B)		'Clery'	3.72±0.24b	14.82±1.62a
		'Joly'	6.87±1.45a	10.63±1.75b
		'Dely'	4.77±0.08b	11.41±0.84b
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	3.64±0.51b	18.64±1.57a
		'Joly'	10.30±2.11a	16.01±0.39ab
		'Dely'	4.97±0.10b	13.26±0.84bc
	Chemical fertilizer	'Clery'	3.80±0.01b	11.00±1.80cd
		'Joly'	3.44±0.21b	5.24±1.33e
		'Dely'	4.56±0.05b	9.55±1.01d
ANOVA				
A			*	*
B			*	*
A x B			*	*

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

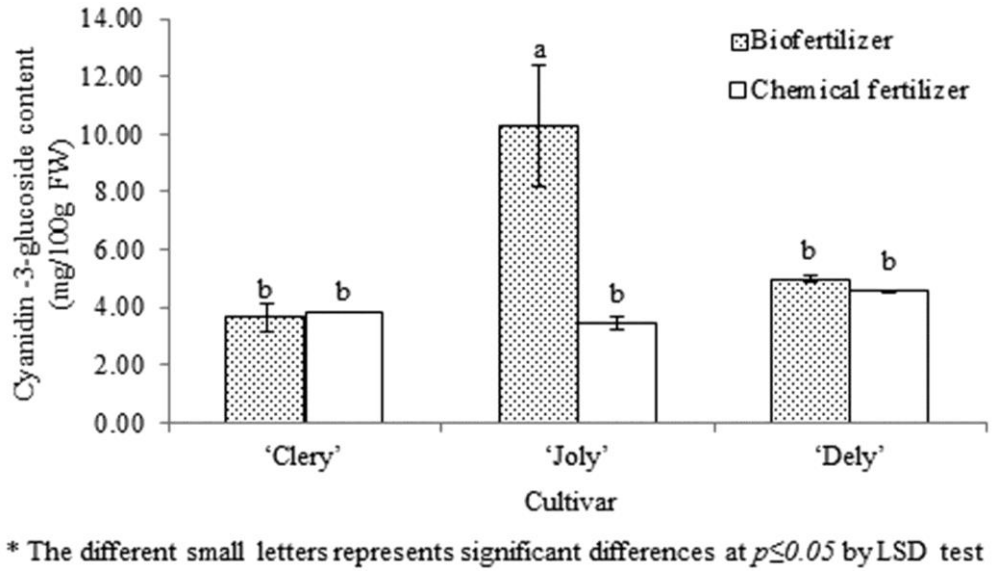

Figure 3. Interaction effect of the fertilizer type and cultivar (A x B) on the cyanidin-3-glucoside content in strawberry fruits.

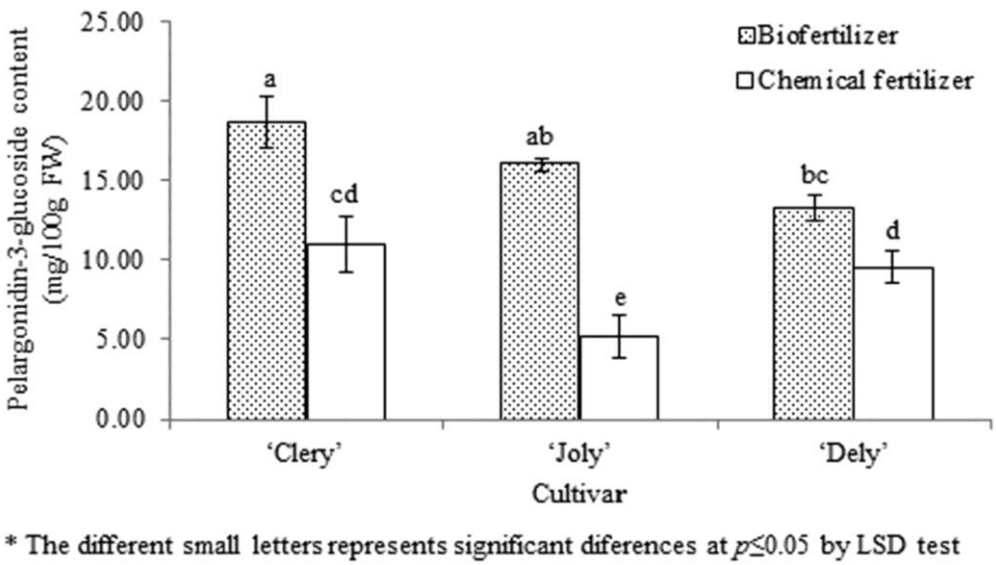

Figure 4. Interaction effect of the fertilizer type and cultivar (A x B) on the pelargonidin-3-glucoside content of strawberry fruits.

Considering the cultivar influence, the statistically highest level of cyanidin-3-glucoside was detected in cv. 'Joly' (6.87 mg/100 g FW) containing approximately a 2- fold higher amount compared to the cv. 'Clery', which was characterized by the highest pelargonidin-3-glucoside content (14.82 mg/100 g FW). The interaction effect of the fertilizer type and cultivar exhibited considerable differences in the cyanidin-3-glucoside content with a positive impact of the biofertilizer in cv. 'Joly' (Figure 3), whereas the content of pelargonidin-3-glucoside was higher in each cultivar subjected to the biofertilizer treatment (Figure 4).

Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC)

The effects of the fertilizer and cultivar, as well as their interactions on the total phenolics and antioxidant capacity in cvs 'Clery', 'Joly' and 'Dely' are shown in Table 7 and Figures 5 and 6.

The highest TPC (301.5 mg/100 g FW) as well as TAC (482.8 Trolox, mmol/100g FW) were observed in the biofertilizer treatments. Significant differences in the levels of TPC were also determined between the cultivars with the highest and lowest content.

Table 7. The influence of the fertilizer type and cultivar on TPC and TAC in strawberry fruits

			Total phenolics (mg/100 g FW)	Antioxidant activity (Trolox, mmol/100 g FW)
Fertilizer (A)		Biofertilizer	301.51±35.47a	482.77±1.29a
		Chemical fertilizer	275.60±42.35b	419.09±16.31b
Cultivar (B)		'Clery'	162.30±18.67c	457.64±10.12a
		'Joly'	508.48 ±7.31a	432.94±18.25a
		'Dely'	194.91±8.58b	462.21±20.41a
Fertilizer x Cultivar (A x B)	Biofertilizer	'Clery'	205.71±19.69b	478.43±2.02a
		'Joly'	503.36±11.47a	487.39±2.02 a
		'Dely'	195.47±8.95b	482.49±0.95 a
	Chemical fertilizer	'Clery'	118.88±19.81c	436.85±16.53a
		'Joly'	513.59±9.64a	378.49±16.61a
		'Dely'	194.34±15.62b	441.93±40.84a
ANOVA				
A			*	*
B			*	ns
A x B			*	ns

Means of 2-year values with three replicates ± standard error.

Values within each column followed by the different small letters are significantly different at the $p \leq 0.05$ by LSD test.

ns - non significant differences.

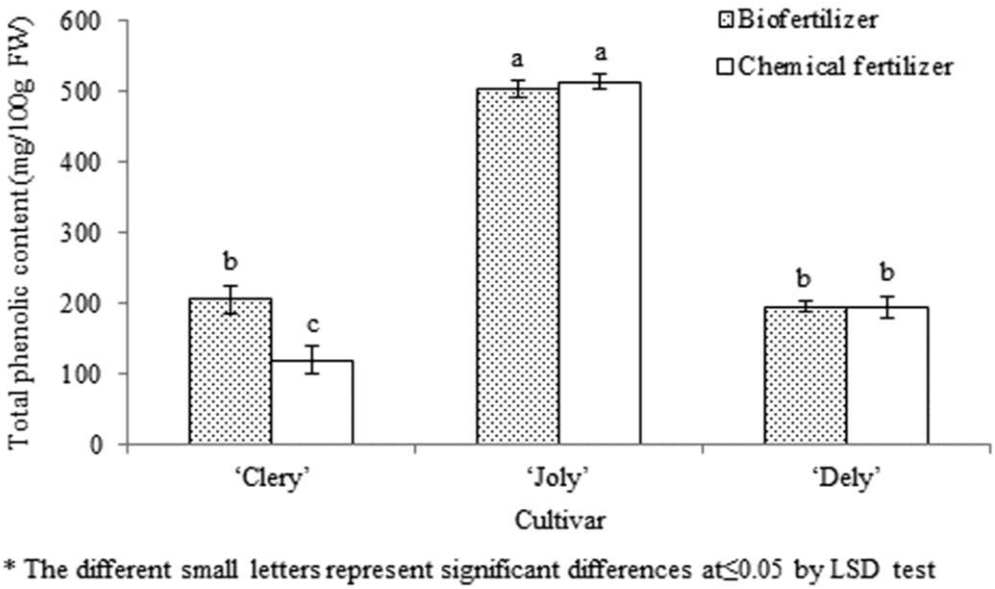

Figure 5. Interaction effect of the fertilizer type and cultivar (A x B) on TPC in strawberry fruits.

'Joly' was the richest in TPC (508.5 mg/100 g FW), whereas a 3-fold lower TPC was found in cv 'Clery' (162.3 mg/100 g FW). Since no significant differences were observed in the corresponding TAC values, this parameter is considered to be mostly uniform among the tested cultivars.

Considering the interaction effect of the fertilizer type and cultivar, the highest and similar TPC values were expressed by cv 'Joly' in both fertilizer treatments (Figure 5).

These values exceed 500 mg per kg of fresh strawberry fruit, being 2 to 3 times higher than those of the cultivars with the lowest TPC ('Dely' and 'Clery'). The 'Clery' cultivar exhibited an almost 2- fold higher TPC level in the biofertilizer treatment (205.7 mg/100 g FW), whereas cv 'Dely' expressed similar values affected by both fertilizer types. Although TPC greatly differed as a result of the interactions impact of fertilizer type and cultivar, no significant differences were observed in the obtained results for TAC.

DISCUSSION

Soil Microbial Counts

Changes in the number of particular systematic and physiological microorganisms groups in soil as well as their activity can be used as important indicators of soil biological productivity and the economic justifiability which is actually governed by the fertilizer type (Hole et al., 2005; Stark et al., 2007; Cerny et al., 2010; Mandić et al., 2012; Pešaković et al., 2013). The results in this chapter inferred that the applied biofertilizer, as an alternative measure of supplementing soil with nitrogen assimilatives, increases total number of microorganisms, the number of *Azotobacters* and oligonitrophils in the soil. Not only is this effect an indicator of a pronounced nitrogen-fixing capacity of strains contained within

the biofertilizer, but is also the indicative of a number of phenomena, i.e., intensification of photosynthesis process, inhibition of phytopathogens development, synthesis of phytohormones (Sukhovitskaja et al., 2004), and other plant growth stimulators, targeted detoxification of heavy metals and high salt concentrations, and exocellular polysaccharide synthesis (Park et al., 2005; Biari et al., 2008). On the other hand, the biofertilizer greatly reduced the number of soil fungi and actinomycetes, probably due to the fact that it consists of strains that can produce antifungal substances. Many of these introduced microorganisms can act like biocontrol agents by controlling or suppressing soil-borne plant pathogens through their competitive and antagonistic activities (Higa and Parr, 1994). Therefore, the decrease in the number of the soil fungi can be attributed to the ability of *Azotobacter* to produce a certain antifungal substance which inhibits the growth of certain soil fungi.

Based on the number of the microorganisms in the soil from the pots used for growing the different strawberry cultivars, the results of our experiment showed that the cultivar significantly affected only the number of *Azotobacters* and oligonitrophils. According to Antoun and Prevost (2005), plant genes play an important role in the interaction between the plant and beneficial microorganisms. Smalla et al. (2001) reported rhizosphere populations of strawberry that were considerably different from those of oilseed rape and potato. Smith and Goodman (1999) also emphasize that the plant genotype affects the response to inoculation with PGPR because it affects root colonization by the introduced bacteria, as well as the total population size of microbial communities on plant and it probably affects the composition of these communities.

Generative Potential and Fruit Quality Traits

Generative Potential

Crop growth and development are closely related to the nature of the soil microflora, especially those in the close proximity to plants roots, i.e., the rhizosphere (Higa and Parr, 1994).

Due to the fact that biofertilizers contain living microorganisms' cells, they improve the soil composition and supply of essential nutrients for increasing productivity (Pešaković et al., 2013). PGPR may induce plant growth by direct or indirect modes of action (Kloeper, 1993; Glick et al., 1999; Persello-Cortieaux et al., 2003). Direct mechanisms include fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron or production of plant hormones that enhance plant growth. Indirect effects are reflected in the fact that PGPR can promote plant growth by improving growth restricting conditions, either by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995; Glick et al., 1999).

With regard to the generative potential, results suggested that the applied biofertilizer induced a higher number of fruits per plant as well as yield per plant. Investigations on the effects of PGPR on yield and some fruit properties of strawberry showed a significant yield increase in the 'Selva' cultivar (Lütfi and Murat, 2009). Güneş et al. (2009) reported similar results in their study of the effects of phosphate-solubilizing microorganisms (*Bacillus* FS-3, *Aspergillus* FS9) on the strawberry yield and nutrient concentrations. In order to investigate the effects of the biofertilizer and bioregulators on the growth, yield and nutrient status of strawberry 'Sweet Charlie', Singh and Singh (2009) reported the highest yield in plants

inoculated with *Azotobacter* and *Azospirillum* in conjunction with 60 kg N ha^{-1} (50% N of the standard dose) and 100 ppm GA_3. In our comparative study, a wide variability in productivity can also be observed among the tested cultivars. 'Clery' has been shown as the superior cultivar in terms of the generative potential including the highest fruit number and yield per plant obtained (36.2 and 0.78 kg, respectively).

Our results indicate that 'Clery' yielded much better than in the study reported by Milivojević et al. (2009), who found a 1.5 times lower productivity for this cultivar grown in bags filled with 15 l of BVB HAASNOOT substrate. An increase of the generative potential observed in our study can be explained by the lower planting density and application of fertilizers. In particular, the biofertilizer treatment induced a slightly higher yield per plant which was probably attributed to the higher solubility and easy availability of inorganic components in such conditions.

In hydroponics production system general advantages are based on the ability to control water and nutrient management inside greenhouse. Conversely, traditional measures performed in the field strawberry production inadequately assess the roles of the microbial community structure and genetic diversity in the soil ecosystem processes that have a direct impact on the soil quality and indirectly influence plant growth and productivity. Therefore, different yields per plant obtained even in the same cultivar could be explained by various growth conditions, especially the substrate characteristics, nutrient protocols and water management. The cultivar selection may also make a major contribution to their fitness for performing well in certain conditions. The cultivars investigated in this work showed a similar trend in the productivity influenced by both the bio- and chemical fertilizer applications. This indicates that besides external factors (cultural practices, environmental factors, etc.), genetic background may have contributed to the similarity recorded in the tested cultivars.

Fruit Quality Traits

Morphometric Fruit Traits

Since fruit size has been discussed as one of the main components of the yield, a large size is preferred for the fresh market and improves the hand-harvest efficiency. In the present study, cv 'Joly' had the largest fruit which is in accordance with the description reported by Martinelli and Leis (2012). The fruit weight recorded for cv 'Clery' was similar to the previously published data of Milivojević et al. (2007), although different production systems were applied in the respective studies.

As regards the effect of bio- and chemical fertilizers on morphometric properties (weight, length, width and fruit shape index) of strawberry, results revealed a stimulating effect of the chemical fertilizer. Similarly to our results, Chepiński et al. (2010) reported that the treatment with multi-component chemical fertilizers at a rate of 400 kg ha^{-1} (10% N, 5% P_2O_5, 10% K_2O) + 30 kg N ha^{-1} (ammonium nitrate) significantly increased the fruit weight in strawberries. Contrary to this, in the comparison of the different fertilization treatments Pešaković et al. (2013) showed that morphometric traits of strawberry fruit (weight, length, breadth and thickness) were the highest in PGPR 1 treatment consisting of a pure culture of the Gram-negative diazotrophic nitrogen-fixing bacteria *Klebsiella planticola*. In the same study, the application of Multi KMg (12:0:43 + 2MgO) resulted in the lowest fruit shape

index. The comparison of the two fertilizer treatments in our study did not show significant differences in the fruit shape index, whereas the cultivar expressed a significant influence on the obtained results. The shape index was the lowest in cultivar 'Dely' (1.01) corresponding to the rounded conical form, while 'Joly' (1.17) and 'Clery' (1.15) tend to have the long conical form.

Chemical Fruit Traits

Individual Phenolic Compounds

Strawberries are an important source of phytochemicals, where in particular the phenolic composition seems to strongly influence the quality of the fruits, contributing to both their sensorial-organoleptic attributes and their nutritional value (Tulipani et al., 2008; Capocasa et al., 2008; Milivojević et al., 2011). Phenolic compounds can be divided into four classes based on their chemical structures: simple phenols, phenolic acids, phenylpropanoids and flavonoids.

The nutritional relevance of flavonoids is demonstrated by an impressive spectrum of health-related effects, while one of the most versatile classes of flavonoids, flavonols, belong to the flavonoid family. Although their presence is minor in strawberry from a quantitative point of view, a relevant interest is focused on flavonols (mainly kaempferol, quercetin and myricetin derivatives) due to their putative higher bioavailability, and the mechanisms of absorption and bioactivity of the main aglycones and glucosides derivatives have been widely studied (Tulipani et al., 2008).

According to Milivojević et al. (2011), kaempferol was the predominant flavonol in strawberry cultivars 'Marmolada' and 'Madeleine', whereas the content of myricetin was 2 to 4 times lower. The amounts of kaempferol detected in our study were the highest in cv 'Joly', meaning that our results were quite different from those reported by Milivojević et al. (2011). This may be explained by the influence of different genotypes, environmental factors (temperature, water deficiency, irrigation, and nutrient stress) and production systems, which lead to the appearance of various forms of kaempferol glycosides.

By analyzing the influence of the different fertilizer treatments, it can be observed that the fertilizer treatment produced the higher kaempferol content. Wang and Lin (2003) also reported differences in kaempferol 3-glucoside-succinate content depending on the applied fertilizer technology. They showed that strawberry plants cultivated with the addition of compost and fertilizer significantly increased the content of kaempferol 3-glucoside-succinate. It is possible that biofertilizer causes changes in chemical, physical and biological characteristics of soil, thus increasing beneficial microorganisms, as well as the nutrient availability and uptake.

The most common hydroxycinnamic acids are *p*-coumaric, caffeic and ferulic acid, while the corresponding hydroxybenzoic acids are *p*-hydroxybenzoic, gallic, ellagic, 3,4-dihydroxybenzoic, vanillic, and syringic acid (Clifford, 1999; Tomás-Barberán and Clifford, 2000; Manach et al., 2004). The dominant phenolic acid identified in our study was ellagic acid which is in agreement with the results of Milivojević et al. (2011). Da Silva Pinto et al. (2008) found that strawberries represent the main source of ellagic acid derivatives in the Brazilian diet, corresponding to more than 50% of all phenolic compounds found in the fruit. Määttä-Riihinen et al. (2004) also reported high levels of ellagic acid in strawberry fruits

emphasizing an increased interest in ellagic acid during the past decade due to its possible antimutagenic, anticarcinogenic, and antioxidative effects.

Compared to different fertilizer type, significantly higher contents of ellagic, gallic and protochatecuic acids were recorded in biofertilizer nutrient application, whereas the concentration of *p*-coumaric acid was found to be stable in both treatments. Wang and Lin (2003) also showed that the fertilizer type affected contents of ellagic acid in strawberry fruit, reporting that the addition of compost as a soil supplement significantly enhanced contents of ellagic acid in strawberry fruit.

Our previous investigation also reported that phenolic acids content is greatly influenced by the cultivar (Milivojević et al., 2011). The present study showed that cv. 'Dely' with the smallest fruit size had the highest level of the examined phenolic acids. Considering the interaction effect of the fertilizer type and cultivar, 'Dely' exhibited a 3-fold higher value of *p*-coumaric acid compared to other cultivars tested. However, cultivar did not express a significant influence on the ellagic acid content.

Among a wide range of the phenolic compounds in strawberry fruits, anthocyanins are quantitatively the most important type (Crespo et al., 2010). Anthocyanins belong to the flavonoid group and are responsible for the bright red colour of strawberry fruits. Despite a great number of anthocyanins that are identified in strawberry fruits, pelargonidin-3-glucoside, pelargonidin-3-rutinoside and cyanidin-3-glucoside represent over 95% of the total anthocyanin bulk present in most strawberry fruits (Lopes da Silva et al., 2007).

In the present study cyanidin-3-glucoside and pelargonidin-3-glucoside were separated and identified. Pelargonidin-3-glucoside has been found to be the major anthocyanin form in the study. This has already been reported by Mikulic-Petkovsek et al. (2013) who confirmed the presence of pelargonidin-3-glucoside and pelargonidin-3-malonylglucoside which combined represented 85-95% of the total analyzed anthocyanins.

With regard to fertilizer application, biofertilizer enhanced the contents of both cyanidin-3-glucoside and pelargonidin-3-glucoside in strawberry fruit. Wang and Lin (2002), also revealed that the fertilizer type significantly influenced the anthocyanin content in strawberries. They concluded that the increased anthocyanins content (in the fertilizer and compost treatment) was associated with increased antioxidant capacities which may allow for quenching of the excited state of active oxygen species. There was a marked difference in the reported anthocyanin content (Olsson et al., 2006; Lopes da Silva et al., 2007; Hernanz et al., 2007).

It can be explained by the influence of various factors, such as genetic differences between the cultivars, the degree of maturity at harvest, cultivation techniques and environmental conditions. The results of our study revealed that cv. 'Joly' ranked the highest in terms of cyanidin-3-glucoside content, whereas no significant differences between the other two cultivars were recorded. Interestingly, the highest level of pelargonidin-3-glucoside was detected in cv. 'Clery'.

Moreover, this cultivar contained a 4-fold higher amount of pelargonidin-3-glucoside than quantity of cyanidin-3-glucoside. Cultivars rich in anthocyanins also contained high amounts of total phenolics, since anthocyanins represent a large share of them.

Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC)

The results of our study inferred greater values of TPC and TAC in the tretment with the biofertilizer. The TPC and TAC increase resulting from the biofertilizer application might be due to highly intensive mineralizing processes in the substrate and increased the physiological functions and activity of plant root. On the other hand, the application of the chemical fertilizer exhibited an inhibitory effect, which is indicated by low values of most phenolic compounds probably associated with changes in the organic matter content.

It is also interesting that cv. 'Joly' showed an increase in TPC, especially if we consider a similar positive trend both in the bio- and chemical fertilizer treatment. The high TPC of the 'Joly' fruit can be arguably explained by the response of the nutrient application as well as genetically controlled accumulation of individual phenolics. Despite the highest level of pelargonidin-3- glucoside detected in cv 'Clery', no significant increase of TPC was observed.

Even though the potential health benefits of phytochemicals found in strawberries have received ample attention in the literature, the mechanisms by which they exert their health-promoting effects are still unclear. Most of the past research has been focused on the antioxidant properties of phenolics (Määttä-Riihinen et al., 2004), but the antioxidant capacity of individual phenolic compounds cannot always be evaluated because of the potential interactions among them. Therefore, the determination of the TAC allows a more realistic evaluation of the protective effects of the analyzed fruit (Milivojević et al., 2011).

In our study, the highest level of TAC was quantified in cv. 'Dely', which was particularly rich in phenolic acids. Besides health benefits, hydroxycinnamic and hydroxybenzoic acids play an important role in determining the genotype-to-genotype differences in the phytochemical composition. According to the literature reports (Tulipani et al., 2008; Cordenunsi et al., 2005), a range of hydroxycinnamates, mainly caffeoyl and ferulic esters and other classes of phenolic acids, are found in strawberry even if present at low concentrations. Although *p*-coumaric, gallic and protocatechuic acids were detected in minor quantities in strawberry fruits, ellagic acid as one of the main phenolic compounds in the present study, proved to be highly interesting.

CONCLUSION

Efficient strawberry nutrition management should ensure both enhanced and sustainable production. The results presented in this chapter reveal that fertilizer type is an important factor significantly affecting strawberry production. Bio- and chemical fertilizers showed their benefits and limitations in terms of biological productivity of soil. The investigation into the number of the different microorganism groups showed that the biofertilizer application not only provides the most favorable conditions for the development of soil microorganisms but also exhibits a pronounced effect on the generative potential, total phenolic and individual phenolic compounds, as well as the antioxidant activity. On the other hand, the chemical fertilizer gives the best results in terms of the morphometric parameters of strawberry fruits.

This chapter also demonstrated variability in yield-related characteristics, fruit quality parameters and antioxidant features of each genotype affected by the different fertilizer treatments. Nowadays these aspects of investigations are considered highly useful for the

commercialization of new cultivars, but mostly as a means of selecting new genotypes with a high nutritional fruit quality, combined with the yield efficiency, which now corresponds to plant adaptability, production efficiency and fruit size.

Based on the obtained results, cvs 'Joly' and 'Clery' can be recommended for sustainable strawberry production, allowing substitution of chemical fertilization by biofertilization. Furthermore, this approach seems to contain a certain potential as an appropriate technique in commercial strawberry production, which may improve soil fertility, yield-attributing characteristics and chemical traits of strawberry fruits.

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Chapter 6

BIOFERTILIZERS AS COMPLEMENTS TO SYNTHETIC AND ORGANIC FERTILIZATION

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ABSTRACT

Biofertilizers are agrobiotechnological products composed by microorganisms that are added to the agricultural crops to stimulate growth and production. Although they are sometimes considered as substitutes of the synthetic fertilizers, they indeed have been shown to act complementarily to chemical and organic fertilizers to improve plant performance. Synthetic fertilizers are added to the crops to support plant growth and foster crop production, but regrettably at most 50% of the synthetic nitrogen fertilizers can be absorbed by the plants. Microorganisms can positively affect plant performance in different ways, but they can also increase the percentage of the synthetic fertilizers that can be absorbed by the plants. In this chapter we will analyze the use of biofertilizers as complements to synthetic and organic fertilizers and the beneficial output of considering a chemical-organic-biological approach in crop production.

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INTRODUCTION

Biofertilizers, also known as bioinoculants, microbial inoculants or soil inoculants are agricultural biotechnology products that contain living or dormant microorganisms (bacteria or fungi, alone or in combination) that are added to agricultural crops to boost their growth and productivity.

The term "biofertilizer" derives from the words, biological and fertilizer, so that it refers to a biological fertilizer. In this context, a biofertilizer contains living microorganisms that have the capacity for improving the nutritional status of the plants. Conversely, organic products such as manure, crop residues, compost and vermicompost, which are also added to the crops to improve their nutrition, are not considered as biofertilizers but as *organic fertilizer*; although they indeed contain microorganisms, their identity and concentration normally remain unknown.

Due to the relative novelty of this technology in México and to the great impulse the government has settled for promoting the use of the biofertilizers in the Mexican agriculture many companies try their products be considered as biofertilizers, despite they do not meet the basic requirement of having axenically grown microorganisms. Also, in the worst cases these products can contain pathogenic microorganisms not only for plants but also for animals and the human being itself.

Currently, there are good perspectives for using biofertilizers as a mean to reduce pesticides and increase crop production, because the ever rising prices of the chemical fertilizers and cumulating evidence for environmental degradation caused by the use of agrochemicals is leading agricultural producers to look for cheaper and safer production practices not only for organic but also for conventional agriculture. Within these strategies, it is very important to consider an integrated plant nutrition system based on the use of low doses of chemical fertilizers, application of organic fertilizers (compost, vermicompost) and inoculation of microorganisms possessing capacities for improving the assimilation of the nutrients contained in chemical and organic fertilizers. In this chapter we will review the benefits and mechanisms by which the microorganisms can improve the nutrient and health status of the plants and show the advantages of considering an integrated plant nutrition system for crop production.

CLASSIFICATION OF BIOFERTILIZERS

The microorganisms possess a wide diversity of mechanisms by which they promote plant growth. Based on these mechanisms we recognize four major groups of plant growth promoting microorganisms:

a) Microorganisms that incorporate nitrogen into the plant-soil system through biological nitrogen fixation

The most efficient nitrogen fixers are bacteria belonging to the genera *Rhizobium, Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium*, *Bradyrhizobium, Azorhizobium* and *Allorhizobium* (Bloemberg & Lugtenberg, 2001).

b) Microorganisms that increase nutrient and water uptake

This category includes the fungi which associate with plants to form the symbiotic relationship known as mycorrhizae, which play an important role in water, phosphorus, zinc, sulfur, and copper uptake (Nelson & Spaner, 2010), and bacteria such as *Azospirillum* spp., which increases the capacity of water and nutrient uptake of plants by stimulating root growth through hormonal action. (Hayat et al., 2010).

c) Microorganisms that increase the availability of nutrients found in the soil in not assimilable forms

This category includes phosphate-solubilizing microorganisms capable of producing phosphatases or organic acids (*e.g. Bacillus megaterium* or *Pseudomonas fluorescens*), sulfur-oxidizing bacteria that convert elemental sulfur or any reduced form of this element into sulfates, which are the usable form by plants, microorganisms producing siderophores, such as certain species of the genera *Pseudomonas*, *Bacillus* and *Flavobacterium* that increase the availability of iron to the plants and microorganisms capables of destroy the structures potassium-bearing mineral (*e.g. Bacillus*, *Pseudomonas*, and *Clostridium* and fungi such as *Aspergillus*, *Penicillium* and *Mucor*; Guevara-Granja, 2010)

d) Microorganisms that possess antagonistic activities against plant pathogens

This mechanism is sustained by the fact that a healthy plant will fed and function better and, consequently, will be able to tolerate more efficiently the effect of nutritional deficiencies or adverse environmental conditions. Different species of *Pseudomonas*, *Bacillus, Serratia, Flavomonas, Curtobacterium* and *Trichoderma*, are included in this group (Aguado-Santacruz, 2012).

Additionally to the previous classification, other categories have been proposed for referencing the mechanisms by which the microorganisms promote the growth of plants:

a) Biopesticides

Include microorganisms that stimulate plant growth by controlling phytopathogenic agents. For example, *Pseudomonas aurantiaca* is an orange colored, Gram negative, growth-promoting bacterium originally isolated from the rhizosphere of potato that produces the compound di-2, 4 diacetylphloroglucylmethane, which is an effective antibiotic against various phytopathogenic soil microorganisms (Esipov et al., 1975; Felker et al., 2005). Additionally, some endophytic microorganisms such as *Cladosporium sphaerosperum, Neotyphodium sp., Phomopsis oblonga, Bacillus subtilis* or *Pseudomonas fluorescens* have

biocontrol activities against insects (Azevedo et al., 2000) and nematodes (Hallmann et al., 1997; Ryan et al., 2009) that are harmful to plants.

b) Phytostimulators or Biostimulators

This category includes microorganisms that promote plant growth usually by hormonal action. It has been elucidated that the main mechanism of the growth promoting bacterium *Azospirillum brasilense* is related to its ability to stimulate root growth through the production of indole acetic acid (IAA; Mascarua-Esparza et al., 1988*).* In addition to its effective capacity for fixing nitrogen, *Gluconacetobacter diazotrophicus* can produce IAA (Fuentes-Ramírez et al., 1993; Bastian et al., 1998) and cytokinins (Jiménez-Salgado et al., 1994), which could magnify the growth promoting activity of this bacterium. Likewise, some species of the genera *Pseudomonas*, *Azotobacte*r and *Bacillus* release indole acetic acid, gibberellins and cytokinins into the rhizosphere of plants, exerting a growth stimulation which is specially marked at the seedling stage of plants (Lugtenberg & Kamilova, 2009).

Vessey (2003) considers that although biopesticides can stimulate plant growth by controlling pathogenic organisms, they do not have a direct effect on the nutritional status of plants and, therefore, should not be considered as biofertilizers. Based on this premise, some authors propose to differentiate the plant growth promoting bacteria (PGPB) into biocontrol-PGPB's and PGPB's depending on whether or not exhibit antagonism against pathogenic organisms (Bashan & Holguin, 1997).

Vessey (2003) also mentions that biofertilizers should include any microorganism that promotes plant growth by increasing the supply of primary nutrient availability to the host plant, either by promoting the resupplying of soil nutrients (*e.g.* through biological nitrogen fixation), increasing the availability of nutrients (*e.g.* solubilization of phosphates) or extending the physical access of the plants to these nutrients (*e.g.* increasing the volume or changing root architecture).

Under this definition, biofertilizers should include microorganisms incorporating new nutrients into the plant-soil system as well as microorganisms increasing the uptake and availability of nutrients. However, this raises a problem because many organisms have more than one mechanism to promote growth and can exhibit complementary biocontrol activities (Raupauch & Kloepper, 2000; Manjula & Podile, 2001; Guo et al., 2004). Based on these premises, some authors propose to use the term in a broader sense to include any biological product (or microorganism) capable of promoting plant growth regardless the mechanism used for this purpose; this is the meaning used for "biofertilizer" in this chapter of the book.

The benefits of using biofertilizers in agriculture include:

- Increased capacity of plants to absorb water and nutrients from the soil.
- Reduced demand of irrigation and fertilization doses in crops.
- Increased growth and seedling establishment.
- Increased rooting of cuttings.
- Increased vigor of seedlings and adult plants.
- Biocontrol of pathogens.
- Reduced time of harvest (in some cases between 7 and 9 days) and extension of the productive time of crops (Dibut & Martínez, 2004).

- Increased crop yields in both, field and greenhouse conditions.
- Increased weight and quality of fruits.
- Compatibility with organic production of agricultural crops.
- Reduction of environmental pollution through reduced use of pesticides and chemical fertilizers (Kennedy, 2001).
- Bioremediation of soils contaminated with petroleum derivatives and heavy metals. It is known that high concentrations of metals in the soil and plants affect crop growth and the symbiotic relationships, and consequently, crop yields by disruption of fundamental physiological processes, such as photosynthesis, respiration, protein synthesis and carbohydrate metabolism (Khan et al., 2009). Different experiments have demonstrated the great potential of plant growth promoting rhizobacteria (PGPR) and mycorrhiza for detoxification of organic pollutants (Lucy et al., 2004, Abdul, 2006). Sarabia-Ochoa et al. (2010) refer different examples of bioremediation by PGPR including lead and zinc (*Azotobacter chroococcum* HKN-5-1, *Bacillus megaterium* HKP1, *Bacillus mucilaginosus* HKK-1), nickel (*Bacillus subtilis* SJ-101), cadmium (*Brevundimonas* sp. KR013, *Pseudomonas fluorescens* CR3, *Pseudomonas* sp. KR017, *Rhizobium leguminosarum* bv. trifolii NZP561, *Mesorhizobium huakuii* subsp. rengei B3), nickel, lead and zinc (*Kluyvera ascorbata* SUD165, *Kluyvera ascorbata* SUD165/26). In particular, the ability of *Burkholderia xenovorans* (formerly *Pseudomonas cepacia*, *Burkholderia cepacia* or *Burkholderia fungorum*) to degrade, chloroorganic pesticides and polychlorinated biphenyls (PCBs) is well documented. Kuiper et al. (2001) developed the concept of rhizoremediation, in which contaminant-degrading rhizobacteria, living on or close to the root, are selected for their ability to assimilate the root exudates rather than the chemical pollutants.

Certain microorganisms possess a wide range of added values. For example, some strains of *Pseudomonas* spp. have biocontrol activities against phytonematodes (Ali et al., 2002; Haas & Kell, 2003) and mollusks that represent a problem in water reservoirs (Molloy & Mayer, 2007). Some strains of *Pseudomonas cepacia* and *Pseudomonas solanacearum* are capable of hydrolyzing fusaric acid, which is the causative agent of wilt by *Fusarium* sp. (Sarabia-Ochoa et al., 2010).

Tsukamurella paurometabola C-924 is a tricalcium phosphate-solubilizing bacterium with nematicidal activity and capacity for producing indole acetic acid, proteases and chitinases. This bacterial strain also possesses antagonistic activities against phytopathogenic fungi such as *Sarocladium oryzae, Alternaria longipes, Pestalotia debaryanum* and *Pythium palmarum,* and stimulates the growth of maize plants under greenhouse conditions (Marín et al., 2013).

Several strains of *Paenibacillus polymyxa* have demonstrated plant growth promotion through biological nitrogen fixation and phosphate solubilization, while being capable of producing hydrolytic enzymes including proteases, 3-glucanases, cellulases, xylanases, lipases, amylases and chitinases, and a wide variety of secondary metabolites including auxins, cytokinins, lytic enzymes, and antibacterial and antifungal compounds. Likewise, *P. polymyxa* causes structural changes in the root of plants and exerts control over different phytopathogenic fungi such as *Botrytis cinerea, Fusarium oxysporum, Pythium* spp.,

Phytophthora palmivora, *Pythium aphanidermatum*, *Micrococcus* spp., *Aspergillus versicolor* and *Phytophthora capsici*, and against different parasitic nematodes of plants, including the root-knot nematode *Meloidogyne incognita* (Benítez-Noyola, 2013).

On the other hand, certain species of the biocontrol fungus *Trichoderma* sp. are efficient producers of many extracellular enzymes used in the food and textile industry. In addition, they also have a great potential for the production of lignocellulosic biofuels due to their ability to degrade complex polysaccharides (Kovacs et al., 2009).

Finally, another advantage of using microorganisms as a means to control plant diseases is that, unlike what happens with chemical pesticides, biofertilizers are less prone to induce resistance because they possess multiple mechanisms to control the pathogens.

ACTION MECHANISMS OF BIOFERTILIZERS

The mechanisms explaining the developmental and productivity responses of plants to the inoculation with beneficial microorganisms can be direct or indirect.

DIRECT MECHANISMS

Through these mechanisms, biofertilizers improve plant growth favoring the nutrition of crops, either by increasing the availability and uptake of nutrients and water via hormonal action or by altering the structure and the absorptive surface of roots.

Biological Nitrogen Fixation

This activity involves the enzymatic reduction of atmospheric nitrogen (N_2) to ammonium (NH_4). In some plants this reductive process is performed in specialized structures (such as the root nodules of legumes) and is catalyzed by the enzymatic complex of the nitrogenase, which consists of two different proteins: dinitrogenase, the molybdenum-iron protein, and dinitrogenase reductase, the iron protein (Seefeldt et al., 2009; Moure et al., 2013).

Synthesis of Hormones

Hormones are natural compounds that in low concentrations are able to affect fundamental morphological and physiological processes of plants. The production of hormones (auxins, gibberellins and cytokinins) has been one of the preferred mechanisms used by different researchers to explain the direct stimulation of growth by microorganisms (Brown, 1974; Patten & Glick, 1996, García de Salamone et al., 2005). In bacteria, particularly Gram-negative, production of indole acetic acid (IAA) is one of the most widespread mechanisms of growth promotion. The precursor of this hormone, the amino acid tryptophan, is one of the compounds mostly abundant in root exudates (Kamilova et al., 2006)

and IAA can be found in up to 80% of the bacteria isolated from the rhizosphere of some plants (Loper & Schroth, 1986).

Synthesis of Vitamins

The production of certain vitamins contributes significantly to the growth promoting activity of certain microorganisms. For example, it has been shown that *Pseudomonas fluorescens* strain 267 produces water soluble vitamins of the B group, which stimulate the growth of red clover, *Trifolium pratense* (Marek-Kozaczuk & Skorupska, 2001). Also, some strains of *Azotobacter* and *Azospirillum* produce B vitamins that increase the rooting capacity of plants and affect soil microbial populations (Rodelas et al., 1993; Revillas et al., 2000).

Regulation of the Ethylene Levels

Ethylene is a plant hormone that can inhibit the development of the root and, therefore, limit the ability of plants to absorb nutrients and water from soil. In higher plants, the enzyme S-adenosyl-L-methionine (SAM) synthase catalyzes the conversion of methionine to SAM (Giovanelli et al., 1980). In response to various types of stress, including mechanical injury, water stress (drought and flooding), salinity, herbicides, among others, the enzyme ACC synthase catalyzes the conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC), which is the immediate precursor of ethylene. Subsequently, ACC oxidase enzyme catalyzes the conversion of ACC to ethylene, carbon dioxide and hydrogen cyanide (John, 1991). This increase in the levels of ethylene in the root causes a delay in root growth. Some microorganisms, *e.g*. different species of the genera *Pseudomonas* and *Bacillus*, possess an enzyme called ACC deaminase, which hydrolyzes 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of ethylene, to form ammonia and α-ketobutyrate (Mayak et al., 1999; Shaharoona et al., 2006; Glick et al., 2007) and, thereby, prevent the formation of ethylene. Consequently, when the ACC deaminase activity increases, the levels of ethylene in the plant decreases and root development is visibly increased (Muhammad et al., 2007).

Siderophore Production

Siderophores are molecules (mainly non-ribosomal peptides) with a high affinity for iron that are produced by various microorganisms and grasses (Neilands, 1952; 1995) to increase the bioavailability of this element. At neutral pH, the iron availability in the soil is limited to plants due to the low solubility of the mineral phases (such as iron oxides) that associate to this element. Siderophores dissolve these mineral phases forming soluble complexes of iron can be then introduced into plant cells by active transport mechanisms. Under anoxic conditions (low oxygen), iron is usually found in the oxidation state of Fe^{+2} (ferrous ion) which is a soluble form. However, under oxic conditions (*i.e*. high concentrations of oxygen), iron is found as ferric ion (Fe^{+3}) that is capable of forming different insoluble minerals. To obtain the iron from these minerals, cells produce siderophores that binds to iron with high

affinity and transport it inside the cells. Siderophores produced by different bacteria and fungi include ferrichrome (*Ustilago sphaerogena*), mycobactin (*Mycobacterium* sp), enterobactin and bacillibactin (*Bacillus subtilis*), ferrioxamine B (*Streptomyces pilosus*), azotobactin (*Azotobacter vinelandii*), pseudobactin (*Pseudomonas* B10), ornibactin (*Burkholderia cepacia*) and coprogen, ferricrocin and palmitoylcoprogen (*Trichoderma spp*). Fluorescent pseudomonads produce a peptide siderophore with high affinity for iron called pyoverdin (Madigan & Martinko, 2005).

Phosphate Solubilization

Phosphorus (P) is, after nitrogen, the most important macronutrient for plant nutrition and a critical element in agricultural and natural ecosystems worldwide. Phosphorus in an essential component of key molecules for organisms, including RNA, DNA, AMP, ADP, ATP and phospholipids.

The productivity of arid regions is particularly low due to scarce and erratic precipitation, but also to the low availability of phosphorus in the soil. The soil phosphorus is readily converted into insoluble complexes such as hydrous oxides (oxides, hydroxides and oxyhydroxides) of iron and aluminum, amorphous and crystalline aluminum silicate and calcium carbonate (Sample et al., 1980).

Although arid soils contain a high concentration of phosphorus, *i.e.* 557-729 kg/ha, depending on soil use only 2.4 to 3.9% is found in available forms for plants. Generally, 15 to 20% (97 to 110 kg/ha) of the total phosphorus is present in organic forms such as phytin, lecithin, phospholipids and other compounds, while the remaining 77-82% is available in inorganic forms such as tricalcium phosphate, and a smaller quantity as iron and aluminum phosphates (Rao & Tarafdar, 2002). Insoluble forms of phosphorus include aluminum phosphates on acid soils, and calcium phosphates on alkaline soils. Fixing reactions in the soil cause that only a small portion (10 to 15%) of the phosphorus applied to the crops as chemical fertilizer or manure can be used by plants during the same year of its application.

Phosphorus deficiency causes a reduction of plant growth, alteration of the leaves to a bluish-green color and formation of acid tasting and small fruits. Strategies to address the low availability of phosphorus include the use of organic sources of this element (phosphoric rock or fish phosphate fertilizers) and the use of phosphate solubilizing bacteria (PSB), which increase the availability of this element for plants. PSB constitute a beneficial bacterial group capable of hydrolyzing both organic and inorganic phosphorus from insoluble sources (Goldstein et al., 2003). PSB secrete both organic acids and phosphatases to convert insoluble phosphates into soluble ions of monobasic ($H_2PO_4^-$) and dibasic (HPO_4^-) phosphate, through the process known as mineral phosphate solubilization.

It is generally accepted that the main mechanism of mineral phosphate solubilization is associated with the production of low molecular weight organic acids, which chelate the cations bound to the phosphate through its hydroxyl and carboxyl groups, favoring its conversion to a soluble form. In addition, some PSB produce phosphatases, such as phytase, which efficiently hydrolyze the organic forms of phosphate. PSB possess the ability to solubilize compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and phosphoric rock; the gluconic acid and 2-ketogluconic are the compounds most commonly referred as phosphate solubilizers. Various enzymes, such as nonspecific phosphatases,

phytases and C-P lyases release phosphorus from soluble organic compounds in the soil. C-P lyases break the bonds C-P in organophosphates (Barea et al., 2005).

The ability of organic acids to increase the availability of P is not only related to acidification of the plant rhizosphere, but also to their ability to form stable complexes with the Al and Fe. Organic acids increase the availability of micronutrients in the soil (Fe, Zn and Mn) by decreasing the pH in the rhizosphere or by chelating these micronutrients.

Solubilization of soil phosphates leads to an increase in the availability of phosphorus and, consequently, to increased absorption of this element by the plants (Gyaneshwar et al., 2002). Likewise, the organic acids participate in the soil in phenomena such as microbial chemotaxis and metal detoxification.

