Characterization of Phosphate Rocks/Fertilizers and Their Effects on Cd Uptake by Komatsuna (*Brassica rapa* var. *perviridis*) and Spinach (*Spinacea oleracea*) Grown on Melanudand and Haplaquept

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燐鉱石 / リン酸質肥料のキャラクタリゼーションとそれらの Melanudand および Haplaquept に栽培したコマツナとホウレンソウのカドウミム吸収に及ぼす影響

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ABSTRACT

Phosphate rocks (PRs) are mainly used for the manufacturing of phosphate fertilizers (PFs). Next to nitrogen, PF is by far the most limiting nutrient for crop production. The characteristics of PRs and PFs with special reference to cadmium (Cd), which are important phosphate fertilizer source, were investigated. In my field of study, PR1 of Wyoming, USA; PR2 of Florida, USA; PR3 of Idaho, USA; PR4 of Quedzem, Morocco; PR5 of Slyudanka, Russia; PR6 of Okinawa, Japan; PF1 China 1; PF2 China 2; PF3 China 3; PF4 Japan1; PF5 Japan 2; PF6 Japan 3 and PF7 Myanmar were selected and compared using X-ray fluorescence (XRF), inductively coupled plasma spectrometry (ICP-MS) and X-ray diffraction (XRD). Elemental analysis results of PR samples showed that they were mainly composed of Ca, P, Si, Al, S and Fe. Total phosphate (P_2O_5) concentrations in all PRs and PFs were 6 to 38% and 12 to 50% on the average. The Cd concentration levels in all PRs and PFs ranged from 0.15 to 507 and 0.04 to 4.04 mg kg^{-1}. XRD analysis revealed that; fluorapatite, hydroxyapatite and carbonate fluorapatite were the dominant minerals. This study confirmed that prominent mineral component of PR3, PR4, PF2 and PF5 showed that higher Cd content was carbonate fluorapatite, suggesting that Cd might be exchanged to Ca and or occluded in carbonate fluorapatite during its formation by sedimentation.

Cd concentration in food product may be controlled to some extent by reducing Cd input in phosphate fertilizers. The pot experiment was studied with five phosphorus sources (China 1, China 2, China 3, Japan 1, and Myanmar 1) applied at 120 kg ha^{-1} of P_2O_5 and control to know influence of fertilizers on Cd in soil and Komatsuna. The highest Cd contents in Komatsuna reached 0.034 and 1.24 mg kg^{-1} by (China 1) application whereas the lowest one resulted 0.029 and 1.08 mg kg^{-1} from (Myanmar) treatment of Melanudand and Haplaquept. It was included that total Cd concentrations in soils were the main controls on Cd contents in plants.

A pot experiment was conducted to investigate the influence of calcium superphosphate and fused magnesium phosphate fertilizers on Cd in soil, Komatsuna and spinach growth and Cd uptake. The phosphate application increased total soil Cd (2.11 to 2.24 mg kg^{-1}) and extractable Cd (1.36 to 1.41 mg kg^{-1}) of Haplaquet. The phosphate application increased total soil Cd (0.48 to 0.58 mg kg^{-1}) and extractable Cd (0.06 to 0.08 mg kg^{-1}) of Melanudand. Result showed that the long-term use of PF would slightly increase the concentration of Cd in soils. Compared with the no phosphate fertilizer treatment, different phosphate fertilizers application increased the Cd concentration in dry leaves by Komatsuna (0.19 to 0.23 mg kg^{-1} grown on Melanudand and 1.58 to 1.28 mg kg^{-1} grown on Haplaquept) and spinach (0.74 to 0.78 mg kg^{-1} grown on Melanudand and 12.1 to 15.2 mg kg^{-1} grown on Haplaquept). The results suggested that the concentration of Cd in Spinach was 10 times higher than that in Komatsuna when it was grown in Cd polluted soil (Haplaquept) and 4 times in unpolluted soil (Melanudand).

A field experiment was conducted on Haplaquept to investigate the influence of calcium superphosphate on soil Cd and uptake of Cd among four Komatsuna cultivars. The superphosphate application increased total soil Cd from 2.51 to 2.75 mg kg$^{–1}$, 0.1 mol L^{-1} HCl extractable Cd from 1.48 to 1.55 mg kg$^{–1}$. Cd input reached 5.68 g ha$^{–1}$ at a rate of 240 kg ha$^{–1}$ superphosphate fertilizer application. Field experiments in two years demonstrated that applications of different levels of calcium superphosphate did not influence Cd concentration in soil and Komatsuna significantly. However, there was significant difference in Cd concentration of fresh and dry Komatsuna leaves among four cultivars in 2008 and 2009. The highest Cd concentration was found in Nakamachi cultivar (2.14 mg kg^{-1} in 2008 and 1.91 mg kg^{-1} in 2009). The lowest Cd concentration was observed in Maruha cultivar (1.51 mg kg^{-1} DW) in 2008 and in Hamami No.2 cultivar (1.56 mg kg^{-1} DW) in 2009. This study recommended the cultivars of Maruha and Hamami

No.2 to grow Komatsuna in Cd-contaminated soil, because of lower Cd accumulation.

燐鉱石 /リン酸質肥料のキャラクタリゼーションとそれらの Melanudand および Haplaquept に栽培したコマツナとホウレンソウのカドウミム吸収に及ぼす影響

リン鉱石（PRs）は主にリン酸質肥料（PFs）製造のために使われる。リン酸は窒素に次ぐ作物生産を最も制限する栄養素である。本研究は、様々なリン鉱石とリン酸質肥料中の Cd に関連して調査したものである。6 種類のリン鉱石（米国 Wyoming 産（PR1、Florida 産（PR2、Idaho 産（PR3、モロッコ Quedzem 産（PR4、ロシア Slyudanka 産（PR5、沖縄産（PR6）ならびに 7 種類のリン酸質肥料である過リン酸石灰および熔成リン肥（中国産（PF1、PF2、PF3）、日本産（PF4、PF5、PF6）、ミャンマー産（PF7）の各種性質を、蛍光 X 線、X 線回折、ICP-MS を用いて比較した。リン鉱石は主に Ca、P、Si、Al、S、Fe から構成されていること、リン酸含量は 6％～38％の範囲にあること、さらにリン鉱石の Cd 濃度は 0.15～507 mg kg-1 の範囲にあることを示した。リン鉱石およびリン酸質肥料の主要な X 線回折ピークは、フッ素リン灰石（fluorapatite：$Ca_5 (PO4)_3 F$), 水酸リン灰石（hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$)、炭酸フッ素リン灰石（cabonate fluorapatite：$Ca_5 (PO4,CO_3)_3F$) に由来するものであった。PR3、PR4、PR5 は Cd 濃度が高く、主要な鉱物が炭酸フッ素リン灰石であったことから、鉱物が生成される堆積過程で Cd が Ca と置換し、閉じ込められたと推察された。

食料品中の Cd 濃度は、リン酸質肥料に由来する Cd のインプットを減らすことである程度抑制できる可能性がある。5 種類のリン酸質肥料を 120 kg P_2O_5 ha-1 の濃度で黒ボク土および灰色低地土に添加し、コマツナの Cd 吸収量をポット試験で比較した。コマツナの Cd 含量は China1（PF1）で最も高く、0.034 mg/kg（黒ボク土）、1.24 mg/kg（灰色低地土）であった。最も低かったのはミャンマー産肥料（PF7）を用いた場合で、0.029 mg/kg（黒ボク土、1.08 mg/kg（灰色低地土）であった。Cd 濃度の異なる肥料を用いた違いに比べると、土壌の種類が異なる方が、よりコマツナの Cd 吸収に影響を与えた。

過リン酸石灰および熔成リン肥を施用した土壌中のCd含量ならびにコマツナ、ホウレンソウの生育とCd吸収への影響をポット試験により調査した。施肥量の増加とともに灰色低地土の全 Cd 含量（2.11→2.24 mg/kg）および抽出可能な Cd 含量（1.36→1.41 mg/kg）が増加した。黒ボク土では、全 Cd 含量が 0.48 から 0.58 mg/kg へ、抽出可能な Cd 含量は 0.06 から 0.08 mg/kg へと増加した。したがって、リン酸質肥料の長期施用により土壌中の Cd 含量がわずかであったが増加することが示された。一方、コマツナの乾物中の Cd 含量は、本研究の範囲内では、リン酸質肥料の施用による明瞭な影響は見られず、黒ボク土では 0.19～0.23 mg/kg、灰色低地土では 1.28～1.58 mg/kg の範囲であった。ホウレンソウの乾物中の Cd 含量は、コマツナ中の Cd 含量の 3～10 倍に達していたが、リン酸質肥料の施用による Cd 含量の増加の影響は見られず、黒ボク土では 0.74～0.78 mg/kg、灰色低地土で 12.1～15.2 mg/kg であった。

過リン酸石灰の施用（0～240 kg ha-1）が土壌中の Cd 含量ならびにコマツナ 4 品種の Cd 吸収に及ぼす影響を、灰色低地土の圃場試験により評価した。過リン酸石灰の施肥により、土壌中の全 Cd 含量は 2.51 から 2.75 mg/kg に、0.1M HCl 抽出 Cd 含量が 1.48 から 1.55 mg/kg へと増加した。240 kg ha-1 の過リン酸石灰の施肥は、1ha 当たり 5.68g の Cd 投入に相当する。2 年間の圃場試験の結果から、リン酸質肥料の施肥によって、土壌中の Cd 含量は有意には増加しないが、コマツナ葉中の Cd 含量は有意に増加した。コマツナ 4 品種の Cd 含量においては、年度の違いも認められたが、最も高いコマツナ葉中の Cd 濃度は「なかまち」で見られ、2008 年は 2.14mg/kg、2009 年は 1.91mg/kg であった。最も Cd 濃度が低かったのは、2008 年では「丸葉」で 1.51mg/kg、2009 年では、「浜美 2 号」で 1.56 mg/kg であった。したがって、Cd 含量の高い土壌では、Cd 吸収能の低いコマツナ品種「丸葉」あるいは「浜美 2 号」の栽培が推奨される。

ACKNOWLEDGEMENTS

First and foremost, I would like to pray to Gautama Buddha, Dhamma, Sangha, my parents and all my teachers.

I would like to express my sincere acknowledgement in the support and help of my supervisors **Dr. Masanori OKAZAKI**, and **Dr. Koki TOYOTA**, Professors, Department of Environment-Symbiotic Producting Systems, Tokyo University of Agriculture and Technology (TUAT), whose sincerity and encouragement I will never forget.

I gratefully acknowledge the funding sources that made my Ph.D. work possible. I was funded by **Monbukagakusho Scholarship** (文部科学省奨学金, *monbukagakushō shōgakukin*) for the great financial supports Scholarship for International Student like me from 2007 to 2012 by providing great contributing for my five and half years survival for studying Japanese language, master and doctor course in Japan.

I also would like to thank heartily **Prof. Toshiro Morikiyo** and **Dr. Katsura Ishida** (Shinshu University) for providing PR5 sample (Slyudanka, Russia) and **Dr. Ryunosuke Hamada**, Professor Emeritus (Tokyo University of Agriculture and Technology) for giving comments and suggestions in the preparation of the papers and thesis.

Grateful appreciation is due to the examiner, **Dr. Tetsu ANDO**, Professor, and **Dr. Ryoichi SATO,** Professor**,** Tokyo University of Agriculture and Technology for his valuable advice and evaluation to the dissertation. For this dissertation I would like to thank my reading committee members: **Dr. Shoji MATSUMURA,** Associate professor and **Dr. Sohzoh SUZUKI,** Professor, Tokyo University of Agriculture and Technology, for their time, interest, and helpful comments.

It is a great pleasure to express my utmost gratitude to **Dr. Takashi MOTOBAYASHI,** Senior Assistant professor, Tokyo University of Agriculture and Technology, for allowing, supporting me to conduct my experiments in the Field Museum Hommachi, Field Science (FS) Center for all years round (2007- 2012) of the study period and for his valuable guidance and suggestions.

I also acknowledge the immense contributions of my Japanese friends for their kind help for Japanese teaching and translation in using every machine and filling up the application forms of my application documents during studying in Japan. I am greatly thankful to my Myanmar friends for their kind help to do my research pot, field experiments cultivation, and their partnership. I would like to express my heartfelt gratitude to my teachers, friends who I meet during my staying in Japan from 2007 to 2012.

My time at **Graduate School of Bio-Applications and Systems Engineering** (**BASE**), **TUAT**, was made enjoyable in large part due to the many friends and groups that became a part of my life. I am grateful for time spent with friends, for our memorable trips into soil survey, research center, conference, symposium, factory, pagoda, ski, mountains, beach, museum and park for their hospitality and memories.

Last but not least, I am heartily thankful to my family, my beloved parents, **U Hla Aung** and late **Daw Sein Ngwe**, my brother and sister in law **U Tin Aung Cho Oo** and late **Daw Khin Soe than**, my sister **Wai Wai Mar**, My nephew **Zay Phyo Tun,** Shwebo Township, Myanmar for their love and encouragement.

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1. GENERAL INTRODUCTION

1.1 Phosphate rocks

1.1.1 The importance of phosphate rocks for phosphate fertilizer

Phosphate rocks (PRs) is a globally accepted but imprecise term describing any naturally occurring geological material that contains one or more phosphate minerals suitable for commercial use. PR is an essential resource for the production of fertilizers. PR is being imported from various sources around the world. Morocco phosphate rock has three quarters of the world phosphate reserves (Bilali et al., 2005). It is the first exporter in the world and the third producer after the U.S.A and Russia (Slansky, 1980). The various phosphate minerals present in PR have diverse origins and chemical and physical properties. The phosphorus content or grade of phosphate rocks is commonly reported as phosphorus pentoxide (P_2O_5). The principal phosphate minerals in PR are Ca-phosphates, mainly apatites. Pure fluorapatite contains 42% P_2O_5, and francolite, the carbonate-substituted form of apatite, may contain 34% P_2O_5. Five major types of phosphate resources are being mined in the world:

- Marine phosphate deposits,
- Igneous phosphate deposits,
- Metamorphic deposits,
- Biogenic deposits,
- Phosphate deposits as a result of weathering.

The world phosphate resources are distributed, according to their type, approximately as follows: 75% from sedimentary marine PR deposits, 15-20% from igneous, metamorphic and weathered deposits, and 2-3 % from biogenic resources (Sengul et al., 2006; Jiang et al., 2007).

Different types of PRs have widely differing mineralogical, chemical and textural characteristics. While there are more than 200 known phosphate minerals, the main mineral group of phosphates is a group of apatites. Calcium-phosphates of the apatite group are mainly found in primary environments (in sedimentary, metamorphic and igneous rocks) but also in weathering environments.

Phosphate minerals occurring in the primary environment include:

- Fluorapatite ($Ca_{10} (PO_4)_6F_2$), found mainly in igneous and metamorphic environments, for example, in carbonatites, and mica-pyroxenites,
- Hydroxy-apatite ($Ca_{10} (PO_4)_6(OH)_2$), found in igneous, metamorphic environments but also in biogenic deposits, e.g. in bone deposits,
- Carbonate-hydroxy-apatites ($Ca_{10} (PO_4,CO_3)_6(OH)_2$), found mainly on islands and in caves, as part of bird and bat excrements, guano,
- Francolite ($Ca_{10-x-y}Na_xMg_y (PO_4)_{6-z}(CO_3)_zF_{0.4z}F_2$). This complex, carbonate substituted apatite is found mainly in marine environments, and, to a much smaller extent, also in weathering environments, for instance over carbonatites.

The chemical reactivity or solubility of PRs is a measure of the PR's ability to release P for plant uptake. The reactivity of sedimentary PRs is relatively high compared to those of igneous and metamorphic origin. The fundamental difference lies in the crystal chemistry of apatite, specifically the degree of isomorphous substitution of phosphate by carbonate. The PRs are concentrated and then chemically processed with sulphuric and phosphoric acid into soluble phosphate fertilizers such as single superphosphates (SSP), and triple superphosphates (TSP), as well as mono-ammonium phosphates (MAP) and di-ammonium phosphates (DAP). Fused Magnesium Phosphates (FMP) are formed by mixing PR sources (usually of low citrate solubility) with

Mg sources such as olivine or serpentine. The mix is then fused in a furnace at about 1500-1600^{o}C.

1.1.2 Phosphate rock as an environmental pollutant of Cd

The PR is, due to its origin, affected by high concentrations of impurities, heavy metals, naturally occurring radionuclides and other so-called trace elements (Martin et al. 1999; Rutherford et al. 1994). PR contains most notably cadmium. PRs tend to contain relatively high concentrations of several heavy metals, but Cd is probably the most important with regard to soil protection and risks to human health. Rutherford et al. (1994) estimate that, on average, PRs are enriched 60 times in Cd compared with shale. The enrichment is thought to be due to substitution of Cd for Ca^{2+} in the apatite crystal. Kongshaug et al. (1992) summarized the average composition of 91% of the world's PR reserves as Cd 25 mg kg^{-1}.

1.2 Phosphate fertilizer

Most of the world's phosphate fertilizers are produced from PR resources and almost all of these resources contain some form of the mineral apatite. Phosphate fertilizers contain traces of cadmium that can be accumulated in soil with repeated application of phosphate fertilizer. The raw materials used for commercial phosphate fertilizer production are mainly of sedimentary, igneous and biogenic origin. Almost all phosphate fertilizers produced from PR contain a trace of cadmium. Large amounts of Cd are contained in phosphate fertilizer (Williams, 1974). Phosphate fertilizers can contain up to 300 mg Cd kg^{-1}, whereas N and K fertilizers generally contain less than 9 mg kg^{-1} (Fergusson, 1990). Phosphate fertilizers are therefore one of the most ubiquitous sources of Cd contamination in agricultural soils throughout the world. All soils receiving these fertilizers will have had an input of Cd, but how much will depend on the composition of the material which, in turn, is determined by type of fertilizer, the source of PR used for its manufacture, and the amount applied.