Some of the acids with phosphate solubilizing capacity secreted by the PSB include oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic, indole acetic and 2-ketogluconic. On the other hand, bacteria that solubilize phosphates through the production of organic acids include the genera *Achromobacter, Aerobacter, Agrobacterium, Azotobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Mesorhizobium, Micrococcus, Pantoea, Pseudomonas, Rahnella, Rhizobium, Streptosporangium* and *Yarrowia* (Paredes-Mendoza y Espinosa-Victoria, 2010).

Potassium Solubilization

Potassium deficiency, also known as potash deficiency, is a plant disorder that is most common on light, sandy soils, because potassium ions (K^+) are highly soluble and will easily leach from soils without colloids (Datnoff et al., 2007). Potassium deficiency is also common in chalky or peaty soils with low clay content and on heavy clays with a poor structure. The main role of potassium is to provide the appropriate ionic balance for metabolic processes occurring in the cytosol, and as such functions as a regulator of various processes including growth regulation (Leigh & Wyn Jones, 1984). Plants require potassium ions for protein synthesis and for the opening and closing of stomata. A deficiency of potassium ions can impair a plant's ability to maintain these processes. Potassium also functions in other physiological processes such as photosynthesis, protein synthesis, activation of some enzymes, phloem solute transport of photoassimilates into source organs, and maintenance of cation:anion balance in the cytosol and vacuole. Typical symptoms of potassium deficiency in plants include brown scorching and curling of leaf tips as well as chlorosis between leaf veins.

Potassium can be immovilized in the soil forming mineral structures such as feldespars or micas. Certain strains of *Bacillus*, *Pseudomonas* and *Clostridium*, and fungi such as *Aspergillus*, *Penicillium* and *Mucor*, solubilize potassium by releasing organic and inorganic acids that react with the potassium bearing-minerals. These microorganisms decompose aluminosilicates and release part of the potassium contained in them (Delgado-Higuera, 2002). Wuxing et al. (2007) demonstrated that *Bacillus mucilaginosus* is able to solubilize K^+ and SiO_2 silicates. This bacterium dissolved solid minerals and mica simultaneously, releasing K^+ and SiO_2, but was unable to solubilize feldspar. *Frateuria aurentia* is a potassium-solubilizing bacteria widely used in the fabrication of biofertilizers that can act in any kind of soil, using carbon, sugars, organic acids and amino acids from the soil or root exudates (Guevara-Granja, 2010).

Sulfur Solubilization

Sulfur is the fourth most important element for plant growth after nitrogen, phosphorus and potassium. Sulfur importance is equal to that of the nitrogen in terms of protein synthesis, while in terms of assimilation by crops is greater than that of the phosphorus (Vidyalakshmi & Sridar, 2007). The original source of sulfur in the earth was igneous rocks, primarily igneous pyrite (FeS_2). Since then, the amount of sulfur in the environment has increased due to volcanic activity and weathering of the earth´s crust in an oxygen atmosphere (Hoffman et al., 1998). Current sulfur sources come from the weathering of soil minerals, atmosphere and sulfur already fixed in the organism's biomass.

The essential processes of the sulfur cycle in nature are:

1) Mineralization of organic sulfur into inorganic forms, such as hydrogen sulfide (H_2S), elemental sulfur, as well as sulfide minerals.
2) Oxidation of hydrogen sulfide, sulfide, and elemental sulfur (S) to sulfate (SO_4^{2-}).
3) Reduction of sulfate to sulfide.
4) Incorporation of sulfide into organic compounds (including metal-containing derivatives).

Transfer of sulfur between organic and inorganic sources within the sulfur cycle is caused entirely by the activity of soil biota, particularly by the microbial biomass, which has the greatest potential for mineralization and the subsequent transformation of the oxidation state of sulfur.

Sulphate is the main form of sulfur assimilated by plants, so this element has to be first converted into this salt to be assimilated by plants (Mahendra, 1988). Sulfur oxidation leading to the formation of sulfate is the most important process in the sulfur cycle that leads to increased soil fertility. Additionally, soil acidification resulting from this oxidation process, help solubilizing nutrients and improve fertility in alkaline soils (Wainwright, 1984). The reduced inorganic sulfur compounds are oxidized exclusively by prokaryotes, although fungi such as *Alternaria tenius, Aureobasidium pullulans, Epicoccum nigrum, Scolecobasidium constrictum, Myrothecium cinctum, Aspergillus* and a number of species of the genus *Penicillium* are capable of oxidizing elemental sulfur and thiosulfate (Vidyalakshmi et al., 2009). On the other hand, the oxidation of sulfur in members of the genus *Eukarya* is conducted by bacterial lithoautotrophic endosymbionts (Nelson & Fisher, 1995).

Prokaryotes have the ability to oxidize hydrogen sulfide, sulfur, sulfite, thiosulfate and different polythionates under alkaline, neutral or acidic conditions (Harrison, 1984; Sorokin et al., 2001). The sulfur-oxidizing aerobic prokaryotes belongs to genera *Acidianus, Acidithiobacillus, Aquaspirillum, Aquifex, Bacillus, Beggiatoa, Methylobacterium, Paracoccus, Pseudomonas, Starkeya, Sulfolobus, Thermithiobacillus, Thiobacillus* and *Xanthobacter,* which are basically mesophilic microorganisms. Photoautotrophic, anaerobic, sulfur-oxidizing bacteria are primarily neutrophilic and mesophilic and belong to genera such as *Allochromatium* (formerly *Chromatium*), *Chlorobium*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodovulum* and *Thiocapsa* (Friedrich et al., 2001).

Sulfur-oxidizing microorganisms are primarily Gram-negative bacteria of the genera *Thiobacillus*, *Thiomicrospira* and *Thiosphaera*, although some heterotrophic bacteria such as

Paracoccus, *Xanthobacter*, *Alcaligenes* and *Pseudomonas* may also exhibit chemolithoautotrophic growth on inorganic sulfur compounds (Vidyalakshmi et al., 2009).

Production of Volatile Compounds

Volatiles constitute a group of compounds that evaporate rapidly at ambient temperature and pressure. Some strains of rhizobacteria belonging to *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Enterobacter cloacae* promote plant growth releasing volatile compounds (Ryu et al., 2003). Growth promoting action by volatile compounds is one of the mechanisms more recently studied in PGPR. Acetoin (3-hydroxy-2-butanone) and 2,3 butanediol are volatile compounds produced by *B. subtilis* and *B. amyloliquefaciens*, which promote growth of *Arabidopsis thaliana in vitro*. Some of these compounds act by regulating the synthesis of auxin and cell expansion (Zhang et al., 2007), but a role has also been proposed in induction of systemic resistance (Farag et al., 2006) and antibiosis (Mitchell et al., 2010). Certainly, this knowledge area requires further research to identify new compounds and elucidate new plant signaling pathways involved in the interactions with plant growth promoting microorganisms. For example, two new volatile compounds, the 2-methyl propanoic acid and 3-methyl butanol, which are synthesized *de novo* during the interaction of *Arabidopsis thaliana* with the bacteria *Bacillus megaterium* and *Stenotrophomonas maltophilia* has been recently identified (Kai et al., 2007; García-Juárez et al., 2010).

Furthermore, in biocontrol fungi (*e.g. Trichoderma* spp.) certain volatile compounds, such as acetone, 2-methyl-1-butanol, heptanal and octanal, increase the antagonistic activity of the beneficial fungi by inhibiting the protein synthesis of pathogen fungi (Humphris et al., 2001). Additionally, *Arthrobacter agilis* UMCV2 is capable of producing volatile organic compounds (VOCs), primarily dimethylhexadecylamine, an amino lipid with antifungal activity, which exerts a strong inhibition on *Botrytis cinerea* and *Phythopthora cinnamomi.* Conveniently, the effect of this VOC on *Trichoderma virens* is very subtle, suggesting the possibility of developing comprehensive strategies for disease control considering this differential action (Velázquez-Becerra et al., 2010).

Synthesis of Pyrroloquinoline Quinone

Pyrroloquinoline quinone (PQQ) is a novel, water soluble and heat stable growth factor in plants that has the ability to carry our redox cycles (Stites et al., 2000). It has been reported that PQQ acts as a reactive oxygen species (ROS) scavenger by directly neutralizing reactive species in *Escherichia coli* (Misra et al., 2004). PQQ acts as a non-covalently bound redox cofactor of several bacterial dehydrogenases. Several gram-negative bacteria are capable of producing organic acids by direct oxidation of aldehydes, which then diffuse in surroundings and help in the acidification of poorly soluble mineral phosphates such as calcium phosphate (Goldstein, 1986; Sashidhar & Podile, 2010). Glucose dehydrogenase (GDH) requires PQQ as a redox cofactor for direct oxidation of glucose to gluconic acid, which then helps in acidic solubilization of insoluble phosphates in soil. There are plant growth-promoting bacteria that use GDH-PQQ holoenzyme for solubilization of both inorganic and /or organic phosphates in soil (Han et al., 2008).

The Gram-negative bacterium *Rahnella aquatilis* is ubiquitous and is characterized by its beneficial metabolism leading to mineral phosphate solubilization, antimicrobial activity, nitrogen fixation and plant disease suppression (Calvo et al., 2007). This bacterium produces PQQ and its mineral phosphate solubilizing capacity is contributed by mechanisms similar to other phosphate solubilizing microbes. Strain HX2*R* of *R. aquatilis* has been used as a biocontrol agent for grapevine crown gall caused by *Agrobacterium vitis*. PQQ mutants of this bacterium become ineffective in its biocontrol activity. Different reports suggest that the GDH-PQQ holoenzyme is involved in production of antimicrobial compounds in *P. fluorescens* (Schnider et al., 1995; de Werra et al., 2009) and *Enterobacter intermedium* 60-2G (Han et al., 2008). Particularly, *E. intermedium*, a phosphate-solubilizing bacterium, has the ability to induce systemic resistance in plants against the soft rot bacterial pathogen *Erwinia carotovora* and the fungus *Magnaporthe grisea*. Mutations in PQQ cause *E. intermedium* lose their biocontrol ability and its capacity for enhancing the systemic resistance of plants (Han et al., 2008).

INDIRECT MECHANISMS

The indirect promotion of plant growth occurs when biofertilizers prevent, reduce or eliminate one or more pathogenic organisms (Glick et al., 1999; Hernández & Charlloux, 2001) through the following mechanisms:

Competition for Space and Nutrients

To exert their beneficial effect on plant growth, microorganisms must be rhizosphere-competent, *i.e.*, should be capable of compete with other microorganisms present in the rhizosphere for nutrients secreted by the root and the physical space available inside or over the root. Only a small part of the root surface is covered by bacteria. Favorite sites for bacterial growth are the junctions between epidermal cells and origin points of the lateral roots. Once soil microorganisms colonize plant roots, they colonize space and consume nutrients that otherwise could be used by plant pathogens (Kloepper et al., 1988, O'Sullivan & O'Gara, 1992).

Siderophore Production

Siderophore synthesis is a dual mechanism for promoting plant growth, since it increases iron availability to plants but also contributes to biological control of phytopathogenic agents. Sequestering iron from the soil and making it available for themselves and for plant cells capable of assimilating the bacterial siderophore-iron complexes, plant growth promoting microorganisms can limit the pool of this element for other microorganisms that are unable to access the sequestered iron (Castignetti & Smarrelli, 1986; O'Sullivan & O'Gara, 1992; Dowling et al., 1996).

Antibiotic Synthesis

Production of antimicrobial compounds has been documented in several plant growth promoting microorganisms (O'Sullivan & O'Gara 1992; Haansuu et al., 1999) and this is the mechanism most commonly associated with the ability of biofertilizers to inhibit phytopathogens (Keel et al., 1992; Chet & Inbar, 1994; Whipps, 1997). The ability of some bacteria to suppress fungal pathogens depends on their ability to produce antibiotics such as pioluteorina, pirronitrina, fenacin-1-carboxylic acid and 2,4-diacetylphloroglucinol (Picard, et al., 2000). Other compounds with pathogen-inhibiting capacities released by bacteria include hydrogen cyanide (HCN) and/or lytic enzymes such as chitinase, β-1,3 glucanase, proteases and lipases (Friedlender et al., 1993; Chet & Inbar, 1994). Though pectinolytic activities are commonly associated with pathogenic bacteria, some species of non-pathogenic bacteria such as *Rhizobium* (Angle, 1986), *Azospirillum* (Umali-Garcia et al., 1980; Tien et al., 1981), *Klebsiella pneumoniae*, *Yersinia* (Chatterjee et al., 1978) and *Frankia* (Séguin & Lalonde, 1989) are also capable of degrading pectins. In general terms, pectinolytic enzymes play a role in the invasion of the roots by bacteria.

Induction of Systemic Resistance

Research on the benefits of microbial inoculants extends beyond their capacities to improve plant nutrition, since microbial inoculants can also trigger the mechanism of systemic acquired resistance (SAR) of plants to different phytopathogenic agents such as *Blumeria graminis*, *Gaeumannomyces graminis*, *Fusarium culmorum* and *Pseudomonas syringae* (Heitefuss, 2001; Waller et al., 2005; Khaosaad et al., 2007; Ramos-Solano et al., 2008). In plants, the SAR is a global resistance response that occurs after plants have contact with a pathogen or a product derived from it. In a broad sense, the systemic acquired resistance in plants is equivalent to the response of the immune system of animals to the attack by pathogens. After an early and localized exposure to certain infectious organisms, SAR actives the resistance mechanisms at the whole plant level against a wide variety of pathogens, (including to that initiating the response), so it is considered as a wide-spectrum response. It has been shown that endophytic colonization of cocoa seedlings by *Trichoderma* activates the plant defense signaling cascades (Bailey et al., 2006). SAR is associated with the induction of a great variety of genes (genes PR's or pathogenesis related) and requires the accumulation of endogenous salicylic acid.

SAR is associated with the ability to induce cellular defense responses more rapidly and to a greater degree than in non-induced plants, a process called "priming." The phenylalanine ammonia lyase (PAL) gene activation and callose deposition are among the main cellular defense responses induced by SAR.

Plant growth-promoting rhizobacteria can effectively induce pathogen resistance by triggering the expression of the hypersensitive response (HR) of plants, enhance lignification and callose deposition, increase hydrogen peroxide production and expression of the defense enzymes β-1,3-glucanase, chitinase, phenylalanine ammonia lyase, peroxidase and polyphenol oxidase (Niranjan-Raj et al., 2006).

Strain 63-28 of *Pseudomonas fluorescens* functions as an activator of disease resistance of plants by inducing the synthesis of callose in tomato (M'Piga et al., 1997), while the application of saprophyte fluorescent pseudomonads in beans resulted in increased lignin content in the root (Anderson & Guerra, 1985).

Serratia plymuthica, strain R1GC4, sensitizes cucumber plants to react more quickly and effectively against the attack by *Pythium ultimum*, through the formation of physical and chemical barriers to impede the penetration of the fungi (Benhamou et al., 2000).

Formation of Biofilms to Prevent the Entry to Pathogens

Frequently, bacteria live in the environment as biofilms, which are highly structured, surface-attached communities of cells encased within a self-produced extracellular polymeric substance matrix (O'Toole et al., 1999; 2000; Branda et al., 2005; Kolter & Greenberg, 2006). Bacterial biofilms established on plant roots could protect the colonization sites and act as a sink for the nutrients in the rhizosphere, hence reducing the availability of root exudate nutritional elements for pathogen stimulation or subsequent colonization on the root (Weller & Thomashow, 1994). Particularly some strains of *Paenibacillus polymyxa* can form biofilms around the root tips of some plants to improve drought tolerance and to prevent the access to pathogens (Timmusk et al., 2005; Singh et al., 2006).

INTEGRATED PLANT NUTRITION SYSTEM (IPNS): COMBINED USE OF BIOFERTILIZERS AND CHEMICAL OR ORGANIC FERTILIZERS

One of the most important requirements in agriculture is the maintenance of soil fertility. Traditionally, nutrient deficiency, especially of N, is corrected by adding fertilizers. However, the high costs of these chemical products limit this practice, especially in developing countries, where the need to increase food production is more urgent (Aguado-Santacruz et al., 2012). It is estimated that crops absorb between 20-40% of the fertilizer applied because the rest is lost in different ways, generating substantial economic losses and environmental pollution such as eutrophication of water bodies, acid rain, destruction of the stratospheric ozone layer and increased greenhouse effect (Duxbury, 1994). On the other hand, some other sources, mainly organic fertilizers (compost, vermicompost and manures) and more importantly the Nitrogen Biological Fixation (NBF), can also contribute significantly to the N soil economy,

The IPNS basic concept is the adjustment of soil fertility for providing the optimal plant nutrients in order to maintain high crop productive levels taking advantage of the benefits from all of the possible sources of nutrients in an integrated manner (Jen-Hshuan, 2006). The implementation of sustainable technologies, such as biofertilization, to complement and enhance the assimilation of nutrients from chemical synthetic fertilizer and organic fertilizer, has been assessed in different studies that report satisfactory performance of different crops cultured under different environments emphasizing the necessity of considering complementary nutritional sources and tools for augmenting the nutrient assimilation by agricultural crops.

Different studies report a better assimilation of nutrients when the chemical fertilization is complemented with beneficial microorganisms. For example, Sundara et al. (2002) found that the application of the PSB *Bacillus megatherium* var. *phosphaticum* increase the availability of P in the soil, improving growth, yield and quality of sugarcane. When used in conjunction with phosphate fertilizers this PSB reduce the required dose of P by 25%. Moreover, it was shown that when applied in combination with phosphate rock, *B. megatherium* can help saving up to 50% of the production costs by replacing superphosphate. The effects of a combined treatment consisting of a multifunctional biofertilizer (mixture of *Bacillus sp., B. subtilis, B. erythropolis, B. pumilus* and *P. rubiacearum*) plus 50% of the recommended chemical fertilization dose was compared with the complete chemical fertilization dose on the growth of lettuce and water celery. The results of this study indicated a 25% increase of lettuce yield and 34% dry matter increase of water celery in the biological-chemical treatment (Young et al., 2003; 2004) as compared to the chemical fertilization treatment alone, indicating that at least 50% of the chemical fertilization can be saved by a complementary approach of multifunctional biofertilizers and chemical fertilizers.

Benítez-Noyola (2013) demonstrated that maize plants fertilized with 90 and 180 Kg N ha^{-1} and inoculated with *Paenibacillus polymyxa* extracted from 20 to 28% more nitrogen and produced more grain than plants that were only chemically fertilized. These effects were explained in terms of increased root growth and nutrient availability.

Obando et al. (2013) found a statistically significant increase of 4% in the nitrogen removal by maize plants chemically fertilized (urea) and biofertilized with *Azotobacter chroococcum* AC1, with respect to plants that were only chemically fertilized.

Compared to individual application of nitrogen fertilization, Das et al. (2004) found greater N_2 nitrogen accumulation in cotton when chemical fertilization was combined with *Azotobacter* M4. The increased nitrate assimilation promoted by *Azotobacter sp*. may be related to a greater elongation of root hairs, which would improve the ability to absorb nutrients and water (Obando et al., 2013).

Dibut et al. (2009) mention that by using *Azotobacter chroococcum* they were able to reduce 30% the recommended dose of nitrogen fertilization (urea) for banana without affecting crop yield because of the atmospheric nitrogen fixing capacity of *A. chroococcum*, which they demonstrated by isotopic techniques (^{15}N). These authors also indicate that inoculation of chickpea plants with *Mesorhizobium cicerii* resulted in beneficial effects on different parameters of growth and development, which permitted a reduction of 70% of the recommended nitrogen (urea) dose without affecting grain yield (2.05 ton ha^{-1}) as compared to control plants fertilized with 100 kg N ha^{-1} that produced 1.98 ton ha^{-1}.

According to Biswas et al. (2000) nitrogen-fixing microorganisms can promote plant growth by transferring the fixed nitrogen to plants or by enhancing the absorption of nutrients through the modulation of the hormonal activity. In this sense, Bashan (1999) mentions that inoculation of plant growth promoting microorganisms, such as *Azospirillum sp.*, results in increased accumulation of nitrogenous compounds by promoting a more effective absorption of nutrients with no apparent nitrogen fixation. Conversely, Shamsuddin (1994), using the ^{15}N isotope technique, found that up to 89% of the N requirement of oil palm plantlets is supplied by the symbiosis with *Azospirillum.* (Mia et al., 2010)

Covarrubias-Ramírez et al. (2005) evaluated the kinetics and efficiency of P uptake in potato plants (*Solanum tuberosum* L.) cv. Alpha, through ^{32}P isotope technique. They demonstrated that the inoculation of *Bacillus subtilis* increased the potato biomass by 31.7%

and P uptake by 27.5%. According to the authors the increase in these variables was the result of a more developed root system, which permitted to expand the exploratory capacity of the plant in the soil.

Naveed et al. (2008) demonstrated the possibility of maintaining grain maize yields by replacing 87 Kg urea ha^{-1} (50%) of the complete N fertilizer dose (175 Kg ha^{-1}) with 300 Kg ha^{-1} of an organic compost elaborated with fruit and vegetable wastes and enriched with 147 g N fertilizer Kg^{-1} compost; a basal dose of P and K (100 and 50 Kg ha^{-1}, respectively) was applied to all field plots. However, when the N-enriched compost was inoculated with different strains of *Pseudomonas* and then applied to the field plots conjunctly with 88 Kg urea ha^{-1} a significantly increase (1.1 ton grain ha^{-1}) in the growth and yield of maize was observed over full dose of N-fertilizer and exhibited superiority over organic fertilizer (0.5 ton grain ha^{-1}). According to the authors, *Pseudomonas fluorescens* strain N3 was particularly effective in promoting growth because of its high capacity of root colonization, chitinase activity and ACC deaminase activity, characteristics that confer this strain a relatively more competitive advantage. Effects of rhizobacteria containing ACC deaminase activity are well known for improving root growth of plants as a result of reduced ethylene synthesis through ACC hydrolysis into NH_3 and α-ketobutyrate in the inoculated roots (Shaharoona et al., 2007). These results imply that inoculation of organic fertilizers with PGPR possessing these traits could help developing improved biological products combining the nutritional characteristics of the compost and the beneficial activities of the rhizobacteria with synergistic effects on growth and productivity of crops.

Khurram et al. (2012) evaluated the effect of *Bacillus* strains possessing ACC deaminase activity and phosphate solubilizing characteristics, either as a single mechanism or a dual mechanism (strains with both features). Under axenic conditions, the bacterial strains with dual plant growth-promoting activities were superior in improving growth of wheat as compared to the strains possessing single trait. Similarly, these dual traits bacterial strains were more effective than single trait strains under soil conditions (pot trial) in increasing root weight (up to 3.9-fold) and root elongation (up to 3.8-fold), dry shoot weight (up to 37.6%), number of tillers (up to 56%), grain yield (up to 38.5%) and P uptake in grain (up to 77.4%) of wheat grown in the presence of P applied as diammonium phosphate (DAP), RP (rock phosphate) or RP-enriched compost. An almost similar trend was observed when the same trial was repeated under field conditions. Inoculation in the presence of RP-enriched compost resulted in promoting various growth parameters almost comparable to that recorded in the case of DAP. It was concluded that the simultaneous presence of two superior plant growth-promoting traits in the bacteria could have an additive effect not only on growth and yield of wheat but also on P uptake. The performance of *Bacillus* strains possessing dual traits was distinctly superior to that of the single trait strains.

Abdullahi et al. (2013) studied the effect of using a biofertilizer (containing *Azospirillum* sp. and *Glomus mosseae*) and poultry manure (PM) on nutrient uptake, plant growth and soil microbial population associated with sesame under field conditions. Plant height, numbers of leaves/plant, numbers of branches/plant, leaf area, shoots and root dry biomass increased significantly due to the application of the biofertilizer and poultry manure singly or in combination over control. Combined application of the biofertilizer and poultry manure at 5 ton/ha (bio-organic treatment) significantly produced the plants with the best growth parameters, nutrient content, and N, P, and K uptakes, and also recorded the highest populations of *Azospirillum* sp. (28.56 X 10^{-6} CFU g^{-1} soil) and AM fungi (69.3 AM spores g^{-}

[1] soil). The positive responses observed in the growth of inoculated plants may be due to the provision of nutrients, especially nitrogen, and growth promoting hormones by *Azospirillum* sp. and greater uptake of phosphorus and other nutrients due to mycorrhizal colonization (Zaidi et al., 2004). The greater availability of nutrients can be attributed to organic manure decomposition or transformation of inorganic substances into available forms by microorganisms. The increased population of *Azospirillum* sp., as well as spore density and *G. mosseae* colonization could be related to the application of manure, which constitutes a carbon source for microbes.

CONCLUSION

The information presented in this chapter shows that plant growth promoting microorganisms can be successfully used as complementary tools to organic and chemical fertilization for improving plant nutrition. It is clear that the confidence of the studies conducted under field conditions will largely depend not only on our knowledge of the nutrient and biological charge of the soil and composts or manures employed as organic fertilizers, and the growth promoter activities of the microorganisms employed as biofertilizers, but also on their particular interactions. This knowledge will be instrumental in the implementation of successful, low-environmental impact and more profitable agriculture production systems (organic or conventional).

Current and predicted costs of chemical fertilizers and cumulative evidence of negative effects of agrochemicals on the environment and human health will follow redirecting the efforts of crop research to look for the implementation of an integrated plant nutrition system in the agricultural production systems. Certainly, environmental and health, but mainly economic concerns will greatly impulse this approach in the next years.

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SECTION IV. NON-CONVENTIONAL FERTILIZERS

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Chapter 7

SILICON FERTILIZERS: AGRICULTURAL AND ENVIRONMENTAL IMPACTS

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ABSTRACT

Silicon (Si) is the second most abundant element of the Earth's surface. Usually the cultivated plants adsorb Si in amounts equivalent or higher to those of essential macronutrients such as nitrogen (N), phosphorus (P) and potassium (K). Silicon fertilization has a double effect on the soil-plant system. First, improved plant Si nutrition reinforces plant protective properties against diseases, insect attack, and unfavorable climatic conditions such as drought, salt, heavy metal or hydrocarbon toxicity. Second, soil treatment with biogeochemically active Si substances optimizes soil fertility through improved water, physical, and chemical soil properties and maintenance of nutrients in plant-available forms. As a result, Si fertilization gives possibility to reduce the application of pesticides (by 40-70%), traditional mineral fertilizers (by 10-40%), irrigation water (by 30-40%) without a reduction in the yield of cultivated plants. On an average, Si fertilization provides the increase in yield by 10-25%. Several types of Si fertilizers are available on the market: natural, synthetic, liquid, or solid. Some industrial by-products can be used as Si fertilizer as well. Special methodologies were elaborated to assess soil Si deficiency and Si fertilizer activity. Besides economical effects (increasing plant productivity, reduction of the traditional agrochemical application rates), Si fertilizers have high environmental impacts. First of all, the application of Si fertilizer provides increasing or restoration of the soil fertility level and reducing the leaching of chemicals from cultivated areas. These effects realized via improvement of physical-chemical and biological properties of the soil. Second, Si fertilizers can be used to control

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heavy metal mobility in the soil-plant system and for reducing the heavy metal accumulation in the crop. Finally, silicon fertilizers can be used for restoration of the hydrocarbon polluted areas and for acceleration of the organic pesticide degradation in the soil. At present, silicon fertilizers are successfully used in the USA, Canada, Australia, Russia, China, India, and other countries. Annually the market of Si fertilizers increases by 20%.

Keywords: Silicon fertilizer, soil chemistry, plant physiology, technology

INTRODUCTION

One of important aspects necessary to provide sustainable agriculture, especially organic farming, is related to the protection of cultivated soil against chemical, physiological, and biological degradation. The conventional agriculture usually leads to soil degradation. To transfer conventional agriculture to organic, high level of soil fertility should be formed.

Silicon is a constituent of many plants, but its role and functions in plant biology remain poorly understood (Liang et al., 2007; Ma, 2003). Beginning in 1840, numerous laboratory, greenhouse, and field experiments have shown benefits of Si fertilization for crop productivity of rice (*Oryza sativa* L.) (15-100%), corn (*Zea mays* L.) (15-35%), wheat (*Triticum aestivum* L.) (10-30%), barley (*Hordeum vulgare* L.) (10-30%), sugar cane (*Saccharum officinarum* L,) (15-40%), cucumber (*Cucumus sativus* L.) (10-40%), strawberry (*Fragaria* spp.) (10-30%), citrus (*Citrus* spp.) (5-15%), tomato (*Lycopersicon esculentum* Mill.) (10-40%), grasses (*Stenotaphrum secundatum* Kuntze, *Cynodon dactulon* L., *Lolium multiforum* Lam, *Paspalum notatum* Fluegge) (10-25%), banana (*Musa paradisiaca*) (20-40%; Matichenkov et al., 2011; Snyder et al., 2006).

Today Si fertilizers are successfully used in USA, Japan, China, India, Australia, Russia, and other countries. During last 15-20 years, the volume of Si fertilizers and Si-rich soil amendments was increasing by 15-20% annually. Hovewer, in spite of economical and environmental benefits, Si fertilization is still rare in the world agricultural practice. The main reason is low information about this element and its role in the soil-plant system.

Si Biogeochemical Cycle

Si is one of the most widely distributed elements in the Earth's crust. Soil is the most silica-enriched layer of the Earth's crust: 20 to 35% of Si is tested in clay soils and 45 to 49% in sandy soils (Kovda, 1985). Mainly, Si compounds in the soil are presented by crystalline or amorphous Si dioxide and various alumosilicates. Quartz is the most distributed form of Si substances on the Earth (Kovda, 1956). This crystalline kind of silica is characterized by high stability to weathering (Russell, 2002). Together with coarse-crystalline silicates (feldspar, plagioclase, orthoclase) and secondary or clay Si-rich minerals (kaolinite, vermiculite, smectite) silica form a soil skeleton (Orlov, 1992). Fine clay minerals and amorphous silica represent biogenic (phytoliths) and abiogenic amorphous forms or hydrated Si dioxide films on the soil particle surface. They exhibit high geochemical activity and affect the chemical soil properties (Kovda, 1985; Jacinin, 1994; Orlov, 1992). All natural waters, including soil

solution, contain soluble silicon substances. These are the products of mineral weathering or dissolving.

Monosilicic acid possesses high chemical activity (Iler, 1979; Lindsay, 1979). Monosilicic acid can react with aluminum, iron, and manganese with the formation of slightly soluble silicates (Lumsdon and Farmer, 1985):

$$Al_2Si_2O_5 + 2H^+ + 3H_2O = 2Al^{3+} + 2H_4SiO_4$$

$$Al_2Si_2O_5(OH)_4 + 6H^+ = 2Al^{3+} + 2H_4SiO_4 + H_2O$$

$$Fe_2SiO_4 + 4H^+ = 2Fe^{2+} + 2H_4SiO_4$$

$$MnSiO_3 + 2H^+ + H_2O = Mn^{2+} + 2H_4SiO_4$$

$$Mn_2SiO_4 + 4H^+ = 2Mn^{2+} + H_4SiO_4$$

Monosilicic acid under different concentrations is able to combine with heavy metals (Cd, Pb, Zn, Hg, and others) forming soluble complex compounds if monosilicic acid concentration is slight (Schindler et al., 1976) and slightly soluble heavy metal silicates when the concentration of monosilicic acid is greater in the system (Lindsay, 1979).

$$ZnSiO_4 + 4H^+ = 2Zn^{2+} + H_4SiO_4$$

$$PbSiO_4 + 4H^+ = 2Pb^{2+} + H_4SiO_4$$

The anion of monosilicic acid $[Si(OH)_3]^-$ can replace the phosphate anion $[HPO_4]^{2-}$ from calcium, magnesium, aluminum, and iron phosphates (Matichenkov, 2007).

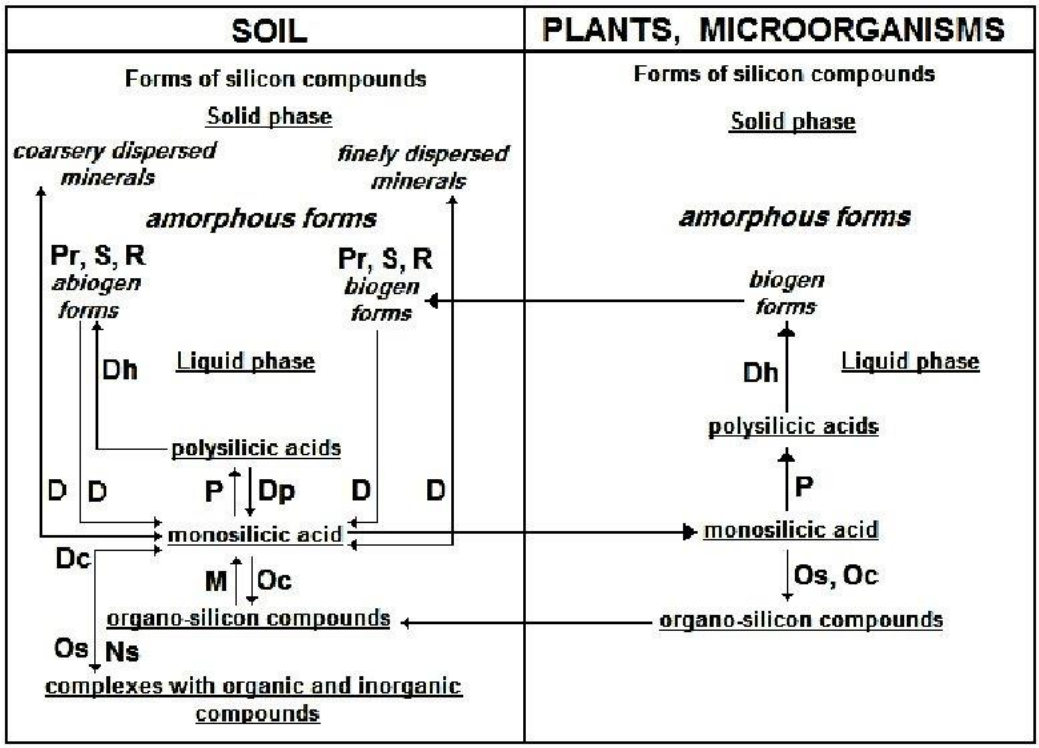
In natural solutions, oligomers of silicic acid are present as well, they contain 2 and more (up to 100) atoms of Si (Knight and Kinrade, 2001). Usually these substances are tested together with monosilicic acid, but they possess some difference in chemistry, compared with monosilicic acid (Matichenkov and Bocharnikova, 2001; Matichenkov et al., 2011). However, the knowledge about this form of soluble Si is very low.

Polysilicic acids with high content of Si atoms (more than 100) are an integral component of the natural solution as well. Unlike monosilicic acid, polysilicic acid is chemically inert and basically acts as an adsorbent and forms colloidal particles (Jacinin, 1994).

$$n(Si(OH)_4) \rightarrow (SiO_2) + 2n\,(H_2O) \text{ or}$$

$$[Si_nO_{2n-(nx/2)}(OH)_{nx}] + mSi(OH)_4 = [Si_{n+m}O_{2n-(n2x/2+2m(2-p)}(OH)_{nx+4m-p}]$$

Polysilicic acids are readily sorbed by minerals and form siloxane bridges (Chadwick et al., 1987). Since polysilicic acids are highly water saturated, they may have an effect on the soil water-holding capacity. Polysilicic acids have been found to be important for formation of soil structure (Matichenkov and Ammosova, 1996).



Note: D – dissolving, Pr – precipitation, P –polymerization, Dp – depolymerization, Dh – dehydratation, S – salt formation, R – replacement of inorganic anions, Oc – formation of organo-silicon compounds, Ns – formation of complexes with inorganic compounds, Os – formation of complexes with organic compounds, Dc – decomposition of complexes, M – mineralization of organo-silicon compounds.

Figure 1. Silicon cycle in system soil–plant–microorganisms (Biel et al., 2008).

Except mono-, oligomers and polysilicic acids, organosilicon compounds are present in soil, water systems, and living organism tissues (Voronkov et al., 1978). The occurrence of organosilicon substances and their classification is discussed now.

Soluble Si-rich substances play an important role in the soil-plant system because of their high biogeochemical activity. There are monosilicic acid, polysilicic acid, organosilicon compounds, and soluble complexes with organic and inorganic compounds. Monosilicic acid is topping soluble Si-rich substance of a soil. Monosilicic acid is adsorbed by plants and microorganisms (Yoshida, 1975). The soil solution monosilicic acid is responsible for the trend of transformation of secondary minerals and concentration of mobile P, Al, Fe, Mg, and Mn (Lindsay, 1979; Matichenkov et al., 2011). Monosilicic acid controls formation of polysilicic acid and organo-silicon compounds in soil and living organisms (Mann and Ozin, 1996; Matichenkov and Bocharnikova, 1994).

At our Planet, biological cycle of silicon is the most intensive in terrestrial ecosystems where plants uptake silicon in the range from 20 to 7000 kg Si/ha/year (Matichenkov and Bocharnikova, 1994). Silicon is the 4th most abundant element in plant biomass after oxygen, carbon, and hydrogen (Bazilevich, 1993; Knight and Kinrade, 2001; Perelman, 1989). As noted above, the plants and soil microorganisms are able to uptake only monomers of silicic acid and its anions (Ma, 2003; Yoshida, 1975). In higher plants, this process takes place

through the roots and leaves. However, the silicon is distributed within the plant irregularly, according to the needs of the organism (see below). Cultivated plants annually remove from soil 30 to 70 kg Si ha^{-1} (Anderson, 1991; Bazilevich, 1993; Ma and Takahashi, 2002; Savant et al., 1999). Usually the concentration of monosilicic acid in the soil is ranged from 5 to 40 mg Si L^{-1}. The removing of these substances results in crushing of the Si biogeochemical cycle in the arable soil and accelerating the following processes: soil texture degradation, soil organic matter decomposition, reduction in soil adsorption capacity, reduction in nutrient plant-availability (Biel et al., 2008; Kulikova, 2012; Matichenkov, 2007, 2008). To restore soil silicon status, Si fertilizers and soil amendments are necessary.

Silicon fertilization has a double effect on the soil-plant system. Firstly, improved plant Si nutrition reinforces plant protective properties against diseases, insect attack, and unfavorable climatic conditions. Secondly, soil treatment with biogeochemically active Si substances optimizes soil fertility through improved water, physical, and chemical soil properties and maintenance of nutrients in plant-available forms.

Si Effect on Plants

Silicon is an integral part of plants. Distribution of silicon between plant organs is not equal and may vary from 0.001% in the pulp of fruit to 10–15% in the epidermal tissue (Ma and Takahashi, 2002). Plants have a special mechanism for selective uptake of monosilicic acid from the soil solution (Ma et al., 2006). Plant tissues are characterized by extremely high concentrations of mono- and poly-silicic acids in the sap and are able to redistribute this element rapidly inside the plant (Biel et al., 2008). The main function of Si in plant is formation the defense system. Silicon provides the protective functions of plants on the mechanical, physiological, chemical, and biochemical levels.

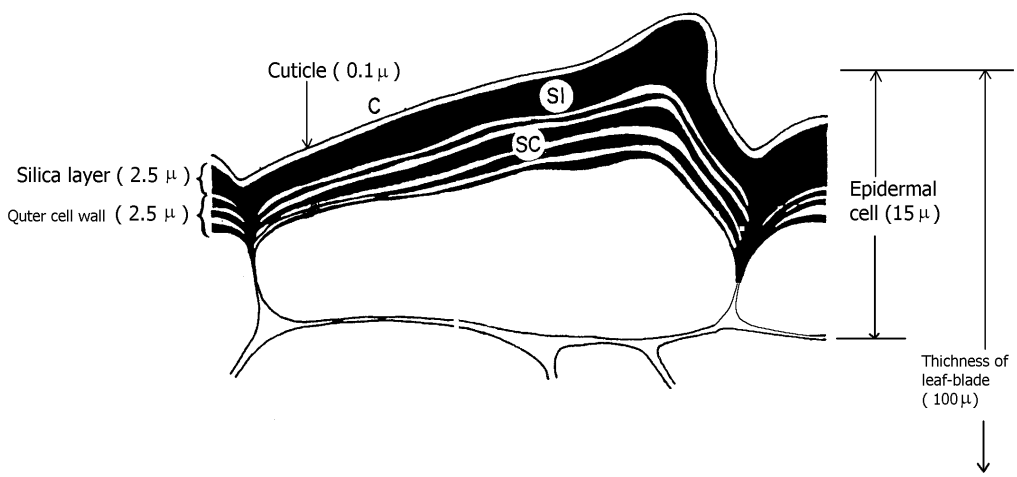


Figure 2. Schematic representation of the rice leaf epidermal cell (Yoshida, 1975).

Mechanical

The accumulation of Si in the epidermal tissue creates mechanical plant protection. Absorbed molecules of monosilicic acid are accumulated in the epidermal tissues (Yoshida, 1975; Figure 2) and form the silicon-cellulose envelope where silicon is bonded with pectin and calcium (Ma and Takahashi, 2002). As a result, the double cuticle layer protecting and mechanically strengthening the plants is formed. The mechanical protection of plants against biotic (fungi and insect attack) and abiotic (lodging) stresses is probably the most investigated and popular for explanation of Si fertilizer direct effect on plant resistance.

Physiological

The physiological effect of Si on plants proceeds via the formation of better developed root system (Kulikova, 2012; Ma and Takahashi, 2002). Monosilicic acid supports stability of chlorophylls molecules and other organelles which reinforce physiological plant stability (Biel et al., 2008; Matichenkov et al., 2008; Snyder et al., 2006). We suggested that increasing of plant drought tolerance by soluble Si realized via physiological mechanism. Due to high concentrations found in the symplast and apoplast of plant, polysilicic acid can keep water and its molecules can serve as a rechargeable water tank (Matichenkov et al., 1994). As shown in greenhouse and field tests, Si fertilization makes possible to reduce water iirrigation application rate by 30-40% without reduction in the yield (Table 1).

Chemical

Salt toxicity is a worldwide agricultural problem. Approximately one-third of the world land surface is arid and semi-arid, of which half is affected by salinity. In the nearest future, under global warming, the problem will increase. Several hypotheses were proposed to explain the beneficial effect of active Si on the plant salt tolerance. They are (i) improved photosynthetic activity, (ii) enhanced K:Na selectivity ratio, (iii) increased enzyme activity, and (iv) increased concentration of soluble substances in the xylem, which results in reduced sodium adsorption by plants (Biel et al., 2008; Matichenkov and Bocharnikova, 2001; Snyder et al., 2006).

Table 1. The effect of Si fertilizers on the weight of 3-week old wheat and the content of active Si in Chestnut Soil

Treatment	Optimum moisture		Water deficiency (50%)	
	Dry weight (g)	Active Si (mg/kg)	Dry weight (g)	Active Si (mg/kg)
Control	0,33	8,45	0,20	6,32
SiO_2 1000 kg/ha	0, 42	18,62	0,31	18,41
Deatomitoes earth 1000 kg/ha	0,38	14,70	0,32	12,32
Monosilicic acid, 100 mg/l Si	0,37	12,82	0,28	11,24
LSD_{05}	0,04	0,62	0,03	0,56

Matichenkov et al., 2011.

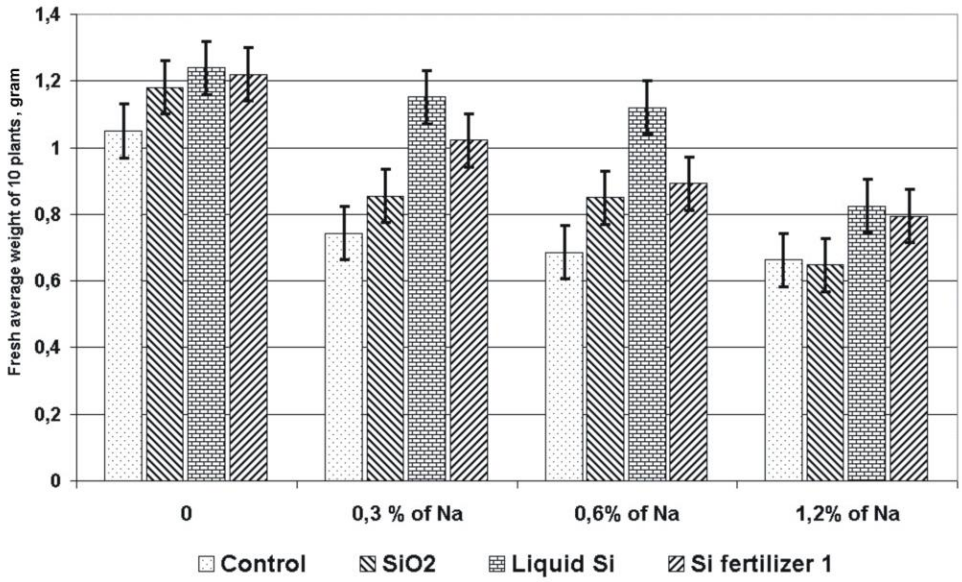


Figure 3. The effect of active Si on the plant salt tolerance of 3-week old barley (Matichenkov, 2008).

Our recent investigation has shown that soluble Si compounds can block or delay Na transport in the apoplast. Monosilicic acid protects chlorophyll molecules against demolition of them by Na (Matichenkov, 2008).

Silicon fertilizers are able to protect chemically plants against heavy metal toxicity (Benavides et al., 2005).

Biochemical

Si plays an important part in all mechanisms described above. But some Si effects on plant protection (for example, increasing frost tolerance) can't be explained by mechanical, physiological or chemical processes. Additional mechanism was hypothesized to exist for the synthesis of specific and non-specific stress-protectors in plant cell, which provided by catalytic properties of polysilicic acid matrix (Biel et al., 2008). The additional pre-suppositions for organic compounds synthesis on gels of polysilicic acids in living cell are the following.