1.2.1 The importance of phosphate fertilizer for crop production

Next to nitrogen, phosphate is by far the most limiting nutrient for crop production. Phosphate is essential for plant growth and maintaining agricultural productivity. It is a nutrient required in relatively large amounts by plants. Plants need phosphorus for growth, utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumen formation. Phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. Phosphorus is, therefore, important in cell division and development of new tissue. Phosphorus is also associated with complex energy transformations in the plant. Adding phosphorus to soil low in available phosphorus promotes root growth and winter hardiness, stimulates tillering, and often hastens maturity.

1.2.2 Phosphate fertilizer as a source of Cd in agricultural field

Cadmium can accumulate in soils from ongoing phosphate-based fertilizer application. Long- term use of high-cadmium fertilizers could cause the toxic metal to accumulate in edible plants and inadvertently enter the food chain as shown in Figure 1. The cadmium content of phosphate fertilizers can be reduced by using low-Cd PR (usually of magmatic origin), or by decadmiation of either PR or phosphoric acid. Using low-Cd rock has been the dominant strategy in countries where cadmium reduction policies are being pursued. Decadmiation is currently too expensive and low-cost technologies are not yet fully developed. In some cases technical restrictions impede the use of either magmatic rock or decadmiation. Apart from reducing fertilizer use, lowering the cadmium content in fertilizers is the only feasible way of reducing the cadmium input to farmland from this source. This can be achieved either by using raw materials (PR) with a lower cadmium content, or by eliminating the cadmium from the PR or the phosphoric acid during the production process. Obviously, the flow of cadmium to agricultural soils can also be reduced by applying lower amounts of phosphate fertilizer and/or substituting fertilizers by other products containing phosphorus e.g. manure.

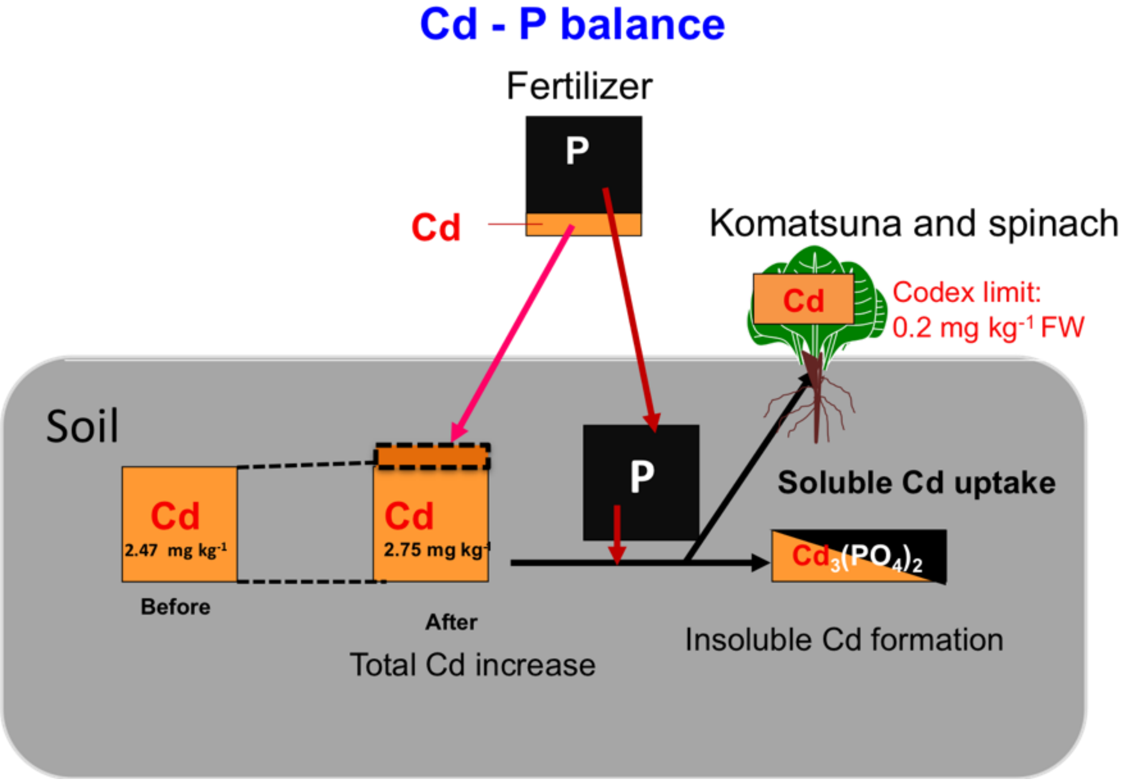


Figure 1. Cadmium and phosphate reaction in soil after application of fertilizer

1.3 Cadmium

1.3.1 Introduction

Cadmium, Cd, atomic number 48, is in a group IIb metal of the periodic table and a mineral mined as part of Zn deposits (Elson and Haas, 2003; Department for Environment, Food and Rural Affairs and Environmental Agency, 2002). Elemental Cd is a bluish-white divalent metal. Its structure deviates from perfect hexagonal close packing by elongation about the six-fold axis. It is relatively volatile with melting and boiling points of 321 and

767^{0}C, responsively, and a heat of vaporization of 26.8 kcal mol^{-1} (Cotton and Wilkinson, 1996). Cd can be a potential threat to a wide range of biota because (i) it is non-essential and non-metabolic, (ii) it is toxic to humans at concentrations lower than those toxic to plants, (iii) it is more mobile and bioavailable than other metals, and (iv) its effects on humans are cumulative. Cd in soil originates either from geological parent materials (Adriano, 1986; Jeng, 1992) or from anthropogenic pathways (Kabata-Pendias and Pendias, 1992; Singh, 1994). It occurs as an impurity in phosphate fertilizers, with which it is applied to agricultural land. Cadmium is also added to agricultural land through treated sewage. Its most common form in soils is the free Cd^{2+} (Department for Environment, Food and Rural Affairs and Environmental Agency, 2002). Most of the Cd found in water up to pH 9.0 is in the divalent cation form (Cd^{2+}). Cd is highly soluble under acidic conditions, but its solubility decreases above pH 9.0 due to the formation of cadmium hydroxide ($Cd(OH)_2$). The presence of organic matter lowers the toxicity of Cd as the metal is adsorbed onto exchange sites of organic matter (Doyle, 1978). Cd strongly binds to sulphydryl (-SH) groups hence the pronounced tendency of Cd to bio-accumulate in the food chain (Zambezi River Authority, 2001).

1.3.2 Cadmium in soils

Cd concentration in soil ranges from low in uncontaminated soils to high in soils, receiving large quantities of Cd through agricultural and industrial activities or in soils naturally rich in Cd. In spite of the level of Cd in soils, the speciation and fate of Cd is controlled to a greater extent by parent material compositing and the intrinsic soil properties. Knowledge of chemical forms or speciation of Cd in solution phase as well as in solid phase in soils leaching rates and transformations between chemical forms in contaminated and non-contaminated soils is potentially valuable in predicting bioavailability. Cd in solution phase may be present as free, hydrated cations or species complexed with organic and inorganic ligands. Complexed Cd may be bound to high molecular weight organic material or to disperse metal colloids such as hydroxy polymers of Fe and Al. Chemical reaction phenomena such as precipitation-dissolution, charged-surfaces, adsorption-desorption, ion–exchange, speciation etc. in the soil system control the concentration of Cd in the solution phase. Soil phase Cd may be present as chemical precipitates in Cd minerals or in association with other soil components. The process of sorption and desorption determines the distribution of Cd between the solid phases of the soil and the soil solution. Many soil components such as metal oxides (Fe and Mn), layer silicates, calcite and hydroxyapatite, organic matter and biological colloids (micoorganisms) are involved in sorption and desorption process (McBride, 1989; Zachara et al., 1992; Stevenson, 1994; Huang and Bollag, 1997).

1.3.3 Cadmium in plants

Cd absorption across the plasma membrane of root cells is controlled by the electrochemical potential difference between the activities of Cd^{2+} in the cytosol and that in the root apoplasms. The large negative membrane potential alone provides more than enough energy to drive Cd^{2+} uptake even at low-solution activities of Cd^{2+}. The kinetics of Cd^{2+} absorption by roots show bi-phasic characteristics with a saturable component at low Cd^{2+} activities in the absorption solution and a linear component at higher Cd activities (Costa and Morel, 1993). Besides Cd^{2+}, absorption of Cd could also occur as inorganic complexes of Cd such $CdCl^{+}$, $CdCl_2$, $CdSO_4$ etc. (Mclaughlin et al., 1997) or as organic complex such as phytometallophore complexes. Cd in xylem sap could move as an inorganic cation (Cd^{2+}) as well as in complexes with organic molecules, but in phloem sap virtually all Cd^{2+} is present in complexed forms (Welch, 1986).

Cd availability to plants is affected by both biotic and non-biotic factors affecting Cd uptake by plant (Table 1).

Table 1. Biotic and non-biotic factors affecting Cd uptake by plants

Biotic factors	Non-biotic factors
Plant species	Soil pH
Crop cultivar	Clay content
Plant tissue	Carbonates
Leaf age	Metal oxides (Fe and Mn)
Root activity	Redox potential
Rooting pattern	Organic matter (types and content)
Rhizoshpere and root associated microorganisms	Complexing ligands
(for example, mycorrhizal fungi)	Soluble salts
	Soil management practices
	Phosphate fertilizer
	Manure
	Lime
	Sewage sludge
	Soil tillage
	Irrigation

Source: Oliver et al., 1993

1.3.4 Cadmium in the environment

Cadmium is present in the earth's crust at an average of 0.2 mg kg^{-1} and usually occurs in association with Zn, Pb and copper sulphide ore bodies (WHO, 1992). Cadmium is used in the steel and plastics industries and is released to the environment through wastewater (WHO, 1993). The main sources of Cd in the environment are due to:

(1) air emission from Zn, Pb and copper smelters and industries involved in manufacturing alloys, paints, batteries and plastics

(2) wastewater from mining

(3) agricultural use of sludge and fertilizers containing Cd

(4) burning of fossil fuels

(5) deterioration of galvanised materials and Cd- plated containers

Wastewater has been reported as a major source of Cd, although the metal is often not detected in sludges (Lisk, 1972). Doyle (1978) reported Cd accumulations of over 1 mg kg^{-1} in the soil, following high rates of application of sludges over a long time. The same author also reported accumulation of 100 mg kg^{-1} under furrow irrigation with sludge in some extreme cases.

1.3.5 Health effects of cadmium

Human uptake of cadmium takes place mainly through food. An exposure to significantly higher cadmium levels occurs when people smoke. Other high exposures can occur with people who live near hazardous waste sites or factories that release cadmium into the air and people who work in the metal refinery industry. The average daily intake for humans is estimated at 0.15 μg from the air and 1 μg from water, while smoking a 20-cigarette pack can lead to inhalation of around 2-4 μg of Cd (Scottish Executive Environmental and Rural Affairs Department, 2002). Johnston and Jones (1995) noted that plant-based foodstuffs were the largest source of dietary Cd and that the relative contribution of soil Cd content in plants was important but largely unresolved.

When people breathe in cadmium it can severely damage the lungs. This may even cause death. Cadmium is first transported to the liver through the blood. There, it is bond to proteins to form complexes that are transported to the kidneys. Cadmium accumulates in kidneys, where it damages filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further kidney damage. It takes a very long time before cadmium that has accumulated in kidneys is excreted from a human body. Other health effects that can be caused by cadmium are:

- Diarrhea, stomach pains and severe vomiting
- Bone fracture
- Reproductive failure and possibly even infertility
- Damage to the central nervous system
- Damage to the immune system
- Psychological disorders
- Possibly DNA damage or cancer development

Occupational exposures to cadmium have caused a variety of clinical conditions and fatalities (Hallenbeck, 1984). The worst case of environmental exposure was the Itai-itai disease produced by consumption of cadmium-polluted rice. The symptoms of Itai-itai disease, were first noted in the Toyama Prefecture Japan in 1912 (Imamura, 2007), and after World War II, an outbreak attracted world-wide scientific and press attention (Aoshima, 1997; Nogawa, 1981; Fan et al., 1998). Due to various reforms, itai-itai disease has largely disappeared from Japan but a recent press report (Yanagisawa and Shimbun, 2008) claims that new cases of the malady are still coming to light in the contaminated area.

Somewhat similar health effects have been observed in China (Nordberg, 2004). The toxicity was due to contamination of soils to the extent of only 0.9-1.5 mg kg^{-1} from a tungsten dressing plant and very likely the high soil-acidity and the high consumption in the locally grown vegetables contributed (HMSO, 1995).

Itai-itai like diseases seem to have occurred only in Asia where it is linked to a staple diet of rice (Tsukahara et al., 2003a,b) grown, in some circumstances on soils with as little as 2 mg kg^{-1} Cd (Cai et al., 1990).

Cadmium accumulates in the kidneys and has a long biological life in humans of 10-35 years (WHO, 1993).

1.3.6 Environmental effects of cadmium

Cadmium waste streams from the industries mainly end up in soils. The causes of these waste streams are for instance zinc production, phosphate ore implication and bio industrial manure. Another important source of cadmium emission is the production of artificial phosphate fertilizers. Part of the cadmium ends up in the soil after the fertilizer is applied on farmland and the rest of the cadmium ends up in surface waters when waste from fertilizer productions is dumped by production companies. Cadmium strongly adsorbs to organic matter in soils. When cadmium is present in soils over 3 mg kg^{-1}, it can be extremely dangerous, as the uptake through food will increase. Soils that are acidified enhance the cadmium uptake by plants. This is a potential danger to the animals that are dependent upon the plants for survival.

1.3.7 Prevention of Cd contamination

Accumulation of Cd in soils and food crops can be limited by reducing Cd input through selection of low Cd fertilizers on one hand and the adoption of efficient management practices to reduce Cd availability to crops on the others. To prevent soil from cadmium contamination of phosphate fertilizer, it is primordial to remove cadmium from phosphate ore. Two techniques are proposed to eliminate cadmium from phosphate ores: calcinations and leaching. Calcination is most effective at temperatures between 850 and 1150^{0}C, either under an inert atmosphere or reducing conditions. The cadmium oxide produced is very volatile but it is also very toxic. Calcination in the presence of chloride salts allows a temperature as low as 700^{0}C to be used Baechle and Wolstein, 1984 but this results in excessive corrosion.

Leaching technique is economical, better than calcinations (Keith Syers, 2001a,b).

Decadmiation, or the removal of Cd from fertilizer during manufacturing, is only technically feasible for fertilizers based on phosphoric acid, where the acid can be treated with ion exchangers, complexing or precipitating agents to selectively remove Cd (Vermeul, 1996). Practical technologies to remove Cd from PR during the manufacture of fertilizers are still not economical. Commercial scale decadmiation has not yet been successful. There was a fairly substantial amount of research on calcination in the 1980s and some of this led to patents. The CERPHOS process, developed in Morocco, decreased the cadmium content of Moroccan PR by 75 per cent, when heated in the range of 750 to 950^{0}C under controlled pressure to reduce and volatalize the cadmium (Kossir and Chik, 1996); the estimated cost was about US$10 tonne^{-1} of phosphate concentrate. It appears that the only calcination facility in the world for removing cadmium was built by Nauru Phosphate Corporation. This plant operated at a capacity of 75 tonnes hour^{-1}, and decreased the cadmium content of Nauru PR from about 600 to less than 120 mg kg^{-1} P (Van Kauwenebergh, 2001). The plant has now been decommissioned. The precise costs of calcination by the different methods are not known but are thought to be in the range of US$10 to 20 tonne^{-1} PR, although the cost for calcining Nauru PR appears to have been higher, given that in 1992 Nauru PR was selling for about US$50 tonne^{-1} whereas the calcined product sold for over US$90 tonne^{-1}.

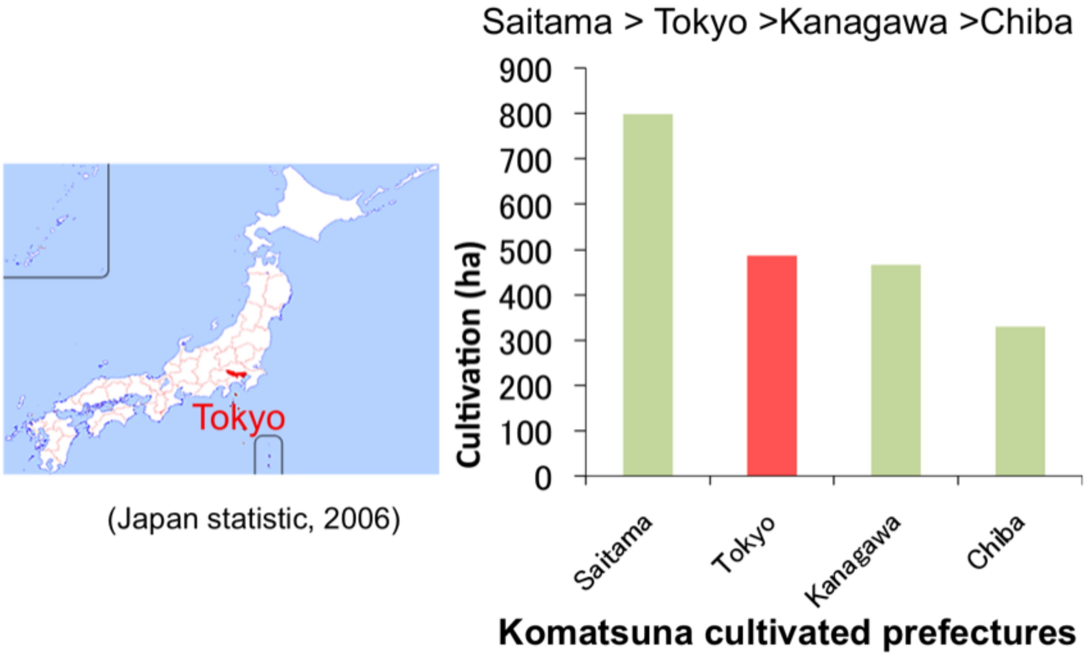
To the knowledge of the author, there is currently no economically viable industrial process for reducing the cadmium content of PR to acceptable levels for the manufacture of phosphorus fertilizers. Also, there have been no recent research publications on the removal of cadmium by calcination. Interest has waned because of economic considerations and because other research has indicated that cadmium removal from phosphoric acid is a more cost-effective route (Van Kauwenbergh, 2001). Cd removal is technically difficult.