- Active Si increases plant resistance against any type of stresses (Ma and Takahashi, 2002; Matichenkov et al., 2008; Matichenkov et al., 2011; Savant et al., 1997; Snyder et al., 2006).
- Any stress initiates increasing Si in plant tissue (Biel et al., 2008; Matichenkov, 2008; Matichenkov and Bocharnikova, 1994; Snyder et al., 2006).
- Plant tissue contains high concentrations of mono- and poly-silicic acids (Matichenkov et al., 2008).
- Optimization of Si plant nutrition increases the antioxidants and stress-ferments amounts in plant tissue (Biel et al., 2008; Matichenkov, 2008).

- Polysilicic acids are used for the synthesis of organic molecules (Banerjee et al., 2001).

As influenced by any stress, nuclear control of the cell is stimulated for identification of a type of stress (Figure 3). After this identification, the signal system of the plant cell initiates metabolism and protein synthesis for the formation of specific and non-specific stress-protectors. Several points in this process are critical for realization of the plant defense. First, there is time period required for stress identification. Second, it is time and energy for the synthesis of anti-stress proteins and anti-stress metabolites. The time and energy deficiency are the main factors of negative influence of stresses on plant productivity and ability to survive.

Possibility to use polysilicic acid for direct catalytic synthesis of organic molecules in the plant cell can strengthen this process by the following. The stress initiates nuclear control for identification of a stress and asking for additional transport of Si into problematic cell. After identification, the synthesis of specific and non-specific stress-protectors is realized by well-known mechanisms and by additional low-energy required (catalytic) synthesis of these substances on polysilicic-acid matrix (Figure 4). With the changes occuring in the global environment, the role of Si in plant protection against stresses will become more and more important for sustainable and ecologically safe crop production.

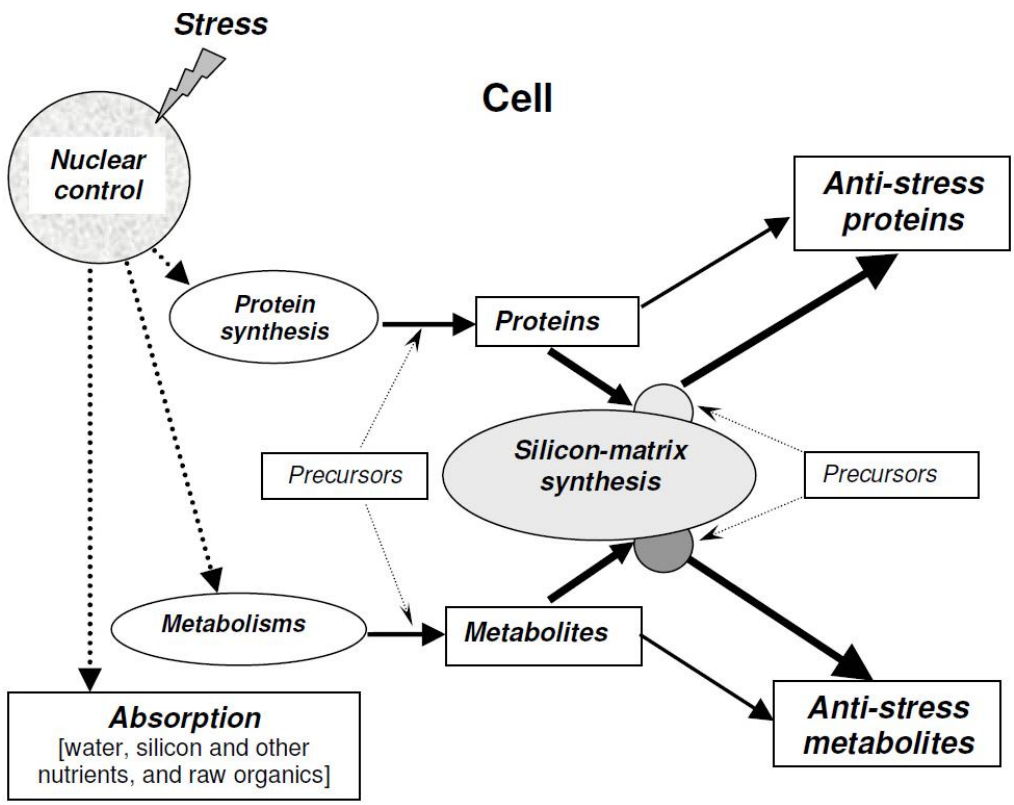


Figure 4. Schematic of stress-protection system of plant cell.

Silicon Effect on Soil

The application of Si soil amendment has positive effect on the chemical and physical properties of cultivated soil. Most investigations of Si soil amendments or Si fertilizers on soil properties concern their interaction with phosphates (Matichenkov, 2008; Matichenkov and Ammosova, 1996).

The thermodynamic calculations showed that the reaction of displacing phosphate-anion by silicate-anion from slightly soluble phosphates and formation of the corresponding silicates is possible (Matichenkov and Ammosova, 1996). The model and field experiments have completely confirmed this suggestion (O'Relly and Sims, 1995).

$$CaHPO_4 + Si(OH)_4 = CaSiO_3 + H_2O + H_3PO_4$$

$$2Al(H_2PO_4)_3 + 2Si(OH)_4 + 5H^+ = Al_2Si_2O_5 + 5H_3PO_4 + 5H_2O$$

$$2FePO_4 + Si(OH)_4 + 2H^+ = Fe_2SiO_4 + 2H_3PO_4$$

Our laboratory and field tests conducted in various countries have demonstrated that the application of Si fertilizer positively effects soil plant-available P and makes possible to reduce the traditional P fertilizer rates by 25-50% (Table 2; Matichenkov, 2008; Matichenkov and Bocharnikova, 2001; Matichenkov et al., 2011).

On the other hand, Si fertilizers or Si soil amendments usually possess good adsorption capacity. Field demonstrations on sandy soils (deep sandy Entisol) in Florida have shown that the application of Si soil amendment provided the reduction in P leaching by 200-300% and keeping P in plant-available forms (Figure 5; Chimney et al., 2007). The leaching of N, K, and organic matter by Si was reduced as well (Matichenkov, 2008).

Table 2. Effect of Si fertilization on plant-available P in soil (Matichenkov, 2007).

Soil and location	Plant-available P (0.1 n HCl-extracted), mg kg^{-1}	
	Before Si application	After Si application
Soddy Podzolic Soil, Moscow region, Russia	37.9±2.7	59.6±1.5
Chestnut Soil, South Russia	28.2±1.4	42.4±3.5
Mullisoil, South Russia	11.0±0.6	14.6±0.6
Alluvial Soil, Jordan River valley	63.8±3.5	104.8±6.8
Grey Soil, Tajikistan	16.2±1.1	36.6±2.5
Calcareous Soil, Andalusia, Spain	15.3±1.0	46.0±3.1
Spodosol, Florida, USA	123±13	142±12.5
Histosol, Florida, USA	13.2±0.3	23.9±1.4

Active Si applied to soil initiates the formation of the secondary clay minerals, which results in increased soil water-holding capacity, exchange capacity and improved soil texture (Table 3; Matichenkov et al., 1994).

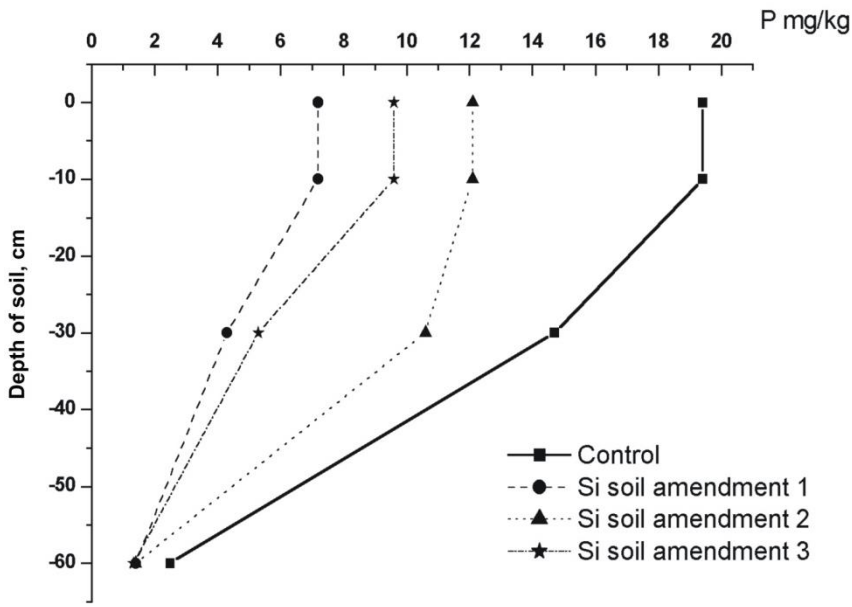


Figure 5. Leacheable (water-extractable) P in sandy soil treated or untreated with Si soil amendments.

Table 3. Influence of Si-rich materials on some soil water-physical properties (Matichenkov et al., 1994)

Ground	Sand	Clay-sand	Sand-clay	Clay
Variant	W%	W%	W%	W%
Control	15.7	27.3	42.0	87.2
Amorphous silica	20.3	29.9	44.8	76.9
Si soil amendment 1	26.7	35.2	42.3	67.2
Si soil amendment 2	31.5	48.7	77.4	84.3
LSD*	1.3	1.5	2.1	1.8

* Least significant difference at 0.05 level.

An alleviative effect of Si on Al toxicity in acid soils has been reported in many crops (Haak and Siman, 1992; Myhr and Erstad, 1996). It is possible to postulate five different mechanisms of the Al toxicity reduction by Si-rich compounds. Firstly, monosilicic acid can increase soil pH (Lindsay, 1979). Secondly, monosilicic acid can be adsorbed on aluminium hydroxides impairing their mobility (Panov et al., 1982). Thirdly, monosilicic acid can form slightly soluble substances with ions of Al (Horigushi, 1988; Lindsay, 1979). Another mechanism for the aluminium toxicity reduction by silicon-rich compounds can be strong adsorption of mobile aluminium on silica surface (Schulthess and Tokunaga, 1996). Fifthly, mobile silicon compounds can increase plant tolerance to Al (Rahman et al., 1998). All of these mechanisms may to work simultaneously, with one prevailing over others depending on soil conditions.

During last century, industry and agriculture have seriously disturbed the natural cycles of heavy metals in the soil-plant system (Adriano, 1986; Benavides et al., 2005). Current and

traditional methods used to regulate and manage HM mobility in the soil, such as changing pH or increasing soil adsorption capacity by adding amendments to the soil, are not so effective because of constant change of the soil matrix, caused by the action of plants, microorganisms, and inflow and outflow of water solutions (Kavamura and Esposito, 2010). Many researches have showed that Si fertilization provides reduction in the heavy metal mobility in the soil and plant protection against heavy metal toxicity (Cunha et al., 2008; Matichenkov, 2008; Treder and Cieslinsky, 2013).

Si in Sustainable Agriculture

In cultivated soils, the balance of nutrients is usually destroyed through their annual harvesting with crop. The Si removal from cropland ranges from 40 to 300 kg Si ha^{-1} (Matichenkov et al., 2011). Increasing Si deficit causes a number of negative consequences for soil and plant. The lack in soluble Si leads to accelerating soil degradation processes manifested as reduction of soil organic matter, decreasing water-holding and adsorption capacities, increasing the Al toxicity. Insufficient plant-available Si in the soil negatively impacts natural plant defense system against biotic and abiotic stresses (Biel et al., 2008; Matichenkov et al., 2011).

At present, the Si fertilizer requirements of the world agriculture are estimated to reach about 700 mln. t. The major problems concerning the Si fertilization are scanty information being disseminated on the benefits of using Si-rich materials as a fertilizer and absence of highly informative Si soil tests to assess the plant-available silicon deficiency.

Si Soil Test

The absence of simple, universal, and informative methods for soil classification on the deficiency of plant-available and active forms of silicon retards practical use of Si fertilizers in the world. The large number of methods of soil testing for plant-available Si have been suggested, they differ in the preparation of the soil samples, type of extractant, procedure of the extraction, and the method that is used for the determination of Si in the extract (Matichenkov, 2007). A serious problem with regard to all methods is the treatment of soil samples prior to analysis. During soil drying, both poly- and mono-silicic acids transform into silicon dioxide, as a result, data obtained on dried soil may not adequatly describe plant-available soil silicon. We offered to determine monosilicic acid (plant-available Si) in water extract from fresh soil sample. To describe Si nutritional status of a soil, an analysis of monosilicic acid is not enough. Monosilicic acid is an active form of Si controlling the Si plant nutrition and primary soil biogeochemical processes. This is ‘actual Si’ which exists in the soil solution at the moment. Besides actual Si, it is necessary to have information about soil Si compounds that can be transformed (dissolved) to actual form in the future. This is ‘potential Si’. The potential Si may be determined by the hydrochloric acid (0.1 n) - extraction method from dry soil.

In practice, to evaluate the silicon nutritional status, united parameter is more suitable. Such complex parameter can be described as ‘active Si’.

Table 4. Soil classification on deficiency of actual and potential Si

Deficiency level	Actual Si	Potential Si	Active Si	Soil
	----------mg kg^{-1} of Si in soil---------			
Not deficient soil (W)	>40	>600	>1000	Virgin Mollisols (Chernozems), volcanic ash soils
Low deficient soil (L)	20-40	300-600	500-1000	Cultivated Mollisols, greenhouse potting mixtures
Deficient soil (D)	10-20	100-300	200-500	Most cultivated soils
Critically deficient soil (C)	0-10	0-100	0-200	Tropical soil, sandy soils, degraded cultivated soils

Our data on various soil examinations has demonstrated 'actual Si' to be in the ratio to 'potential Si' as 1:10 (Matichenkov, 2007). The following formula for active Si was suggested.

$$\text{Active Si} = 10*\text{Actual Si} + \text{Potential Si}$$

Several greenhouse investigations showed a close relationship between the active Si (calculated parameter) and the Si concentration in rice leaves. To assess the need in Si fertilization, the soil classification on deficiency of actual, potential, and active Si was suggested (Table 4).

- *Not deficient soil* - Si fertilization or Si-rich soil amendments are not required. Sometimes the application of Si-rich soil amendments would have beneficial effect via acting Si compounds on soil properties and NPK behavior in the soil-plant system.
- *Low deficient soil* - Si fertilization is necessary for Si-accumulating plants (cereals, grasses). Si-rich soil amendments are required for optimizing P plant nutrition.
- *Deficient soil* - Si fertilizers and Si-rich soil amendments have stable and significant effect on all crops and increase soil fertility. A standard rate of Si application is necessary.
- *Critically deficient soil* - lack of active Si has a negative effect on crop productivity and environment. High rates of Si fertilizers or Si soil amendments are necessary.

Types of Si Fertilizers

Several types of silicon fertilizers may be distinguished: synthetic, plant remains - based, natural Si-rich minerals, and Si-rich industrial by-products. Synthetic Si fertilizers are present commonly in a liquid form; they are effective in initiation or activation of the plant natural defense system. However, these fertilizers have low effect on soil properties and don't provide sufficient plant Si nutrition because the application rates are very small (0.25-10 L ha^{-1}).

Table 5. Effects of zeolite, diatomaceous earth and advanced Si fertilizer on yield of selected crops (Matichenkov, 2008)

Cultivated plants	Zeolite		Diatomaceous earth		Advanced Si fertilizer	
	Effect, %	Rate, kg	Effect, %	Rate, kg	Effect %	Rate, kg
Potatoes	62	600	12.9	1000	65	300
Water melon	30.8	300	-	-	-	-
Cucumber	33	825	24.6	2000	82	400
Wheat	42.5	400	-	-	55	100
Barley	30	250	-	-	52	100
Banana	-	-	11.7	1000	-	-
Sugarcane	-	-	20.5	1000	-	-

Plant remains-based fertilizers or plant ash can be used as silicon sources, however, the demand for silicon fertilization generally exceeds that which can be supplied by plant residues. Ones of the most popular and widely used silicon fertilizers are Si-rich industrial by-products or slags. But they may contain heavy metals, associated with their origin and processing. The high rates needed to supply silicon may result in heavy metal concentrations greater than allowed. For example, the application of the slag in Florida (the Everglades Agricultural Area) resulted in significant increase in the Pb content in sugar produced from local sugar cane. In spite of ecological control, the use of industrial slags as Si fertilizers creates high risk for heavy metal pollution.

The best type of Si fertilizers is natural minerals high in plant-available Si. There are zeolites, diatomaceous earth, bentonite et al. The major problem with this type of the Si fertilizers (soil amendments) is a high application rate required. The diversity of rates with different fertilizer types can be attributed to the different solubilities of silicon compounds. Table 5 shows comparison effects of zeolite (Khotynetsky deposit, Russia) and diatomaceous earth (Palkarra deposit, Australia) on plant growth.

The effeciency of the natural Si-rich minerals as Si fertilizers depends on their solubility and other properties (Kulikova, 2012). To estimate potential value of a source, we suggested the Si determination in water and weak acid extracts (Matichenkov et al., 2011).

Advanced Si Fertilizer

The main problems in the practical application of Si fertilizers or Si-rich soil amendments are:

a) low efficiency, as a result, high rates are required (the rates of by-products from metals industry used as Si source or Si-rich minerals such as diatomite, zeolite or others range 300 to 6000 kg ha^{-1});
b) high cost of Si-rich soil amendments per hectare. For example, natural Si fertilizers usually have cost 300-600 US$ per ton with application 2 t ha^{-1};
c) a short-term effect of liquid Si fertilizers;

d) poorly understood mechanisms of Si action in the soil-plant system. For this reason, wrong rates and techniques for application are used;
e) low information about the role and functions of Si in the soil-plant system.

The evolution of any fertilizer generally tracks from raw materials low effective in the soil-plant system to advanced technology products elaborated on a basis of chemical, biological, and physical processes and specific mixture with other ingredients. Si fertilizer advancement has a similar regularity. The future of Si fertilizers seems to be connected with activated Si-rich natural minerals and synthetic liquid Si-rich substances. In our study, the broadcast application of 2-4 t ha^{-1} of Si-rich minerals provided a similar effect as the application of 5-100 kg of modified mineral-based Si fertilizer (Table 5).

To attain the combination of high efficiency, ecological safety, and low cost is the target for science and business in regard to Si fertilization.

CONCLUSION

Silicon fertilization provides the following agronomic and environmental benefits:

1) Si increases crop production and quality;
2) Si increases plant drought and salt tolerance;
3) Si protects plant against diseases, insect and fungi attack;
4) Si promotes restoration of degraded soils and increases soil fertility;
5) Si increases soil resistance to wind and water erosion;
6) Si alleviates Al toxicity in acid soils;
7) Si improves plant P nutrition;
8) Si reduces P, N, and K leaching from cultivated areas;
9) Si reduces the mobility of heavy metals.

The main problem concerning the Si fertilizer implementation in the world is scanty information being disseminated on the benefits of using Si-rich materials as a fertilizer.

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Chapter 8

NON-TRADITIONAL AMELIORANTS

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ABSTRACT

Clay minerals, especially bentonite clay, play a huge role in a variety of industries, however, in agriculture their use is currently limited to undeserved. In a series of laboratory and field experiments established the high efficiency of these non-traditional ameliorants on fertility of low humus sod-podzolic soils. Entering them to the soil significantly have increased the amount of total exchangeable bases, improved structure, protected the nutrient from being washed out, and mineralization of humus. All this contributed to increasing productivity on 12-40 %. The lower the fertility of initial soil, the higher the useful effect observed from entering bentonite clay. Through the use of original technology of entering ameliorants the top five-centimeter layer of soil (making mulch layer) was able to achieve decrease of traditional rates of clay application from 100-200 t-ha^{-1} to 4.8 t-ha^{-1} without reducing their effectiveness.

Keywords: Bentonite clays, ameliorant, soil fertility, mulching, mulch layer, reclamation, clay minerals, sod-podzolic soils, soil moisture, soil consistency, soil feature, crop yield

INTRODUCTION

Clay minerals are valuable natural resources, which found its application in various fields of human activity. It is now known a large number of clay minerals that differ from each other in crystal-chemical structure and chemical composition.

A special place among the ranks of clay minerals has occupied montmorillonite, derives its name from the place of Montmorillon in France, where was first opened.

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It belongs to a class of layered aluminosilicates having high dispersibility (crystal size less than 1 micron) and therefore having a very large specific surface. The largest number of montmorillonite contained in bentonite clays - more than 70% of the rock mass. The name of bentonite clay or bentonite due to its large deposit near Fort Benton in the US.

Beside montmorillonite in bentonite composition includes other less common minerals of smektite groups: beidellite, nontronite, saponite, volkonskoite, and others. Of the non-clay minerals presents detrital quartz, amorphous silica, glauconite, iron hydroxides in the form of films and subtle inclusions, sometimes zeolite and ilmenite (Goryushkin, 2006).

The crystal lattice of all smectite consists of three layers that make up the package: the extreme upper and lower layers of the package consist of tetrahedron and $AlSiO_4$ called tetrahedral. Between the tetrahedral layers there is a layer composed of octahedrons Al and Fe, called octahedral. Three-layer packet has a negative charge due to the substitution of trivalent elements (Al, Fe) in the octahedron layer by divalent elements (Mg, Fe) or tetravalent Si to trivalent Al in the tetrahedral layer (Gradusov, 1976).

Owing to the negative charge on the surface of the package are situated the positive one-, two-and trivalent cations. It mainly, Na, K, Ca, Mg and Fe. Features of crystal-chemical structure of montmorillonite and presence on its surface of the ion-exchange cationsgives it a number of unique features:

1 High dispersion. Cause to the very small size of the crystals of the mineral, which ensures a large specific surface.
2 Hydrophilicity and swellability. This is due to the presence of mobile, able to expand the crystal lattice. Depending on the chemical composition of the mineral when hydration it increases in volume by 2-20 times. The most significant swelling mont-morillonite containing an increased amount of exchangeable sodium.
3 The high absorption capacity. The cation exchange capacity of montmorillonite is one of the highest among the other minerals and is equal to 80-120 mmol-100 g^{-1} of minerals.
4 The high binding capacity. Is a good soil-aggregate stabilizer. In conjunction with humic acids it forms in soil water-resistant aggregates.
5 Nontoxicity and chemical resistance. This property makes it indispensable in manu-facturing, construction and many other fields.

Chemical composition of bentonite clay is one of the most important characteristics. It gives considerable information on the nature of rock, its adsorption properties and the ability to swell. Chemical composition of bentonite is directly related to the mineral composition and by the presence of inclusions which accompanying with clay rocks.

Furthermore, the chemical composition of the montmorillonite has fluctuated signify-cantly: SiO_2–53.5-73.5%; Al_2O_3–3.5-27.8%; Fe_2O_3–2.2-32.0%; CaO–0.2-2.6%; MgO–1.5-6.2 %; K_2O–0.2-0.6%; Na_2O–0.1-1.9% (Gorbunov, 1978).

According to the chemical composition there are two types of bentonite clays:

- alkaline - with a predominance of exchangeable sodium (most valuable to the industry);
- alkaline-earth - with a predominance of exchangeable calcium.

In nature, the most common are calcium-magnesia (alkaline earth) species. Calcium and calcium-magnesian bentonites can be translated into the category of sodium by treatment with solutions of sodium salts. These sodium bentonites are called activated, and the process is called ion exchange replacement by activation.

In the pure form of bentonite clay is used rarely, mostly in industry and other areas of the economy are used bentonite mud powder. This material is obtained by drying and small crushing bentonite clay. Bentonite mud powder is added in the polymeric material, admixed to the concrete, which considerably increases the strength of adhesion between theirs dissimilar phases. On the basis of bentonite powder produced the most affordable drilling mixtures having high performance and providing excellent results in a vertical or horizontal drilling. Bentonite mud powders in combination with refractory materials are the main raw material for the manufacture of natural sand blend. Depending on the proportions of mud powder and perlite such mixtures may have different properties. Sand blend based on bentonite clay is differ high durability, optimum gas-tight, herewith easy formed and environmentally friendly. Bentonite clay is also known as bentomat – material used for waterproofing of various objects. This material is very easy to operate and can be used in most weather conditions, including at low temperatures. Equally important is the use of bentonite clays in the preparation of means for cleaning oil-products, various oils, wines, as well as raw materials for the production of heat and waterproofing materials (Petrov, 1980).

The largest deposits of bentonite are located in the US. (Black-Giles, Sanders-Defiance), Canada, Great Britain, Armenia and Ukraine. Russia also has significant reserves of raw bentonite and its territory is home to more than 300 deposits of bentonite and bentonite-containing clays (Volga region, Transcaucasia, Central Asia, Far East, etc.).

But only a small part of them (about 10%) explored in detail, and their reserves are considered All-Union Geological Fund. Stocks in these deposits are defined in the amount of 544.8 million tons (including forming, adsorption, etc.). Stocks of most valuable alkaline bentonite clays are only 50 million tons (Gradusov, 1976). The largest producers and major exporters of bentonite are now the United States, Greece, Japan, Italy, Argentina, Spain.

Thus, bentonite clays have a number of unique properties that defined their wide application in various fields of human activity. However, in agriculture their current use unfairly restricted. The most widely studied their role as the major secondary minerals that make up the soil-forming material, and many kinds of soils (Gorbunov, 1978; Gradusov, 1976). There are also a number of studies on the use of bentonite as a filler in various pesticides (Petro, 1980), and in animal breeding as a valuable animal feed additives (Antipov et al., 2010; Burihonov, 1992).

Promising sector of the use of clays is reclamation light (sandy and sandy loam) soil. This agricultural practice long been known to farmers, but the information on the effectiveness of quite contradictory and fragmented. This is due to the different composition of clays in use and different environmental conditions (soil, climate, and production).

This direction of the use of bentonite clays in agriculture has been studied by the author from 1991 to 1997. According to the results of research was defended the Candidate's dissertation in 1997 (Lednev, 1997). At present being studied a new direction using of clay minerals - as effective ameliorants for remediation of soils contaminated with heavy metals.

Bentonite clay is a multifunctional ameliorant and therefore it has diverse effects on soil properties. Most researchers studied the high and very high doses of this ameliorant 100 – 200 t-ha^{-1} (Buzmakov and Lamzin, 1971; Lgotski, 1979; Sharafeeva, 1980).

We have developed a new way of entering it - mixing bentonite clay with upper five-centimeters layer of the soil, namely the creation of mulch on the surface layer (Patent № 21-64060 RUC2). This method allowed to drastically reduce the dose of entering ameliorant without reducing its effectiveness. We studied three doses of application of 4, 8, and 16 t-ha^{-1}.

In most cases, the application dose 8 t-ha^{-1} was provided the most effective. Dose of 4 t-ha^{-1} shown mathematically significant effect only with simultaneous application of bentonite clay and limestone flour in a dose of 4 t-ha^{-1}. Consider the effect of a mulch layer of bentonite clay on the basic properties of sod-podzolic soils.

EFFECT OF BENTONITE CLAY ON PHYSICOCHEMICAL PROPERTIES

The main effect of bentonite clay on physicochemical properties manifested in a significant increase in the absorptive capacity of the soils, especially in relation to the cation (Figure 1). This is due to the high content of montmorillonite in bentonite clay.

Montmorillonite has, as already mentioned, very high cation exchange capacity (CEC) – 90-120 mmol-100 grams^{-1} of the mineral. In our experiments we studied bentonite clay from Biklyan deposit (Tatarstan, Russia), it had the CEC - 96 mmol-100 g^{-1}. Moreover, during the receipt of the this ameliorant, feedstock ground in the roller-pendular mill and sieved through a sieve set, the hole diameter of the last – 0,074 mm. Prepared in this way bentonite clay contains 50-60% fraction of silty particles.

For sod-podzolic soils with a small total exchangeable bases (on average 10-15 mmol-100 g^{-1} soil), increase CEC is a very important positive effect of the ameliorant. The increase in the absorptive capacity allow to reduce washing away of mineral nutrients from the topsoil, more efficient use of mineral fertilizers, increase the buffer capacity of soils.

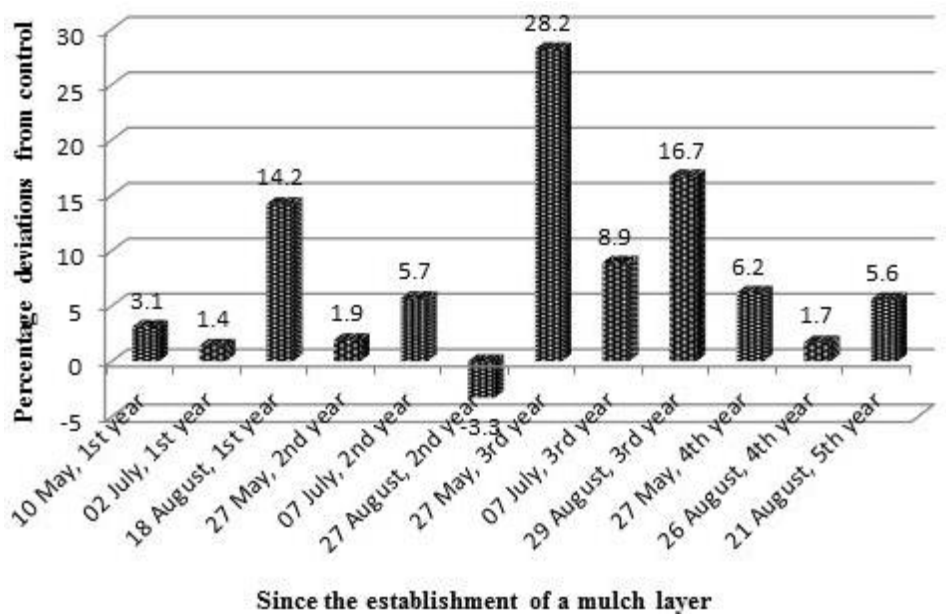

Figure 1. Effect of bentonite clay ploughed in the 0-5 cm soil layer on the total exchangeable bases of the arable layer, % deviations from control (no ameliorant).

The data in Figure 1 show that the bentonite clay shaft increases the total exchangeable bases from 1-2% to 28%, the positive effect was maintained throughout the five years of observation, and on average with all terms of definitions it was 7.5%. It should be noted that in this and subsequent diagrams summarizes data from three field experiments which are on different areas and in different years carried out that significantly increases objectivity results. In order to be able to compare the results from different sections, on each experiment was calculated deviation from the control (variant without entering ameliorants), and only then was found the arithmetic mean of three experiments. A similar, but less significant increase in the total exchangeable bases observed by other authors (Lgotski, 1979; Sharafeeva, 1980).

Lesser effect of bentonite clays these authors explained by stirring ameliorant with all arable horizon, resulting to superfluous dilution of its active substance and thus reduce efficiency. Thus, according to our studies, stirring 8 t-/ha^{-1} of bentonite clay with a layer soil 0-20 cm has decreased the total exchangeable bases to 1.2-2.0 mmo-100 g^{-1}, compared with the variant where it was stirred with a layer 0-5 cm. In order to achieve the result obtained when creating a mulch layer, application rate of this ameliorant should be increased to 50-100 t-ha^{-1}.

Stirring 8 t-ha^{-1} of bentonite clay with a soil layer of 0-5 cm somewhat reduced the soil acidity. His influence on this indicator does not always proved statistically, though the average for five years of observation, there was an increase pH_{KCl} by 2.1% (Figure 2) and a decrease of hydrolytic acidity by 5.4% (Figure 3) as compared with control without mulch material. Reducing the soil acidity is explained by faintly alkaline reaction of this ameliorant (pH_{KCl} - 6.4) and very high base saturation.

Similar results the interaction of bentonite clay with soils that have an acid reaction were obtained by other researchers (Lgotski, 1979; Sharafeeva, 1980).

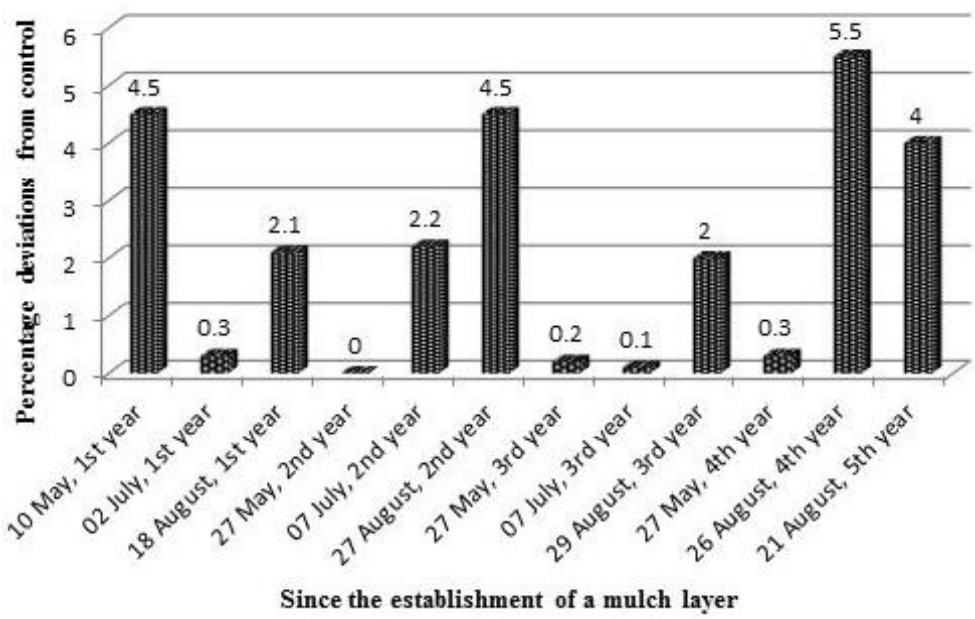

Figure 2. Effect of bentonite clay ploughed in the 0-5 cm soil layer on exchange acidity of the arable layer, % deviations from control (no ameliorant).

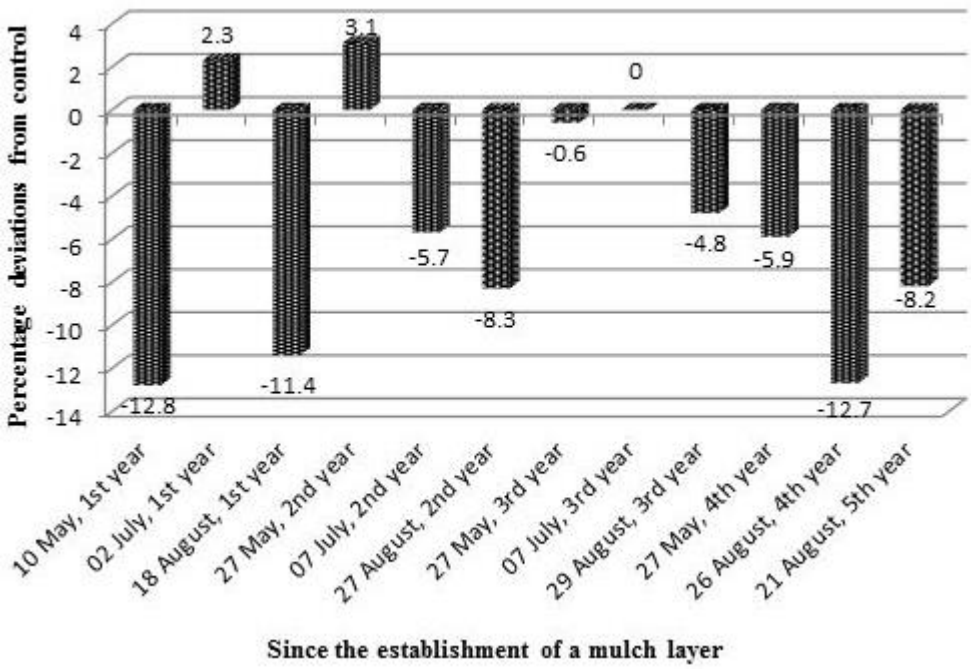

Figure 3. Effect of bentonite clay ploughed in the 0-5 cm soil layer on hydrolytic acidity of the arable layer, % deviations from control (no ameliorant).

EFFECT OF BENTONITE CLAY ON THE CHEMICAL PROPERTIES

Creating a mulch layer of bentonite clay contributed to a slight increase in the content of organic matter in the arable layer (Figure 4). The average for five years its amount, compared with the control, increased by 4.4% with a high authenticity level. The rise of humus content is explained stabilizing influence exerted by clay minerals on organic matter of soil.

Lgotski (Lgotski, 1979) found that bentonite clay increases the accumulation of simple organic compounds, from humus andfulvic acids. It should be noted that the application of high doses of this ameliorant leads to slower decomposition of organic substances and humification of crop remains in the soil, which is not always a positive process.

During the growing season was observed dynamics of change in the content of organic matter, its maximum increase was observed at the end of the growing season, after entering into the soil with fresh organic crop residues, the minimum – in the months of June and July (the peak of its mineralization process). While stirring 8 t-ha^{-1} bentonite with 0-20 cm layer of soil, increase of the content of organic substances in our experiments was not observed.

Bentonite clay and methods of its incorporation into the soil have a different effect on the content of mineral nutrients in the soil. So, mixing it with a soil layer 0-5 cm has increased in the arable horizon of labile phosphorus content of 6.4% with a high authenticity level (Figure 5).

Apparently, this is due to improvement of phosphatic regime and a positive sorption of phosphates by bentonite clay. The mechanism of absorption anions by montmorillonite is very complex and still not sufficiently explored.

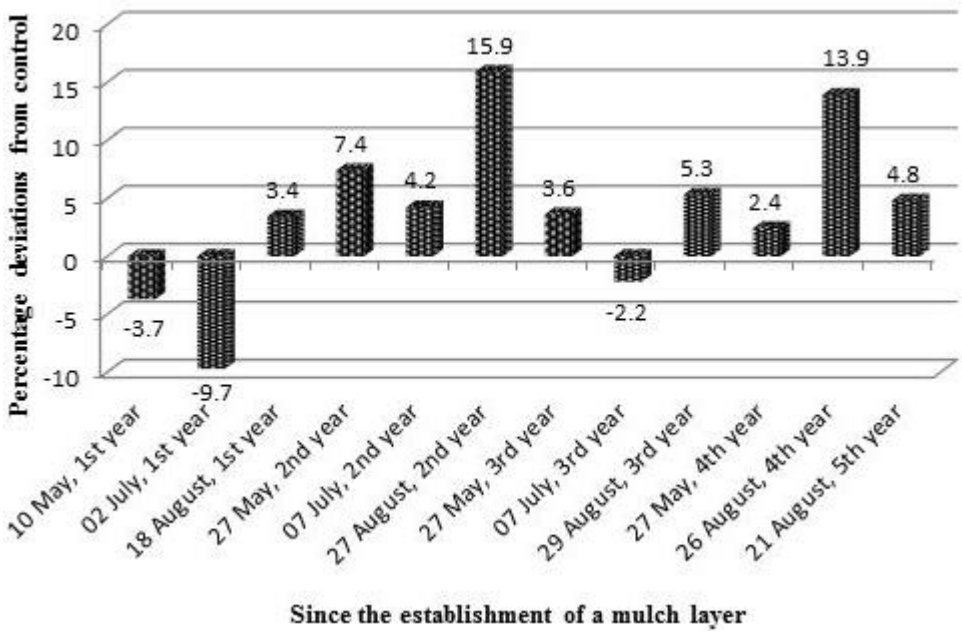

Figure 4. Effect of bentonite clay ploughed in the 0-5 cm soil layer on the content of organic matter in the arable layer, % deviations from control (no ameliorant).

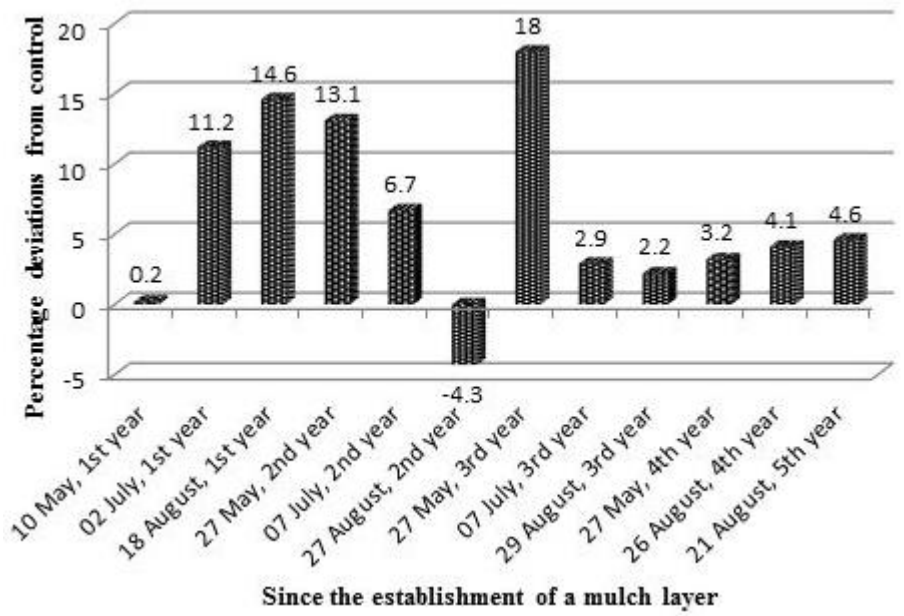

Figure 5. Effect of bentonite clay ploughed in the 0-5 cm soil layer on the content of labile phosphorus in the arable layer, % deviations from control (no ameliorant).

The mixing of bentonite clay in the soil layer of 0-5 cm did not show a clear-cut effect on the exchangeable potassium content. Differences with control over the observation period varied in different directions, and in most cases do not prove statistically (Figure 6).

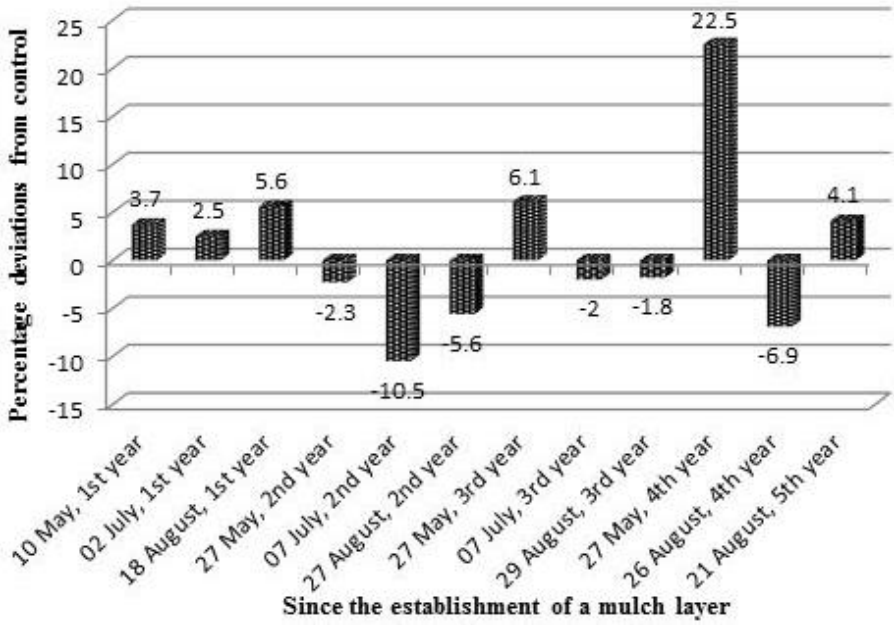

Figure 6. Effect of bentonite clay ploughed in the 0-5 cm soil layer on the content exchangeable potassium in the arable layer, % deviations from control (no ameliorant).

Bentonite clay, stirring it with the soil layer 0-20 cm, more efficiently has absorbed the potassium ion. This has promoted to increase the number of exchangeable potassium in the arable horizon by 4-15 % compared with the control (without ameliorant) and protected him from washing away down the profile. The process of absorption of potassium ion by montmorillonite observed by other researchers (Gorbunov, 1978; Goryachev, 2012; Lgotski, 1979; Mattson, 1934).

The obtained data on the effect of bentonite clay on the nitrate nitrogen content are contradictory. Based on the available number of observations is not possible to draw definite conclusion, but other authors proved the possibility of absorption of this ion (Gorbunov, 1978; Iryanova, 1987).

EFFECT OF BENTONITE CLAY ON AGROPHYSICAL PROPERTIES

One of the basic indicators of soil fertility are an agrophysical properties, which often limit the productivity of crops. Entering bentonite clays had a positive effect on these properties, especially on the structure of the arable layer (Table 1). The best indicator of dispersity factor, which characterizes the durability of the microstructure (the higher the dispersity factor, the less durable microstructure of the soil), was observed in the variant with mixing bentonite clay with a soil layer of 0-5 cm and was below that variant with the entering its in the soil layer 0-20 cm by 8.1%. The mixing bentonite clay with a soil layer of 0-5 cm increased the aggregative index of the arable layer by 5.2 % compared to the deep placement of this ameliorant and by 10.6% compared with the control (without ameliorant). Increasing the aggregative index means improving the water stability of the structure.

Table 1. Effect of bentonite clay and the depth of its embedment on indicators of soil structure

Variant	Depth of sampling, cm	Stirring in the 0-5 cm soil layer	Stirring in the 0-20 cm soil layer
Dispersity factor			
The control (without ameliorant)	0 – 5	16.6	15.4
	5 – 10	17.0	16.0
	average	16.8	15.7
Bentonite clay, 8 t-ha^{-1}	0 – 5	11.0	14.5
	5 – 10	16.2	15.2
	average	13.6	14.8
The aggregative index			
The control (without ameliorant)	0 – 5	34.3	26.2
	5 – 10	39.2	42.9
	average	36.7	34.6
Bentonite clay, 8 t-ha^{-1}	0 – 5	36.2	31.3
	5 – 10	45.0	46.0
	average	40.6	38.6
Granulometric factor of structure			
The control (without ameliorant)	0 – 5	10.3	12.9
	5 – 10	10.2	11.8
	average	10.3	12.3
Bentonite clay, 8 t-ha^{-1}	0 – 5	16.1	14.7
	5 – 10	11.1	12.4
	average	13.6	13.5

The entering of bentonite clay in soil increased its granulometric factor of structure that characterizes the potential ability of the soil to the conditioning, but he did not change with the depth of embedment ameliorant.