1.4 Crops

Leafy vegetables are an important part in the human diet (Anjana et al., 2007). Vitamin C is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body (Lee and kader, 2000). Vitamin C is the most important vitamin in fruit and vegetables for human nutrition. More than 90% of the vitamin C in human diets is supplied by fruit and vegetables. Among the edible plant parts, highest cadmium levels were found in lettuce and spinaches, and were followed by levels in brassica tops, radish and carrot tubers, pea seeds and oat grains (Matt and John, 1970).

1.4.1 Komatsuna

Komatsuna (*Brassica rapa* L. var. *perviridis*) is a type of leaf vegetable. It is a variant of same species as the common turnip. It is grown commercially in Japan, and Taiwan. It is also known as Japanese Mustard spinach and is usually stir-fried, pickled, boiled and in salads. It is an excellent source of calcium (Queensland Government, 2010). It was chosen as experimental plant for this study because this is a popular vegetable in Japan. Tokyo is one of the highest production places of Komatsuna as shown in Figure 2. In Fuchu city, the main production of vegetable is Komatsuna.



(Japan statistic, 2006)

Figure 2. Komatsuna cultivation in Japan

1.4.1 Spinach

Spinach (*Spinacea oleracea* L.) is an edible flowering plant in the family of Amaranthaceae. It is native to central and southwestern Asia. It is also popular vegetable in Japan as well as in many other countries. It is widely cultivated in the world as one of the most popular vegetables. It is an annual cool season crops and in temperate area it can be grown all year around. It is a green, leafy vegetable and it can be eaten boiled, baked, and raw a baby leaf as well. It represents a good source of vitamins as well as minerals such as calcium and magnesium (NRC, 1989). Spinach is low in calories and a good source of vitamin C, vitamin A and mineral especially iron (Toledo et al., 2003).

1.5 Soils

Soil of Saiwaicho is Cumulic Andosols (Melanudand) and soil of Hommachi is Gray lowland soils (Haplaquept).

1.5.1 Melanudand

Melanudand (Andisols) with thick humus horizons, Melanic and Fluvic epipedons occur widely in mountainuous area of Japan. These two epipedons have formed through intermittent contributions of volcanic ash deposition (Shoji et al, 1993). Melanudand is formed in layers of volcanic ash. The dark surface is a good example of a melanic epipedon. A central characteristic of Andisols is their development from volcanic ejecta and/or volcaniclastic materials that have colloidal fractions dominated by short-range orered minerals and/or Al-humus complexes (Takahashi and Shoji, 2002). Andisols in Japan also show the highest accumulation of organic carbon among mineral soil (Nanzyo et al. 1993).

1.5.2 Haplaquept

The Haplaquept are the Aquepts from the zone where soil temperature at the 50 cm depth differs by more than 5^{o}C between winter and summer. They are dominant on all larger alluvial and deltaic plains of that zone, and also occur extensively in inland valleys and on lower slope. These soils commonly occur on alluvial plains except natural levees and backswamps in eastern Japan and are used mainly for paddy cultivation (The Group of Japanese Pedology, 1990).

1.6 Objectives of the study

Fertilizer application is one of the most importance ways to improve the yields and qualities of crops in agricultural activities. However, it can influence the accumulation and transfer of cadmium in soil-plant system. It is one of the sources of soil cadmium (He et al., 2005) especially phosphate fertilizer. A few studies have been carried out to investigate the effect of phospahte fertilizers on the uptake of Cd, one of the most toxic elements, in leafy vegetable. This thesis deals with impacts of different phosphate fertilizer application to

Komatsuna and spinach cultivation on Cd uptake. The objectives of this thesis are:

(1) To investigate Cd contents of PRs and PFs from different countries

(2) To elucidate the relationship between the Cd content and CFA

(3) To examine the effect of different Cd content in PFs on Cd concentration and uptake by Komatsuna grown on Melanudand and Haplaquept

(4) To identify the effects of application of calcium superphosphate and fused magnesium phosphate on Cd uptake between Komatsuna and Spinach grown on Melanudand and Haplaquept

(5) To investigate the effect of application of calcium superphosphate levels and cultivars on Cd uptake by Komatsuna

The Codex Committee on Food Additive and Contaminant (CCFAC) has proposed limits for Cd concentrations in different crops such as rice, wheat, potato, root and tuber, leafy vegetables and others (Codex Alimentarious Commission, 2005) as shown in Figure 3. The Codex Committee (Codex, 2005) adopted 0.2 mg kg^{-1} FW (Fresh Weight) of Cd concentration in leafy vegetable such as Komatsuna and Spinach in 2005 based on 7 µg per kg body weight per week of Provisional Tolerable Weekly intake (PTWI) after long discussion in Joint FAO/WHO Expert Committee on Food Additive (JECFA).

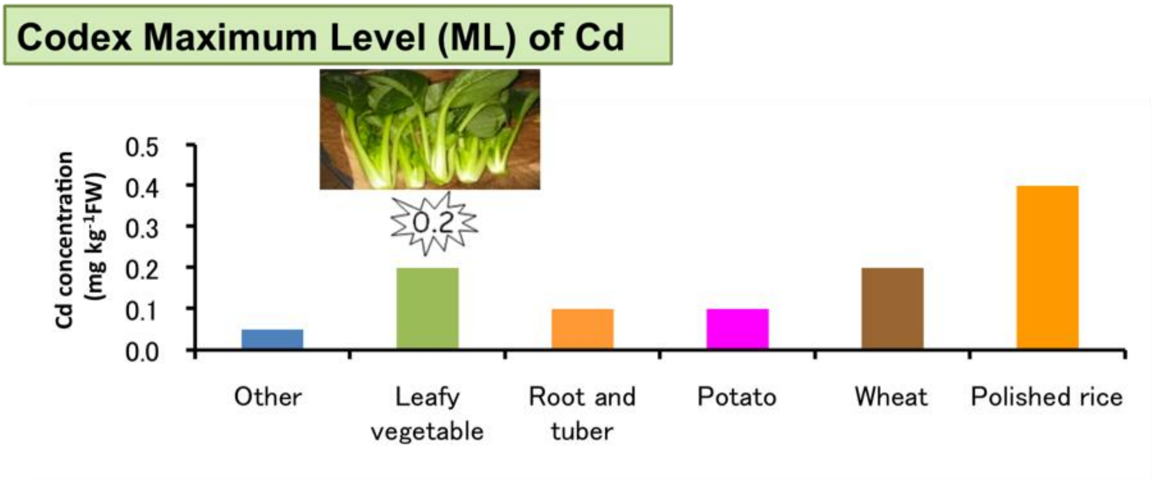


Figure 3. Cd concentration of Codex maximum level of different crops

1.5 Outline of this thesis

Chapter 2 describes mineral, chemical composition and cadmium concentrations of different PRs which is published in Microchemical Journal 104 (2012) 17-21. Data from the experiment (Chapter 2) is also included cadmium concentration in different PRs from other studies to compare our study. Chapter 3 deals mineral characteristic and cadmium concentration in different phosphate fertilizers that use in our experiment and comparison of cadmium concentration in fertilizers from other studies. After analyzing of Cd in phosphate rocks and fertilizers, the effect of different phosphate fertilizer treatments on Cd uptake by Komatsuna are emphasized in Chapter 4 because there is one source of anthropogenic activities in agricultural field as well known theories but not yet established in Komatsuna. The Chapter 4, the information about the cadmium concentration in soil and plant, which is quantified, is mentioned. The Chapter 5 deals effect of different levels of calcium superphospahte and fused magnesium phosphate on Cd uptake by Komatsuna. The Chapter 6 deals effect of different levels of calcium superphosphate and fused magnesium phosphate on Cd uptake by spinach. The Chapter 7 deals the Influence of phosphate fertilizer application levels and cultivars on cadmium uptake by Komatsuna which is published in Soil Science and Plant Nutrition journal 58 (2012) 492-502. The overall impacts of different phosphate fertilizer application on Cd uptake by Komatsuna and spinach are discussed in Chapter 8 in which crop response and soil contamination after harvest are dealt with. Finally conclusion of the thesis is drawn in the Chapter 8.