The positive effect of bentonite clay on the microstructure of the soil explained by the very high dispersity of the particles of which it is composed, therefore entering it into the soil resulted in an increase in its silty fraction - the best aggregate stabilizer.

Creating a mulch layer of bentonite clay has contributed not only to better conditioning, but also reduce the density of the upper part of the arable layer (5-8%).

All this, in turn, significantly increased the water permeability of the arable layer during the growing season by 4.6-9.5%. At the same time, the mulch layer served as an obstacle to the evaporation of soil moisture. Besides, the bentonite clay is characterized by a very large moisture capacity. Integrated positive impact mulch layer of bentonite clay conditioned the increase in the arable soil layer productive moisture content of 18.8% on average over the period of observation (Figure 7).

EFFECT OF BENTONITE CLAY ON GRAIN CROP YIELD

The most objective assessment of any agricultural practice expresses in an increase or decrease of crop yields. This also applies to stirring bentonite clay in a dose of 8 t-ha^{-1} in the soil layer of 0-5 cm.

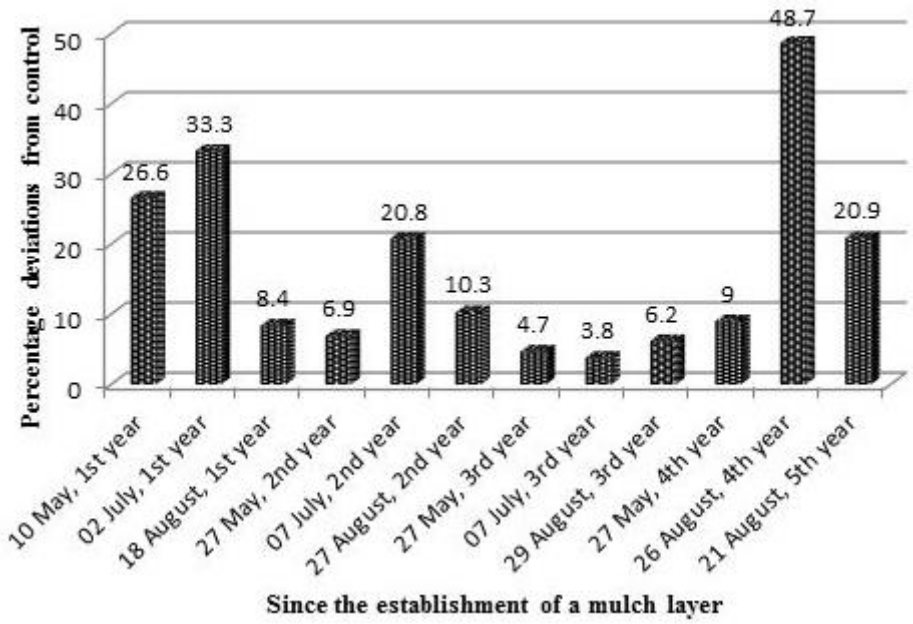

Figure 7. Effect of bentonite clay ploughed in the 0-5 cm soil layer on the available moisture content in the arable layer, % deviations from control (no ameliorant).

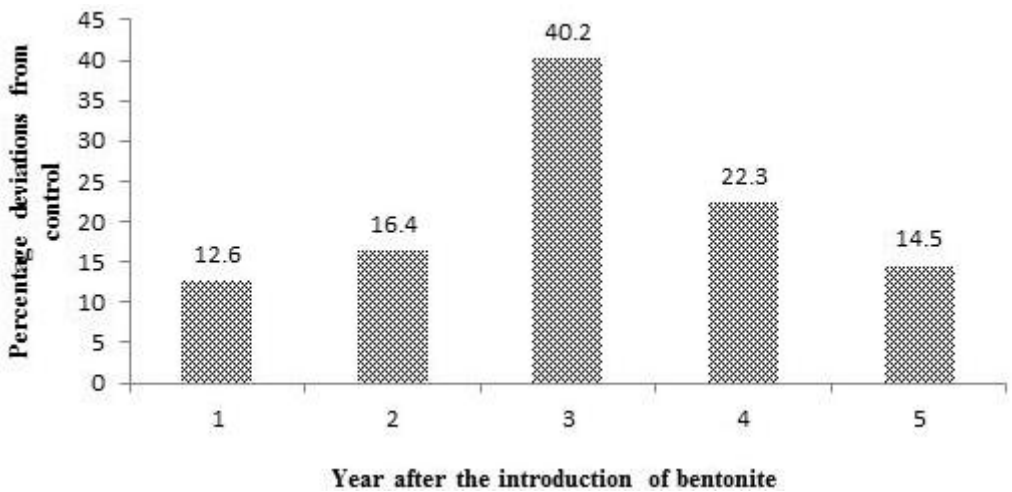

Figure 8. Effect of bentonite clay ploughed in the 0-5 cm soil layer on crop yields, % deviations from control (no ameliorant).

In all years of observation creating a mulch layer of this ameliorant contributed statistically significant increase grain yields (Figure 8). By year the raise of crop yield varied greatly, as dependent on the biological characteristics of culture, the weather conditions and the period since the establishment of mulch layer. The most responsive cereal crop to enter bentonite clay in the soil layer of 0-5 cm was barley. The least of all responsive culture was winter rye, as it had a well-developed root system and it less dependent on soil fertility.

Mulching has the greatest impact on crop yields in dry growing season, especially if the observed soil drought.

The stirring of bentonite clay at a dose of 8 t-ha^{-1} in the 0-20 cm soil layer also increased grain yield, the amount of raise ranged from 2.3 to 10.6% of control (without ameliorant).

EFFECT OF BENTONITE CLAY TO REDUCE THE MOBILITY OF HEAVY METALS IN CONTAMINATED SOILS

The unswerving growth of industrial production and population will inevitably lead to an increase in anthropogenic impacts on the environment and, in particular, on the soil.

A particular danger causes chemical contamination of soil by various pollutants and primarily heavy metals as they not only reduce soil fertility, but also can accumulate in plants and then enter the food chain to animals and humans and cause variety diseases.

In the European part of Russia among the most common and dangerous pollutants are lead and cadmium. The main part of the most contaminated soils located in major industrial centers and their surroundings, in mineral deposits, along the highways.

Restoration of soil fertility contaminated with heavy metals is impossible without a specially designed system of actions for reclamation. Chemical, physical, physicochemical and biological methods are used to carry out this type of work. Each of them has its positive and negative sides.

Reclamation of soils contaminated with heavy metals, via clay minerals, including bentonite clay, is based on physicochemical absorption of pollutants by colloidal part of ameliorants. Consumed by heavy metal ions are less mobile and much less absorbed by plants.

Laboratory experiments showed that bentonite clay showed a statistically significant effect on the reduction of the mobility of heavy metals only rate of application $\geq$ 50 t-ha^{-1} and qualitative stirring it with a layer of contaminated soil. Studies in the field experiments have shown that the application of bentonite clay at 50 t-ha^{-1} in arable horizon contaminated sod-podzolic soils has decreased degree of mobility of lead at 23-42%, cadmium - by 5-21%. Doze of ameliorant 100 t-ha^{-1} reduced degree of mobility of lead at 44-53%, cadmium - by 18-32% (as aextractant used 1 molar solution of calcium chloride).

Recultivation using as ameliorant bentonite clay is especially effective on sandy and sabulous soils, as it additionally provides a complex positive influence on many agrochemical and agrophysical properties.

CONCLUSION

Bentonite clay is a promising ameliorant, providing a complex positive impact on a number of agrochemical and agrophysical properties. It performs many functions soil humus (absorbs and retains water in its composition, mineral nutrients, effectively adheres the soil particles together, increases the total exchangeable bases, buffer capacity of soil, etc.).

Therefore, it is most clearly shows its positive features on low-humous soils, especially light granulometric texture.

To reduce the rates of application of bentonite clay (up to 8 t-ha^{-1}) while maintaining the effectiveness we propose stirring it with the soil layer of 0-5 cm.

In this case, the bentonite clay has two functions: it is an ameliorant and forms on the surface of the arable layer protective mulch layer. The exception is the reclamation of soils contaminated by heavy metals; in this case, bentonite clay is needed to be quality stirred with arable layer.

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Chapter 9

EFFECTS OF NANOFERTILIZERS ON PLANT GROWTH AND DEVELOPMENT, AND THEIR INTERRELATIONSHIP WITH THE ENVIRONMENT

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ABSTRACT

Bionanotechnology, which represents all facets of research with regard to the interactions of biology and nanoparticles, has firmly established itself as one of the principle and focused subdisciplines within nanotechnology. Fertilizer, which is a key nutrient source for food, biomass, and fiber production in agriculture, is by far the most important source of nutritional elements and molecules. At the same time, however, nutrient recovery by crops remains relatively low (e.g., about 50% for N). Since a

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nanoparticle is a particle with at least two dimensions below 100 nm, nanofertilizers could soon offer a technological solution to the nutrient-loss problem, thereby aiding technologically-minded farmers and subsistence farming. Nanofertilizers refer to nanoscale-dimension products that deliver nutrients to crops. These nutrients can be i) encapsulated inside nanomaterials such as nanotubes or nanoporous materials, ii) coated with a thin protective polymer film, or iii) delivered as particles or emulsions. It has been reported that nanoparticles and nanotubes in numerous crops (sunflower, common bean, and maize, among others) have enhanced germination and seedling growth, physiological activities including photosynthetic activity and nitrogen metabolism, mRNA expression and protein level, and positive changes in gene expression, indicating their potential use for increasing crop yields. Nevertheless, additional research is necessary in order to understand the effect of nanofertilizers on the genetic, physiologic, and morphologic changes in crops, as well as their effect on soil microbial communities, symbioses, physicochemical soil properties, and pollution. One may speculate that the creation and improvement of fertilizers at nanoscale dimensions could have a profound impact on energy, the economy and the environment. Speculation notwithstanding, the scientific, technical, and agricultural projects linked with nanofertilizers must include side effects in order to accurately determine progress and shape a sustainable future.

Keywords: Nanomaterials, crop yield, nanofertilizers, soil fertility, plant nutrition

INTRODUCTION

Although no clear definition of *nanofertilizer* exists, it is commonly defined as those materials of nanoscale dimensions (i.e., < 100 nm) and specific function, which are added to a soil to supply one or more plant nutrients essential to the growth of plants. It is well known that 16 nutrient elements are required to grow crops (Table 1). Three essential nutrients, known as structural elements [carbon (C), hydrogen (H), and oxygen (O)], are absorbed from atmospheric carbon dioxide and water. The other 13 nutrients are absorbed from the soil; these are usually grouped as primary nutrients, secondary nutrients, and micronutrients. Additionally, as shown in Table 1, seven elements [Nickel (Ni), Selenium (Se), Vanadium (V), Sodium (Na), Silicon (Si), Cobalt (Co), and Aluminium (Al)] are known as beneficial elements for plants, i.e., they are not required by all plants but can promote plant growth and may be essential for particular taxa (Hawrylak-Nowak, 2009; Pilon-Smits et al., 2009; Sae-Lee et al., 2012; Saco et al., 2013). However, the beneficial effects of low doses of Al, Co, Na, and Se have received little attention compared to the toxic effects that typically occur at higher concentrations (Pilon-Smits et al., 2009).

The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K); these are commonly found in blended fertilizers or equivalent grades. Indeed, crops utilize primary nutrients more frequently than other nutrients; therefore, primary nutrients are applied at higher rates than secondary nutrients and micronutrients. Secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S); these are required by crops in smaller amounts. The least frequently used nutrients are the micronutrients: iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo), and chlorine (Cl).

Table 1. Essential plant nutrients

Number	Nutrient	Form absorbed by plants	Average concentration in plant shoot dry matter
Structural nutrients			
1	Carbon (C)	CO_2	44.0%
2	Oxygen (O)	O_2	44.0%
3	Hydrogen (H)	H_2O	6.0%
Primary major nutrients			
4	Nitrogen (N)	NO_3^-, NH_4^+	1.5%
5	Potassium (K)	K^+	1.0%
6	Phosphorus (P)	$H_2PO_4^-$, HPO_4^{-2}	0.2%
Secondary major nutrients			
7	Calcium (Ca)	Ca^{+2}	0.5%
8	Magnesium (Mg)	Mg^{+2}	0.2%
9	Sulfur (s)	SO_4^{-2}	0.1%
Micronutrients or trace minerals			
10	Iron (Fe)	Fe^{+2}, Fe^{+3}	100 ppm (mg kg^{-1})
11	Manganese (Mn)	Mn^{+2}	50 ppm
12	Boron (B)	$H_2BO_3^-$	20 ppm
13	Zinc (Zn)	Zn^{+2}	20 ppm
14	Copper (Cu)	Cu^{+2}	6 ppm
15	Molybdenum (Mo)	MoO_4^{-2}	0.1 ppm
16	Chlorine (Cl)	Cl^-	0.2%
Beneficial element			
17	Nickel (Ni)	Ni^{+2}	0.5 ppm
18	Selenium (Se)	SeO_4^{-2}, SeO_3^{-2}	Trace
19	Vanadium (V)	VO_3^-, VO^{+2}	Trace
20	Sodium (Na)	Na^+	Trace
21	Silicon (Si)	$Si(OH)_4$	0.1%
22	Cobalt (Co)	Co^{+2}	Trace
23	Aluminum (Al)	Al^{+3}	20 ppm

The nature of plants makes them vulnerable to a wide range of natural environmental insults, both biotic and abiotic, in addition to an increasing number of anthropogenic factors, including pesticides and pollutants. However, with the continuous improvement of agriculture technology, especially the application of chemical fertilizers, crop yields have greatly increased in the past sixty years (Fan et al., 2012). Recent research on nanoparticles in a number of crops has found enhanced germination and seedling growth, photosynthetic activity, nitrogen metabolism, mRNA expression, and protein levels, as well as positive changes in gene expression; these observations clearly indicate the potential use of nanoparticles for crop improvement (Kole et al., 2013). Nanoparticles are defined as particles with at least two dimensions below 100 nm (i.e., 0.1 μm). They are generated during combustion processes and diverse, naturally occurring abiotic and biotic chemical processes. Their commercial production has increased steadily because they are used in many novel applications such as catalysts, semiconductors, drug carriers, and cosmetics; the development of additional novel materials is likely (Dietz and Herth, 2011). Food and agricultural

production are among the most susceptible fields of application of nanotechnology. It is predicted that nanotechnology will revolutionize agriculture through new tools for disease treatment and detection, smart delivery systems, sensors, and better management devices. However, the rapid development of nanotechnology could release a massive amount of engineered nanoparticles, which may cause adverse effects on edible plants (Hong et al., 2013).

The purpose of this study was i) to discuss the use, occurrence, effect, and type of nanofertilizers on plants and assess the need for future research on crop development, with specific emphasis on crop yields and the environmental impact; ii) to evaluate the germination of twelve plants under increasing nanoparticle concentrations [magnetite (Fe_3O_4) and hematite (Fe_2O_3)]; and iii) to determine the effect of nanoparticles of magnetite, hematite, ferrihydrite ($FeOOH•xH_2O$), titanium dioxide (TiO_2), and zinc oxide (ZnO) on particular plant characteristics during the early stages of common bean, maize, and sunflower growth under greenhouse conditions.

MATERIALS AND METHODS

Experimental Site

This study was conducted from January to July 2013 under greenhouse conditions by the '*Laboratorio de Interacciones Planta-Ambiente*' del Cinvestav-Saltillo, located in Saltillo, Coahuila, Mexico. Specifically, the area is located in the southeastern state of Coahuila, centered at 25°31' N, 101°37' W, at an altitude of 1,600 m above sea level, and with a mean annual temperature of 18 °C. To a large extent, the climate of Coahuila is dry and semi-warm to extremely warm, with some variants throughout the regions. The average temperature in January, the coldest month, was 12 °C; in June and July, the hottest months, it was 23 °C. The average annual rainfall is 369 mm, with the majority of it occurring during September and October. Based on the Köppen climate classification, this is a semi-arid, hot climate (BSh). Additionally, according to the FAO/UNESCO soil classification system, the soil is a Haplic Xerosol with a pH of 7.3, electrolytic conductivity of 4.8 dS m^{-1}, water-holding capacity (WHC) of 865 g kg^{-1}, total N content of 0.7 g N kg^{-1} soil, and an organic carbon content of 1.5 g C kg^{-1} soil.

Biological Material

This study highlights the effects of nanoparticles on the seed germination of basil (*Ocimum basilicum* L.), sweet alyssum (*Alyssum maritimum L.*), parsley (*Petroselinum sativum* Hoffman), snapdragon (*Antirrhinum majus* L.), oregano (*Origanum vulgare* L.), coleus (*Coleus blumei* Benth), lettuce (*Lactuca sativa* L.), floss flower (*Ageratum houstonianum* Mill), common bean (*Phaseolus vulgaris* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), and oxeye daisy (*Chrysanthemun leaucanthemum* L.). All of the seeds, except common bean, maize, and sunflower, were purchased from '*Germinal, S.A. de C.V.*' (D.F., Mexico). The sunflower and maize seeds were provided by '*Universidad*

Autónoma Agraria Antonio Narro', Coahuila, Mexico, while the common bean seeds were donated by '*INIFAP-Celaya*', Mexico. The seeds were all kept in the dark at 4 °C until use.

Germination in Pure Culture

For each individual treatment, 200 seeds of each species were soaked for 1 h in 5 mL of either i) 0.0, 0.3, 0.5, 1.0, 1.6, and 3.0 g nano-Fe_3O_4 L^{-1}; ii) 1.0, 1.6, and 3.0 g nano-Fe_2O_3 L^{-1}, or iii) deionized water as a control without surface disinfection. Once the seeds were placed in the treatment solution, each jar received a five-second shake three times over the course of the hour to ensure that all the seeds were thoroughly and evenly coated with the solutions. One piece of filter paper was placed in each 100 × 15 mm Petri dish, and 10 mL of the appropriate treatment test solution was added. The seeds were then transferred onto the filter paper, with 25 seeds per dish. The Petri dishes were covered and sealed with tape before being placed together randomly in a stove set to 33 °C for 38 days. Three replicates of each treatment were prepared, for a total of 30 Petri dishes per each plant species.

We measured the seed germination rate, shoot length, and root length for all replicates after 38 days of incubation, with the exception of lettuce, common bean, maize, and sunflower, which were measured on the 16th, 6th, 6th, and 6th days, respectively, due to their quick germination and growth rates. The seed germination was defined as the moment when the radicle or plumule emerged from the seed coat; the seed germination rate (GR) was calculated as the proportion of the total seeds that germinated. The shoot length (SL) and root length (RL) were measured by a slide caliper. From these data we calculated three ratios.

(a) Treatment effects on germination:

$$\frac{GR_{TRT} - GR_{CONTROL}}{GR_{CONTROL}}$$

where GR_{TRT} is the germination rate of the treatment sample and $GR_{CONTROL}$ is the germination rate of the control.

(b) Treatment effects on leaf length:

$$\frac{LL_{TRT} - LL_{CONTROL}}{LL_{CONTROL}}$$

where LL_{TRT} is the leaf length of the treatment sample and $LL_{CONTROL}$ is the leaf length of the control.

(c) Treatment effects on root length:

$$\frac{RL_{TRT} - RL_{CONTROL}}{RL_{CONTROL}}$$

where RL_{TRT} is the root length of the treatment sample and $RL_{CONTROL}$ is the root length of the control.

Cultivation of Plants in the Greenhouse

Sixty subsamples of 300 g soil, i.e., five nanoparticles × three replicates × four concentrations, were added to square, plastic pots whose length, width, and height were 8 × 8 × 7 cm, respectively. Specifically, five treatments, comprising nano-Fe_3O_4, nano-Fe_2O_3, nano-$FeOOH•xH_2O$, nano-TiO_2, and nano-ZnO at four concentrations of 0.0, 1.0, 3.0, and 6.0 g L^{-1} were applied to the soil during irrigation; in essence, we sprayed each plastic pot with 250 mL of 0.0, 1.0, 3.0, or 6.0 g nano L^{-1} solution throughout the experiment. We planted three different seeds for this experiment: sunflower, maize, and common bean; therefore, we used a total of one hundred eighty plastic pots, i.e., five nanoparticles × three replicates × four concentrations × three crops. The seeds were placed at a depth of 3 cm in each pot. Five days after planting, the seedlings were thinned to one plant per plastic pot, and the pots were placed under greenhouse conditions for 20 days. Although a plastic container was placed under each pot to collect drained liquid, the irrigation was well controlled, so no leaching was observed. Twenty days after sowing, three plastic pots were selected at random from each treatment and concentration. The entire soil column was removed from the pot, and soil samples were taken at depths of 0.0–3.5 cm and 3.5–7.0 cm, with care not to damage the root structure. The roots were then separated from the shoots, and the root and shoot lengths were measured. The roots and shoots were dried at 70 °C, weighed, and analyzed for Ti, Fe, and Zn. The soil samples from 0.0–3.5 cm and 3.5–7.0 cm were analyzed for pH, EC, Ti, Fe, and Zn at 0 and 20 days, and the amount of chlorophyll was quantified every two days. The climatic conditions in the greenhouse were not determined. However, during the experiment, a similar greenhouse was installed with equipment to monitor its temperature and moisture content. Its measurements were 32 °C and 47%–50%, respectively.

Chemical Analyses

The pH was measured in 1:2.5 soil or wastewater sludge/H_2O suspension using a 716 DMS Titrino pH meter (Metrohm Ltd. CH.-901, Herisau, Switzerland) fitted with a glass electrode (Thomas, 1996). The EC was determined in a 1:5 soil/H_2O suspension as described by Rhoades et al. (1989). The organic C in the soil was measured in a total organic carbon analyzer TOC-V_{CSN} (Shimadzu, USA). The inorganic C was determined by adding 5 mL 1 M hydrochloric acid (HCl) solution to 1 g air-dried soil and trapping the CO_2 evolved in 20 mL 1 M NaOH. The total N in the soil, root, and shoot was measured by the Kjeldahl method, using concentrated H_2SO_4, K_2SO_4, and $CuSO_4$ to digest the sample (Bremner, 1996). Soil particle size distribution was defined by the hydrometer method as described by Gee and Bauder (1986). The WHC was measured based on 6.5 kg soil that was placed in a PVC tube (length 50 cm and diameter 16 cm), water-saturated, stoppered with a PVC ring, and left to stand overnight to drain freely. The WHC was defined (Gardner, 1986) as described below:

$$WHC = \left(\frac{Soil_{water-saturated} - Soil_{drying\ at\ 105\ °C}}{Soil_{drying\ at\ 105\ °C}} \right) * 100$$

where the units of WHC are expressed in g kg^{-1}. The amount of chlorophyll was measured with a Minolta SPAD-502 Chorophyll meter (Markwell et al., 1995). The Fe, Ti, and Zn were determined by inductively coupled plasma mass spectrometry (ICP–MS).

Statistical Analysis

The data were subjected to an analysis of variance and means compared with the Tukey test using Statistical Analysis System (SAS) software version 8.0 for Windows (SAS Institute, 1989). Soil and plant characteristics were subjected to a one-way analysis of variance using a general lineal models procedure (PROC GLM) to test for significant differences between treatments ($P < 0.05$). All analyses were performed using the SAS statistical package (SAS Institute, 1989).

RESULTS AND DISCUSSION

Nanoparticles are adsorbed by plant surfaces through natural nano- or micrometer-scale plant openings (Dietz and Herth, 2011). During the germination and seedling emergence, these nanoparticles could decrease some plant characteristics. We found no significant effects of nanoparticles on the germination rate when seeds of basil, coleus, sweet alyssum, common bean, sunflower, and maize were sprayed with magnetite (Fe_3O_4) or hematite (Fe_2O_3) nanoparticles at increasing concentrations (Figure 1). However, Dehkourdi and Mosavi (2013) showed that an increase in the concentration of nano-anatase caused a significant increase in the percentage of germination, germination rate index, root and shoot length, fresh weight, vigor index, and chlorophyll content of seedlings of parsley (*Petroselinum crispum*). Parsley's seed germination takes much longer than that of most vegetables. It is quite difficult to be germinated (the germination percentage is at 55%-75%, it is lower than the average germination percentage), especially under unfavorable environmental conditions. Their results showed that an increase in the concentration of nano-anatase caused a significant increase in the percentage of germination, germination rate index, root and shoot length, fresh weight, vigor index, and chlorophyll content of seedlings. The best concentration of nano-anatase was 30 mg mL^{-1}.

In recent months, nano priming has become a new method for increasing seedling vigor and improving both germination percentage and seedling growth. The effects of nano-TiO_2 (rutile) and non-nano-TiO_2 on the germination and growth of naturally aged spinach seeds were studied. An increase of these factors was observed at 0.25–4‰ nano-TiO_2 treatment (Zheng et al., 2005). Although Feizi et al. (2012) reported that nano-TiO_2 in a suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2, in high concentrations, it had inhibitory or no effects on wheat. Similar results were reported by Castiglione et al. (2011), who studied the effect of nano-TiO_2 on the seed germination, development, and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. They found that root elongation was affected only after treatment with the highest nano-TiO_2 concentration (4.0 parts per thousand), while further significant effects were detected,

showing a mitotic index reduction and concentration-dependent increase in the aberration emergence, which evidenced a nano-TiO_2-induced genotoxic effect for both species.

Carbon nanotubes have shown promise as regulators of seed germination and plant growth (Khodakovskaya et al., 2012). Begum et al. (2012) evaluated the possible phytotoxicity of multi-walled carbon nanotubes (MWNTs) at 0, 20, 200, 1,000, and 2,000 mg L^{-1} with red spinach, lettuce, rice, cucumber, chili, lady's finger, and soybean based on root and shoot growth, cell death, and electrolyte leakage at the seedling stage. They reported that red spinach and lettuce were most sensitive to MWNTs, followed by rice and cucumber, while very few or no toxic effects were observed for chili, lady's finger, and soybean.

We found that, compared with the control, magnetite and hematite significantly increased the seed germination rate of oregano when the seeds were irrigated at 1.6 and 1.0 g L^{-1}, respectively (Figure 1). In addition, solutions of 1.6 and 3.0 g magnetite L^{-1} as well as 1.0, 1.6, and 3.0 g hematite L^{-1} substantially increased the seed germination rates of parsley and snapdragon, compared with the control (Figure 1). We found that hematite's effects were equal to or greater than magnetite's effects on seed germination rate. We also found that plant species differ in their susceptibility to different kinds of nanoparticles, as witnessed by the seed germination rate. Low concentrations of magnetite, i.e., 0.3, 0.5, and 1.0 g L^{-1}, did not stimulate certain physiological changes during the seed germination as observed by the germination rates of parsley and snapdragon. This observation notwithstanding, our results indicated that an appropriate concentration of nanoparticles could promote the seed germination of some plants. Additionally, Kumar et al. (2013) found a considerable correlation between the expression of key plant regulatory molecules, microRNAs, seed germination, growth, and the antioxidant potential of *A. thaliana* on exposure to gold nanoparticles. These results suggest that the increasing release of nanoparticles into the environment could have positive effects on the seed germination rates in some cultivated plants. Furthermore, there was no evidence of nanotoxicity in plants when low or high nanoparticle doses were evaluated.

We determined that, compared to the control treatment, the nanoparticles of magnetite, hematite, ferrihydrite, zinc oxide, and titanium dioxide had a significant effect on a number of plant characteristics during the early stages of common bean, maize, and sunflower under greenhouse conditions ($P < 0.05$; Table 2). Seedling emergence, fresh weight of the shoot, and chlorophyll content decreased significantly ($P < 0.05$) in maize, common bean, and sunflower plants when the soil was irrigated with hematite nanoparticles (Table 2). However, the effect was insignificant when other nanoparticles were used during the irrigation process.

Titanium dioxide, a well-known photocatalyst, has been widely studied for its possible enhancement of photosynthesis (Narayanan et al., 2013). Su et al. (2007) showed that suitable concentrations of nano-anatase TiO_2 can improve the oxygen evolution in spinach plants. This phenomenon may be attributed to the increase in light absorbance and energy transfer among the amino acids. Similar results were demonstrated with nano-TiO_2 on wheat plants (Moaveni et al., 2011). However, our results showed that the chlorophyll content was significantly equal to the control treatment when plants were irrigated with TiO_2 (Table 2). Jacob et al. (2013) revealed that the exposure of common bean and wheat to TiO_2 nanoparticles did not affect biomass production, but did substantially increase root Ti sorption and uptake.

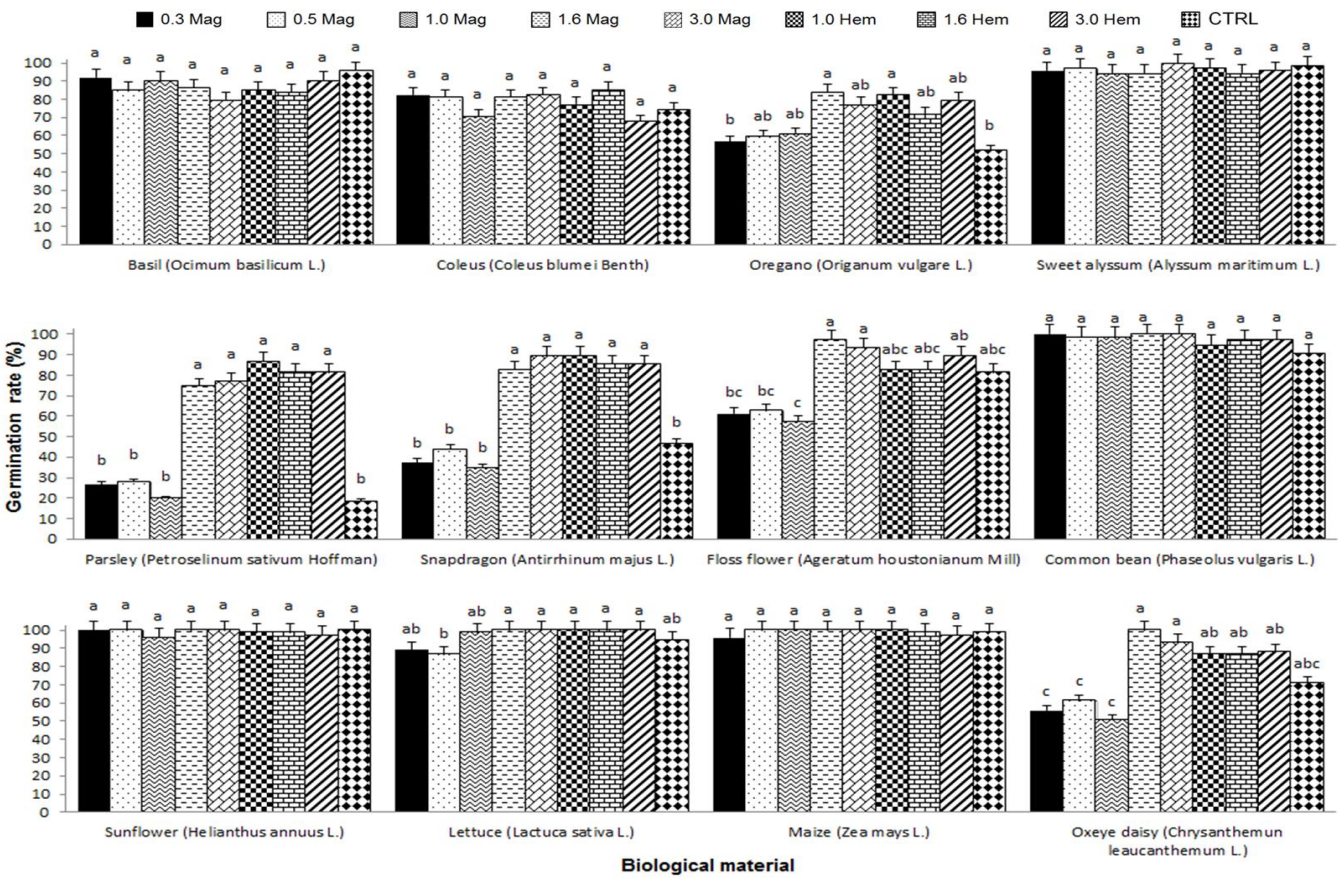

Figure 1. Germination rate of twelve plants under increasing nanoparticle concentrations [magnetite (Mag; Fe_3O_4) and hematite (Hem; Fe_2O_3)]. 0.3 Mag, 0.3 g nano-Fe_3O_4 L^{-1}; 0.5 Mag, 0.5 g nano-Fe_3O_4 L^{-1}; 1.0 Mag, 1.0 g nano-Fe_3O_4 L^{-1}, 1.6 Mag, 1.6 g nano-Fe_3O_4 L^{-1}; 3.0 Mag, 3.0 g nano-Fe_3O_4 L^{-1}; 1.0 Hem, 1.0 g nano-Fe_2O_3 L^{-1}; 1.6 Hem, 1.6 g nano-Fe_2O_3 L^{-1}; 3.0 Hem, 3.0 g nano-Fe_2O_3 L^{-1}; and CTRL, deionized water. The experimental setup was conducted in a stove set to 33 °C for 38 days, with the exception of lettuce, common bean, maize, and sunflower, which we measured on the 16th, 6th, 6th, and 6th days, respectively, due to their quick germination and growth rates.

Table 2. Effect of nanoparticles on plant characteristics of common bean (Phaseolus vulgaris L.), maize (Zea mays L.), and sunflower (Helianthus annuus L.)

Characteristics	Nanoparticles					Control	LSD[¥]
	Magnetite	Hematite	Ferrihydrite	Zinc oxide	Titanium dioxide		
Seedling emergence (units)	2.25 a[∞]	1.77 b	2.15 a	2.37 a	2.17 a	2.14 a	0.3
Plant height (cm)	18.9 a	18.2 a	16.2 b	17.6 ab	19.0 a	17.3 b	1.6
Length of roots (cm)	11.7 b	14.4 a	12.0 a	16.6 a	14.4 a	13.4 a	5.0
Fresh weight of shoot (g)	0.94 a	0.66 b	0.86 a	0.95 a	0.77 a	0.84 a	0.4
Fresh weight of root (g)	0.22 ab	0.24 ab	0.26 a	0.21 ab	0.16 b	0.17 b	0.09
Dry weight of shoot (g)	0.064 b	0.075 b	0.081 b	0.104 a	0.080 b	0.080 b	0.04
Dry weight of root (g)	0.040 b	0.051 ab	0.056 ab	0.057 ab	0.044 b	0.060 a	0.02
SPAD (units)	29.0 a	26.4 b	30.5 a	30.9 a	29.7 a	29.9 a	2.1

[¥] LSD, least significant difference ($P < 0.05$).

[∞] Values with the same letter are not significantly different between nanoparticles, i.e., within the rows ($P < 0.05$).

Data were pooled among the three crops. All data presented were the mean of three plants cultivated in soil from three different plots and from three consecutive experiments conducted under greenhouse conditions, and were sampled 20 days after sowing.

Plant height increased significantly ($P < 0.05$) when crops were irrigated with magnetite, hematite, or titanium dioxide nanoparticles, compared with the control treatment. However, magnetite had no significant effect on the chlorophyll content compared with the control treatment. Contrary results showed that magnetite nanoparticles negatively influenced the photosynthetic pigment biosynthesis by diminishing the chlorophyll content by up to 50% when sunflowers were cultivated in culture medium (Ursache-Oprisan et al., 2011). Additionally, the plant heights of maize, common bean, and sunflower were not affected by any of the tested nanoparticles (Table 2).

The length of roots decreased significantly in maize, common bean, and sunflower plants when these plants were irrigated with magnetite, but there was no significant effect when other nanoparticles were used during the irrigation event. Mushtaq (2011) studied the effect of magnetite and TiO_2 on cucumber. He found several inhibitory effects, including reductions in root growth and seed germination percentages. In the germinating test for all cases, he observed some perturbations of the normal functions with respect to control.

Ferrihydrite and zinc oxide produced a significant increase ($P < 0.05$) in the fresh weight of roots and the dry weight of shoots, respectively, compared with the other treatments (Table 2). In both maize and cabbage, Pokhrel and Dubey (2013) found that measures of germination and root elongation revealed low nano-ZnO toxicity, compared to free ions. Kumari et al. (2011) demonstrated that ZnO nanoparticles can be a clastogenic/genotoxic and cytotoxic agent on root cells of *Allium cepa*.

Magnetite and titanium dioxide showed a significant decrease ($P < 0.05$) in the dry weight of roots, compared with the other treatments. Song et al. (2013) showed that TiO_2 nanoparticles were absorbed into the stems, leaves, and fruits of tomato plants. In addition, they found that exposure to titanium dioxide nanoparticles resulted in acute toxicity upon germination and significantly decreased root elongation at each concentration tested. Neither ferrihydrite nor zinc oxide nanoparticles affected maize, common bean, or sunflower (Table 2).

Further studies of the environmental and ecological effects of nanoparticles and strategies to mitigate its inappropriate agricultural use are required. In addition, scientific, technical, or agricultural projects linked with nanofertilizers must include environmental side effects such as pollution, greenhouse gas emissions, and ecological damage in order to ensure a sustainable future.

CONCLUSION

The increasing applications of different nanomaterials in the myriad nano-enabled products and their potential for environmental pollution have raised environmental, health, and safety concerns. Knowledge of nanoparticles in soils and investigations on nanoparticle–crop interactions are still rare and in the rudimentary stages. Nevertheless, nanoparticles in agricultural systems may potentially be used as appropriate candidates for change in the growth, development, productivity, and quality of plants. However, despite the many advantages of nanoparticles, there are still concerns that their introduction into soil in order to enhance plant yield may have adverse environmental effects, such as an environmental pollution source or a food-chain pollution source. Indeed, the phytotoxicity research on

nanoparticle–crop interaction has yielded confusing results, ranging from strong toxicity to positive effects. This study may help to further understand the interaction of nanoparticles with the environment prior to their use in agriculture.

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SECTION V. IMPROVING FERTILIZER APPLICATIONS

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Chapter 10

MODIFICATION OF THE PROPERTIES OF NATURAL RUBBER BY COPOLYMERIZATION WITH A BIOPOLYMER FOR A CONTROLLED RELEASE OF UREA FERTILIZER

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ABSTRACT

One of the problems associated with the use of fertilizers in conventional agriculture is the loss of fertilizer through volatilization and leaching by rain, which can cause serious environmental problems away from the original site of application. Thus, one solution to this problem would be to encapsulate fertilizers using biopolymer membranes to control the diffusion of water and the release of the water-soluble active fertilizer. The objective of this chapter is to develop a new product for the controlled release of a urea fertilizer, using a modified natural rubber (NR) with another biopolymer, and to demonstrate its performance in water and soil for an agriculture field. The approaches for preparing the capsules and the release of the urea fertilizer from the capsule are discussed. Factors e.g., the modification methods for NR, the NR content, the numbers of NR layers, the ratio between the NR and biopolymer on the optimum release rate of urea from the capsules obtained from the coating were subsequently investigated. The properties of NR were improved by chemical modification, e.g., crosslinking, grafting and epoxidation reactions of NR. In parallel, the NR was blended with other polymers, e.g., poly(vinyl alcohol) (PVA), polyacryamide, starch (St) or sodium alginate (SA) and cellulose fiber to increase its agricultural usefulness. The rate of the fertilizer release from the capsule decreased with an increase of the NR coating layer and the NR concentration in polymer membrane and polymer blend ratios. The rate of the swelling ratio for these samples was responsible for the release of fertilizer from the capsule in an aqueous phase. When the capsules were coated with modified NR latex, it was found that the release rate of urea was significantly decreased and the duration of release was prolonged for roughly

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30 h in an aqueous medium. In addition, the modified rubber was easily applied to encapsulate the urea fertilizer. The resulting polymer membrane of the capsule was degraded in natural soil after use. The advantages of this value-added NR product and the biopolymer are its environmental friendliness and low production cost.

Keywords: Encapsulation, Fertilizer, Natural rubber, Modification

1. INTRODUCTION

Fertilizers are one of the essential agrochemical agents required to supply nutrients to sustain plants and promote their abundant and fruitful growth. The current agricultural systems may be unable to supply the exploding world population in the future. Farmers depend heavily on fertilizer to improve their crop yields. Many crops in tropical countries such as rubber, rice, corn and vegetables are illustrated in Figure 1. There are three major components of fertilizers: nitrogen, phosphorus, and potassium. However, the efficiency of the use of the fertilizers is poor (Riyajan, et al., 2012). Less than half of the fertilizer applied to the crop soil is actually recovered in crops. In conventional agriculture many of the losses of fertilizer occur through volatilization or leaching by rain, and this can lead to some important environmental problems. In addition, the parameters that affect the efficiency of fertilizer use are the nature of the soil, the climate, the technique of application of the fertilizer and the nature of the crops. One way to improve the efficiency of fertilizer utilization could be to encapsulate the fertilizers within a polymer membrane that would control the rate of release of the water-soluble active agents (Riyajan et al., 2012). One such polymer membrane for controlling the release of fertilizer can be made by modifying natural rubber (NR) with a biopolymer (Riyajan, et al., 2012a) and demonstrate its ability to control its release in the water and soil of an agriculture field. Factors e.g., modification methods to the NR, the NR content, the NR layer, the ratio between NR and biopolymer on the release rate of urea, the form of the fertilizer confined within such capsules that can be made with an enclosing membrane formed from an NR biopolymer membrane have now been investigated. The properties of NR were altered by chemical modification, e.g., crosslinking, grafting and epoxidation. In parallel, NR was blended with other polymers, e.g., poly (vinyl alcohol) (PVA) (Riyajan et al., 2008), polyacryamide (Riyajan and Wongsa, 2013b), starch (Riyajan et al., 2010) or sodium alginate (SA) Riyajan et al., 2009b), and natural fibers (Riyajan et al., 2013c) to increase its use in agriculture The results of some of these experiments have been described in this manuscript.

2. MODIFICATIONS OF NR

NR, consisting mainly of 93–95% *cis*-1,4-polyisoprene is an elastomer, produced from latex of the rubber tree. Natural rubber latex (NRL) is a brand of NR and it consists of two main components including hydrocarbon bonds (30-35% rubber) and non-rubber components such as water, protein, carbohydrate, fatty acid and other chemical agents (Riyajan and Santipanusopon, 2010b). Fresh latex from the *Hevea brasiliensis Muell. Arg* tree contains 30–35% rubber. After collection, the NRL is usually stabilized with NH_3 to aid its dispersion in a

liquid medium for long-term storage. Before use, it undergoes concentration by continuous centrifugation so that it contains ~60% rubber. The diameters of the NR particle range widely from 0.01 to 5 μm. NR is widely used in many fields such as tires, medical gloves, condoms and other products because it has excellent dynamic properties, with a high tear and abrasion resistance. Before its use, it is vulcanized by one of three methods that include the use of sulfur, peroxide and irradiation. Considering the limitations of cured rubber after use, the biggest problem is that it is difficult to degrade in nature and this has led to environmental pollution. Other drawbacks of NR are its low weathering and oil resistance. This is due to the NR molecule, which contains carbon-carbon double bonds and is a nonpolar polymer. Therefore, this chapter describes the modification of the properties of NR so that it could control the release of fertilizer for an agricultural application. To overcome the limitations of NR and expand its applications, modification is crucial and three main methods have been employed (Figure 2). The first is chemical modifications that include cyclisation, degradation, epoxidization and grafting. Chemical modification can introduce other groups or atoms onto the NR molecular chains, and thus derive polymers such as epoxidized natural rubber (ENR) (Riyajan et al., 2009_a), hydrogenated NR (Piya-areetham et al., 2013), and grafted NR (Riyajan et al., 2013a,c).

The second is blending with a hydrophilic polymer such as poly (vinyl alcohol) (PVA) (Riyajan et al., 2009a) and sodium alginate (SA). The third method is by addition of an additional chemical agent e.g., filler or fiber from a natural source such as sugar cane (Riyajan et al., 2013c).

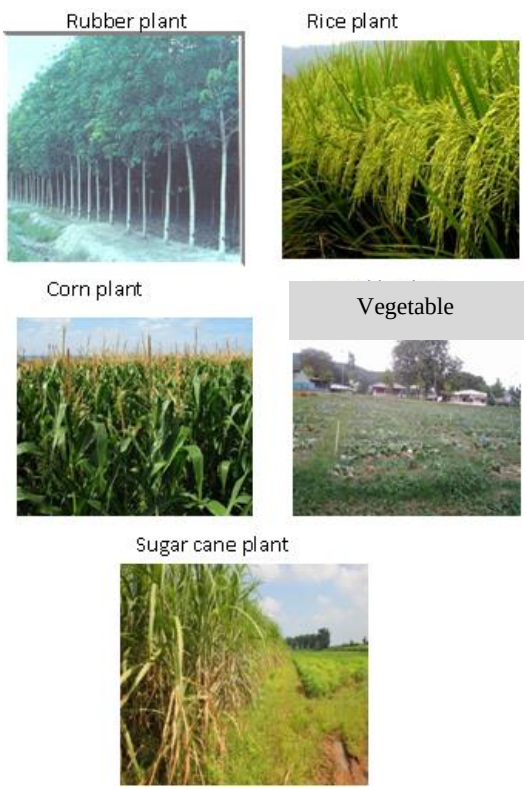

Figure 1. Photographs of crops in a tropical country.

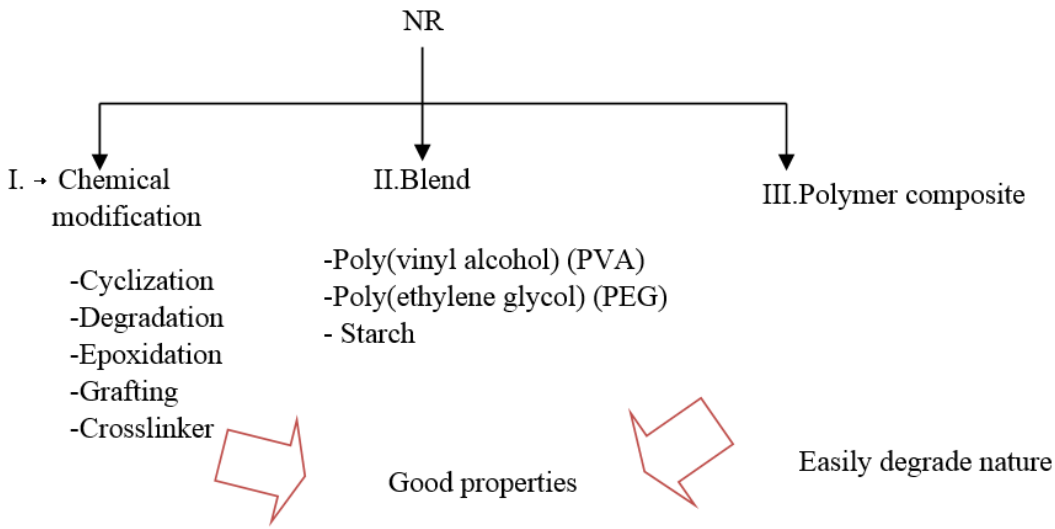

Figure 2. Summary of modification of NR via chemical modification, blending and the addition of a chemical agent.

The three forms of NR that have been used for modifications include latex (Riyajan et al., 2012a), solution (Nakason et al., 2004) and melt (Xavie et al., 2001). Compared to modifications in solution, the modification in melt is more advantageous in terms of energy savings and the environment. However, the disadvantages of chemical modification in the melt form are the possibilities of an easy side reaction, because of the heterogeneous properties of modified rubber. In addition, the investigations with regard to modification in form have been carried out using brabender-type internal mixers, a two-roll mill, a single-screw extruder and a twin-screw extruder. Recently, modifications to its latex form have been widely used for improving the properties of rubber owing to the friendly environment, high productivity and an available process such as a dipping process. Water is no doubt the most environmentally friendly solvent. When modification is carried out in the latex form, the process of modification of the properties of NR would be much more efficient due to there being no use of organic materials. Thus, modification in the latex phase is attractive. However, its usage in the organic reaction processes has limitations because many organic materials do not dissolve in water. In addition, many reactive intermediates and catalysts are decomposed by water. This is the case for Lewis acid catalyzed reactions, which are of great current interest because of their unique creativities and selectivity and it can be achieved using mild conditions (Hayashi et al., 2011).

Prevulcanizsation

Vulcanization is a chemical modification of NR that converts natural rubber or related polymers into a more durable material by using curing agents e.g., sulfur, peroxide and other chemicals (Datta et al., 2007). These curing agents modify the NR molecule by forming crosslinks (bridges) between individual NR chains as shown in Figure 3. The outstanding property of vulcanized NR is that it is less sticky, operates well in any outdoor environment

and has superior mechanical properties. At the present time, the cured NR is widely used in tires, for shoe soles, hoses, conveyor belts and hockey pucks. Prevulcanized latex (PVL) is defined as a process in which the crosslinking of the NR occurs with only discrete particles which are dispersed in a liquid medium, namely serum and it is a very convenient raw material for the latex goods manufacturing industry. Production of sulfur PVL is carried out by heating raw NR latex with various compounding ingredients such as an accelerator, activator and sulfur until the required degree of crosslinking is completed. After the drying process of PVL, NR latex results in a crosslinked film. To date, there are many methods for investigation of the extent of crosslinking including the swelling ratio (Riyajan et al., 2003), stress-relaxation (Sombatsompop, 1998), NMR (Wootthikanokkhan and Clythong, 2003) and other techniques.

Cyclisation

Cyclisation is a simple reaction that can transform *cis*-1,4-polyisoprene to fuse as a six-membered ring structure, using an acidic reagent as shown in Figure 4. The acidic reagents used to change the chemical structure of NR include trimethyl silyl triflate (Riyajan and Sakdapipanich, 2009c) benzotrichloride (Riyajan et al., 2007a) and sulphuric acid (Riyajan et al., 2006) systems. The advantage of these catalysts is that the cyclized product can be dissolved in an organic solvent. Moreover, the cyclised NR is a tough, hard and brittle material. Therefore, it can be used to reinforce resinous materials. When the cyclised NR is blended with other polymers, the polymer blend has a high modulus, high hardness and low specific gravity. In addition, it is used for photo resistant applications, pigments for printing and as a raw material for producing membranes. It is clear that the modulus of the NR increases with the increase of cyclised NR content in the NR matrix (Riyajan et al., 2007a).

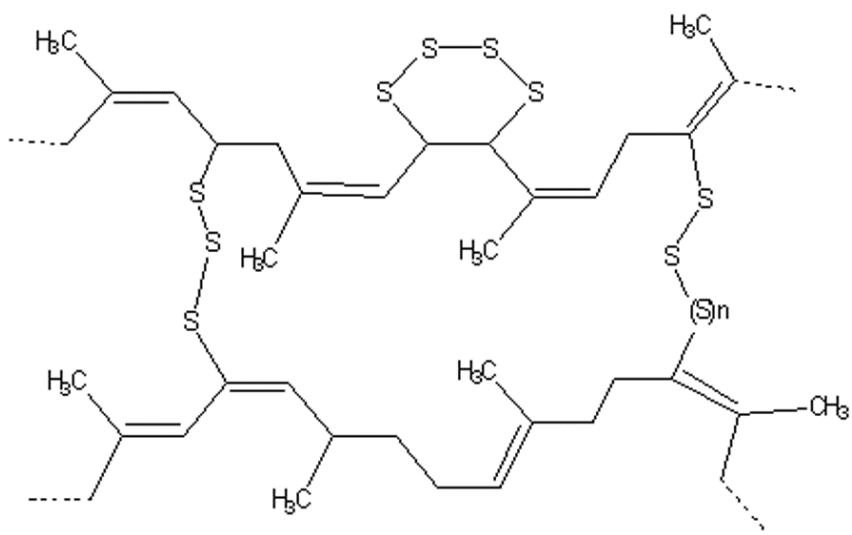

Figure 3. Production of Prevulcanizsed natural rubber latex with sulfur and other compound ingredients during heat. (http://en.wikipedia.org/wiki/Vulcanisation, 2012).

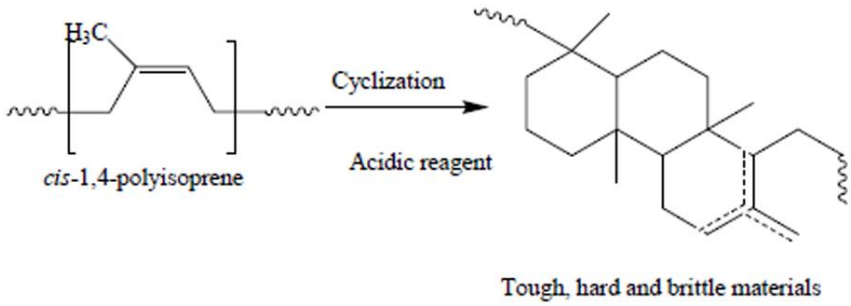

Figure 4. Cyclisation of natural rubber by using an acid catalyst.

Degradation

NR itself has high molecular weight; its molecular weight is roughly 1 million g mol^{-1}. It is good in some applications but it is limited for use as an adhesive, plasticizer and lubricant. The very high molecular weight of NR can be decreased by chemical degradation (Riyajan, 2007b). Chain scission has been developed to decrease the molecular weight of the rubber as shown in Figure 5. In previous work, mechanical mastication, photo-degradation and thermal-chemical degradation were proposed to degrade NR. The terminal units of the degraded NR were found to be hydroxyl groups while the oxidative degradation of the rubber resulted in a liquid rubber with formyl and epoxy groups. When we compared the mastication and the photo-degradation with the oxidative degradation, it was obvious that the oxidative degradation in the latex stage had many more advantages to decrease the viscosity and to allow for better process ability.

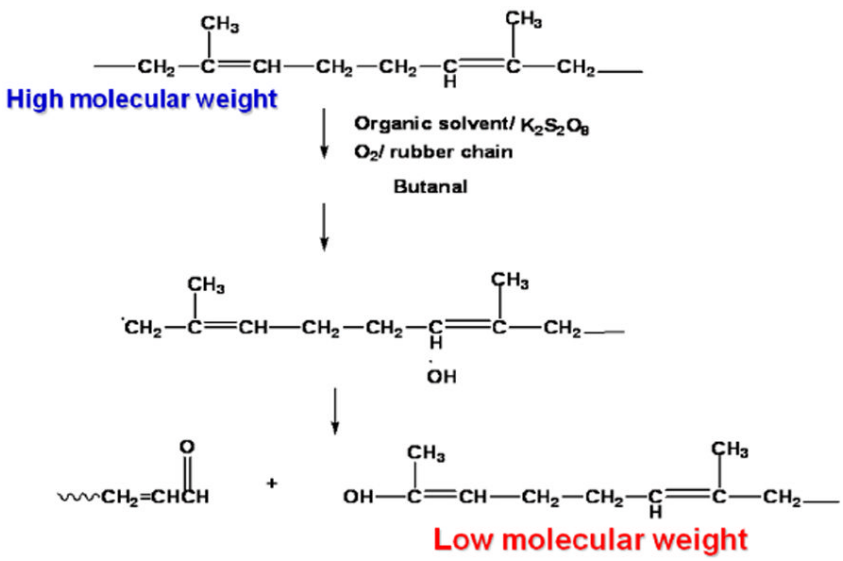

Figure 5. Chemical degradation of natural rubber by using $K_2S_2O_8$ and butanol.

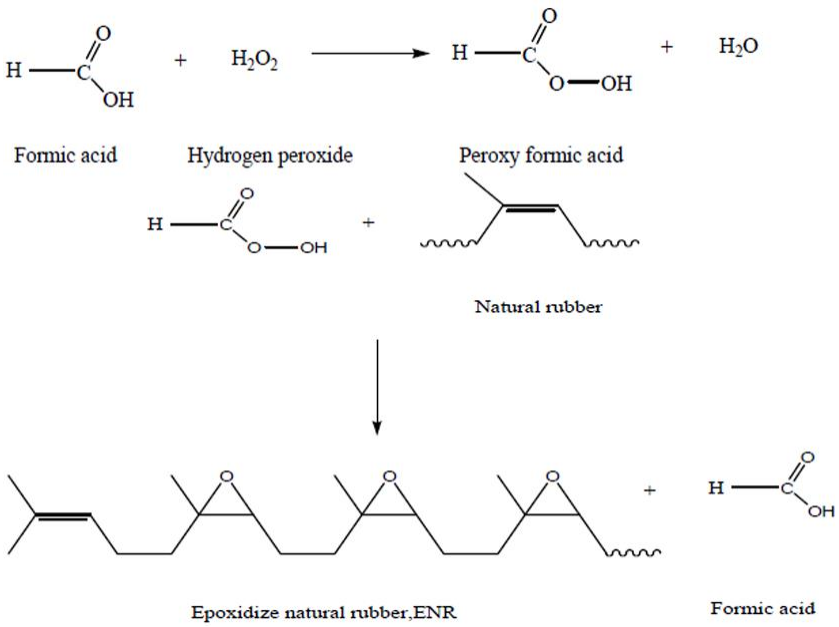

Figure 6. Epoxidized reaction of NR by using H_2O_2 and formic acid.

Epoxidation

The epoxidation of NR to produce ENR is a chemical epoxidation using modified natural rubber prepared by converting a part of the carbon-carbon double bonds in the NR molecules into functional epoxy groups and is normally carried out in the latex form (Riyajan et al., 2009a). By adding the mixture of formic acid and hydrogen peroxide, we obtain ENR having epoxide groups randomly distributed along the NR molecule as shown in Figure 6. Compared with NR, the damping, polarity and glass transition temperature of ENR are increased. But ENR shows a reduction in swelling in hydrocarbon oils and gas permeability. The possible applications of ENR are as an adhesive (Riyajan and Phupewkeaw, 2012c) and compatibilizer (Rajasekar, 2009) for blends.

Grafting Reaction

The organic resistance and biodegrability of NR are improved by grafting a copolymer. In this work, the NR molecule was grafted with biopolymers such as chitosan (CTS) (Riyajan and Sukhlaaied, 2013a) cassava starch (Riyajan et al., 2012a) and cellulose fiber (Riyajan et al., 2013c). In addition, the chemical structural of NR was grafted with maleic anhydride for use as a polymer compatibilizer. The possible grafting reaction between ENR and CTS is displayed in Figure 7.

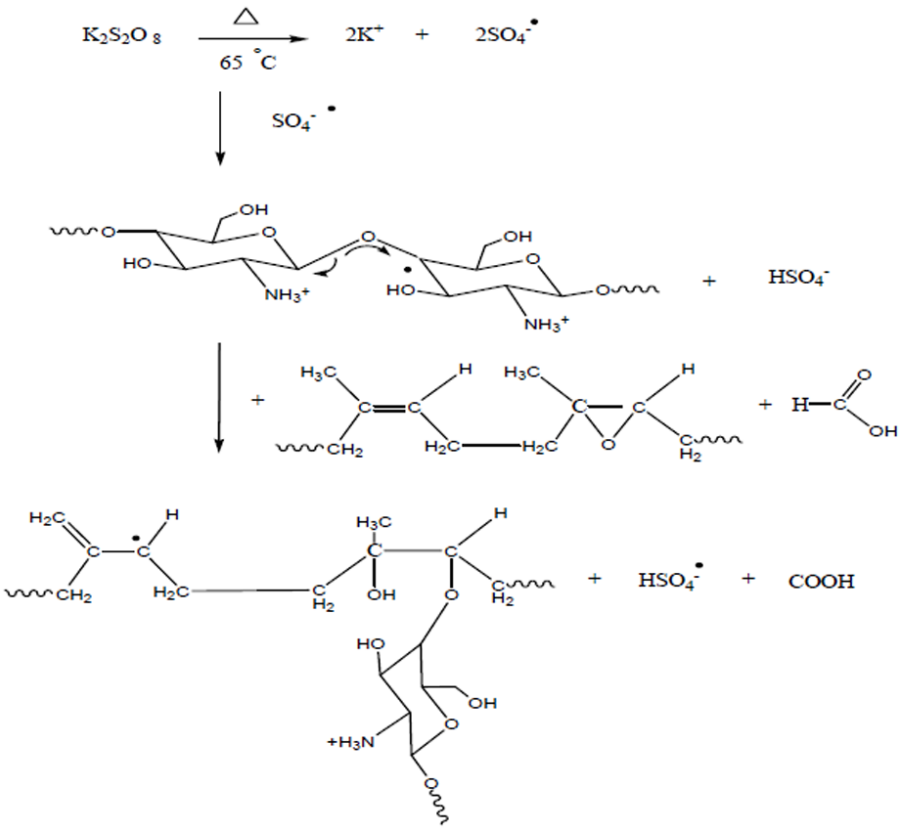

Figure 7. Epoxidized natural rubber–g-chitosan from ENR latex and CTS by $K_2S_2O_8$ as an initiator.

First, potassium persulphate was heated when it decomposed into free radicals, which then reacted with both chitosan molecules and ENR to form chitosan and ENR free radicals. At the same time, short chitosan chain free radicals continued to react with the hydroxyl groups of the ENR molecule to produce ENR-g-CTS. After the synthesis of ENR-g-CTS, it was used to form a polymer membrane to encapsulate the fertilizer. The mechanism of the $K_2S_2O_8$ reaction with starch was that when it was heated it changed into a $K_2S_2O_8$ free radical. Then, the $K_2S_2O_8$ free radical reacted with a starch molecule. The degradation of starch occurred as shown in Figure 8. The free radicals from starch attacked the carbon-carbon double bonds of NR, which were activated by $K_2S_2O_8$ leading to the NR-g-St formation as shown in Figure 8.

A possible chemical grafting between MA and NR is presented in Figure 9. BPO decomposed into free radicals, which then reacted with the carbon-carbon double bonds of MA and NR molecules. In addition to the grafting of MA onto the *cis*-1,4-polyisoprene of NR, the crosslinked product from the NR molecules through MA bridging could be obtained. Moreover, BPO radicals might break sulfur-prevulcanized natural rubber (SPNR) molecules into short chains during the modification of SPNR. In addition, oxygen radicals occur by the activation of oxygen with heat and this could react with the double bond of the SPNR molecules.

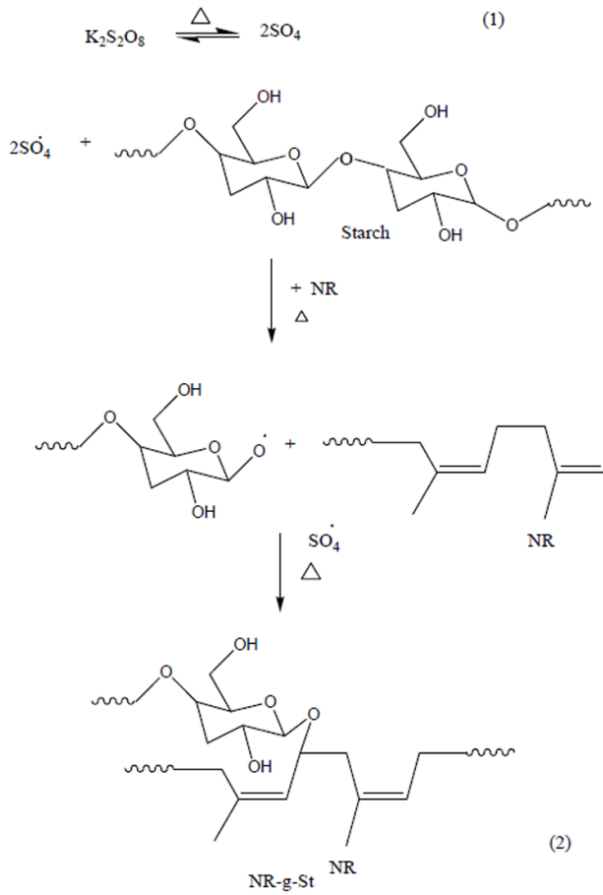

Figure 8. NR–g-St from natural rubber latex and starch by $K_2S_2O_8$ as an initiator.

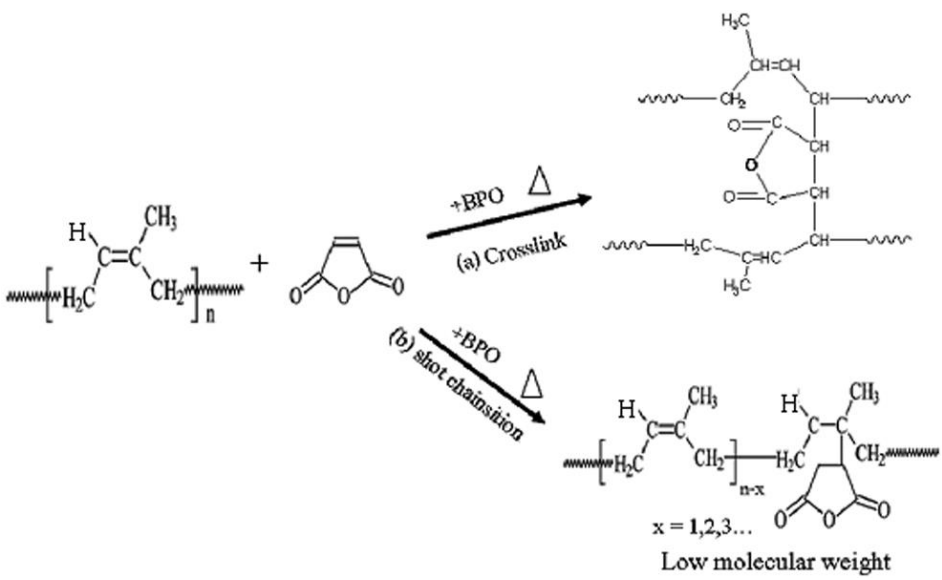

Figure 9. Possible mechanism for preparing maleated SPNR from SPNR latex and MA.

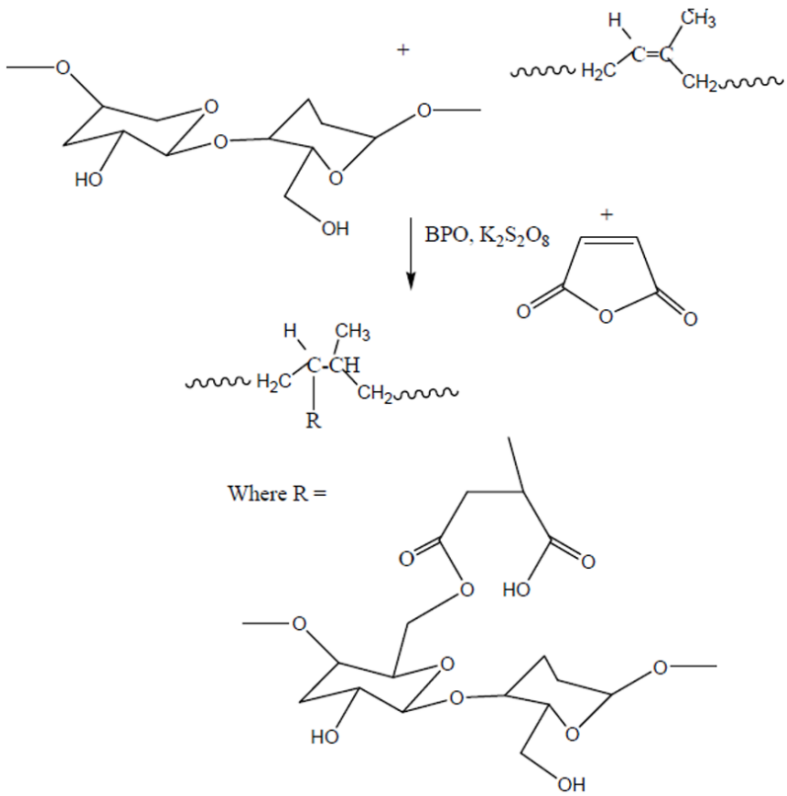

Figure 10. A possible mechanism of grafting maleated SPNR with cellulose in the presence of potassium persulfate.

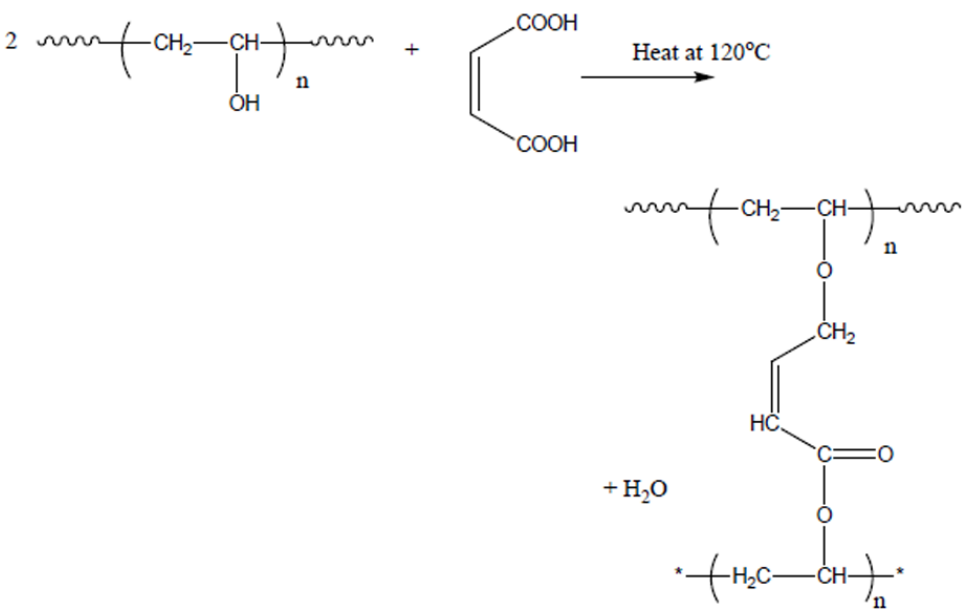

Figure 11. Possible reactions of crosslinked PVA at 120 ^{o}C (Gohil et al., 2006).

The possible mechanism of grafting M-SPNR with cellulose fiber by using BPO and $K_2S_2O_8$ at 80°C is presented in Figure 10. Free radicals from BPO attacked the carbon-carbon double bonds of MA, which then reacted with the carbon-carbon double bonds of the SPNR molecules activated by $K_2S_2O_8$ leading to the formation of M-SPNR. When the M-SPNR reacted with cellulose fiber, an M-SPNR-g-cellulose fiber was generated. In addition, the crosslinks between the M-SPNR and cellulose fiber might occur through MA bridging.

Polymer Blends

A polymer blend is a heterogenous material that consists of two polymers. The properties of NR are modified with a hydrophilic polymer such as PVA as described earlier. NR (Riyajan et al., 2008) or ENR (Riyajan et al., 2009a) latex was blended with 5%w/w of a PVA aqueous solution in the presence of different concentrations of maleic acid as a crosslinking agent. The possible chemical reaction of the blend between PVA and maleic acid at 120°C is displayed in Figure 11. After heating, the hydroxyl group of PVA is crosslinked with the carboxylic group from MA to form the crosslinked PVA through esterification.

The swelling of any polymer blend film in a solvent depends upon the diffusion coefficient of the solvent, the relaxation rate of the amorphous regions of the polymer chain and its degree of crystallinity. In polymer gels, there are two different categories, i.e., a physical gel and a chemical gel. In a physical gel the junction points of the network arise due to the physical bonding like Van Der Waals interactions, hydrogen bonding, the presence of crystallites, etc.; hence, in a good solvent such networks exhibit a large degree of volume change owing to the facile penetration of the solvent. The PVA selected for the present study possessed a degree of hydrolysis of 98%. The swelling ratio of a semi-interpenetrating sample could be practically used to approximate the crosslinking density in the sample. The swelling ratio of the maleic acid crosslinked PVA was based on the NR/PVA blend having different maleic acid contents but all were cured at 120ºC for 60 min and studied in water after keeping the samples immersed in solvents for 5 days. The polymer blend based on NR and PVA showed a lower swelling ratio after being cured with maleic acid (Figure 12), when compared with the uncured measurement. This indicated the chemical reaction that occurred between the PVA and maleic acid. When the amount of maleic acid increased in the blend, the swelling ratio of the polymer blend slightly increased. This was due to the solubility of the excess maleic acid. The swelling ratio of the sample in water decreased as a function of the amount of maleic acid in the sample as shown in Figure 13. The water resistance of the ENR/PVA blend system also increased with an increasing amount of maleic acid in the sample after curing. The swelling ratio of the semi-IPN ENR/PVA blend with 10% w/w maleic acid was 130%. When the maleic acid contents in the sample were increased from 10 to 30% w/w, the swelling ratio of the ENR/PVA blend was 80%.

The morphology of the ENR/PVA blend was observed under TEM as shown in Figure 14. The images show that the PVA molecules penetrated into the ENR particles. The tensile strength of the ENR/PVA blend was higher than that for the NR or ENR due to the increase in miscibility between the ENR and PVA. The properties of polymer blend were also improved after the addition of maleic acid to the blend. The tensile strength of the NR/PVA blends as a function of curing times and the maleic acid contents are presented in Figure 15. The results indicated that the tensile strength of the polymer blend based on the ENR/PVA blend

increased with the increasing maleic acid content in the blend. This might be used as evidence for the existence of chemical crosslinks between maleic acid and PVA. However, it was also clear that the tensile strength of ENR/PVA blend slightly increased with the curing time. This can be explained by a certain amount of cross-linking of the PVA being generated by activation of the double bonds of the maleic acid during curing. It should be emphasized that the tensile strength values of the polymer blend with maleic acid was high when compared to the polymer blend without the maleic acid.

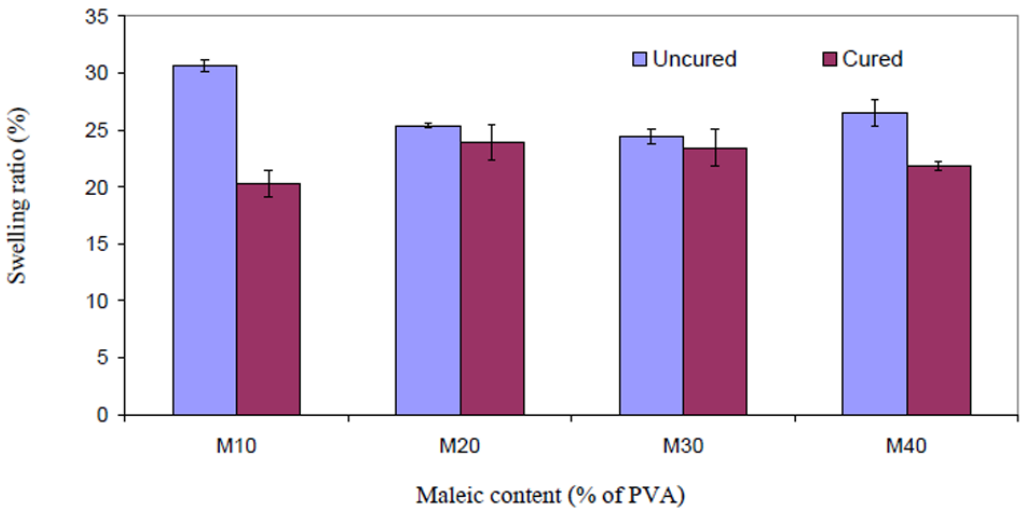

Figure 12. Swelling ratio of the NR/PVA blend with different maleic acids before and after vulcanizsation at 120°C in water.

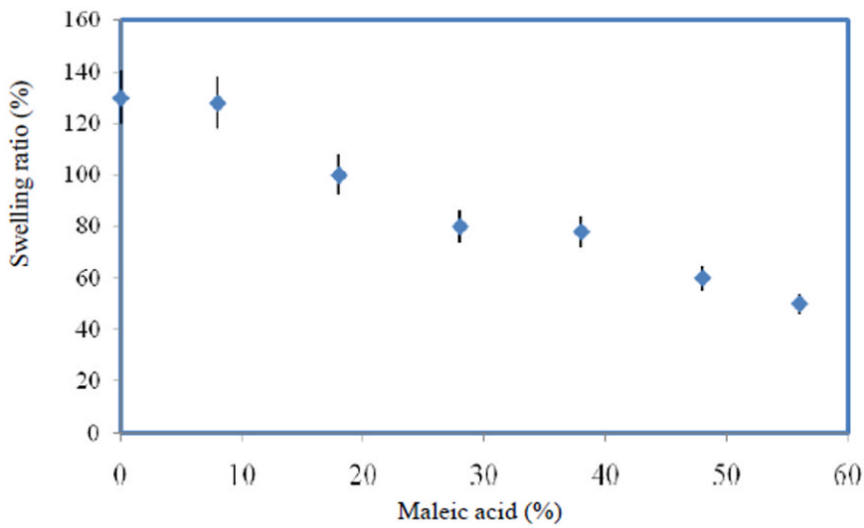

Figure 13. Swelling ratios of the ENR/PVA blends with different maleic acids after vulcanizsation at 120°C in water.

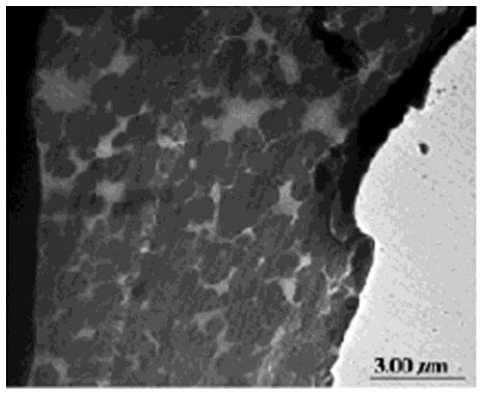

Figure 14. TEM images of ENR/PVA blends crosslinked with maleic acid (MA).

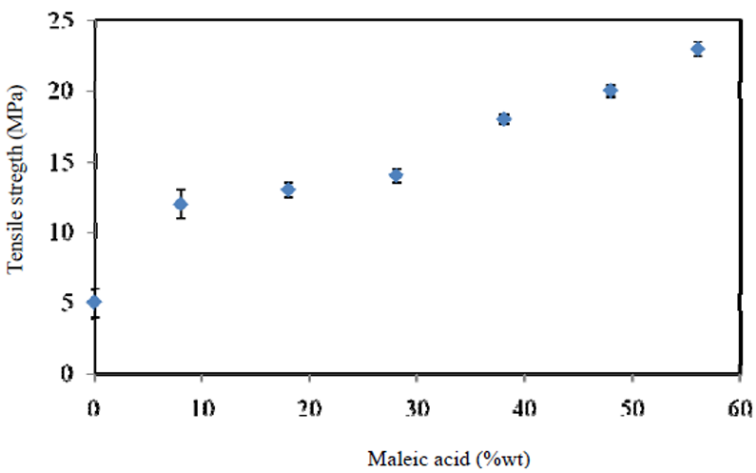

Figure 15. Tensile strength of semi-IPN based on ENR/PVA with maleic acid contents.

The polarity of ENR was improved by blending with polyethylene glycol (PEG) and St, which was then crosslinked with isophorene diisocyanate. The results showed that the tensile strength of the ENR/PEG blend increased with the starch (St) content and then decreased as a function of the St as shown in Figure 16. The highest tensile strength of the ENR/PEG blend was found to be at 20% St. This is due to a good chemical interaction between the polymers in the blends.

With an increase of the St content in the blend, the swelling ratio of the ENR/PEG blend crosslinked with isophorene diisocyanate dramatically decreased because of the chemical interaction between the polymer blends (Figure 17). The crosslinking density of the samples was estimated by the swelling ratio both in water and hot water. The swelling ratio of the maleic acid crosslinked blend, based on the ENR/PVA/starch blend having different maleic acid and starch contents, all cured at 120ºC for 60 min., was studied in water after keeping the samples immersed in solvents for 5 days. The blends, based on the ENR and PVA, in both the presence and absence of starch had a lower swelling ratio after being cured with maleic acid as shown in Figure 18. This result indicated that a chemical reaction occurred between the PVA or starch network with maleic acid.

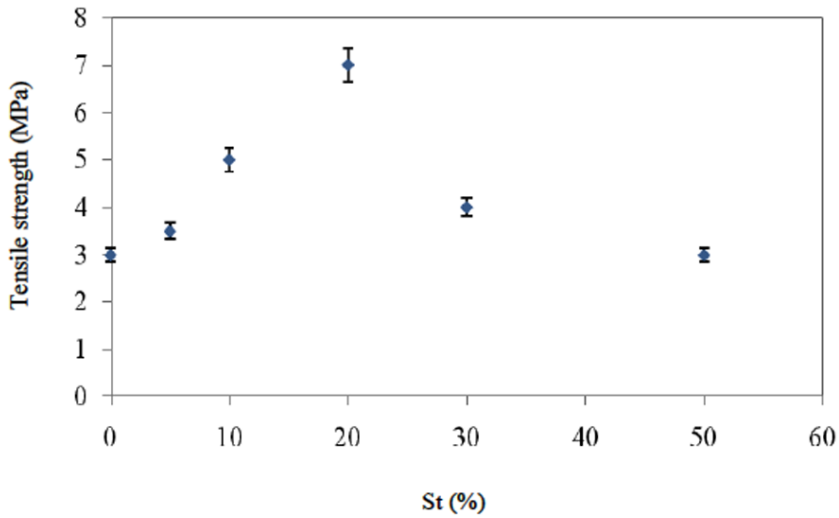

Figure 16. Influence of St on the %age swelling ratio of the ENR/PEG blend crosslinked with isophorene diisocyanate.

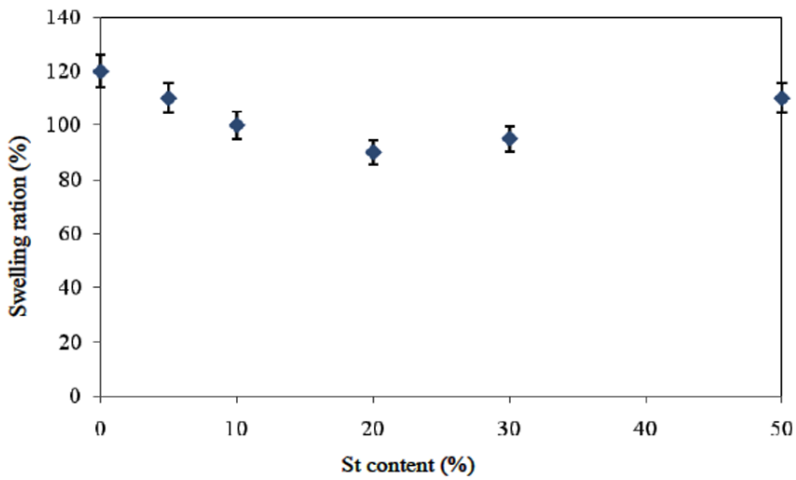

Figure 17. Influence of St on the % swelling ratio of ENR/PEG blend crosslinked with isophorene diisocyanate.

The crosslinking density of samples was estimated by the swelling ratio both in water and hot water.

In addition, this implied that maleic acid became crosslinked with starch and PVA after vulcanization. One explanation for this was that more crosslinking occurred between the maleic acid and starch or the PVA as the maleic acid content was increased up to 60%. When the water temperature was increased from 30^{o}C to 70^{o}C, the swelling ratio of the polymer blend sample containing 60% maleic acid increased compared to that at 30^{o}C after 5 h of immersion in the water medium (Figure 18). When the starch content in the sample was increased, a lower water resistance of the polymer blend sample was observed. After vulcanization, the swelling ratio of the polymer blend sample containing 60% maleic acid

dramatically decreased presumably because of the greater crosslinking between the maleic acid and the PVA or starch molecules.

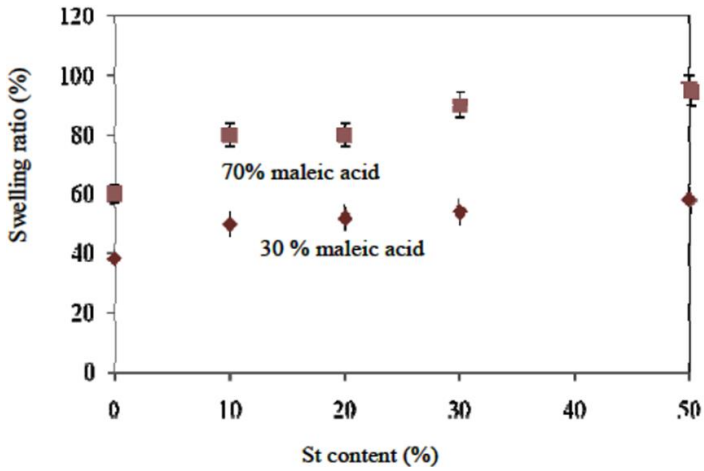

Figure18. Swelling ratio (%) of the ENR/PVA blend with different starch and maleic acid contents (30 and 70% w/w) in water.

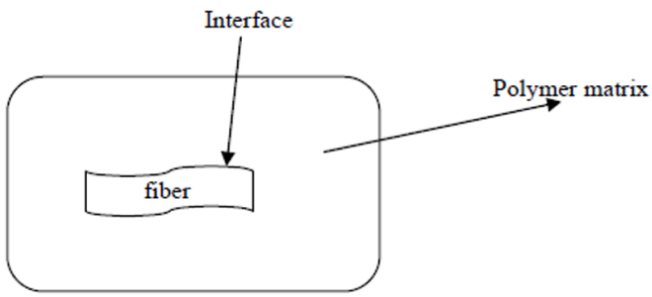

Figure 19. Model of a polymer composite composed of two main components including the polymer matrix and fiber.

Polymer Composite

The properties of NR can be modified by the addition of fiber when it is called a "composite". A polymer composite is a heterogenous material composed of two main components e.g., the matrix and a fiber (Figure 19).

The NR was mixed with sugar cane leaves (Riyajan et al., 2012), tea waste (Riyajan and Sukhlaaied, 2012) and baggasse fibers (Riyajan and Intharit, 2011). For example, the resulting composite beads could be used to remove Pb^{2+} from wastewater by absorption. This novel absorbent was prepared by blending an SA solution with a NR latex matrix and coconut waste (Cofiber). After being crosslinked by calcium chloride, the beads were highly stable, flexible, spherical shaped and easy to use in an environmental issue. The starch/natural rubber composite was prepared by blending the modified starch, which was modified by esterification with NR latex (Wang et al. 2009). The crystal structure of starch in the composite disappeared after the modification by esterification, and the starch particles with an

average size of around 200 nm was homogenously dispersed throughout the NR matrix. The enhanced thermal stability and mechanical properties of the modified St/NR composite was mainly due to the improved phase interface interactions between the rubber and starch. The NR was modified by grafting with the dimethylaminoethyl methacrylate (DMAEMA) to form a latex with cationic water-soluble polymeric 'hairs' of the polyDMAEMA, they acted only as a filler in the starch films, but with the modified NR, the mechanical properties of the films were significantly altered (Rouilly et al., 2004). The elastic modulus was greatly decreased and the strain at breaks greatly increased. Freeze-fracture TEM micrographs indicated that there was a strong interaction between the surface of the modified NRL and starch. The polyDMAEMA chains were more hydrophilic than the starch, and the addition of the grafted latex resulted in a 20° drop of the water contact angle of the formed film, and a 25% increase of the water absorption compared to the native starch; however the unmodified NRL, caused the opposite effect. A thermoplastic starch/NR polymer blend was obtained using natural latex and cornstarch with an intensive batch mixer at 1b50^{o}C, and an NR content that varied from 2.5 to 20% (Carvalho et al., 2003). There was a reduction in the modulus and the tensile strength, and the blends became less brittle than the thermoplastic starch alone. Increasing the plasticizer content made it possible to have a greater amount of rubber present. The influence of the silica and polymer blend ratio on the swelling ratio of the polymer blend in the presence of 10% starch in a water medium is shown in Figure 20. It is clear that the swelling ratio of the polymer blend dramatically decreased with an increasing silica content in the sample. This was because the chemical network structure formed by the combination between the SiO_2 with PVA or the St in the polymer blend prohibited the water molecules from dissolving and improved the water uptake of the film. When the NR was increased from 30% to 50%, the degree of the swelling ratio of the polymer blend decreased further. An explanation for this result was that the NR consisting of 1,4-*cis*-polyisoprene chains exhibited a hydrophobic behavior to prevent water uptake. Therefore, the swelling ratio of the polymer blend decreased.

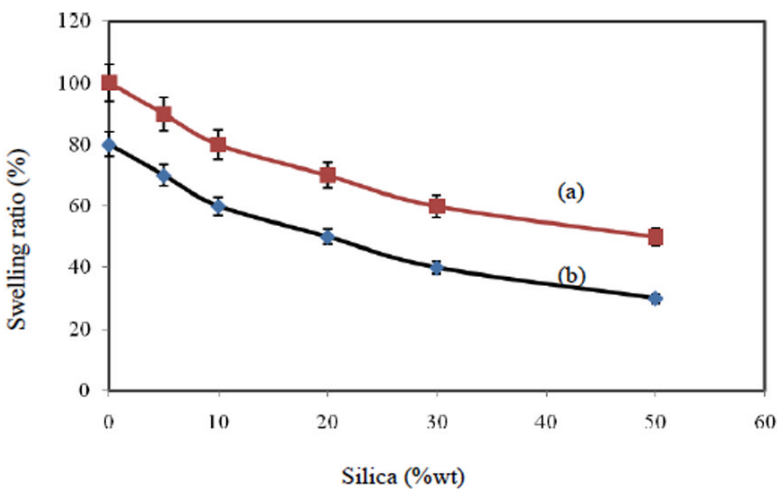

Figure 20. Swelling ratio (%) of the (a) 35:35:30 PVA/St/ENR blend and (b) 25:25:50 PVA/St/ENR blend with different silica contents in water.

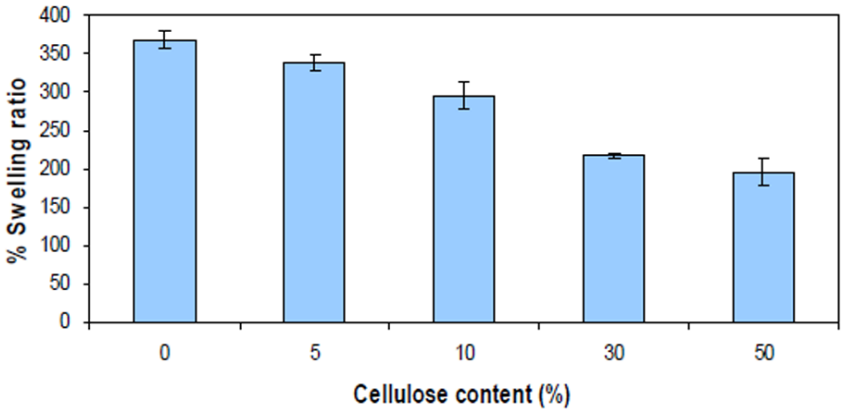

Figure 21. Swelling ratio (%) of the 8:2:3 PVA/St/ENR blend in water.