CHAPTER 2. MINERALS, CADMIUM AND CHEMICAL CHARACTERISTICS OF DIFFERENT PHOSPHATE ROCKS

2.1 Abstract

Phosphate rocks (PRs) can be a source of cadmium pollution in soil and food chain. Mineral, cadmium and chemical characteristics of phosphate rocks (PRs), which are important as phosphate fertilizer sources, were investigated. In my field of study PR1 of Wyoming, USA, PR2 of Florida, USA, PR3 of Idaho, USA, PR4 of Qued Zem, Morocco, PR5 of Slyudanka, Russia and PR6 of Okinawa, Japan were selected and compared using X-ray fluorescence (XRF), X-ray diffraction (XRD) and inductively coupled plasma mass spectrometry (ICP-MS). Elemental analysis results of PR samples showed that they were mainly composed of Ca, P, Si, Al, S and Fe. Total phosphorus concentration in all PRs is 6 to 38% on the average. As a result of XRD analysis, the main peaks of the samples were found to be fluorapatite ($Ca_5(PO_4)_3F$) , hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$) and carbonate fluorapatite ($Ca_5(PO_4,CO_3)_3F$). The Cd concentration levels in all PR samples ranged from 0.15 to 507 mg kg^{-1}. PR4 samples exhibited about two times higher Cd concentration than those of the literature so far published. This study also confirmed that PR3 and PR4 exhibited higher Cd content and prominent mineral component of carbonate fluorapatite suggesting that Cd might be exchanged to Ca and/or occluded in carbonate fluorapatite during its formation by sedimentation.

2.2 Introduction

Phosphate rock (PR) is a general term which refers to rock which is most commonly of the apatite group {Ca5 $(PO_4)_3$ [F, OH or Cl]}. PR is naturally occurring mineral assemblages containing high concentrations of phosphate minerals, which can be commercially exploited, either directly or after processing (Notholt and Highley 1986; Bartels and Gurr 1994). It is natural mineral deposit of phosphorus and calcium, which belongs to the species of the apatite family. About 95% of the world PR consumption, which approach 150 million tons annually, is consumed in fertilizer industry (Abouzeid 2008). The world phosphate resources are distributed, according to their type, approximately as follows: 75% from sedimentary marine deposits, 15-20% from igneous, metamorphic and weathered deposits, and 2-3% from biogenic sources (bird and bat guano deposits) (Sengual et al. 2006; Jiang et al. 2007). About 90% of these deposits are used as raw material for manufacturing of phosphate fertilizer (Zapata and Roy, 2004). Composition of PRs mostly depends on their type and origin. PR belongs mainly to sedimentary, slightly to igneous and negligibly to metamorphic rocks. Sedimentary rocks contain high concentration of heavy metals (Hurst 1989). It is used for the production of phosphorus based fertilizers, acids, detergents and many products of common use. More than 40 countries all over the world produce PRs. Most important of these countries, arranged in descending order of their production capacities, are: USA, Morocco, China, Russia, Tunisia, Jordan, Brazil, Israel, Syria, South Africa, Egypt, Australia, Senegal, India, Togo, and Canada. The United States is the world's largest producer and consumer of PRs and the leader in fertilizer production and exports (Chen et al. 2007). In addition, phosphate minerals occurring in the primary environment include: fluorapatite ($Ca_{10}(PO_4)_6F_2$), found mainly in igneous and metamorphic environments, e.g., in carbonates, and mica-pyroxenites, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), found in igneous, metamorphic environments but also in biogenic deposits, for example, in bone deposits, carbonate hydroxyapatites ($Ca_{10}(PO_4,CO_3)_6(OH)_2$, found mainly on islands and in caves, as part of bird and bat excrements, guano, francolite (Ca_{10-x-y} $Na_xMg_y(PO_4)_{6-z}(CO_3)_zF_{0.4z}F_2$). This complex, carbonate-substituted apatite is found mainly in marine environments, and, to a much smaller extent, also in weathering environments (Straaten 2002). Cadmium (Cd) is one of the trace elements highly enriched in PR. PR contains a great variability of heavy metals and trace elements, which upon dissolution in the soil may be harmful at certain concentrations. Special concern is given to the contents of Cd, As, U, Th, Pb, Se and Tl. Sedimentary rocks have higher Cd concentrations than the igneous deposits, typically in the range of (3-150 mg kg^{-1}) can be found in some rocks. Igneous deposits are generally very low in cadmium content. The average Cd concentration in PR (18 µg g^{-1}) is 60 times its average content in shale's (0.3 µg g^{-1}) (Altschuler 1980). The amounts of Cd vary widely not only among various PR sources but also even in the same deposit (Stocia et al. 1997). As there is currently no commercial means of removing Cd completely during fertilizer manufacturing process, the only measure to reduce Cd in product is to use relatively low Cd concentration PR to make fertilizer. The geochemistry of Cd in PR has many aspects. The composition of the PR depends on the genesis conditions, lithology, stratigraphy and geographical provenance. It is important to know the PR cadmium assemblages not only for reasons of agronomic or fertilizer technology, but also for environmental issues. Cd is a biologically mediated trace constituent of seawater and is distributed analogously to the nutrients such as phosphate.

Chemical characterization of PR is required to be checked and account for Cd concentration because quantification of these Cd relative to nutrient value of rock can help in designing and improvement of the chemical process used for production in industry. PR of commercial grade has been exploited in several countries in Middle East and in North Africa. Morocco, which are the major phosphate producer and have the greatest reserve, followed by South Africa and U.S. The distribution of Cd in the environment is governed by the properties of metal and influences of environmental factors. The toxicity of Cd and the fact that the major route of Cd addition to soils is through phosphate fertilizers (Nomiyama 1975; Cook and Freney 1988; Merry and Tiller 1991) adds another incentive to this study. As far as we searched, there are no papers available concerning Cd contents and minerals component of PRs. The objectives of this study are to investigate cadmium contents of PRs from different countries and to elucidate the relationship between the Cd content and carbonate fluorapatite.

2.3 Materials and Methods

2.3.1 Sample collection and preparation

Samples of PR1, PR2, PR3, PR4, PR5 and PR6 were collected from Wyoming (USA), Florida (USA), Idaho (USA), Qued Zem (Morocco), Slyudanka (Russia) and Okinawa (Japan) respectively. The sample was crushed, ground to powder, homogenized and kept in an oven at 110 ºC for 1 day to remove moisture if any. The 6 samples of PRs were analyzed in order to estimate chemical and mineral composition, the concentration of Cd.

2.3.2 Elemental analysis

Quality control was assured by the use of four replicates analyses performed on all samples, the use of reagent blanks, standard reference and internal control samples were also analyzed in order to monitor analytical accuracy and precision. All the samples were also analyzed for their major elements (Na_2O, MgO, Al_2O_3, SiO_2, P_2O_5, SO_3, Cl, K_2O, CaO, TiO_2, V_2O_5, MnO, Fe_2O_3) by X-ray fluorescence analysis (PANalytical, Axios). The sample to cellulose ratio was 1:3.

Plate 1. X-ray fluorescene

2.3.3 Scanning electronic microscopy

The morphology of the PRs was studied by a Keyence VE-9800 scanning electron microscope (SEM). The PRs were made in to small pieces by hammer and put it into slide and took the photo by SEM.

2.3.4 Digestion procedure for Cd

For the analysis of total Cd, one gram of each powdered sample was weighed on an analytical balance and placed in a 200 mL conical flask; 10 mL of concentrated HNO_3 was added to each sample. The flask was heated below boiling temperature and 5 mL $HClO_4$ was poured and the sample was again heated. On the appearance of brown vapour, more $HClO_4$ was added once more and the sample mixture was reheated. This step was repeated until emission of brown vapor stopped. The solution was boiled for evaporation to near dryness and 15 mL of 2 mol L^{-1} HNO_3 was added in it to dissolve all the digested salts. Digestate was cooled and filtered with No.5C Advantec filter paper. Residue was washed with deionized water to get the final concentration of 0.3 mol L^{-1} HNO_3. Certified reference standard (JSAC 0402) was used to check the analytical method and the instrument accuracy.

2.3.5 Cadmium analysis

Standard solutions for cadmium were prepared using high purity standard solution of 0, 0.01, 0.1, 1.0, 10 µg L^{-1}. These standard solutions were used for making a standard calibration curve for Cd to be determined. Analysis of PR for Cd concentration in the digested solutions was carried out with inductively coupled plasma mass spectrophotometry (ICP-MS Thermo, X Series II). Analytical conditions for Cd measurement are listed in (Table 2). Certified reference standard material JSAC 0402 was used to check the analytical method and the instrument accuracy. Deionized-water was used throughout and was directly obtained from a Milli-Q system for ICP-MS analysis.

Plate 2. ICP-MS Theromo, X Series H

Table 2. Analytical condition of ICP-MS analysis

(1) Ion source of ICPOutput	27.12 MHz 1.42 kW Forward, < 5W Refection
Output	
Ar Flow	
Cool Gas (L min^{-1})	1.3
Aux. Gas (L min^{-1})	0.8
Neblizer Gas (L min^{-1})	0.8
(2) Vacuum Interface	
Aperture size	1.0 mm (sampling cone), 0.6 (skimmer cone)
Expansion	2.0 x 10^{0} mbar
Analyzer	1.0 x 10^{6} mbar
(3) Bias of lenses	
Extraction (V)	-120
Lens (V)	-94
Len 1 (V)	-1190
Len 3 (V)	-185
Forcus (V)	-3.7
D1 (V)	-39
D2 (V)	-104
DA (V)	-90
Pole Bias (V)	-16
Haxapole Bias (V)	-13.2
(4) Parts of Mass Spectrometer	
Scanning Mode	peak jump mode (3 points per peak)
Dwell Time	10 msec
Number of sweeps	30
Ion Detection	pulse counting
Dead Time	55 nsec
Cd wavelength	228.804 nm
Detection limit	0.001 µg kg^{-1}
Detector	liquid state detector

2.3.6 X-ray diffraction analysis

To examine the structure of PRs, the samples were analyzed by X-ray Diffraction (XRD). For XRD analysis, the samples were dried and pulverized to less than 75 µm by using a ball mill (MM 301, Retsch) in order to induce the preferred orientation of particles horizontally loaded to the sample holder. The

XRD (Rigaku, Ultima IV) was operated at 40 kV and 30 mA and the Cu Kα radiation (0.543088 nm) was selected as a primary beam. The goniometer was operated under the following conditions: start angle 10°, end angle 70°, step size 0.02° with a scan speed 2° m^{-1} at room temperature.