Figure 21 shows the effect of the cellulose fiber on the swelling ratio of the PVA/St/ENR blend. Before adding the cellulose fiber, the swelling ratio of the 8/2/3 PVA/St/ENR blend was 360%. It was clear that the swelling ratio of the polymer blend decreased as a function of the cellulose fiber content. This was due to the interaction between the hydroxyl groups from St, PVA and ENR and the cellulose fiber.

3. ENCAPSULATION

Encapsulation is a technique in which the reactive agents, such as a herb, and fertilizer were enclosed within some other materials such as a natural or synthetic material (Riyajan, 2011). Here, we used a modified NR for use as the polymer membrane (namely, encapsulation model and matrix model) for an encapsulated fertilizer (Figure 22). The product from this technique is a capsule consisting of two phases the active reagent and the polymer matrix. The active agents were the fertilizer and herb while the polymer matrix was the modified NR, as mentioned above.

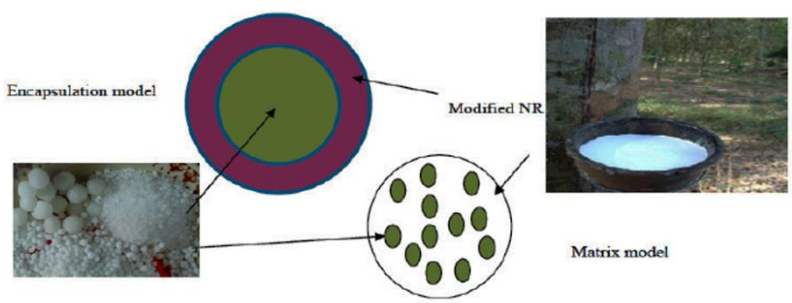

Figure 22. Model of a fertiliserfertilizer capsules product using natural rubber latex measured by a digital camera.

3.1. Method

The NR-g-St/urea mixture films were prepared by a solution-casting method. The graft copolymer was an NR-g-St latex 25 mL (solid 5.0 g) and urea (2.0 g). The mixture was stirred for 0.5 h at room temperature and then cast on a glass plate. In order to compare with the natural St matrix, the St/urea film was made by the following method: the starch 5 g and 25 mL of distilled water were placed in a glass beaker and stirred mechanically for 20–30 min to form a dispersion. The beaker was maintained in a thermostated oil bath at about 90^{o}C for 20–30 min while being stirred to gelatinize the starch. Then, the urea particles were thoroughly mixed with the gelatinized St paste with a glass rod. Finally the mixture was poured onto a glass plate (Chen, 2008) to form the film.

3.2. Preparation of Capsules by the Precipitation Technique

The urea granules were dissolved in concentrated NR latex which were then blended with the SA solution. Then the mixture was precipitated in a 90% aqueous solution of acetic acid using a dropper. The capsules that formed were quickly immersed in the distilled water in order to leach out acetic acid and then dried. The urea beads formed are shown in Figure 23 and are spherical in shape. The amount of urea released from the NR-g-St or starch matrix were measured at 191 nm by UV–Vis spectrophotometer (UV-1601, Shimadzu). About 1 g of capsule sample, weighed exactly, was extracted in distilled water to form a homogeneous solution. The total urea in the solution was extracted for 72 h with distilled H_2O and its mass was determined spectrophotometrically. At definite intervals of time, the conical flasks were shaken well and a 10-mL aliquots were taken for the analysis of urea using UV (Shimadzu UV-1601) at 191 nm. The experiments were performed in triplicate in order to minimize the error of variation. The cumulative release of urea from the capsule beads was estimated.

Figure 23. Photograph of fertiliserfertilizer beads obtained from natural rubber latex blended with the SA solution.

Table 1. Summary of literature review concerning encapsulation of fertiliserfertilizer by using rubber

Condition	Results	References
NR grafting cassava starch, latex, urea.	Rate of urea release, CSt content in graft copolymer.	Riyajan et al. (2012)
Creaming skim natural rubber latex, Blending with polystyrene, gamma radiation, Sodium alginate.	Non-spherical capsules containing a homogeneous urea dispersion.	Tangboriboonrat et al. (1999)
Rubber matrix type, the concentration of sodium alginate used as the capsule coating agent, and the initial concentration of urea.	The lowest rate of urea released from the capsules coated urea-unvulcanized rubber, from which the release was prolonged for ~ 50 days.	Tangboriboonrat and Sirichaiwat (1996)
		Helaly et al. (1993)
Styrene butadiene rubber ammonium nitrate.	The rate of releasing fetilizers from capsule was controlled by the concentration of fertilizer, temperature of the enviroment and pH medium. The urea release form capsule is prolonged for two months.	Helaly et al. (1990)
Urea-rubber matrices as slow-release fertilizers.		Hepburn et al. (1989a)
		Hepburn et al. (1989b)
Urea fertilizer by rubber. Processing and vulcanization procedures.		Hepburn et al. (1989c)

3.3. Encapsulated FertiliserFertilizer with Natural Polymer and Synthetic Polymer

It is well known that 40-70% of the nitrogen fertilizers applied to agriculture fields are lost so it has been suggested that slow release nitrogen fertilizers need to be developed to try to avoid losses. Previous workers have studied the use of natural polymers e.g., cellulose (Riyajan, 2009), starch (Riyajan, et al., 2012), and sawdust as polymer membranes for the encapsulation of the fertilizer. A brief literature review is summarized in Table 1. Among them, natural rubber (NR) has been widely used as a polymer membrane for controlling the release of fertilizer because it has good water resistance.

Tangboriboonrat and co-workers produced an encapsulated urea fertilizer using the natural rubber latex (Tangboriboonrat and Sirichaiwat, 1996) and skim natural rubber latex (Tangboriboonrat et al., 1999) for the controlled release of urea. They studied many factors that affected the release rate of urea such as the type of rubber matrix, the concentration of SA used as the capsule coating agent, and the initial concentration of urea. The maximum efficiency of the encapsulated urea in capsules was 80%. After the capsule coating was made using unvulcanized rubber, the lowest rate of urea released from the capsules was achieved using coated urea-unvulcanized rubber. The completion of the urea release from the capsule was roughly 50 days. In the case of the skimmed NR latex, the creamed skimmed latex was blended with 15-45 phr SA and then its blend was used for encapsulation of urea fertilizer. They reported that the non-spherical capsules contained a homogeneous urea. They used SEM to try to explain why there was a need to use a split feeding mixing technique to

properly encapsulate the urea particles to produce the controlled release of the fertilizer from the bead matrix (these reasons have not been provided here). The lowest rate of urea released from the matrix was found in the sample with the highest crosslinked density (Tangboriboonrat and Sirichaiwat, 1996).

Fertilizers were entrapped in styrene-butadiene rubber as the polymer matrix for the controlled release of fertilizers (Helaly et al., 1993). The rate of releasing fertilizers from the capsule was controlled by the concentration of fertilizer, the temperature of the enviroment and the pH of the medium. The urea released from the capsule was prolonged for two months. After the addition of clay to the capsules, there was a decrease in the release rate of the nitrogen. The effect of the filler on the apparent activation energy of the amount of ammonium nitrate released into the water was also reported. After the urea was encapsulated in the rubber matrix, the release of urea from the capsules increased with temperature. This was due to diffusion, which allowed for a temperature-dependence of both the diffusion coefficient in water and the saturated concentration of urea. The rubber matrix may prevent both the high leaching losses and the seedling damage associated with a high concentration of free urea. The effect of the size of the encapsulated urea bead on both their release characteristics in moist soil and N-supply to plants was also reasonably well predicted by the diffusion model. At the same time, the slow-release form of urea was apparently able to supply nitrogen to plants over the span of a full growing season.

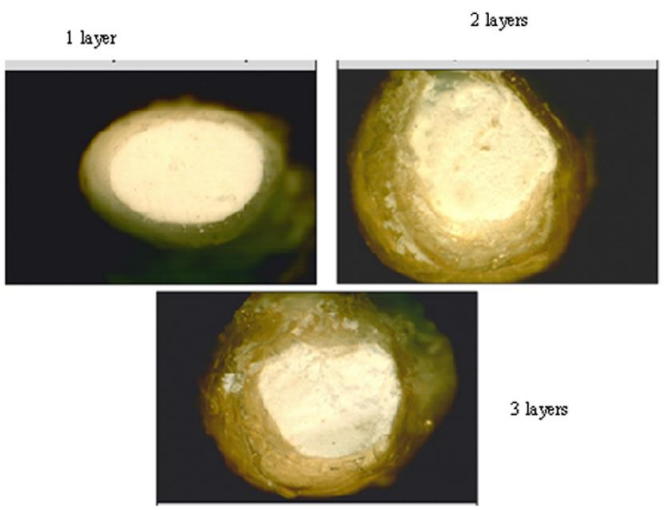

Figure 24. Photographic images of the urea capsule coated with different layers of modified PVA.

3.4. Encapsulated FertiliserFertilizer with the Modified PVA

The urea released from the beads was subjected to a number of physical and chemical parameters including those related directly to the release from the beads into the water medium, the release conditions (temperature) and those resulting from changes to the characteristics of the controlled release device (beads). The photographic images of the urea capsule coated with the modified PVA (DPVA) at different layers are provided in Figure 24.

The effect of the different coating layers of DPVA on the release rate of urea from beads is presented in Figure 25. These curves show the release of neem from the capsules, which were controlled by the number of NR coating layers and the crosslinked density. It is clear that with an increase in the NR coating layer from 1 to 3 layers, the rate of diffusion of the fertilizer from the beads greatly decreased. After washing with water, the urea was exposed to the surface of the DPVA films and could be completely removed. By measuring the urea concentration in the water, the urea released from the films could be determined using UV-Visible spectroscopy. The release profile from urea without the (DPVA) coating is also shown for comparison. It is obvious that the urea release rate was reduced significantly by the DPVA coating, which is consistent with the results of the swelling study. The DPVA film is very strong, rigid and hard to swell, so the diffusion through this coating is the rate limiting step for swelling and urea release. The release was prolonged by the addition of any DPVA layers on the capsule surface. The cumulative release of urea from the capsule was derived from the immersion times of 2, 24, 72 and 240 h in aqueous medium and found to be 31, 69, 81 and 100%, respectively. When the DPVA coating on the capsule was increased from 1 to 3 layers, the cumulative release of urea from the capsules that had been stored under the same conditions was 8, 29, 36 and 60%, respectively. It is to be noted that with an increase in the DPVA coating, the capsule matrix became denser and resulted in a decrease in the rate of urea diffusion through the swollen beads, especially the beads with the third-DPVA coating. This result was in agreement with Chen and co-worker (Chen, 2008). They found that St-g-poly (L-lactide) (PLLA) (Chen, 2008) was an effective material for encapsulating urea for controlling its release. The St-g-PLLA exhibited a relatively low swellability, a large encapsulating capacity, and a slow-release rate and the water-resistance of the matrix could be improved by increasing the PLLA graft efficiency on the St granules.

3.5. FertiliserFertilizer Encapsulated with the PVA/St/NR Blend

The PVA/St/NR blend was applied as a polymer membrane to encapsulate the fertilizer. The problems of urea are runoff and leaching to the environment and vaporization, leading to low utilization efficiency for the plants as previously mentioned. When the PVA/St/NR blend was used as the matrix to encapsulate the urea particles, the encapsulated urea with this polymer blend still remained strong and was resistant to water. Thus, the swelling ratio of the PVA/St/NR blend was also estimated and results are shown in Figure 26. The swelling of the polymer blend decreased as a function of the NR content. The lowest value of the swelling ratio of the sample was found in the sample in the presence of 5%w/w NR. These phenomena may perhaps be explained by the difference in swellability of PVA/St/NR blend matrices at high NR because the PVA/St/NR blend with a low NR could be easily swollen by water. The urea in the swollen PVA/St/NR blend at a low NR value can diffuse rapidly and can be released quickly. After modifications of the PVA, both the swellability of the matrix films decreased so that the release rate of the urea was reduced with the increasing NR content portion in the blend. After the preparation of the polymer blend, it was used as a polymer membrane for controlling the release of the fertilizer. The urea released from the beads was subjected to a testing of a number of physical and chemical parameters including those directly related to the release medium, the release conditions (temperature) and those resulting from changes in the characteristics of the device to control the release (beads). The

effect of the release rate of urea from the beads coated with the different layers of PVA/St/NR blend is presented in Figure 27. It is obvious that the release rate for urea was reduced significantly by an increase in the modified PVA coating layer, which is consistent with the results of the swelling study. The modified PVA film is very strong, rigid and hard to swell, so the diffusion through this coating is the rate-limiting step for the swelling and urea release. These phenomena may perhaps be explained by the difference in swellability of the PVA/St/NR blend matrices as shown in Figure 26. The bead coated polymer blend with a thin coating layer can diffuse rapidly and be quickly released.

The cumulative release of urea from the capsule was derived from different immersion times at 3, 10, 20 and 30 h in an aqueous medium. The release rate was 8, 60, 81 and 95%, respectively. When the polymer blend was coated onto the capsule with an increase of 1 to be 3 layers, the urea was cumulatively released from the stored capsule was 2, 29, 59 and 82%, respectively, under the same conditions. It must be noted that with an increase in the PVA/St/NR coating, the capsule matrix became denser, which resulted in a decrease in the rate of diffusion of urea through the swollen beads, especially the beads with a third-modified PVA/St/NR blend coating. This result supported the results of Li Chen and co-worker (Chen, 2008). The St-g-PLLA exhibits a relatively low swellability, a large encapsulating capacity, and a slow-release rate. The water-resistance of the matrix could be improved by increasing the efficiency of the PLLA graft onto the St granules as described above.

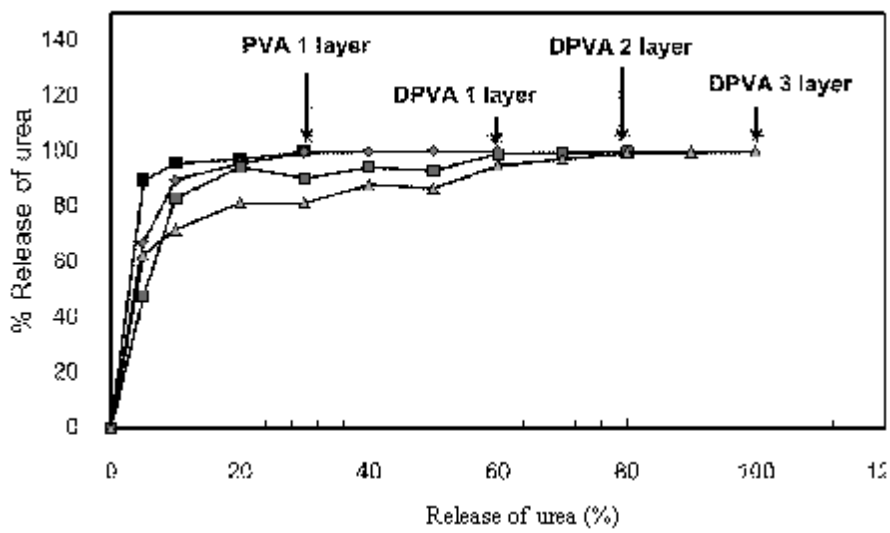

Figure 25. Percentage of the cumulative urea release from beads coated with PVA and DPVA.

3.6. Encapsulated FertiliserFertilizer with NR-g-St

First, the urea exposed on the surface of the bead could be removed completely by washing with water. It was clear that the urea release rate was significantly reduced by the NR/St blend coating, compared to the St coating, which is consistent with the results of the swelling study as displayed in Figure 29. The NR/St blend membrane is very strong and hard

to swell in water, thus the diffusion through this coating is the rate-limiting step for swelling and urea release. These phenomena may perhaps be explained by the different swellabilities of the NR/St blend matrices since St can easily swell with water. The urea in the swollen St can diffuse rapidly and can be released quickly due to weak interactions between the urea and the St membrane. The urea cumulatively released from the capsules coated with St was almost complete within 6 h (Figure 30). After the NR was grafted with St, the urea released from the beads dramatically decreased compared to the other samples as observed from the % urea cumulative release. This is due to the graft copolymer between the NR and the St, which has a very strong water barrier. When the NR/St blend and NR-g-St was coated onto the capsule, the cumulative urea released from the capsule stored under the same conditions for 48 h was 85 and 60%, respectively. In conclusion, it should be noted that with the NR-g-St coating, the capsule matrix becomes denser and resulted in a decrease in the rate of diffusion of urea through the swollen beads due to the chemical interaction between the NR and the St through the grafting interaction. This result is supported by our previous work (see section 3.5).

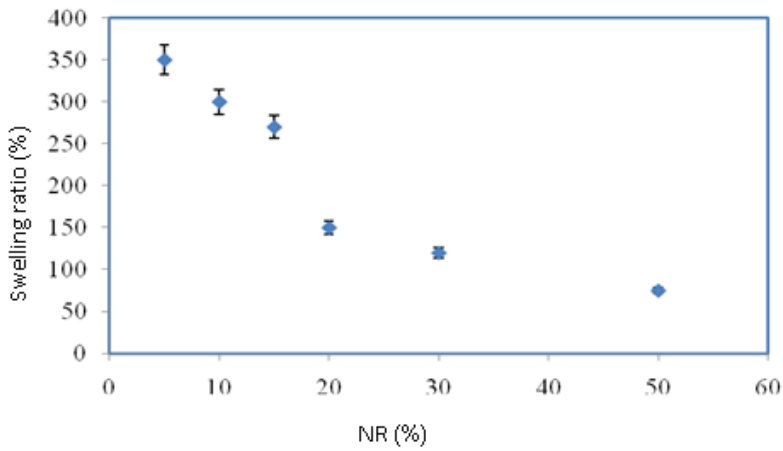

Figure 26. Swelling ratio (%) of the 5:5 PVA/St blend with different NR contents in water.

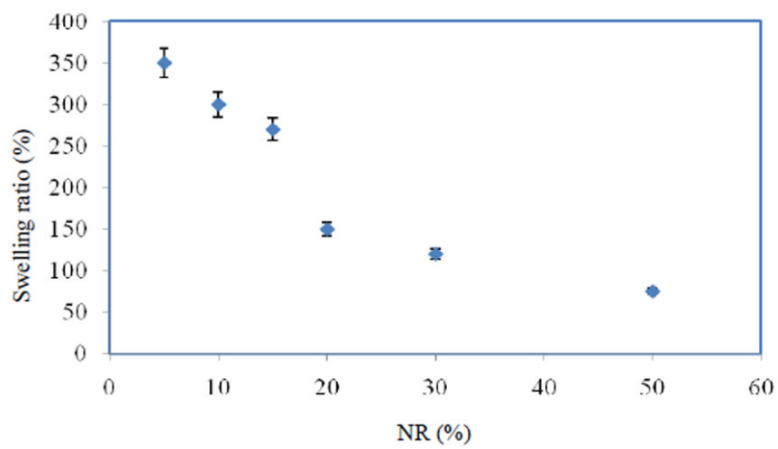

Figure 27. Cumulative amounts of urea released (%) from the capsule with a modified PVA coating layer.

The mechanism of encapsulation of the urea is described in Figure 31. The NR-g-St has a core-shell structure with a hydrophobic NR as the core and the hydrophilic grafted St as a shell. The hydrophobic NR core formed a wall-like barrier inside, while the hydrophilic starch part and the urea particles were encapsulated inside the matrix (Figure 30).

The mechanism of encapsulation of the urea is described in Figure 31. The NR-g-St has a core-shell structure with a hydrophobic NR as the core and the hydrophilic grafted St as a shell. The hydrophobic NR core formed a wall-like barrier inside, while the hydrophilic starch part and the urea particles were encapsulated inside the matrix (Figure 30).

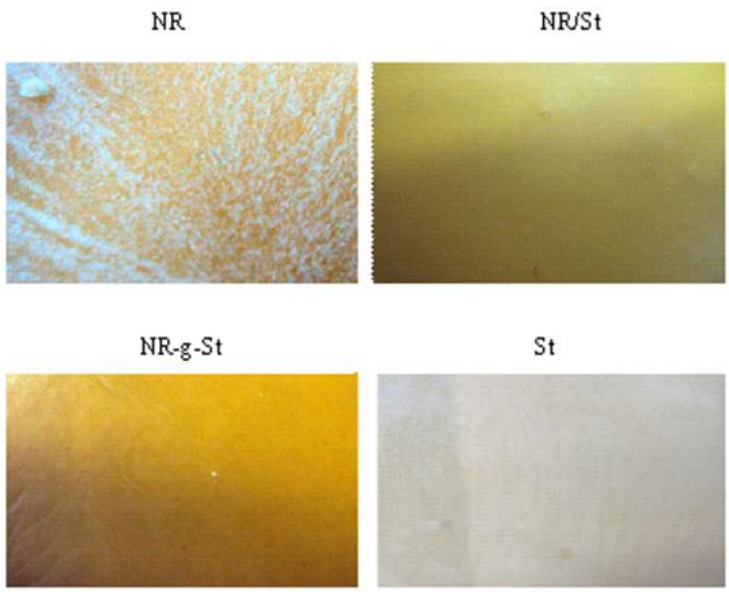

Figure 28. Photo images of the capsules coated with different polymers NR, NR/St blend, NR-g-St and St.

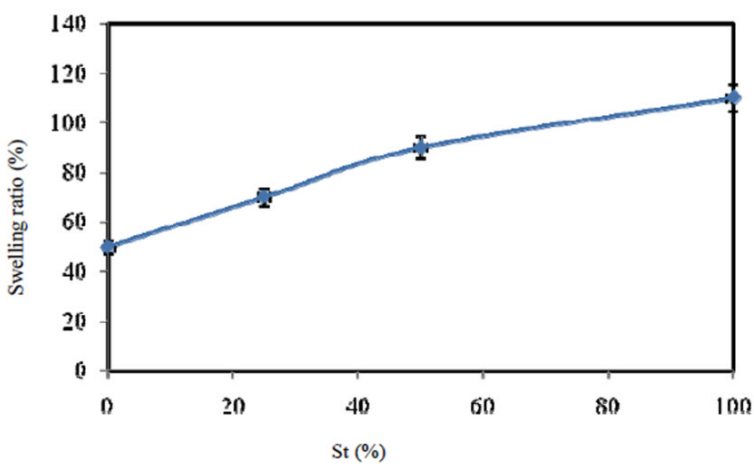

Figure 29. Swelling ratio (%) of the 5:5 PVA/St blend in water with different NR contents.

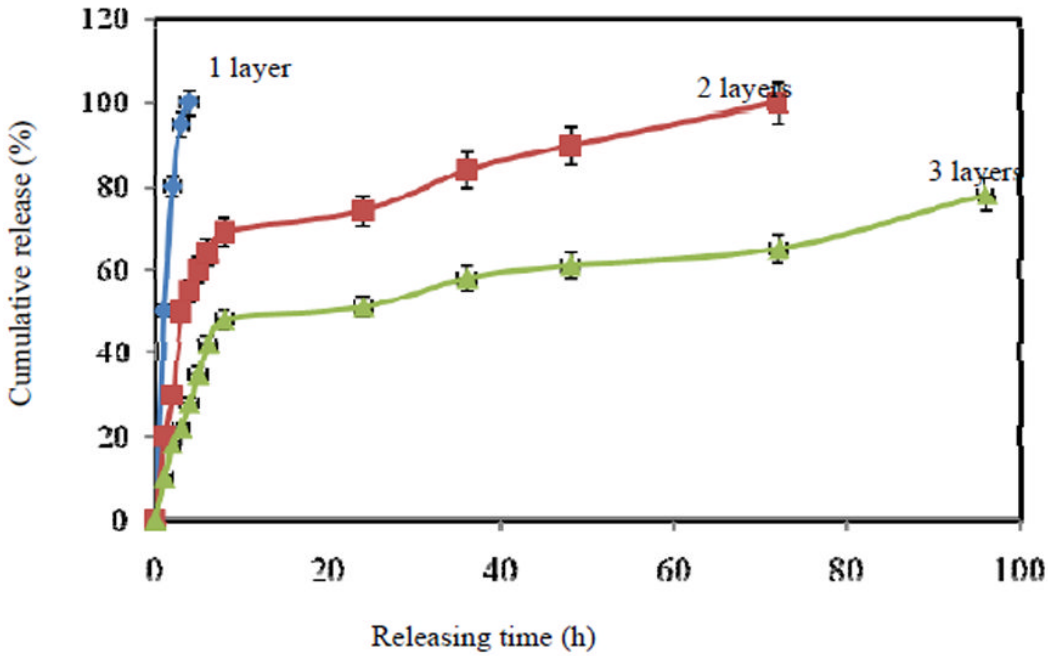

Figure 30. The cumulative urea released (%) from the capsules coated with different polymer types.

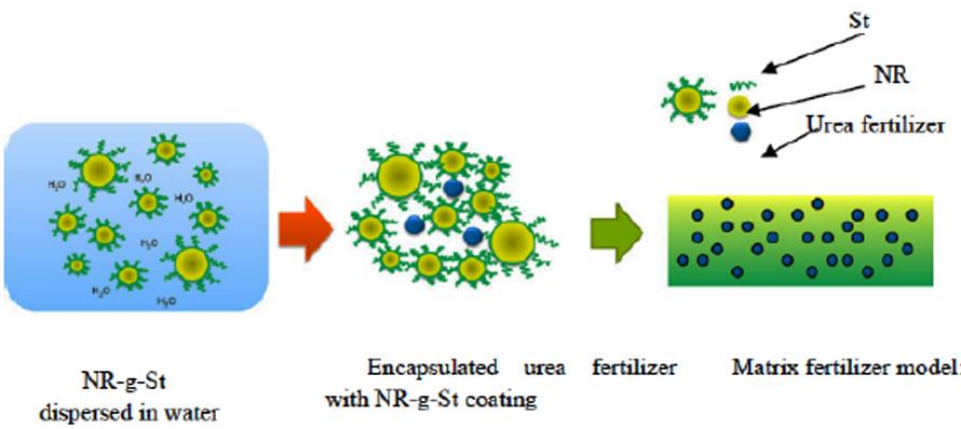

Figure 31. Possible mechanism for encapsulation of the urea fertiliserfertilizer with NR-g-St.

3.7. NR-g-CPAM

Cationic polyacryamide (CPAM) was successfully grafted onto the NR molecule in its latex form by introducing the initiator-KPS to the NR latex particles in addition to the CPAM solution. ATR-FTIR spectroscopy revealed that the CPAM was chemically attached to the NR particles, as observed by the bands at 3300~3500 cm^{-1} that refer to amine groups and at 1659 and 952 cm^{-1} and the amide quaternary and ammonium groups. In addition, the chemical structure of NR-g-MCPAM was confirmed at 180 (carbonyl group) and 176 (ester group) ppm by using solid state ^{13}C NMR. The thermal stability of the NR and CPAM was

significantly altered after modification with CPAM due to the grafted copolymer as observed from the TGA. The benefits of this absorbent are that it is highly stable, flexible and easy to use in the environment. The effect of NR-g-CPAM on the rate of release of urea from the beads coated with different layers is presented in Figure 32. It is obvious that the rate of urea release was reduced significantly as a function of the NR-g-PAM coating, which is consistent with the results of the swelling study. The NR-g-PAM film is very strong, rigid and hard to swell, so the diffusion through this coating is the rate-limiting step for swelling and release from the NR-g-CPAM. Using additional NR-g-CPAM layers on the capsule surface prolonged the release. The cumulative urea released from the capsule with a 1 layer coating, produced after 5, 10, 30 and 90 h immersion in aqueous medium, was 25, 72, 80 and 90%, respectively. It must be noted that with an increase in the NR-g-CPAM coating, the capsule matrix becomes denser resulting in a decrease in the rate of urea diffusion through the swollen beads, especially the beads with the third NR-g-CPAM coating. The possible mechanism for the release of urea from the capsule is displayed in Figure 33. There could be many methods for the release of urea from the capsule e.g., erosion of the polymer, diffusion and stress driven. After the beads were immersed in a water medium, the urea fertilizer was diffused from the beads due to the swelling of the wall of the water barrier.

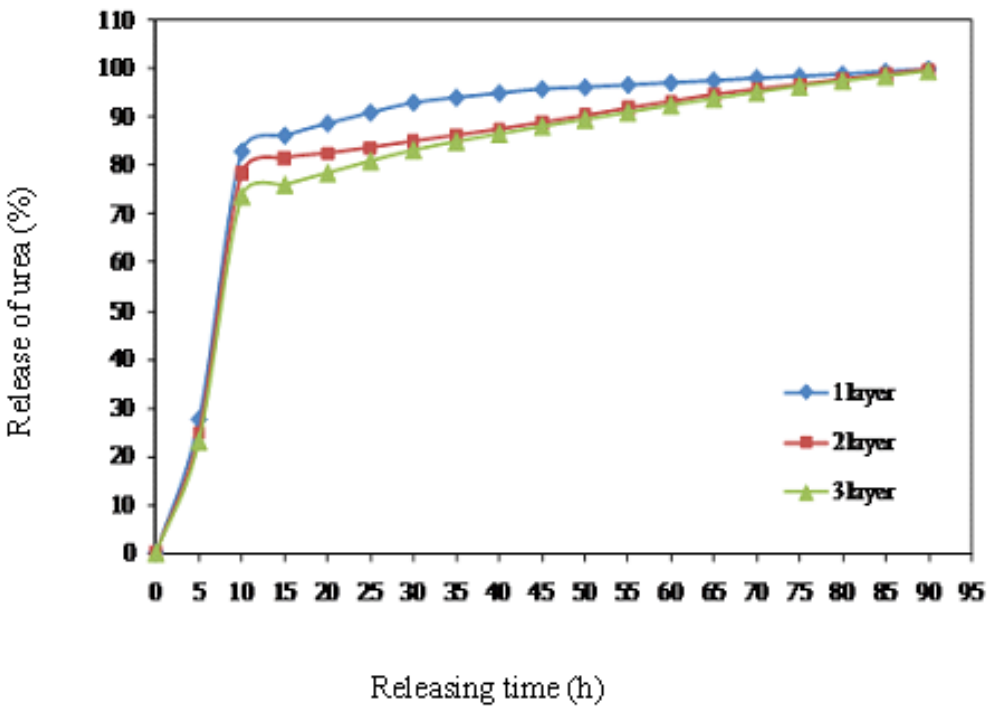

Figure 32. Relationship between the release rate of the urea fertiliserfertilizer and the release time from the beads obtained from different coatings.

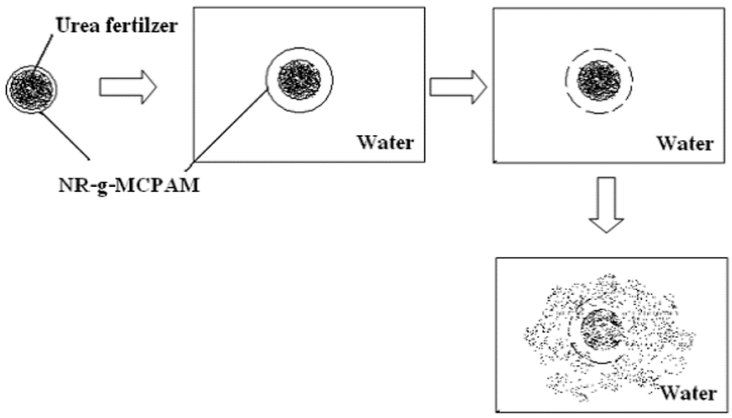

Figure 33. Possible mechanism of the release of urea from the encapsulated urea fertiliserfertilizer with NR-g-CPAM in a water medium.

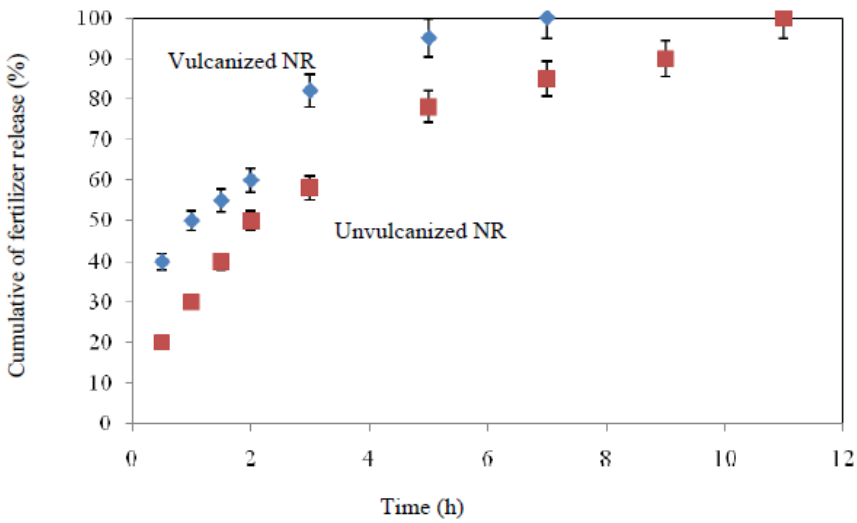

Figure 34. Effect of vulcanizsation of NR latex on the % cumulative loss of urea fertiliserfertilizer in from beads stirred in water medium.

3.8. Effect of Unprevulcanizsed or Prevulcanizsed NR

This part describes the effect of crosslinking the NR with sulfur to form vulcanized rubber on the cumulative release (%) of urea from the beads compared to an unvulcanized sample and the results are provided in Figure 34. It is obvious that there was a high rate of urea released from the two samples within 9 h. The rate of urea released from the capsule, coated with unvulcanized NR, was higher than those coated with unprevulcanized NR. One explanation for these results was that the unvulcanized NR showed a special ability to wet the urea during the production of the bead. This phenomenon resulted from a poor dispersion of urea in the vulcanized NR matrix and hence it more easily diffused from the bead in the water medium. These results are in agreement with a previous work (Tangboriboonrat and

Sirichaiwat, 1996). This was explained in section 3.3. They found that after the capsule was coated with unvulcanized NR, the lowest rate of urea released from the capsules was achieved using the urea coated by unvulcanized rubber compared to the non-vulcanized NR as the polymer matrix and the complete release of urea from the capsule was roughly 50 days. Another study, by Hepburn and co-workers (Hepburn, et al., 1989a) studied the experience of incorporating the urea in unvulcanized NR and vulcanized NR matrix by using the two-roll mill method. They reported that the rate of urea released from beads made with unvulcanized NR was faster than that of beads made from vulcanized NR due to the different dispersions between the urea and the rubber matrix.

4. BIODEGRADATION

NR, biopolymers can be degraded by aerobic soil bacteria mainly in the order Actinomycetales and genus Streptomyces. The initial attack occurs at the C=CH bonds to produce C=O and CHO groups by radical-generating enzymes (Figure 35). Attack by these enzymes is often restricted by poor access to the substrate probably because of the hydrophobicity of the polyisoprene chains. When NR is modified chemically, or by mixing with a biopolymer, the enzymes have more access to the C=CH bonds so the rate of degradation is often enhanced. There is very little information yet on the further degradation pathways to CO_2 and H_2O but they are likely to involve the normal β–oxidation metabolic pathways. Three different enzyme mediator systems, consisting of radical-generating enzymes and their substrates, acting as radical precursors have been investigated with regard to the biodegradation of polyisoprene and rubber material. Although these enzymes may have a different physiological function, these studies have demonstrated that biochemically generated radicals are capable of attacking C=CH bonds in the polyisoprenoids. After use, the polymer membrane from the capsule can be degraded in natural soil. In this experiment, water (50 mL) was poured into the soil every week for 1 month. Each week, the specimen was carefully taken out, washed with distilled water and dried at 45°C for 2 days before being weighed. The % weight loss of the residual sample was calculated from the equation (2).

$$\%\text{Weight loss } (\%) = \frac{(W_e - W_1)*100}{W_1} \qquad (2)$$

Where W_e is the weight of the residual sample after being buried in soil for various times. Until fairly recently the mechanisms involved in the degradation of natural polyisoprenoids have been mostly unknown but now there has been considerable progress in our understanding of microbial rubber degradation. Analyses of the degradation products have indicated a possible biodegradative pathway for this abundant and technically vital polymer. Two nonhomologous enzymes involved in this process and their respective genes were recently identified in species of the genera Streptomyces and Xanthomonas. This should allow for more detailed molecular and biochemical studies to determine the mechanisms by which natural and synthetic polyisoprenoids are degraded. These enzymes have been studied in the mycelium-forming actinomycetes in the genera *Streptomyces actinoplanes* as well as in*Micromonospora* (Jendrossek et al., 1997; Linos et al., 2000; Linos and Steinbüch et al.,

2002) and *Nocardioform actinomycetes* in the presence of *Gordonia mycobacterium* and *Nocardia* (Arenskötter et al., 2004). In the case of Gram-negative bacteria or proteobacteria, there has been one *Xanthomonas* sp. strain 35Y and *Pseudomonas aeruginosa* AL98 (Linos et al., 2000; Jendrossek and Reinhardt 2003).

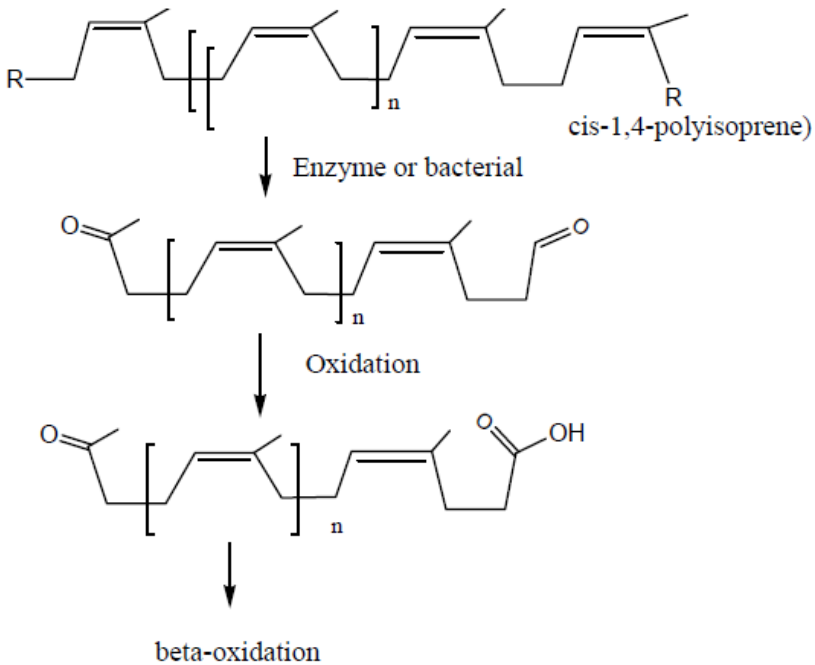

Figure 35. Possible biodegradation pathway for *cis* 1,4-polyisoprene (Linos et al. 2000).

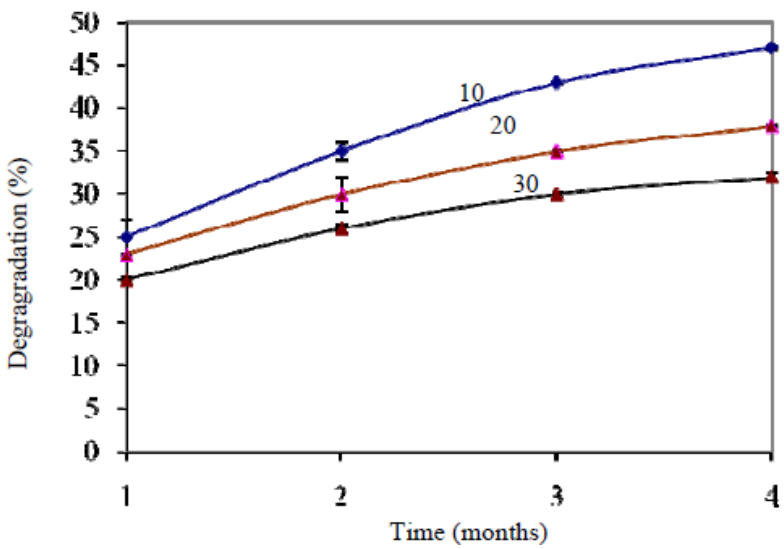

Figure 36. Biodegradation of ENR crosslinked with 9% GA at 10, 20 and 30 min of immersion time in soil.

The decomposition of the cured ENR samples, immersed in 9% w/w GA solution for 10, 20 or 30 days buried in soil was investigated and the percentage of weight loss as a function of buried time was determined as presented in Figure 36 (a), (b) and (c), respectively. The results showed that the weight loss (%) of the cured ENR dramatically decreased with an increase of burial time. The rate of biodegradability of cured ENR for 10 days was faster than that of ENR cured for 20 and 30 days (Figure 36). It was explained that the higher cross-linked density of the cured ENR in the latter cases impeded the penetration of bacteria into the sample. It is well known that high molecular weight NR (~10^6) *cis 1,4*-polyisoprene is responsible for the more difficult biodegradation compared to St (Riyajan et al., 2012a). NR can be slowly degraded in nature by specific microorganisms i.e., 31 Streptomyces strains, 5 Micromonospora strains, 3 Actinoplanes strains, 2 Nocardia strains, 1 Dactylosporangium strain, and some fungi (Bhatt et al., 2008). The total aerobic activity began by splitting the polymer chains at the C=CH bonds followed by the metabolism of the depolymerization products with mixed microbial populations leading to their eventual mineralization (Bhatt et al., 2008). Certainly, the biodegradability of rubber by microorganisms decreases after curing (Jendrossek et al., 1997). As a result of the ester linkage between ENR and GA, ENR cured with GA would be easier to decompose by microorganisms and hydrolysis reactions in the soil compared with those cured with sulfur. The natural soil environment contains fungi, bacteria and moisture (Jacob et al., 2004). The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the modified NR. In addition, the moisture in natural soil and the added water at weekly intervals for 3 months allowed the degradation process to penetrate into the samples. Three physical forces: physical, chemical, and biological modify the polymer surfaces and create new surfaces for reacting with chemical and biochemical agents, a critical phenomenon for the degradation of solid polymers (Kamal and Huang, 1992). Depolymerization of many biopolymers such as starch, cellulose, and hemicelluloses (Kamal and Huang, 1992) can be initiated by the many different microorganisms in the soil, which can produce many different enzymes (Alexander, 1977). The cellulose fibers and St in the NR composites are therefore more easily degraded than NR and are the first to be hydrolyzed before the microorganisms can utilize the NR as a nutrient source (Abrahama et al., 2012).

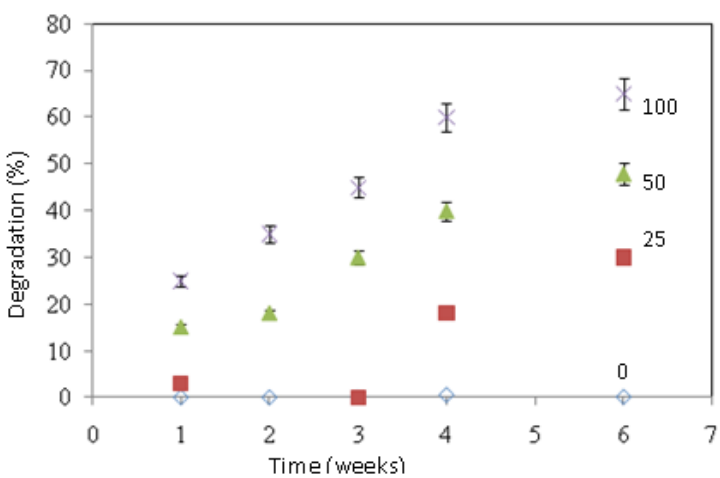

Figure 37. Degradation (%) of NR, NR-g-St with different St (%wt) in soil.

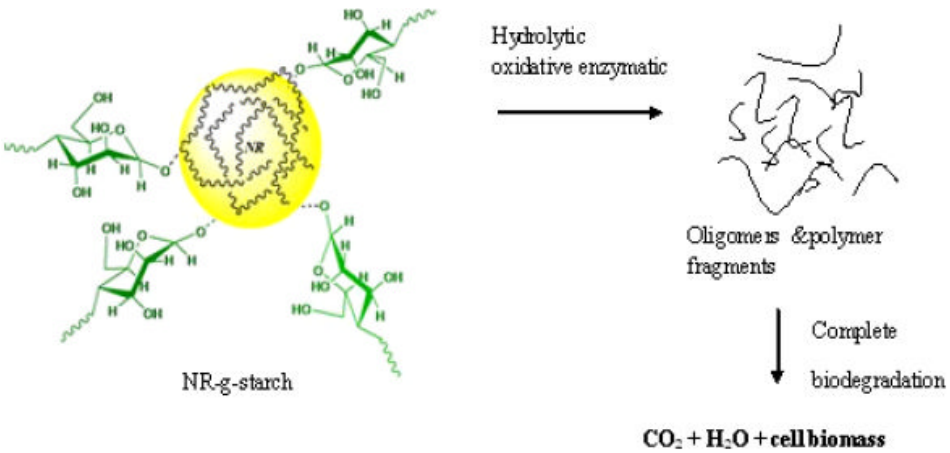

Figure 38. Possible mechanism of biodegradation of NR-g-St in soil.