Plate 3. X-ray diffraction (Rigaku, Ultima IV)

2.3.7 Statistical methodology

Cd concentrations in different PRs are reported by computing a 95% confidence interval for the mean. In order to compare Cd concentration among different PRs, means were also analyzed using a Tukey's (HSD) test at 0.05 levels. Data analysis was done using standard packages of the JMP 8.01 procedure in the Statistical Analysis System (SAS 2008).

2.4 Results

2.4.1 Elemental composition of phosphate rocks

The elemental concentrations analyzed in the present study were as follows.

Table 3. Elemental analysis (wt. %) in phosphate rocks from different countries by X-ray fluorescence

Elements	PR1	PR2	PR3	PR4	PR5	PR6
CaO	44.8	52.1	39.3	71.0	60.1	55.4
P_2O_5	28.4	32.7	6.12	24.4	37.7	33.6
SiO_2	9.27	8.48	36.5	0.895	0.847	0.930
Al_2O_3	7.29	1.81	8.15	0.285	0.0647	5.94
SO_3	0.795	1.08	0.233	1.19	0.337	0.305
Fe_2O_3	6.39	1.69	3.09	0.353	0.0835	1.53
Na_2O	2.15	1.06	1.81	1.05	0.203	0.656
MgO	0.165	0.462	2.23	0.304	0.0525	0.408
Cl	0.0606	0.0355	0.0085	0.0236	0.219	0.123
K_2O	0.173	0.159	1.16	0.0304	0.0023	0.288
TiO_2	0.0765	0.0745	0.583	0.0133	0	0.128
V_2O_5	0	0.0176	0.069	0.0361	0.0032	0.005
MnO	0.0805	0.0435	0.116	0.0051	0.0223	0.0751
Total	**99.7**	**99.7**	**99.4**	**99.6**	**99.6**	**99.4**

CaO, P_2O_5, and SiO_2 were the most abundant species in all phosphate rocks samples which values are as high as 39 to 71 %, 6.1 to 38 %, and 0.85 to 37 % respectively. The lowest concentration of calcium (CaO) was detected (39 %) in PR3 and the highest (71 %) in PR4. Concentrations over 40 % were found in PR1, PR2, PR5 and PR6. The lowest concentration of phosphorus (P_2O_5) was detected (6.1 %) in PR3 and the highest (38 %) in PR5. Phosphate rocks with P_2O_5 concentration over 20% were found in PR1, PR2, and PR4. The lowest concentration of silicon (SiO_2) was detected in PR5 (0.85 %), and the highest (37 %) in PR3. PR1 (9.3 %), PR2 (8.5 %) and PR6 (0.93 %) show significant concentrations (Table 3). The lowest concentration of aluminum (Al_2O_3) was detected (0.065 %) in PR5 and the highest (8.2 %) in

PR3. Also there are some significant concentrations in PR1 (7.3 %), PR6 (5.9 %) and PR2 (1.8 %). The lowest concentration of sulfur (SO_3) was detected (0.23 %) in PR3 and the highest (1.2 %) in PR4. Also PR2 had a notable concentration (1.08 %). Likewise significant concentrations were found (0.80 %) in PR1 and (0.305 %) in PR6. The lowest concentration of iron (Fe_2O_3) was detected (0.0835 %) in PR5 and the highest (6.4 %) in PR1. Also high Fe_2O_3 concentration was found in PR3. Likewise, concentrations higher than 1.5 % were found in PR2 and PR6. The lowest concentration of sodium (Na_2O) was detected (0.203 %) in PR5 while the highest (2.2 %) was found in PR1. Other high concentrations were detected in PR2 (1.06 %) and PR4 (1.05 %). The lowest concentration of magnesium (MgO) was detected (0.052 %) in PR5 while the highest (2.2 %) was found in PR3. Other significant concentrations were detected in PR2 (0.46 %), PR6 (0.41 %), and PR4 (0.30 %). The lowest concentration of chlorine (Cl) was detected (0.0085 %) in PR3 and the highest (0.22 %) in PR5. Concentrations over 0.023 % were found in PR1, PR2 and PR4. The lowest concentration of potassium (K_2O) was detected (0.0023 %) in PR5 and the highest (1.16 %) in PR1. Other significant concentrations, over 0.15 %, were found in PR2, and PR6. The lowest concentration of titanium (TiO_2) was detected (0.0 %) in PR5 and the highest (0.58 %) in PR3. Concentrations higher than 0.074 % were found in PR1, PR2 and PR6. The lowest concentration of vanadium (V_2O_5) was found (0.00 %) in PR1 and the highest (0.069 %) in PR3. Values up to 0.0050 % were detected in PR6. The lowest concentration of manganese (MnO) was detected (0.0051%) in PR4, and the highest (0.12 %) in PR3. Concentrations up to 0.081 % were found in PR1, PR2, PR5 and PR6 (Table 3).

2.4.2 Cd in phosphate rocks

The average concentrations of Cd in the PRs in the world are given in (Table 5). Cd concentration is from 0.15 to 507 mg kg^{-1}. PR4 shows the highest Cd content of 507 mg kg^{-1} compared with other PRs. PR5 had the lowest Cd content (0.15 mg kg^{-1}) (Table 4). Also PR3 had a notable concentration (199 mg kg^{-1}). Likewise a significant concentration (29.5 mg kg^{-1}) was found in PR6. Cd concentration of 1.45 and 3.31 mg kg^{-1} was found in PR1 and PR2.

The global range of Cd concentrations in PRs is 0.035 – 243 mg kg^{-1}, while the concentration determined in the present study lies over the global range of Cd in phosphate rock especially in PR4 (Table 4). A potential Cd accumulation in soil is estimated in the long term due to the high application rate of phosphate fertilizers. A detailed chemical characterization of rock is required to be checked and accounts for Cd concentration, because the quantification of Cd relative to nutrient value of rock can help in designing and improving chemical process used for production in industry. The Cd concentration is reflected in the textural and compositional characteristics of the PR, as well as in the degree of amalgamation of the PR strata. In order to evaluate the potential effects on human health and in the ecosystems it is important to carry out detailed studies on the elemental characteristics, Cd concentration and minerals in different phosphate rocks. The presence of high amount of Cd in the studied PR3, PR4 and PR6 samples is a major concern due to high toxicity of Cd. Cd is classified as a Class 1 human carcinogen (Group B1) by the International Agency for Research on Cancer (IARC, 1993) and the World Health Organization (WHO, 1992). According to Cd concentrations in PR1, PR2 and PR5 sample, the concentrations was low enough and was not considered as threat on either a health or environment (Table 4).

Table 4. Cd content and dominant minerals in different phosphate rocks

	Cd content (mg kg^{-1})	Intensity of peak at 2 θ: 29 degree (CPS)	Dominant Minerals
PR1 (Wyoming USA)	1.45	176	Fluoapatite
PR2 (Florida USA)	3.31	193	Hydroxyapatite
PR3 (Idaho USA)	199	1521	Hydroxyapatite, Carbonate fluorapatite
PR4 (Morocco)	507	2858	Carbonate fluorapatite
PR5 (Slyudanka Russia)	0.15	136	Hydroxyapatite
PR6 (Okinawa Japan)	29.5	358	Carbonate fluorapatite Hydroxyapatite

Table 5. Global pattern of cadmium content in phosphate rocks

Phosphate rock origin	Cd con. (mg kg^{-1}DW)	Reference
USA	3-186	(1,2,3,4,5,6,7,8,9,10)
Morocco	3-165	(2,3,4,5,8,9,10,11)
Peru	2-186	(4,5,15)
Russia	0.1-<13	(3,6,8,9,10,11)
North Africa	60	(3)
South Africa	2.0-<13	(3,5,8,9,10)
Israel (Arad)	12.0-32	(4,5,8,9,10)
Pakistan, Abbottabad city, Hazara Division	7.2	(12)
Brazil	4	(13)
Jordan, Amman	6-<30	(3,5,8,9,10,14)
Togo	44-179	(2,4,8,9,10)
Tunisia, Gafsa	53-173	(2,5,8,9,10)
Algeria	22.5-62.6	(4,8,9)
Senegal, Tobene	37.1-221	(4,8,9,10)
Syria	6.1-52	(4,8,9,10)
China (Yunam)	4	(5)
Austrlia (Christmas Island)	7-43	(5)
Mexico	8	(5)
Egypt (Quseir)	8-74	(5,8)
Banaba (Ocean Island)	99	(5)
Nauru	100-243	(5,8)

Sources: (1) Everitt (1993); (2) Batarseh (2009); (3) Edgell (1988); (4) Bowen (1966); (5) Bech (2010); (6) Alloway (1999); (7) Mckelvey et al. (1986); (8) Davister (1996); (9) Botschet and Van Balken (1999); (10) Demandt (1999); (11) Kongshaug (1992); (12) Conceica (2006); (13) Chen (2007); (14) Sabiha-Javied (2009), (15) Turekian (1961).