In addition, the oxirane groups of ENR can play an important role in enhancing the biodegradability (Yew et al., 2006). In addition, the biodegradation of ENR cured for 20 min was lower than that cured for 30 min due to the higher crosslinking in the sample. These results agree with the swelling result. Samples of the NR-g-St were buried in soil and water was sprayed onto the soil every 5 days. The weight of the samples was then measured every 7 days to establish the reduction in weight. The effect of the St content on degradation is shown in Figure 37. It was found that the degradation increased with an increasing St content because St is a biopolymer, which allows it to easily degrade naturally. It is obvious that the rate of biodegradation of NR is low compared to St. The NR used in this experiment was obtained from the latex of *Hevea brasiliensis,* which is a high molecular weight polymer of *cis*-- 1,4 polyisoprene as described above**.** It is apparent that the degradation of the NR-g-St was greater with a higher St content as observed from both the weight loss and the color change. The rate of degradation of the NR-g-St increased dramatically within 1 month. However NR, which degrades in nature due to the action of specific microorganisms, is a slow process and the growth of bacteria utilizing rubber, as their sole carbon source is also slow. The possible mechanism of degradation of NR-g-St is represented in Figure 38. The enzyme from microorganisms first destroy the chemical structure of the modified NR that provide the short polymer chains, which are enough to permeate through the cell walls to be utilized as carbon and energy sources, namely Depolymerization. After the end of biodegradation, the by-product of biodegradation of the specimen is CO_2 and H_2O, or methane if the process is anaerobic, and also results in an increase in microbial biomass. The different end by-product that occurs depends on the degradation pathways as shown in Figure 38.

CONCLUSION

Between 40-70% of the nitrogen fertilizers applied to agriculture fields are lost from the farmlands. This could be significantly reduced by the use of slow release nitrogen fertilizers. Thus, the encapsulation technique, using a modified NR, may help to solve this problem.

Factors such as modification methods for NR, the absolute NR content, number of NR layers, and the ratio between NR and the biopolymer on the release rate of urea from capsules obtained from coating were subsequently investigated. The properties of NR were improved by chemical modification, e.g., crosslinking, grafting and epoxidation reaction. In parallel, NR was blended with other polymers such as PVA, St or SA, and cellulose fiber to increase its agricultural use. There are at least two methods for the preparation of encapsulated fertilizer, e.g., a solution-casting method and a precipitation technique. The rate of fertilizer release from the capsule decreased with an increase of the NR coating layers and the NR concentration in polymer membrane and polymer blend ratios. The swelling ratio for this sample corresponded to the fertilizer release rate from the capsule in an aqueous phase. When the capsules were coated with the modified NR latex, it was found that the release rate of the urea was significantly decreased and the duration of release was prolonged for roughly 30 h in an aqueous medium. In addition the urea in the swollen polymer blend with a low amount of NR can diffuse rapidly and can be released quickly. A graft copolymer between NR and a biopolymer for coating, the capsule matrix of the bead becomes denser resulting in a decrease in the rate of diffusion of urea through the swollen beads due to chemical interactions between the NR and the biopolymer through the grafting interaction. The rate of urea release from the capsule coated with unvulcanized NR was higher than that of a coating with unvulcanized NR due to a special ability to wet the urea during the production of the beads and a poor dispersion of urea in the vulcanized NR matrix. Hence, urea is easily diffused from the bead in the water medium. In addition, the modified rubber could be effectively applied for encapsulation of the urea fertilizer. The resulting polymer membrane of the capsule was degraded in natural soil. Two nonhomologous enzymes involved in this process and the respective genes were recently identified in species of the genera *Streptomyces* and *Xanthomonas*. The enzyme from the microorganisms first destroys the chemical structure of the modified NR to produce short polymer chains. The main advantages of the value-added NR products and a biopolymer are that they are more environmentally friendly with a low cost.

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Chapter 11

EFFECT OF VARIOUS MULCH MATERIALS ON SOD-PODZOLIC SOIL AND FIELD CROPS YIELD

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ABSTRACT

The author suggests a new way to increase soil fertility by forming a mulch layer on the soil surface. The layer is a mixture of a mulch material and the soil layer of 0-5 cm. The paper studies the use of various mulches, including traditional fertilizers and ameliorants (peat and straw), and non-traditional ones (bentonite clay and lime). Mixing mulches with the soil layer of 0-5 cm significantly increased their efficiency, as they acted as both fertilizers and mulch materials. It has been proved that all mulch materials selected for the study had a comprehensive positive effect on the soil. In most cases, they decreased acidity, increased the amount of exchangeable bases, organic matter, mineral nutrients, and the amount of available moisture in the plow horizon. Forming a mulch layer increased crop yields by 10-40% compared with the control (without mulches).

Keywords: Mulch layer, mulch materials, ameliorants, fertilizers, soil properties, yield, soil fertility.

INTRODUCTION

Most of the world soil resources, including the resources of Russia, have a number of characteristics that are negative from the agronomic point of view. Finding ways to increase their fertility is indeed a very urgent task. This is usually achieved through a whole complex of agro-technical, agro-chemical, ameliorative and other activities. However, with more intense agricultural production, there is an increase in the technogenic load on the soil, the number of operations, the depth of soil cultivation, and the intensity of soil pulverization. This, in turn, has a greater negative effect, such as dispersion of soil aggregates, mineralization of organic matter and water loss due to evaporation. Besides, it leads to

excessive spudding of the plow horizon, compression of the underlying layers, much greater water and wind erosion, and a growing dependence on adverse weather conditions.

One of the promising methods of reducing the negative impact of unfavorable weather and human factors on soil is the application of a mulch layer.

A mulch layer gives the soil universal protection, suppresses the kinetic energy of falling raindrops, ensures good drainage, prevents the formation of rainfall runoffs, and reduces evaporation. Mulch stimulates the activity of microorganisms, increases the number of earthworms making numerous channels in the soil, keeps the warmth in autumn and winter, reduces the temperature in summer, increases water stability of the aggregates, improves the bearing capacity of the soil and increases the amount of nutrients available to the plants. In agriculture, mulching is the most effective method, which stimulates the accumulation and retention of moisture and has a positive impact on many other soil properties.

The term "mulching" is currently used in two senses. In the broad sense, it refers to any artificial impact on the topsoil, even its ordinary cultivation.

In the narrower sense, mulching means coating the topsoil with certain material or incorporation of the material into the topsoil. In this study we focused on the incorporation of different mulch materials (in the soil layer of 0-5 cm).

In recent years, mulching has received greater attention worldwide. There have been numerous scientific publications (Romanenko, 2007; Klocke et al., 2009; Jodaugiene et al., 2010; Azadegan et al., 2011; Bagirov, 2001, and others), exhibitions and fairs of various foreign and domestic equipment for minimum tillage and no-tillage mulching systems.

RESEARCH METHODS

The author carried out a series of laboratory and field experiments from 1991 to 1997 to do the research. This paper summarizes data from three field experiments. Experiment 1 was conducted from 1991 to 1995, Experiment 2 – from 1994 to 1996, and Experiment 3 (production) was performed in 1996. The experiments were done to agro-soddly-podzolic lightly washed-off light and medium loamy soils. The agrochemical characteristics of these soils are shown in Table 1.

Table 1. Agrochemical characteristics of the soils before the experiment (plow horizon)

Experiment #	pH_{KCl}	Hr	S	V, %	Humus, %	P_2O_5	K_2O
		mmol/ 100g				mg/kg	
1	4.9	3.4	11.4	77	1.1	133	102
2	5.8	2.2	11.8	84	1.7	159	165
3	5.3	2.6	25.5	91	2.5	38	60

Weather conditions in the experiment years varied considerably. The growing seasons in 1991, 1992, 1995 and 1996 were very dry, while in 1993 and 1994 they were characterized by excessive humidity.

The experiments were designed to study the effect that four mulch materials (straw, low-moor peat, bentonite clay and limestone powder) had on soil properties and crop yields.

Mineral mulch materials were studied at the following proportions: 4 t/ha, 8 t/ha and 16 t/ha; peat – 20 t/ha, 40 t/ha and 80 t/ha; straw – 5 t/ha and 10 t/ha. We studied the effect of the mulch materials both in the pure form and in the form of binary mixtures.

The mulches were studied in the crop rotation. Experiment 1: annual grasses, winter rye, barley, oats, vetch-oats mixture. *Experiment 2:* annual grasses, barley oats. Experiment 3: barley.

All mulches were applied to the soil surface once at the beginning of the observation period (in May) and mixed with the soil layer of 0-5 cm by medium disc harrows. The only exceptions were the variations with straw in Experiment 1, when straw was applied twice – at the beginning of the first and the fourth growing seasons. After the mulches were applied, only subsurface tillage was used to preserve their integrity.

As a result of this work, a candidate dissertation (Lednev, 1997) was defended in 1997 and a new method of applying ameliorants and fertilizers was developed (Patent 2164060 RUC2). Certain elements of this technology were used in further research when conducting different field experiments.

The study found that, in the majority of cases, low and average proportions of the mulches (bentonite clay – 8 t/ha, limestone flour – 4 t/ha, peat – 40 t/ha, straw – 5 t/ha) were most cost-effective. Increase in yield due to the application of a larger proportion does not repay the incurred costs. The effect of plain (single) mulches was similar to the effect of binary mixtures, but their application did not need the additional action of mixing the ameliorants. Therefore this paper will only discuss the data for plain mulches applied at their optimal proportion (i. e. bringing the greatest economic benefit).

For convenience, most of the factual data below are presented in the form of graphs. It should be noted that for all graphs there are summary data of the three field experiments given for different plots and years, which significantly increases the objectivity of the findings. To be able to compare the results for different plots, the deviation from the control (without ameliorants) was calculated in each experiment, and only then the arithmetic mean of the three experiments was calculated.

1. Effect of Mulches on Physical and-Chemical Properties of the Soil

One of the main negative features of podzolic soils is their high acidity and low content of bases in the soil adsorption complex. Mixing the ameliorants and the fertilizers under study with the soil layer of 0-5 cm had a positive impact not only on the top of the plow horizon, but also on its entire depth (Figures 1, 2, 3). Limestone powder, as expected, exerted the greatest influence on the physical and chemical properties. All through the observation period, it decreased both the exchange and hydrolytic acidity of the plow horizon (by the average of 16.7% and 21.8% respectively throughout the observation period) and increased the amount of exchangeable bases (by the average of 23.5%) compared to the control without mulch. This effect of liming the soil is well known and is based on the neutralization of mineral and organic acids and the displacement of hydrogen and aluminum ions from the soil adsorption complex. What is important in our study is that the shallow application of limestone powder did not decrease its effectiveness, compared with the traditional method of applying, but reduced the cost of liming.

Mixing bentonite clay 8 t/ha with the soil layer of 0-5 cm also reduced soil acidity, and although the influence of betonite clay on this indicator was not always proved statistically, after five observations there still was an increase in pH_{KCl} by 2.1% (Figure 1) and a reduction in the hydrolytic acidity by 5.4% (Figure 2) on average compared to the control without mulches.

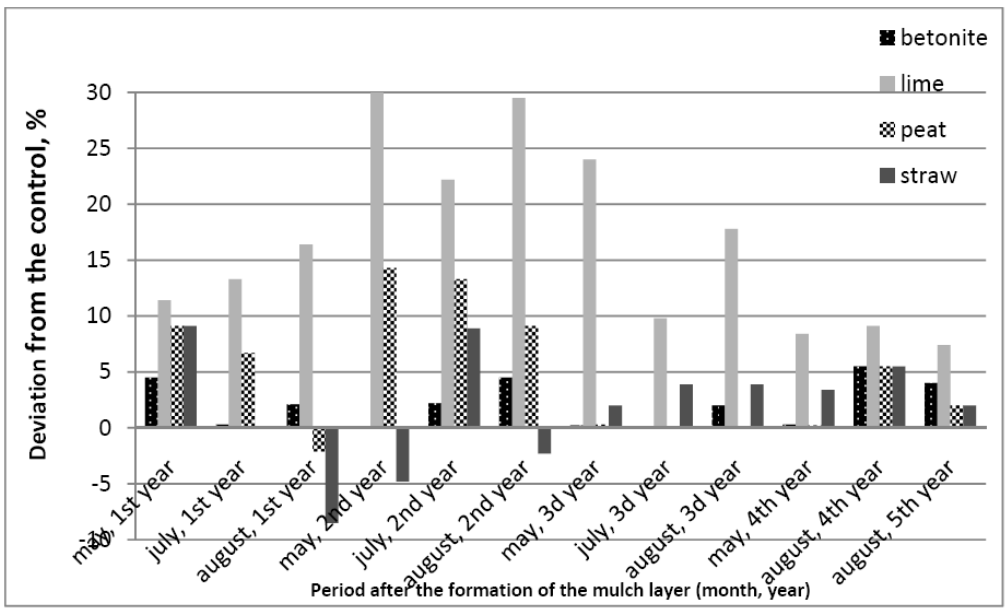

Figure 1. Effect of mulches on the exchangeable acidity of the plow horizon.

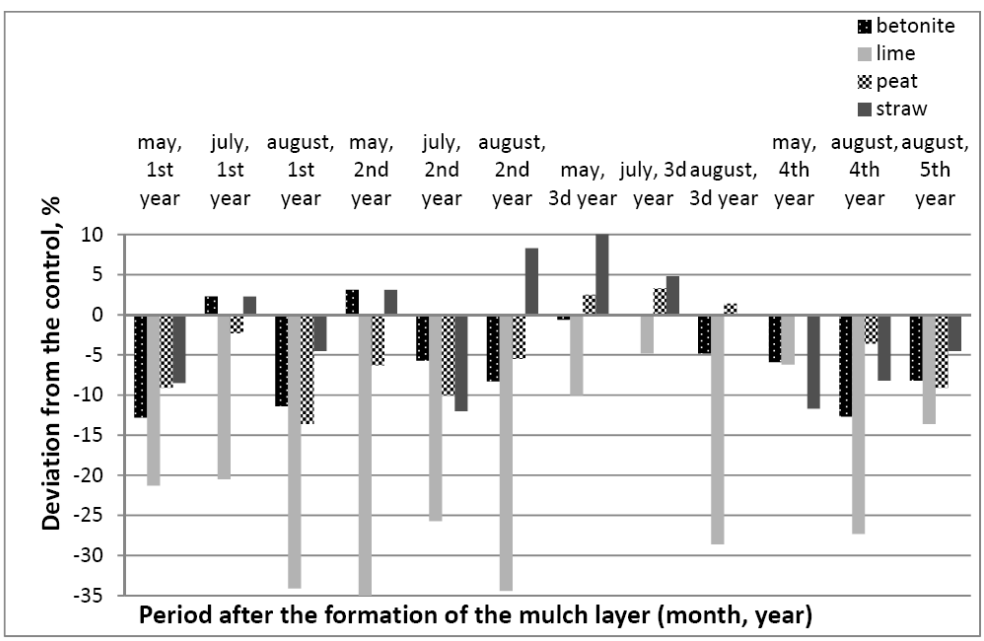

Figure 2. Effect of mulches on the hydrolytic acidity of the plow horizon.

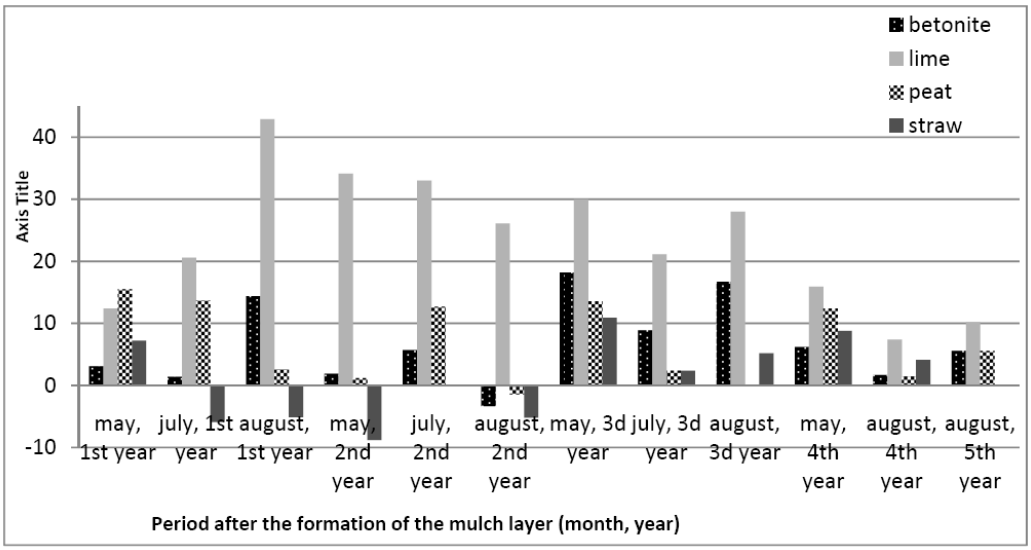

Figure 3. Effect of mulches on the amount of exchange bases in the plow horizon.

The reduction in the soil acidity is explained by the faintly alkaline reaction of this ameliorant (pH_{KCl} – 7.4) and a very high base saturation. Similar effect of bentonite clay on acid soils was observed by other researchers (Sharafeeva, 1980).

Another positive effect of bentonite clay is the increase in the amount of exchange bases from 1-2% to 18% compared with the control (without mulch). It is remarkable that the positive effect maintained throughout the five years of observation, being 6.7% on average. This was possible due to the high content of montmorillonite, a mineral with a very high cation exchange capacity of 90-120g mmol/100g of the mineral.

The effect of peat on physical and chemical properties of soil is determined by its origin, botanical composition and decomposition degree. Usually peat is acidic, due to its chemical composition, i. e. the high content of specific and nonspecific organic acids and the presence of labile aluminum. The amount of hydrolytic acidity in peat varies widely from 1-2 to 150-160g mmol/100g BDH. The most acidic is high-moor peat. Its application without prior liming can cause soil acidification and reduce crop yields. In low-moor peat, organic acids are largely neutralized by calcium and other base cations. Therefore the acid-alkaline balance of the soil may even shift to the alkaline side. In our experiments we tested low-moor peat with the acidity of 6.4 pH_{KCl} units. Mixing it with the soil layer of 0-5 cm at the proportion of 40 t/ha led to a slight decrease in acidity of the plow horizon, but this effect was not stable and was observed mainly during the first two years after the application.

The effect of peat on the amount of exchangeable bases was more apparent. Its incorporation in the soil layer of 0-5 cm increased this indictor to 1-2% ... 13-15% compared with the control, and the average for the five years of observation was 6.7% (Figure 3). This is due to the fact that peat is a natural ion-exchange material with high absorption capacity, which consists of the exchange, chemical, physical, mechanical and biological absorption capacity.

The exchange capacity is determined by the chemical nature of peat and, above all, the content of functional groups. It depends on the decomposition degree of peat and the content

of humic acids, fulvic acids, amino acids, proteins and other organic compounds. In this way up to 65-85 % of ions are bound. The chemical absorption is associated with structural changes of organic compounds in peat. As a results, ions are bound in inactive forms. In this manner about 10-30% of ions are bound. Physical, mechanical and biological absorption of ions by peat under normal conditions is irrelevant (Lishtvin et al., 1984). In our experiments we used peat with a total cationic capacity of 160-180 mmol/100g BDH.

The use of post-harvest residues, including crops straw, as mulch materials has a long history and a wide practical application in all agricultural zones all over the world. It is connected with the low cost and general availability of the material, the ease of its use and its high efficiency.

The effect of straw mixed with the soil layer of 0-5 cm at the proportion of 5 t/ha on the physical and chemical properties of the plow horizon is contradictory. In some cases, it led to soil acidification and reduced the amount of exchangeable bases in the plow horizon, while in other cases it reduced the acidity and increased the amount of exchangeable bases. The marked variations of these parameters are explained by the strong dependence of the straw decomposition rate on the environmental conditions. In the case of slow decomposition, there is a significant amount of acidic intermediate decomposition products, mainly in the form of organic acids (acetic, propionic, butyric, salicylic, etc.). However, this does not happen under favorable environmental conditions. Application of straw on the top of the plow horizon accelerates its mineralization, so there was a slight decrease in the soil acidity and an increase in the amount of exchangeable bases during the five years of observation.

2. Effect of Mulches on Chemical Properties of the Soil

A characteristic feature of podzolic soils is their low humus content, which often limits the ecological state and productivity of soils. As expected, the greatest impact on this indicator was achieved by the application of organic mulches (peat and straw).

Incorporating peat in the soil layer of 0-5 cm at the proportion of 40 t/ha increased the content of organic matter in the plow horizon by 5–23%. During the five years of observation it was on average 15.1% higher than in the control (without mulch) (Figure 4).

This well-known effect was possible because about 80-95% of the peat dry substance is comprised of various organic compounds. The organic matter of peat consists of carbohydrates (cellulose, hemicellulose, bitumen, lignin), protein and humic acids. Each group of the organic compounds plays a different role when peat is applied. Easily hydrolysable fractions are wonderful energetic material for the development of soil microflora. They decompose quickly and enrich the soil with mineral elements, primarily nitrogen and carbon dioxide. Hardly hydrolysable and non-hydrolysable fractions are highly resistant to biodegradation. Nevertheless, they transform gradually and make up a specific part of organic matter in the soil. The humic part is of the greatest value to agriculture. It has been found to consist of acidic compounds and contain aromatic complexes and proteinaceous components that split off amino acid in the hydrolysis (Tishkovich, 1993). Humic acids in peat, like humus in soil, are involved in the regulation and maintenance of redox reactions in the soil, improve root formation, increase the permeability of membranes and stimulate respiration enzymes and other vital processes of plants (Khristeva, 1977).

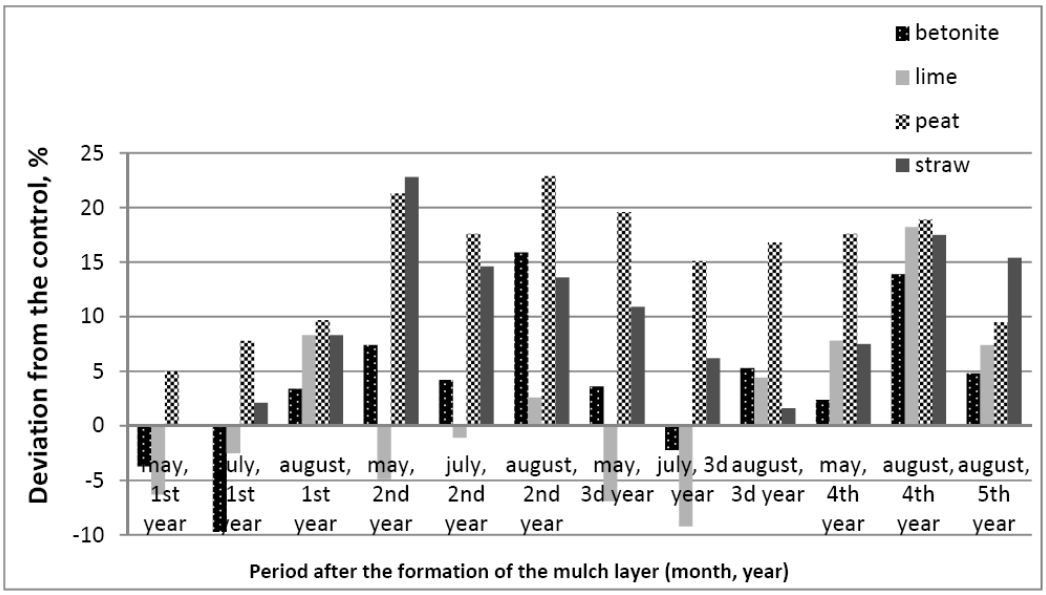

Figure 4. Effect of mulches on the content of organic matter in the plow horizon.

Peat has the highest rate of humification, which is significantly higher compared with other types of organic fertilizers: straw, manure or green manure. The surface application of peat contributed to further strengthening of the humification process. According to our data and data from other literature, in the case of deep application the results similar to those we had in our study are only achievable if the proportion is increased to 80-120 t/ha.

Straw also demonstrated high efficiency in improving the content of organic matter in the soil. Its incorporation in the soil layer of 0-5 cm at the proportion of 5 t/ha increased organic matter in the plow horizon by 2–23%, which exceeded the control (without mulches) by 10% on average during the five years of observation (Figure 4).

Straw is the most common and cheapest source of organic matter in the soil. Dried straw contains 84-86% of solids and 14-16% of water. The chemical composition of the dry substance varies widely depending on the type of crops, weather and soil conditions. In general, it has a high content of nitrogen-free compounds and a low content of protein and ash.

It should be mentioned that in our experiments straw was added twice – in the first and fourth years of study. This is due to the fact that straw mineralizes very quickly when incorporated at a shallow depth, so its action lasts only two or three years. This explains the two peaks in the graph when the content of organic matter in the soil increased in the second and fourth years of observation.

Applying a mulch layer of bentonite clay slightly increased organic matter in the topsoil (Figure 4). Compared with the control, its amount grew by the average of 4.4 % at a high confidence level during the five years. The increased humus content can be explained by the stabilizing effect of clay minerals on organic matter. I. Lgotski (1979) found that bentonite clay increases the accumulation of simple organic compounds, primarily fulvic acids from humus. In our experiments, mixing 8 t/ha with the soil layer of 0-20 cm did not increase the content of organic substances.

Applying a mulch layer of limestone powder slightly reduced the content of humus in the plow horizon in the first three years and then, in the fourth and fifth years, increased it slightly. This contradictory effect of liming is explained by the positive impact it has on the two opposite processes of organic matter transformation: mineralization and humification. The predominance of one process over the other is determined by a number of external factors. The five years of observation revealed only a slight increase in the humus content by the average of 1.5% at a low confidence level compared with the control.

The effect of mulches on the content of mineral nutrients in the soil depended on their chemical composition. The greatest effect on the amount of labile phosphorus and exchange potassium in the soil was achieved by the application of peat at 40 t/ha to the top of the plow horizon, which increased the content of the above elements by the average of 14.7 and 10.7%, respectively during the five years of observation (Figures 5 and 6).

In both cases this increase was statistically provable at a high level of confidence. Mulching with peat also increased the content of nitrate nitrogen in the plow horizon (by the average of 8.3% during the five years), but this increase was not always proved statistically by years.

The increase in the content of mineral nutrients in the soil was due to the fact that they were part of the mulch's organic matter and were released in the process of its mineralization. At the standard humidity of 60%, one ton of low-moor peat contains 20-30 kg of nitrogen, 2-3 kg of phosphorus and 1-2 kg of potassium (Tishkovich, 1993).

It should be noted that deep incorporation of peat increased the content of nutrients by a lower value (3-7% on average compared with the control without peat). This proves that mixing peat with the soil layer of 0-5 cm makes it not only a valuable organic fertilizer, but an effective mulch material that improves the nutrient status of the soil on the whole.

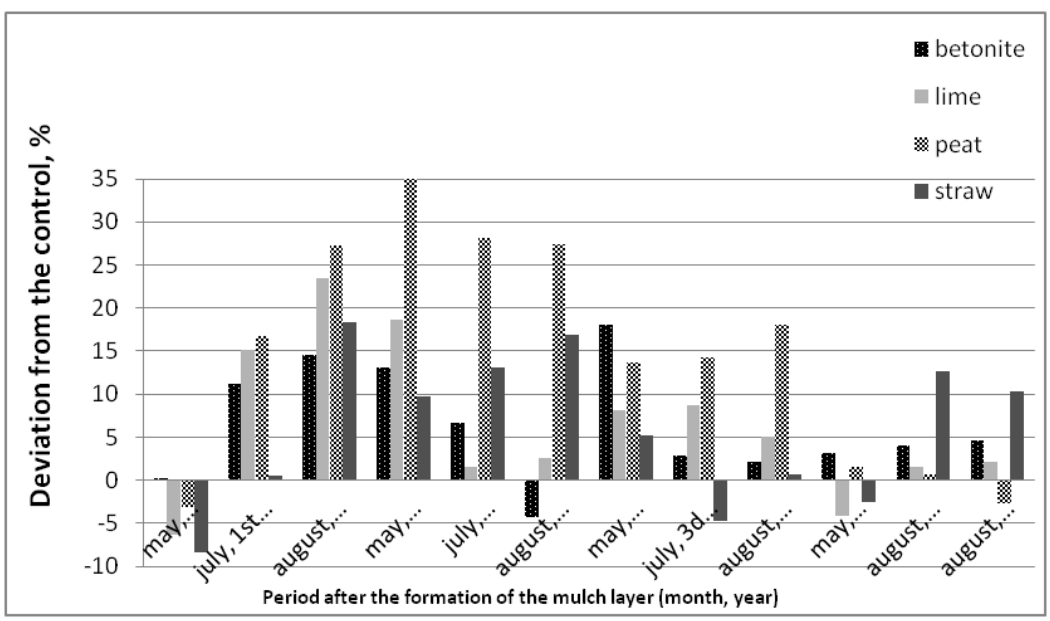

Figure 5. Effect of mulches on the content of labile phosphorus in the plow horizon.

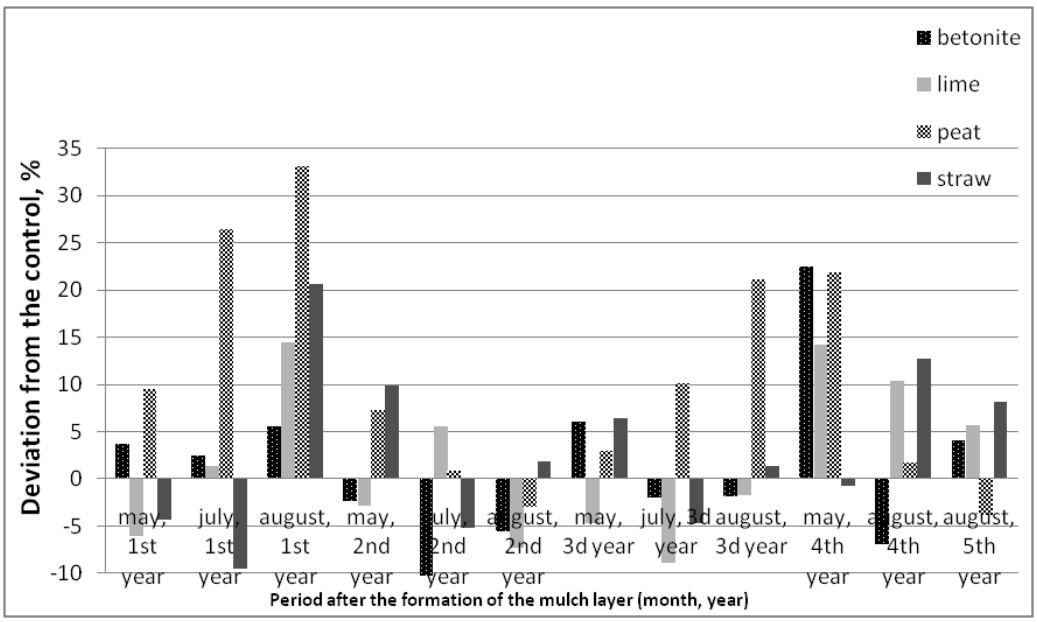

Figure 6. Effect of mulches on the content of exchange potassium in the plow horizon.

Incorporating straw in the soil layer of 0-5 cm increased the content of labile phosphorus by the average of 6% and the content of exchange potassium by the average 3% during the five years (Figures 5 and 6). These elements were released in the process of mineralization of straw's organic matter. Unlike peat, this effect of straw lasted only two years. Besides, its application even reduced the amount of labile phosphorus and potassium in the plow horizon at first. This is explained by the immobilization of these elements by the decomposing microflora. The substance subject to particularly strong immobilization was nitrogen, the amount of which was in most cases lower than in the control throughout the observation period.

Mineral mulches (bentonite clay and limestone powder) also increased the content of the exchange potassium and especially labile phosphorus in the plow horizon. However, this effect was less marked, and it was only due to the improved nutrient status of the soil.

3. Effect of Mulches on Physical Properties of the Soil

The study has found a comprehensive positive effect of mulches on all major agrophysical soil properties. Mulching can restore natural water-regulating mechanisms affected by plowing. Mulch, in a broad sense, acts as A_0 horizon (plant litter or sod) of virgin soils. Mulch passes water very well and prevents the draining of the topsoil. So it increased the stocks of productive moisture in the plow horizon. The greatest effect in this respect was produced by peat – on average it exceeded the control by 17.7% during the five years (Figure 7). The positive effect of using peat as mulch was determined by the fact that it formed a loose well-structured layer on the top of the plow horizon. Mixing 40 t/ha of peat with the soil layer of 0-5 cm reduced the density of this layer by the average of 5-6% (from 1.28–1.32 g/cm3 in the control to 1.20–1.26 g/cm3 when peat was applied) and increased the

aggregation rate (indicator defining water stability of microaggregates) 2.3 units to 29.8 units. The increased soil moisture was also due to a very large water absorption capacity of peat. One kilogram of bone dry peat can absorb and retain from 5 to 30 kg of water (Tishkovich, 1993).

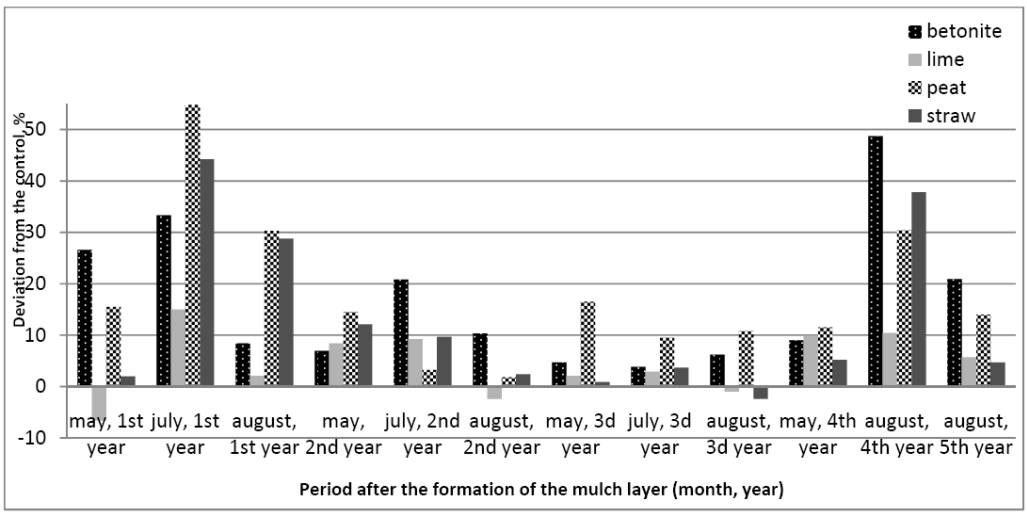

Figure 7. Effect of mulches on the content of productive moisture in the plow horizon.

Mulching with bentonite clay had a similar effect on the retention of productive moisture to that of peat. It exceeded the control by 16.6%. This was due to the fact that the minerals contained in clay had a good structure-forming and moisture-retention capacity. Adding 8 t/ha of bentonite clay to the top of the plow horizon reduced its dispersion factor (indicator that determines the strength of the microstructure) from 28.3 (in the control) to 22.0 units, increased the aggregation rate from 2.3 to 25 units and increased the particle-size index of structural properties (indicator showing the potential ability of the soil to form structure) from 7.7 to 7.9 units.

Mulching with straw showed good results in terms of productive moisture retention, exceeding the control by 12.4%. This effect was caused by a number of reasons. First, soil permeability grew by 10-25 %, which decreased runoff dramatically. Lack of soil crust and a higher layer of water on the soil surface resulted in accelerated infiltration. Secondly, applying straw to the surface reduced evaporation since it had lowered the speed of the surface wind, the soil temperature and the water vapor diffusion resistance. Third, on clear days we observed surface and subsurface dew under the mulch of straw. According to A. N. Kashtanov (1974), during the growing season dew can accumulate up to 85 mm of additional water in the soil.

The ability of post-harvesting residues used as mulch to reduce evaporation significantly was noted by many authors (Klocke Stock et al., 2009; Bagirov 2011, etc.). It should be mentioned that this ability of straw to reduce unproductive evaporation was gradually falling with its greater mineralization and was statistically valid only for two years.

Limestone powder had the smallest impact on the content of productive moisture in the plow horizon, exceeding the control by only 4.6 % on average at a middle confidence level. The effect of lime on moisture retention is based on the improvement of the structure of the

top of the plow horizon (the aggregation rate increased from 2.3 units in the control to 17.0 units when 8 t/ha of lime was incorporated in the soil layer of 0-5 cm) and a slight decrease in its density (5.3% on average).

It is worth noting that lime showed very high efficiency when it was applied in combination with bentonite clay. In terms of the influence on the content of productive moisture in the plow horizon, this combination of mulches was as efficient as the organic mulches, but the effect was more stable and lasted longer.

4. Effect of Mulches on Crop Yields

The most objective comprehensive assessment of the effect mulching has on soil properties is based on an increase or decrease in crop yields. It has been found that all mulches selected for the study caused a statistically significant yield increase. The increase varied by years, since it depended on the type of the mulch material, biological characteristics of crops, weather conditions during the vegetation period and the post-mulching period (Figure 8).

The most consistent positive effect on yields was seen when using peat as mulch. It caused an increase in the crops yield by the average of 23.1 % compared with the control (without mulches) during the five years of observation. The effect of this mulch material lasted for all five years of observation, without significant variations over the years. The exception was the third year of its use, when the crop yield exceeded the control by 42 % during the vegetation period. This is explained by the weather conditions, namely the extremely dry June, which enabled us to see the positive effect of mulching most clearly.

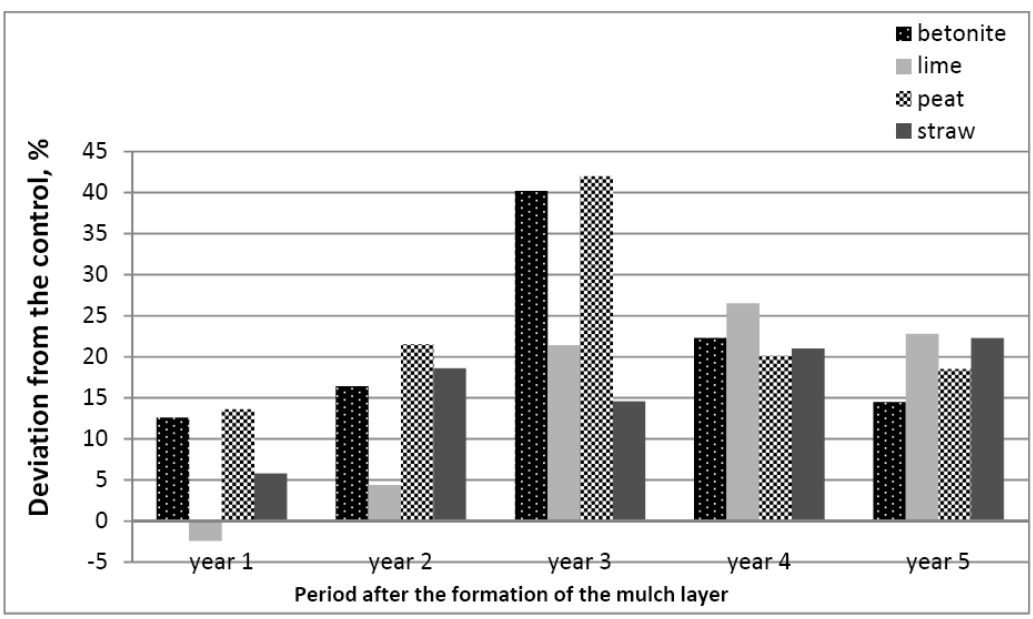

Figure 8. Effect of mulches on the crop yield.

The effect of bentonite clay was similar to that of peat. It increased the crop yields by the average of 21.2% compared with the control during the five years of observation. The action of this mulch material also lasted all five years of observation without significant variations (except for the third extremely dry growing season).

Mulching with straw increased crop yields by 16.5% on average during the five years. Its effect, including the effect on this indicator, did not last more than two or three years, so straw was applied twice during the five-year observation period. It should be noted that despite the mulch material had to be applied twice, its application was most cost-effective. This was due to a number of reasons. First, along with being an effective mulch material, straw was a good organic fertilizer.

When applied appropriately with NPK, it was as effective as the appropriate amount of manure (Shikula et al., 1991). Second, leaving straw on the field makes a number of production processes unnecessary, which reduces the time spent by 20.5%, the fuel consumption by 47.5%, and the labor costs by 40.3 % on average (Klokov, 1984; Romanenko, 2007; Klocke et al., 2009). The use of straw as a mulch material reduces labor costs about by 10 times compared with the cycle of preparation and application of manure, plus it prevents the loss of organic matter (Lomakin, 1991).

The crop that was most responsive to mulching was barley. The least responsive was winter rye, as it has a well-developed root system and is less dependent on soil fertility and the content of productive moisture.

Mulching had the greatest impact on crop yields in dry growing seasons, especially in soil drought.

CONCLUSION

All mulch materials selected for the study had a comprehensive positive effect on the soil. In most cases they produced the following effects in the plow horizon: the acidity decreased while there was an increase in the amount of exchangeable bases, the content of organic matter and mineral nutrients, and the amount of available moisture. The greatest influence on the soil physical and chemical properties of the soil was exerted by limestone powder used as mulch, and the greatest influence on the soil chemical and water properties was exerted by peat used as mulch.

Mixing ameliorants (including organic fertilizers) with the soil layer of 0-5 cm significantly improved their efficiency as they acted as mulch and ameliorants (fertilizers) at the same time.

Shallow application of ameliorants (organic fertilizers) allows for the necessary agricultural activities while maintaining the mulch layer during the crop rotation, which significantly extends the scope of mulching in arable farming.

Formation of the mulch layer increased crop yields by 10-40% compared with the control. Peat and bentonite clay had the greatest effect on this important agronomic indicator. Straw was the most cost-effective mulch material, despite the shortest period of action (not more than two or three years).

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Chapter 12

RADIOLOGICAL IMPACT OF FERTILIZERS: PRODUCTION AND USE

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ABSTRACT

The production of fertilizers based on phosphate is considered a Naturally Occurring Radioactive Material (NORM) industry. The raw materials involved in it are not radioactive per se, but the content of naturally occurring radionuclides (uranium, thorium, and their descendants in the decay chain) in it is high or it can be enhanced during the industrial processes involved. Therefore the fertilizers produced and some by-product and wastes generated, such as phosphogypsum, have enhanced contents of those radionuclides. Their accumulation and disposal can pose radiological hazards. The use of fertilizers in agriculture provides plant not only with essential nutrients needed for their growth, but also the radionuclides present in them can be uptaken and distributed within the plant. The intensive use of fertilizers can also observed in rivers close to areas in which intensive farming is carried out. The run-off waters can enhance the levels of inorganic compounds in these waters and their uranium content, which can surpass the impact of other NORM industries in the area. Fertilizers can also be used as part of remediation projects to reduce the uptake of anthropogenic radionuclides deposited as a consequence of nuclear accidents. It is based on the saturation of the plant with stable cations chemically analogues to the radionuclides. In the case of radiocaesium, the use of potassium based fertilizers reduced the plant uptake.

Keywords: NORM, uranium, radium, phosphogypsum, natural radioactivity

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INTRODUCTION

The industrial production of fertilizers uses raw material that contains high concentrations of naturally occurring radionuclides. Therefore it has been considered as a NORM (Naturally Occurring Radioactive Material) industry, and its processes, by-products, end products, and wastes must be controlled due to the radiological hazard to the general population that they may pose (IAEA, 2003). Phosphate ores are the most important raw material, which can present enhanced concentrations of uranium and thorium, and their descendants in various degrees of equilibrium, depending on its origin. During the industrial treatment of these ores to produce fertilizers, the equilibrium within the natural decay chains is disrupted and the naturally occurring radionuclides are fractioned according to their physico-chemical properties and the industrial procedure. As a result, the fertilizers produced and the by-products and wastes generated are also enriched in naturally occurring radionuclides, depending on the procedure and the by-product considered. In order to assure the adequate radiological protection to workers and the general population, the pathways in which they are exposed to those radiations must be evaluated. External exposure to γ-radiation, inhalation of radon and their transfer into foodstuff are among the most important pathways to man. The release of wastes, mainly phosphogypsum, into the environment can also have a radiological impact, especially in aquatic environments. In terrestrial ecosystems, the fertilizers can also be used to reduce the transfer of anthropogenic radionuclides to plants, by supplying them with the stable element chemically analogue.

DETERMINATION OF RADIONUCLIDES

Radionuclides are unstable isotopes that usually lose their excess energy by the emission of ionizing radiation. The emission probability is different for each radionuclide and is expressed in terms of its half-life. The half-life of a radionuclide, $T_{½}$, is the time it takes for the activity to decrease by a half. There are three main types of ionizing radiation: α, β, and γ, whose main properties are the following:

- α-particles are helium nuclei (2 protons + 2 neutrons). They have high values of mass and kinetic energy, the latter within the range 3.95–8.78 MeV[1]. Their interaction with matter is so strong that even a sheet of paper is enough to stop them, with all their energy being lost in crossing its thickness. Most of α-emitting radionuclides are naturally occurring: ^{238}U, ^{226}Ra, ^{210}Po, ^{232}Th, etc.
- β-particles have various origins depending on the characteristics of the nucleus (β^-, β^+, EC or electron conversion, …), but the final result is the emission of electrons or positrons within a wide range of energies (0.01859–3.54 MeV). Due to their low mass and charge compared with α-particles, they can penetrate more deeply into matter, requiring a few centimetres of aluminium to stop them. Some β-radionuclides are: ^{3}H, ^{210}Pb, ^{90}Sr, ^{40}K, ^{137}Cs etc.

[1] An electron volt (eV) is a unit of energy equal to the amount of kinetic energy gained by a single unbound electron when it accelerates through an electric potential difference of 1 V. 1 eV = 1.609·10-9 J.

- γ-radiation consists of high-energy photons, from a few keV to less than 3 MeV. Their penetrating power is greater than the previous particles, and they require several centimetres of lead to be absorbed. This type radiation is frequently emitted immediately after α- or β-radiation. ^{40}K, ^{137}Cs, and ^{226}Ra, among others, are also γ-emitting radionuclides.

Systems to detect radionuclides are different for each type of radiation, because they have different characteristics. Due to the low penetrating power of α and β particles, it is necessary to perform radiochemical separation procedures for their proper assay. In the case of γ-radiation, however, such radiochemical separations are usually unnecessary. The determination of α-emitting radionuclides is usually by means of silicon detectors within a vacuum chamber. Gas-flow proportional counters and LSC (liquid scintillation counting) are used to determine both β-particles and α-particles. The determination of γ-emitting radionuclides is by means of NaI(Tl) and HP(Ge) detectors.

SOURCES OF RADIONUCLIDES IN THE ENVIRONMENT

The radionuclides occurring in the environment can be classified into two main groups: natural and anthropogenic (or man-made). The naturally occurring radionuclides comprise those belonging to the natural radioactive decay series, ^{40}K, and cosmogenic radionuclides, which are formed by the interaction of high-energy cosmic rays with atomic nuclei in the atmosphere. There are three natural radioactive series present in nature: thorium, uranium, and actinium, also denominated A = 4n, A = 4n+2, and A = 4n+3, where A is the mass number of the radionuclide. These natural series begin with ^{232}Th (thorium series), ^{238}U (uranium series), and ^{235}U (actinium series), and finish with ^{208}Pb, ^{206}Pb, and ^{207}Pb, respectively. The half-lives of these radionuclides are usually very large, $4.468 \cdot 10^{9}$, $7.04 \cdot 10^{8}$, and $1.405 \cdot 10^{10}$ y for ^{238}U, ^{235}U, and ^{232}Th, respectively. Figure 1 shows the decay chain of the uranium series as a way of example. The isotope ^{40}K ($T_{½} = 1.277 \cdot 10^{9}$ yr) is also a naturally occurring radionuclide with a long half-life, and constitutes 0.012% of potassium. The distribution of these radionuclides in the geosphere depends on the distribution of the geological media from which they derive and the processes which concentrate them at a specific location in specific media (IAEA, 2003). They can also be enhanced locally by human activities, and may be a cause for significant radiological concern because of NORM (Naturally Occurring Radioactive Material) industries. These industries use raw materials with high contents of natural radionuclides, and their bioavailability in their products, by-products, and residues may be enhanced due to physicochemical changes or to the form in which they are managed. Mining industries (uranium, iron, copper, …), the production of fertilizers from phosphate rocks, and burning non-nuclear fuels (coal, oil, gas) are some examples of NORM activities (IAEA, 2003).

Anthropogenic radionuclides were first introduced into the environment as a consequence of the atom bomb blasts during World War II. During the 1950s and 1960s, there were a great number of atmospheric nuclear weapons tests, which released huge amounts of a multitude of radionuclides into the atmosphere. Due to atmospheric circulation, they became distributed worldwide and ultimately were deposited onto the soil (UNSCEAR, 2000).

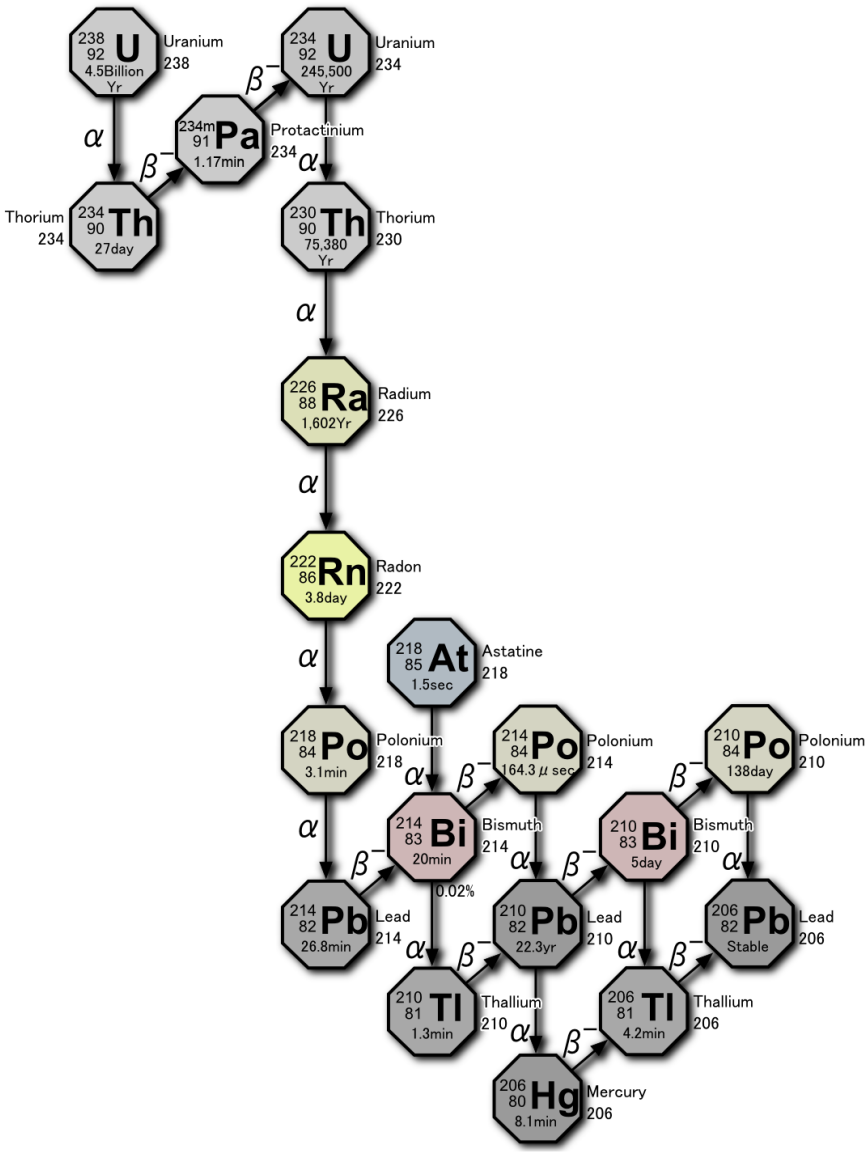

Figure 1. Decay chain of the natural uranium series (4n+2). Graphic from (Tosaka, 2008).

This phenomenon is usually known as global fallout. Most of these radionuclides were short-lived and have already decayed, as is the case with ^{131}I ($T_{½}$ = 8.02 d). However, ^{137}Cs and ^{90}Sr have longer half-lives (30.07 and 28.79 yr, respectively), and are actually the most important man-made radionuclides released into the environment. The estimated quantities of activity released were 948 and 622 PBq for ^{137}Cs and ^{90}Sr, respectively. Some radioisotopes of plutonium were also released but in lower amounts – 10.87 PBq for $^{239+240}$Pu. The annual deposition of ^{137}Cs and ^{90}Sr due to global fallout is shown in Figure 2. There was a maximum around 1962, in which year the greatest number of tests were carried out. As the majority of tests took place in the northern hemisphere, the deposition there was greater than in the southern hemisphere.

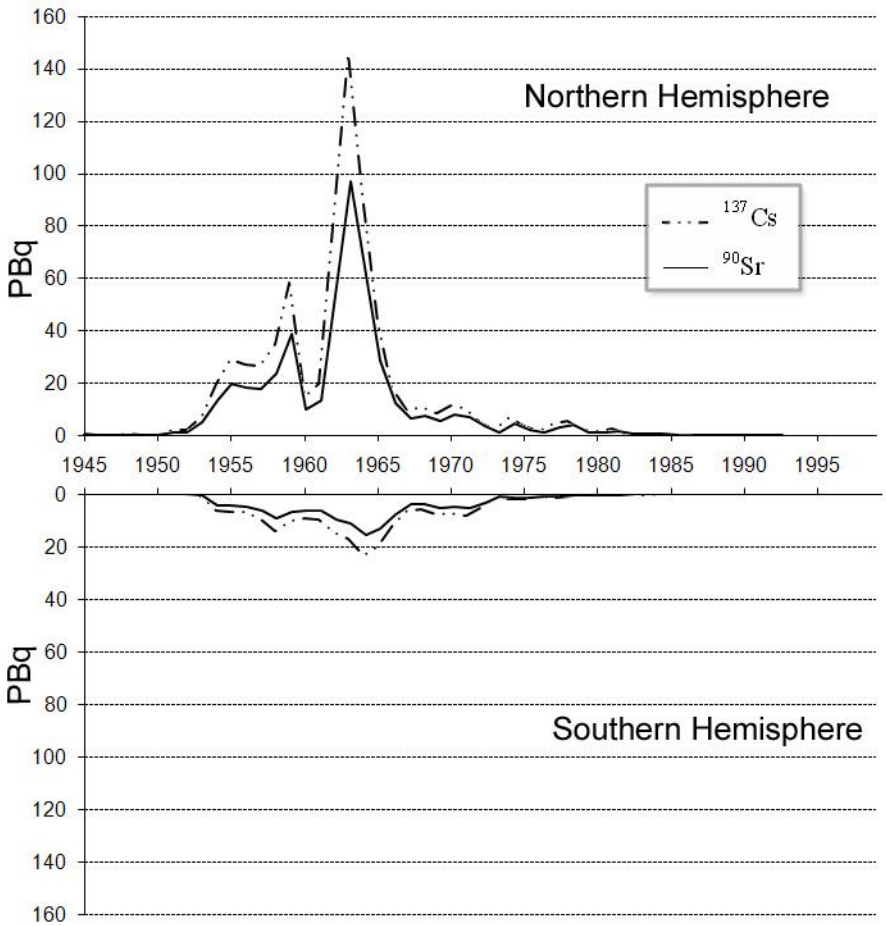

Figure 2. Annual deposition of ^{137}Cs and ^{90}Sr, expressed in petabecquerels (PBq), in the northern and southern hemispheres produced by atmospheric nuclear tests. Data from UNSCEAR (UNSCEAR, 2000).

The deposition was inhomogeneous in latitude because of circulation patterns and airflows in the stratosphere and troposphere. It was greater in the temperate regions, with a maximum deposition of ^{90}Sr in the 40–50° latitude bands, and lower in the equatorial and polar regions (UNSCEAR, 2000).

There is another natural series named neptunium (A = 4n+1), which was extinct because the half-life of ^{237}Np ($T_{½} = 2.14 \cdot 10^6$ yr) is shorter than the Earth's age. However, it was re-introduced into nature by the atmospheric release of anthropogenic ^{241}Pu ($T_{½}$ = 14.4 yr) and ^{241}Am ($T_{½}$ = 432.2 yr), predecessors of ^{237}Np.

Other sources of radionuclides in the environment are related to releases from nuclear fuel reprocessing factories, plutonium fabrication plants, nuclear waste storage sites, and accidents in nuclear reactors such as Windscale, Three Mile Island, Chernobyl, and, more recently, Fukushima. The most serious accident was Chernobyl (26 April 1986), which marked a point of inflexion for radioecology and environmental radioprotection. Large quantities of radionuclides were released, about 54, 85, and 10 PBq for ^{134}Cs, ^{137}Cs, and ^{90}Sr,

respectively (UNSCEAR, 2000). The most heavily contaminated areas (>3700 kBq·m^{-2} of ^{137}Cs) were located mostly within the 30 km radius around Chernobyl. However, it also seriously affected other countries such as Finland, Sweden, Norway, Germany, and Austria, where there were areas in which about 40-185 kBq·m^{-2} of ^{137}Cs were deposited (UNSCEAR, 2000). The importance of the Fukushima deposition in Europe was lower than the Chernobyl one (Masson et al., 2011).

INDUSTRIAL PRODUCTION OF FERTILIZERS

Radioactivity in Raw Material

The industrial production of fertilizers is mainly based on the processing of phosphate rock in order to produce phosphate based fertilizers. The phosphate rock reserves are located in different countries worldwide. Table 1 shows the content of some naturally occurring radionuclides from the uranium (^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, and ^{210}Po) and thorium series (^{232}Th and ^{228}Ra), and ^{40}K in phosphate rocks in different countries. The ^{238}U content in phosphate rock presents the highest activity level and a great variation range, within 10 – 13745 Bq·kg^{-1}. The ^{232}Th content in phosphate rocks was usually lower than that of ^{238}U, within a range 0.8 – 753 Bq·kg^{-1}. The ^{40}K content was the lowest, within the range 1.4 – 360 Bq·kg^{-1}. The radionuclides immediately following ^{238}U (^{234}U and ^{230}Th) usually show similar activity levels than ^{238}U, suggesting that they are close to secular equilibrium in the phosphate rock. The ^{226}Ra was not necessarily in secular equilibrium with its predecessor, ^{238}U, in all phosphate rocks, being the ratio ^{226}Ra/^{238}U within the range 0.6-2.0 (Papastefanou et al., 2001). The ^{210}Pb and ^{210}Pb were usually in secular equilibrium with ^{226}Ra. Table 1 does not fully show the degree of equilibrium because not all descendants are systematically determined in every paper.

The great worldwide range of uranium in phosphate rocks, about four orders of magnitude, can be attributed to the different origin of phosphate rocks used, which can be classified in: i) volcanic/igneous origin; ii) sedimentary origin; and iii) biological origin, the accumulated droppings of marine birds, which generated deposits of guano. The uranium content in sedimentary phosphate rocks are higher than those of volcanic/igneous origin (Papastefanou et al., 2001; IAEA, 2003; Righi et al., 2005; Falk et al., 2006). Table 2 shows the range of ^{238}U, ^{226}Ra, and ^{232}Th reported for phosphate rocks in which their origin was specified. The ^{226}Ra in sedimentary phosphate was higher than in volcanic ones due to the higher content of ^{238}U. The content of ^{232}Th in sedimentary rocks was also higher in volcanic phosphate rock. The enhanced uranium content in sedimentary ores can be attributed to the ionic substitution of calcium by uranium into carbonate and apatite crystals - $Ca_5(PO_4)_3(F,Cl,OH)$-, or by its direct absorption (Rutherford et al., 1994; IAEA 2003).

Other raw materials also used as fertilizers are known as potash. The term potash comprises various salts containing potassium in water-soluble form: KCl, K_2SO_4, K_2CO_3, and other potassium compounds. Due to their high potassium content, the main naturally occurring radionuclide is ^{40}K, which is 0.012% of stable potassium content.

Table 1. Range of activity level of naturally occurring radionuclides, expressed in $Bq \cdot kg^{-1}$ in phosphate rock from different countries. The uranium and thorium contents reported in ppm were converted to activity levels using the factors $9.28 \cdot 10^{-5}$ and $2.46 \cdot 10^{-4}$ $g \cdot Bq^{-1}$ for ^{238}U and ^{232}Th respectively

Country	U series						Th series		^{40}K	References
	^{238}U	^{234}U	^{230}Th	^{226}Ra	^{210}Pb	^{210}Po	^{232}Th	^{228}Ra		
Algeria	511-1295	---	---	250-1150	---	---	6.2-733	---	1.4 –260	(Fourati et al., 1988; Pantelica et al., 1997; IAEA, 2003; Roselli et al., 2009; Cevik et al., 2010; Lakehal et al., 2011)
Australia	15-900	---	---	---	---	---	5-47	28-900	---	(IAEA, 2003)
Brazil	14-1868	320-1841	270-1694	53-1518	< 19-1698	---	56-753	57-1550	< 45-87	(IAEA, 2003; Saueiaet al., 2005; Saueia et al., 2006)
Canada	1293	---	---	---	---	---	26	---	---	(Luther et a., 1993)
Chile	40	---	---	---	---	---	30	---	---	(IAEA, 2003)
China	150-1100	---	---	150	---	---	25	---	---	(Azouazi et al., 2001; Falk et al.,2006)
Egypt	244-1520	---	---	700	---	---	10-49	1700	32-186	(IAEA, 2003, Mourad et al., 2009; Cevik et al., 2010; El-Taher et al., 2010; Falk et al., 2006)
Finland	10	---	---	---	---	---	10	---	110	(Mustonen,1985)
Former Soviet Union	44-915	---	---	126	---	---	78-100	---	55	(Fourati et al., 1988; Pantelica et al., 1997; IAEA, 2003; Roselli et al., 2009; Falk et al., 2006)
Israel	1497-1800	---	---	1801	---	---	2-6.8	---	---	(Pantelica et al., 1997; Papastefanou, 2001; IAEA, 2003)
Jordan	450-1850	---	---	958	---	---	---	---	---	(Papastefanou, 2001;IAEA, 2003)
Morocco	1005-1700	1043-1680	1490-1670	1025-1700	1440-1660	984-1303	5-200	16-22	< 36 -119	(Barišić et al., 1992; Bolívar et al., 1995; Bolívar et al., 1996; Pantelica et al., 1997; Azouazi et al., 2001; IAEA, 2003; Bolívar et al., 2009; Roselli et al., 2009; Cevik et al., 2010; Falk et al., 2006)
Nigeria	---	---	---	617	---	---	---	---	324	(Okeji et al., 2012b)
Northern Africa	446-2530	---	---	737-1650	---	---	---	7.7-12.5	22-91	(Bituh et al., 2009)
Pakistan	62-771	---	---	59-744	---	---	19-112	---	104-360	(Khan et al., 1998; Javied et al., 2010)
Senegal	705-1996	745	---	1025-1370	---	984	67	9-12	< 30	(Barišić et al., 1992; Bolívar et al., 1995; Bolívar et al., 1996; IAEA, 2003)
South Africa	100-200	---	---	---	---	---	483-564	---	---	(IAEA, 2003)
Sudan	147-13745	149-14877	152-12487	153-15377	---	87-15084	0.8-22.3	---	13.7-332	(Sam et al.1999)

Table 1. (Continued)

Country	U series						Th series		^{40}K	References
	^{238}U	^{234}U	^{230}Th	^{226}Ra	^{210}Pb	^{210}Po	^{232}Th	^{228}Ra		
Syria	750-1500	---	---	445-1636	---	---	6.5-14	---	117	(Fourati et al., 1988; Pantelica et al., 1997; Roselli et al., 2009; Cevik et al., 2010; Falk et al., 2006)
Tanzania	20-11000	4377-4996	30-4661	5000-5800	---	---	7-1100	500-717	286	(Makweba et al., 1993; IAEA, 2003; Falk et al., 2006)
Togo	690-1360	1069	---	1010-1591	---	931	110	---	< 35	(Bolívar et al., 1995; Bolívar et al., 1996; Papastefanou, 2001; IAEA, 2003)
Tunisia	304-1700	---	---	250-1530	---	---	28-92	---	50	(Fourati et al., 1988; Pantelica et al., 1997; IAEA, 2003; Cevik et al., 2010)
Turkey	368	---	---	403	---	---	8	---	173	(Erdem et al., 1996; Cevik et al., 2010)
USA	260-4800	---	1300	200-4800	---	---	3.7-78	---	---	(Pantelica et al., 1997; IAEA, 2003; Azouazi et al., 2001; Falk et al., 2006)
Worldwide range	10-13745	149-14877	30-12487	53-15377	<19-1698	87-15084	0.8-753	7.7-1700	1.4-360	Present work

Table 2. Naturally occurring radionuclides in phosphate rocks, expressed from Bq·kg^{-1}, from sedimentary and volcanic origin. Data from (Guimond et al., 1989; Makweba et al., 1993; Papastefanou et al., 2001; IAEA, 2003; Falk et al., 2006)

Type	^{238}U	^{226}Ra	^{232}Th
Sedimentary	60 – 11000	200 – 5800	7 – 1100
Volcanic	70 – 200	200	100 – 400

Radionuclide Fluxes in the Production Processes

There are different industrial processes to manufacture phosphate fertilizer from raw phosphate ore. The most commonly process used, ~ 90 %, is the wet process (Rutherford et al., 1994), in which the phosphate ore is acid leached, usually by means of H_2SO_4, in order to produce phosphoric acid. The general chemical reaction for fluorapatite is shown in equation 1.

$$Ca_{10}F_2(PO_4)_6 + 10\ H_2SO_4 + 20\ H_2O \longrightarrow 10\ CaSO_4 \cdot 2H_2O + 6\ H_3PO_4 + 2\ HF \quad (1)$$

In this process, a great quantity of phosphogypsum ($CaSO_4 \cdot xH_2O$) is generated, approximately 4–5 T per ton of P_2O_5 produced (IAEA, 2003). The annual production of phosphogypsum is estimated within the range $(3–280)\cdot 10^6$ T (Rutherford et al., 1994; Bolívar et al., 1995; Burnett et al., 2001). The degree of hydration of the phosphogypsum depended on the acid concentrations and the temperature of the operating procedure, being $\cdot 2H_2O$ the most common (Rutherford et al., 1994).

This production method based on the chemical attack disrupts the decay chain scheme within the phosphate ore. Each naturally occurring radionuclide goes along with a given product according to their chemical properties. The majority of uranium and thorium goes mainly with the phosphoric acid produced (Rutherford et al., 1994, Erdem et al., 1996, Bolívar et al., 2009). In fact, the ^{238}U content showed a positive correlation with the purity of the P_2O_5 obtained in different steps (Righi et al., 2005; Bolívar et al., 2009). The association of the naturally occurring radionuclides with the P_2O_5 produced decreased in the following order (Poole et al., 1995; Bolívar et al., 2009):

$$^{238}U \approx {}^{234}U > {}^{230}Th > {}^{210}Pb \approx {}^{210}Po > {}^{226}Ra$$

The association of these naturally occurring radionuclides with the phospogypsum follows the same order, but in reverse. About 60-100 % of the ^{226}Ra and 90-100% of the ^{210}Po was associated mainly with the phosphogypsum (Rutherford et al., 1994, Hull et al., 1996). Table 3 shows the range of these radionuclides reported in phosphogypsum produced in different countries worldwide. It can be observed that the phosphogypsum presented higher contents of ^{226}Ra, ^{210}Pb, and ^{210}Po than uranium and thorium. In some occasions a significant percentage of the uranium may be associated with phosphogypsum. Its content in phosphogypsum was mainly due to unreacted phosphate rock (Hull et al., 1996, Bolívar et al., 2009). The uranium content was also observed to decrease with successive washings of the phosphogypsum, which partially removed that unreacted material (Bolívar et al., 2009).

Table 3. Range of activity level of naturally occurring radionuclides, expressed in Bq·kg^{-1} in phosphogypsum from different countries. The uranium and thorium contents reported in ppm were converted to activity levels using the factors 9.28·10^{-5} and 2.46·10^{-4} g·Bq^{-1} for total uranium and thorium, respectively

Country	U series						Th series		^{40}K	References
	^{238}U	^{234}U	^{230}Th	^{226}Ra	^{210}Pb	^{210}Po	^{232}Th	^{228}Ra		
Australia	510	----	----	451-500	----	----	10	----	20	(Beretka et al., 1985)
Bangladesh	----	----	----	234	----	----	21	----	108	(Alam et al., 1997)
Brazil	< 2-61	40-89	32-978	24-940	66-1163	----	19-257	29-273	< 45	(Saueia et al.,2005; Saueia et al., 2006)
Canada	59-143	----	----	----	----	----	4	----	----	(Luther et al., 1993)
Croatia	56-89	----	----	309-772	----	----	----	3.6-6.2	6.2-107	(Bituh et al., 2009)
Egypt		----	----	596	----	----	6.2	----	2.1	(Mourad et al., 2009)
Greece	19-400	----	----	550-2500	430-514	----	2.7-3.3	3.3-80	5-13	(Papastefanou et al., 2006;Stamatis et al., 2010)
Hungary	587	----	----	1093	----	----	6.9	68	18	(Fourati et al., 1988)
India	< 10-60	----	----	505-539	483-594	418-560	----	----	----	(Burnett et al.,1996)
Korea	----	----	----	432-844	----	----	4.6-11.3	----	8.6-37.9	(Song et al., 2011)
Morocco	----	----	----	1420	----	----	----	----	----	(Azouazi et al., 2001)
Nigeria	----	----	----	335	----	----	4	----	200	(Okeji et al., 2012b)
Spain	24-650	260-650	1.1-830	9.1-1110	6-640	----	0.05-34	<1.5-16.9	20-50	(Mas et al., 2006; Dueñas et al., 2007; Bolívar et al., 2009)
Syria	39-38	----	----	259-381	482-532	927-1107	----	----	----	(Al-Attar et al., 2011)
South Africa	17-73	----	----	45-48	76-132	----	205-284	----	----	(IAEA, 2003)
Tanzania	----	----	----	3219	----	----	----	----	41	(Makweba et al., 1993)
Turkey	----	----	----	283-588	----	----	< 5-32.8	----	< 27-116	(Yücel et al.,2004)
USA	22-451	68-403	90-513	269-1358	343-1853	383-1765	11	----	----	(Hull et al., 1996; IAEA, 2003)
Worldwide range	<2-650	40-650	1.1-978	9.1-3219	6-1853	383-1765	0.05-284	<1.5-273	2.1-200	Present work

Table 4. Range of activity level of naturally occurring radionuclides, expressed in $Bq \cdot kg^{-1}$ in different types of fertilizer. The uranium and thorium contents reported in ppm were converted to activity levels using the factors $9.28 \cdot 10^{-5}$ and $2.46 \cdot 10^{-4}$ $g \cdot Bq^{-1}$ for total uranium and thorium, respectively. TSP: Triple Super Phosphate. SSP: Single Super Phosphate. MAP: monoammonium phosphate. DAP: diammonium phosphate

Fertilizer type	U series			Th series		^{40}K	References
	^{238}U	^{226}Ra	^{210}Pb	^{232}Th	^{228}Ra		
NPK	66-1710	1.5-451	78-741	28-307	<2-10	25-4100	(Mustonen et al., 1985; Barisic et al., 1992; Righi et al., 2005; Saueia et al., 2005; Chandrajith et al., 2011; Chauhan et al., 2013)
TSP	62-7024	122-5022	198	16-698	10-23	91-362	(Makweba et al., 1993; Barisic et al., 1992; Alam et al., 1997; Khan et al., 1998; Saueia et al., 2005; Chandrajith et al., 2011; Javied et al., 2011)
SSP	95-3879	499-3394	1255	7-434		87-491	(Makweba et al., 1993; Alam et al., 1997; Khan et al., 1998; Saueia et al., 2005; Chauhan et a., 2013)
MAP	91-3217	8-620	37	75-231	3-11	118	(Barisic et al., 1992; Khan et al., 1998; Saueia et al., 2005)
DAP	97-374	75-578	----	28-198	----	44-74	(Alam et al., 1997; Khan et al., 1998; Saueia et al., 2005; Chauhan et al., 2013)
Nitrophos	130-298	308-501	----	15-163	----	----	(Khan et al., 1998)
Urea	70-273	5.4-73	----	202	----	3-7.9	(Alam et al., 1997; Chandrajith et al., 2011; Chauhan et al., 2013)
Potash	----	17-34	----	95	----	12628-17143	(Alam et al., 1997; Chauhan et al., 2013b)
Zinc sulphate	----	4.8-37	----	25	----	16-245	(Alam et al., 1997; Chauhan et al., 2013b)

It also depended on factors that influenced the uranium partitioning, such as redox potential, digestion temperature, sorption of humic substances and clay, and coprecipitation with fluorides (Hull et al., 1996).

There are other methods of wet processing phosphate rocks using HCl and HNO_3. When using HCl, the by-products generated are CaF_2 and $CaCl_2$. The CaF_2 is insoluble and precipitates as sludges, $CaCl_2$ while is soluble. As ^{226}Ra is chemically analogue to calcium, $^{226}RaF_2$ and $^{226}RaCl_2$ are also formed as by products, being the first one insoluble and the latter soluble (Paradiens et al., 2001; IAEA, 2003). Using HNO_3, produces $Ca(NO_3)_2$ and H_3PO_4 in solution, which can be used to obtain a complex fertilizer, avoiding the generation of phosphogypsum (Righi et al., 2005). Calcium oxide is the main waste stream and uranium and radium is expected to be found in them. Further research is needed in order to assess the fluxes of naturally occurring radionuclides in these processes (IAEA, 2003).

The production of elemental phosphorus can also be achieved by means of thermal processing. In this process, phosphate rock is melted in a furnace at high temperature (~1400 ºC) with silica (sand), coke and iron compounds. Elemental phosphorus and CO_2 are driven off as gases. The ^{210}Pb and ^{210}Po content of the original phosphate ore are released into the gas stream (Papastefanou et al., 2001; IAEA, 2003).

The gas effluent is filtered through electrostatic dust filters, and the ^{210}Pb-^{210}Po content in the collected dust is about 1000 $Bq \cdot kg^{-1}$. The solid residue, known as slag, consists mainly in ferrophosphorus and calcium silicate, and its quantity is about 85% of the phosphate ore. The majority of the uranium and radium in the phosphate ore is attached to the slag. The naturally occurring radionuclides in the furnace slag ranged within 407-1517 $Bq \cdot kg^{-1}$ for ^{226}Ra, 444-2072 $Bq \cdot kg^{-1}$ for ^{238}U and 9-41 $Bq \cdot kg^{-1}$ for ^{232}Th (IAEA, 2003).

Radionuclide Content of Fertilizers

Table 4 shows the range of naturally occurring radionuclide content in several fertilizer types reported worldwide. The phosphate based fertilizers (NPK, TSP, SSP, MAP, DAP, and Nitrophos) presented a higher range of uranium content, which reflected its range reported for phosphate rock due to differences in their origin. Their radium content is usually lower than the uranium because of the different chemical behaviour in the wet production processes. The NP or P fertilizers presented lower ^{40}K content than NPK fertilizers or potash, in which the potassium content is significant. Urea, an N fertilizer, and other fertilizer not based on phosphate presented low content of naturally occurring radionuclides.

Environmental Impact of Residues

Phosphogypsum is the main residue of the industrial production of fertilizers, because the acid leaching of phosphate rocks with sulphuric acid is the most used process. The management of this waste was carried in different ways. Most of them were accumulated in stacks, although waste discharges into rivers and sea also occurred until they were banned (OSPAR, 1996). The accumulation of phosphogypsum in stacks can pose radiological hazards mainly due to the external exposure of γ rays (mainly ^{226}Ra, ^{232}Th, and ^{40}K), the dust resuspension, and the exhalation of radon. The increase of the external irradiation exposure in

the phosphogypsum stacks was within the range (0.082-2.5) mSv/y (Rutherford et al., 1994; Mas et al., 2006; Al-Altar et al., 2011). This range of variation reflects the range of ^{226}Ra in phosphogypsum, 9.1-3219 Bq·kg^{-1} (see Table 3). The external exposition exposures reported can surpass the limit of 1 mSv·yr^{-1} for general population (EC, 1996), and therefore they are usually monitored, and changes in the working conditions are introduced, such as the reduction time allowed to remain in a given location. The external exposure can also be minimized by the construction of protection zones around the stacks, and the use of trees around to form wind barriers (Mas et al., 2006; Al-Altar et al., 2011). The effective dose in a Spanish fertilizer industry, corrected by occupational factor, was 1.6 mSv·yr^{-1}, lower than previously found (2.15-2.5 mSv/y) due to the additional shielding of the water circulation system (Mas et al., 2006).

The dust generated by wind erosion of the stacks was not supposed to be a serious problem in temperate environments, because a crust is usually developed on top of them (Rutherford et al., 1994). However, in other dryer climates with high wind speeds, with maximums over 100 km/h, the surface soil (0-20 cm) surrounding the phosphogypsum stacks presented higher content of naturally occurring radionuclides in the predominant wind direction than in deeper soil (20-40 cm) (Al-Altar et al., 2011), implying that phosphogypsum particles were resuspended and deposited on surface soil.

The presence of enhanced ^{226}Ra content in phosphogypsum turns it into a source of radon, which is it decay product. The radon exhalation rates from <0.004-3.6 Bq·m^{-2}·s^{-1} (Rutherford et al., 1994; Dueñas et al., 2007). The exhalation coefficient depends on the porosity, density, moisture level, temperature, and ^{226}Ra content of the phosphogypsum stack (Dueñas et al., 2007; Lee et al., 2012). Cracks on the phosphogypsum crust also enhance the radon exhalation (Rutherford et al., 1994). As the phosphogypsum stacks are usually located outdoors, the radon accumulation close to them would not probably be high, about 1.1-15 Bq·m^{-3} while in open ground was about 3.7-5 Bq·m^{-3} (Rutherford et al., 1994). However, it can pose a risk when houses or other closed buildings are built on reclaimed phosphogypsum stacks, so radon can accumulate indoors (Papastefanou et al., 2006). The exhalation rate can be reduced by the soil covering used for the stack restoration. Using a soil layer of 25 cm decreased the exhalation from the range 0.042-0.500 Bq·m^{-2}·s^{-1} for unrestored sites to 0.011-0.339 Bq·m^{-2}·s^{-1} for restored ones (Dueñas et al., 2007). Structures built on reclaimed phosphate land presented a radon concentration within the range 4-500 Bq·m^{-3}, with a weighted average of 40 Bq·m^{-3} (UNSCEAR, 1988). About 5% of the phosphogypsum production is used a building material in the manufacture of cement, wallboards and plaster (UNSCEAR, 1998). Due to its high ^{226}Ra content, it can be a source of indoor radon, and is banned (IAEA, 2003).

Before the banning of direct offshore discharges (OSPAR, 1996), about 20% of the phosphogypsum generated in a Spanish fertilizers industry (Absi et al., 2004) and about 2 ton per year in the Netherlands (Köster et al., 1995) were discharged in aquatic ecosystems. However, there is a high degree of uncertainty about the quantity of NORM discharges because they were not always reported (Betti et al., 2004). These discharges had a significant radiological impact on the ecosystems. They increased the naturally occurring radionulide content in water and sediments close to the evacuation points (Köster et al., 1985; Martínez-Aguirre et al., 1994; Köster et al., 1995). The particulate matter in suspension in water also contained fine particles of phosphogypsum, enriched in ^{226}Ra and ^{210}Po, within the ranges 830-2460 Bq·kg^{-1} and 1048-1524 Bq·kg^{-1} respectively (Bolívar et al., 1996). The impact was

also observed when considering the ^{226}Ra in water and sediments, showing an increase in its content. In the cases of industries located close to the seashore, tidal activity also influenced the ^{226}Ra content in water. Samples collected in low tide had higher content than those in high tide (Periáñez et al., 1993). Up to 35% of the total activity in water of ^{238}U and 20-30 % for ^{232}Th were associated with the particulate matter suspended in water filtered through 0.45 μm pore size (Martínez-Aguirre et al., 1994). The ^{210}Pb also showed a tenfold increase in the dissolved fraction, although it decreased quickly downstreams (Martínez-Aguirre et al., 1997). In the case of the use of the HCl wet method, the ^{226}Ra content in contaminated banks was within the range 2300-5400 Bq·kg^{-1}; whereas its content in unaffected ones was about 13 Bq·kg^{-1} (Paridaens et al., 2001).

After the banning of the direct discharges (OSPAR, 1996), modifications were introduced in the industries and improved treatment of effluents were considered, for example close water systems were used to transport the phosphogypsum to the stacks (Betti et al., 2004; Bolívar et al., 2009). The banning had also impact on the naturally occurring radionuclides in water and sediments. A self-cleaning process was reported in Spain and North West England (Villa et al., 2009). In the Spanish case, during the period 1999-2002, the ^{226}Ra content in water showed a continuous decrease from (66 ± 3) mBq·L^{-1} in 1998 to (4±2) mBq·L^{-1} in 2002 (Absi et al., 2004). The ^{226}Ra content in sediments also showed a decrease trend and a homogenization process due to the transportation and mixing of sediments as consequence of currents and tides (Absi et al., 2004; Villa et al., 2009). The sediment content of ^{226}Ra decreased from 700 to 60 Bq kg^{-1}, and that of ^{210}Pb from 370 to 100 Bq·kg^{-1} (Villa et al., 2009). The effective decreasing half-time was within the range 0.16-0.84 yr in water and 5.03-5.99 yr in sediments for ^{226}Ra, while for ^{210}Pb was within the range 2.38-14.94 yr (Villa et al., 2009).

AGRICULTURAL USE OF FERTILIZERS

Radiological Hazard Due to Use of Fertilizers

The fertilizers, especially the phosphate based ones, present high contents of naturally occurring radionuclides (see Table 4). The application of fertilizers to soil increased the content of those radionuclides. The use of several phosphate fertilizers ranging 5-50% P in the form of P_2O_5 in rates 7.4-12.4 kg·m^{-2} for long periods of time increased the ^{226}Ra, ^{40}K, and ^{232}Th content in soils from 12-26, 222-376, and 39-72 Bq·kg^{-1} respectively to 33-100, 227-503, and 51-116 Bq·kg^{-1} respectively (Ioannides et al., 1997). The dose due to external exposure as a consequence of fertilizer application can be considered as negligible, about 37.4-125.8 μSv·yr^{-1} (Santawamaitre et al., 2010), much lower than the 1 mSv·yr^{-1} for the general population. However, radionuclides along with nutrients do not remain immobile in soil. They can be leached by run-off water into surface and groundwater resources, being this one of the causes of eutrophication of water bodies (Badruzzaman et al., 2012). Other chemical species, such as phosphates, nitrates and sulphates, also showed increased levels in water bodies contaminated by use of fertilizers (Zielinski et al., 1997; Baeza et al., 2011; Kamel, 2012). Uranium occurs usually in water bodies contaminated by fertilizers, and the ratio ^{234}U/^{238}U has been considered as a potential marker for tracking nutrient sources

(Zielinski et al., 1997; Badruzzaman et al., 2012). Increases in the uranium content by factors 1.4-5.3 have been observed in a river receiving the run-off water of farming lands using fertilizers (Baeza et al., 2011). In that case, the uranium contribution of run-off was higher than other NORM industry (coal-fired power plant) located upstreams. It was also correlated with the Mg^{2+}, Ca^{2+}, SO_4^{2-}, and NO_3^- content in water, which origin was the fertilizer used, mainly NPK S (MgS) 6-10-18 S(3-36) with potassium from sulfates. The uranium association with the run-off water can be due to its higher water-soluble fraction, about 10%, than other naturally occurring radionuclides in that fertilizer (Baeza et al., 2011).

Another exposure pathway from the naturally occurring radionuclides is their transfer to foodstuff, which can be consumed by humans. The uranium, radium, and thorium content in corn, leaves, grain and wheat grown in lands using NPK fertilizer for years have been reported similar to those grown in non-fertilized (IAEA, 2003). Phosphogypsum can also be used as agricultural amendments of soils (about 1-2% of production), which also increased their radioactivity content, mainly ^{226}Ra, from 37-54 $Bq \cdot kg^{-1}$ to 50-479 $Bq \cdot kg^{-1}$ of ^{226}Ra (Papastafanou et al., 2006). Rice grown on soil amended with phosphogypsum and not amended presented similar ^{226}Ra content, within the range 0.36-1.98 $Bq \cdot kg^{-1}$, leading to an annual effective dose by ingestion about 0.86 $\mu Sv \cdot yr^{-1}$ (Papastafanou et al., 2006).

Fertilizers As Countermeasures for Anthropogenic Radionuclides

Fertilizers have been used as agricultural countermeasures to inhibit or at least decrease the transfer of anthropogenic radionuclides into produces. Their use is based on the saturation of the soil solution with the additional supply of nutrients chemically analogues to the released radionuclides from the fertilizers (Nisbet et al., 1993). The most important anthropogenic long-lived radionuclide released into the environment is radiocaesium (see Fig. 2), which is chemically analogue to potassium, being both alkaline elements. Radiostrontium is the following anthropogenic radionuclide mostly released into the environment, and chemically analogue to calcium, both alkaline earth elements.

The application of potassium based fertilizers was able to reduce the transfer of radiocaesium to plants about 40-60% (Jacob et al., 2009; Rosén et al., 2011). There are a great variety of fertilizers used: potash either as K_2SO_4 (Whicker et al., 1999; Zhu et al., 2000b) or KCl (Mocanu et al., 2001; Salt et al., 2001); and NPK type fertilizers in different ratios (Kaunisto et al., 2002; Camps et al., 2004; Jacob et al., 2009). The application rate was within the range 100-200 kg $K \cdot ha^{-1}$ (Zhu et al., 2000b; Mocanu et al., 2001). Higher application rates, 2000-2500 kg $K \cdot ha^{-1}$, were used in soils with low potassium content (Salt et al., 2001; Robison et al., 2009). Regarding NPK fertilizers, maximum reduction was reported for the NPK ratio 1:1.5:2 (Jacob et al., 2009). The reduction of the radiocaesium transfer to plant was observed during long periods of time, 10-34 yr after fertilization (Kaunisto et al., 2002; Robison et al., 2009; Rosén et al., 2011). These fertilizers supply additional potassium which decreases the ^{137}Cs:K ratio in the soil solution (Nisbet et al., 1993; Zhu et al., 2000b). At lower concentrations of potassium in soil solution ($<10^{-2}$-10^{-1} μM), the root uptake mechanism is unable to distinguish between Cs^+, Rb^+, and K^+ (Nisbet et al., 1993; Shaw et al., 1993; Zhu et al., 2000a). However, at higher concentrations a critical threshold was observed, above which the root uptakes K^+ preferentially to Cs^+. In the case of wheat, it occurred around 20 μM (Shaw et al., 1993). The addition of potassium also had effect on other

radionuclides, decreasing the ^{241}Am and ^{244}Cm content in plants; while for $^{239+240}$Pu, ^{232}Th, and ^{238}U presented no effect (Whicker et al., 1999). The NH_4^+ content in the soil solution had also influence on the bioavailability of ^{137}Cs. Its increase led to an increase of the ^{137}Cs content in the soil solution by a factor of 3-4 (Nisbet et al., 1993). It can also displace K^+ from exchange sites in soils, increasing its concentration in the soil solution (Nisbet et al., 1993). The application of NH_4^+ and manure can also reduce the uptake of ^{137}Cs, due probably to the release of potassium and other ions from the manure when NH_4^+ is applied (Fuhrmann et al., 2003). The addition of phosphate stimulates root growth and may also increase the caesium uptake (Shaw et al., 1993)

The reduction of the uptake and transfer of radiostrontium to plants is carried out by means of calcium addition to soil. This addition is usually in the form of lime, which is a general term for inorganic compounds containing calcium. The success of this countermeasure is more limited than that of radiocaesium, about 20% of ^{90}Sr (Lembrechts, 1993). The highest reductions were obtained in soils with low calcium content (Shaw et al., 1993). Although in some occasions, this limited success is because the rates used, 1.6-15.6 ton $Ca \cdot ha^{-1}$, were not able to modify significantly the calcium content in the soil solution (Vidal et al., 2001; Camps et al., 2004). It has also been observed that it depended on the type of soil. The addition of lime can be beneficial for mineral soils and possibly deleterious for organic ones (Nisbet et al., 1993). Liming in excess can also fail to further reduce the radiostrontium uptake (Lembrechts, 1993). The addition of lime to acidic soil can increase the pH and modify also charges in clay (Massas et al., 2010). Therefore, the radiocaesium bioavailability can vary because it is mainly controlled by its attachement to clay minerals in the soil (Ohnuki, 1994), becoming to fixed to them (Zibold et al., 2009; Massas et al., 2010). The addition of potassium fertilizers increased both the calcium and strontium content in the soil solution in the same way. So the ratio Ca:^{90}Sr remained approximately the same (Nisbet et al., 1993b). The phosphate amendments might also be effective to suppress the radiostrontium uptake due to the probable formation of insoluble strontium phosphate (Shaw et al., 1993).

CONCLUSION

The industrial production of fertilizers, based mainly of phosphate ores, is considered a NORM activity, due to the high activity levels of naturally occurring radionuclides present in the raw material. Their occurrence depended on the origin of the phosphate rock. Sedimentary phosphate ores presented higher ^{238}U, ^{226}Ra, and ^{232}Th contents than others. During the industrial procedure, the naturally occurring radionuclide in the ore is fractioned into different by-products and wastes. Phosphoric acid and fertilizers are enriched in uranium and thorium; while wastes, mainly phosphogypsum, are enriched in radium, ^{210}Pb, and ^{210}Po. As a consequence of these enhanced radionuclide contents, they can pose a radiological hazard.

Regarding phosphogypsum, different hazards can be considered according to the waste management. Their release into rivers increased the radium content in water and sediments downstream of the discharging point. After the banning of these discharges, a self-cleaning process of the affected aquatic ecosystems has been reported due mainly to the redistribution of the contamination by tides and currents. The accumulation of phosphogypsum in stacks

can increase the external exposure for workers due to its high radium content. Changes in the working routine, such as limitations of the residence time and the improvement of waste management, can reduce this pathway. The dust resuspended from the stacks is most significant in dry windy climates than in temperate ones, in which a crust is usually developed. The radon exhalation from the phosphogypsum stacks is almost negligible, since they are usually located outdoors and the radon is diluted in the surrounding atmosphere. However, the constructions built reclaimed stacks are sensitive to an enhancement of indoor radon. The use of phosphogympsum as building material is not recommended because it can also be a source of indoor radon.

The agricultural use of fertilizers increased the naturally occurring radionuclide content in soil. But the external exposure due to this increase can be considered as negligible. Their transfer to different vegetal foodstuff was also low, comparable with non-fertilized plants. However, their use can increase the eutrophication of waterbodies along with an increase of their uranium content. Another interesting use of fertilizers is their ability to reduce the transfer of anthropogenic radionuclides to plants, acting as countermeasures. It was based on the saturation of stable elements, chemically analogue to radionuclides, in the soil solution. The use of potassium based fertilizers reduced the uptake and transfer of radiocaesium; while the use of calcium, generally as lime, reduced that of radiostrontium.

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