2.4.3 Scanning electron microscopy analysis

The surfaces of the phosphate rocks from different countries were examined by scanning electron microscope (SEM). Micrographs at the magnification of 3000 are shown in (Plate 4). The results showed that the samples were different shape among the different phosphate rocks. Comparison of these micrographs indicated that the surface appearance of the phosphate rocks was different.

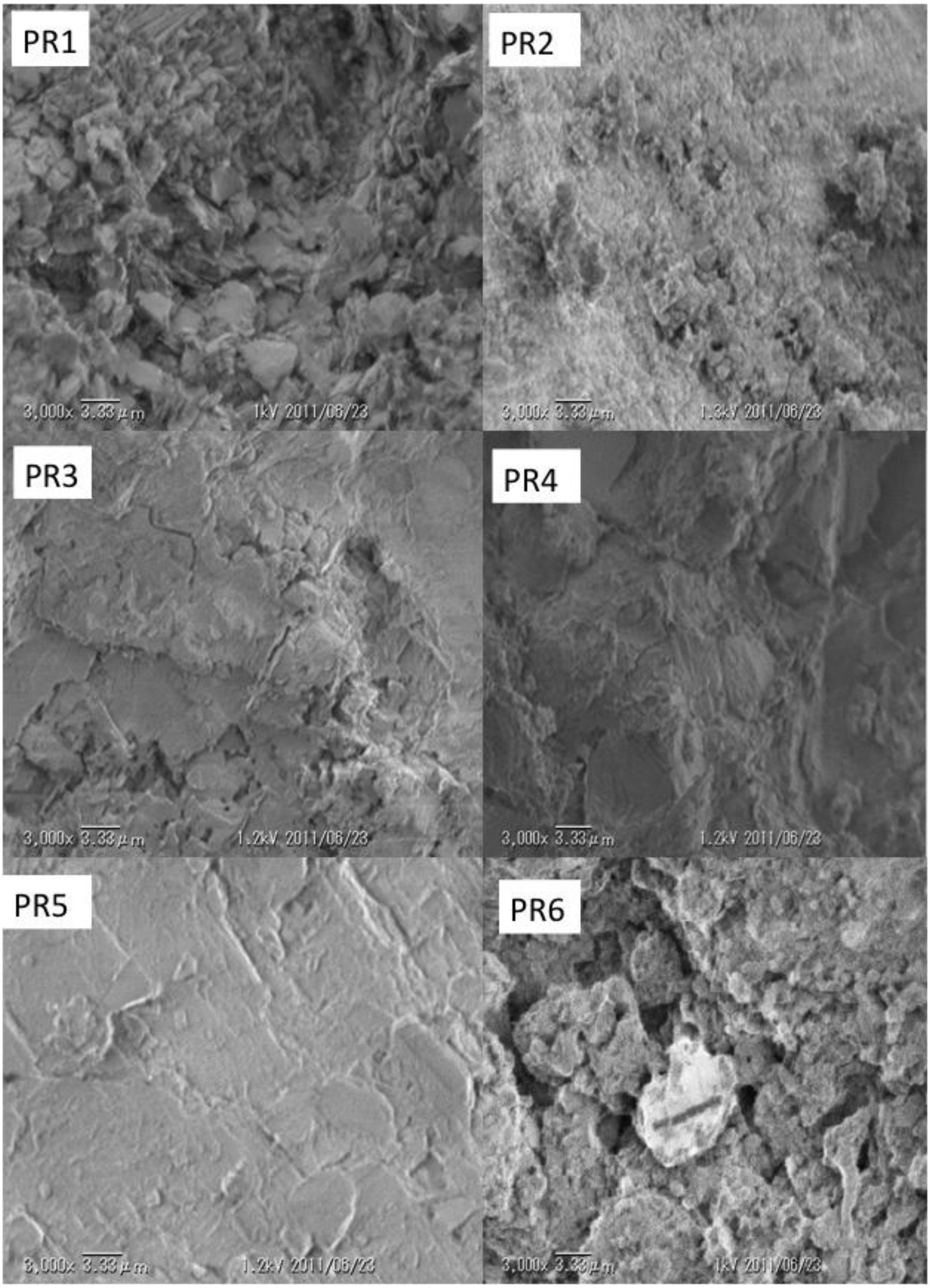


Plate 4. SEM (x 3000) micrograph of phosphate rocks from different countries

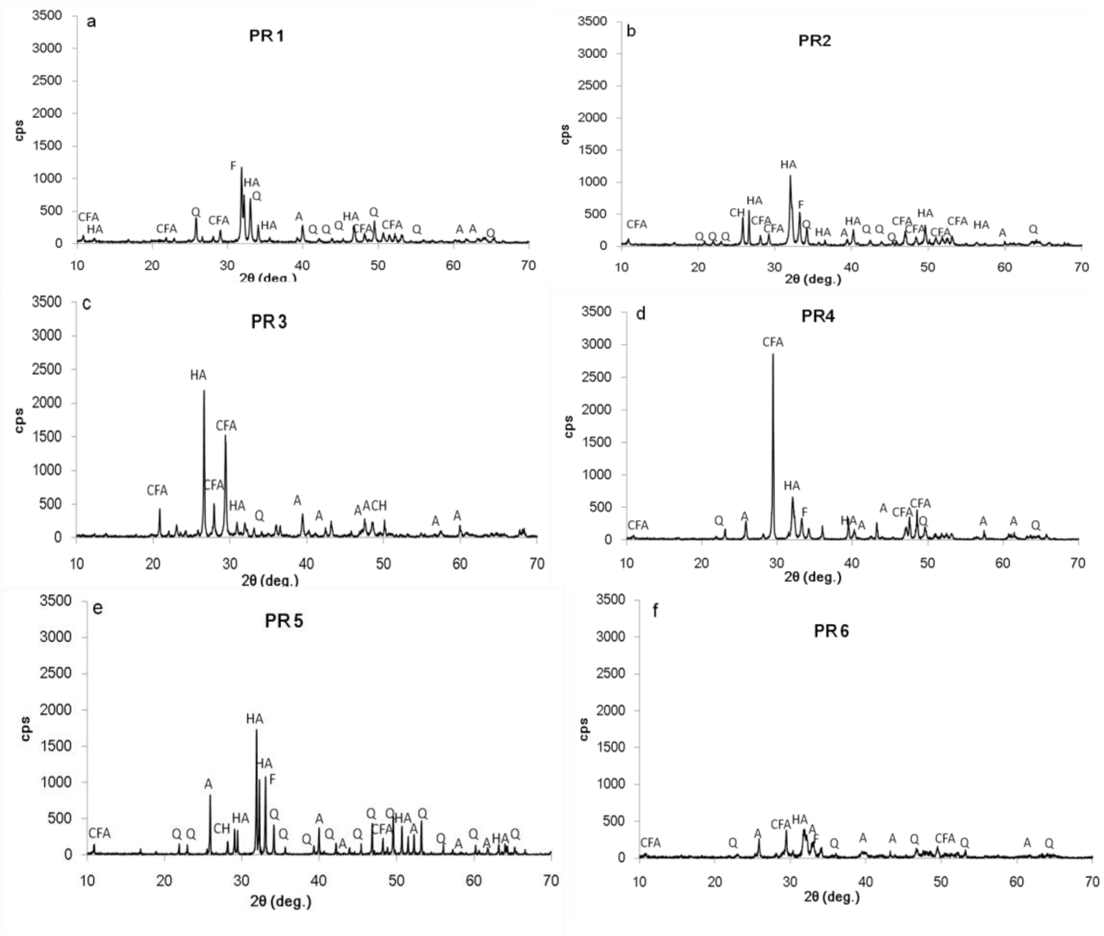


Figure 4. X-ray diffraction diagram PR from different countries

A= Apatite, CFA= Carbonate fluorapatite, CH= Carbonate hydroxyapatite,

F= Fluorappatiet, HA= Hydroxyapatite, Q= Quartz,

a= PR1, b= PR2, c= PR3, d= PR4, e= PR5, f= PR6

2.4.4 The mineral composition of phosphate rocks analysed by X-ray diffraction

The 2θ angles of 31.86°, 32.18° and 33.02°, typical peaks of fluorapatite and hydroxyapatite were detected in PR1 (Fig 4a). The presence of hydroxyapatite, fluorapatite and carbonatehydroxyapatite was observed at the angles of 32.02°, 26.62°, 33.22° and 25.82° in PR2 (Fig 4b). The presence of hydroxyapatite, and carbonate fluorapatite was observed at the angles of 26.62°, 29.38° and 29.44° in PR3 (Fig 4c). Carbonate fluorapatite and hydroxyl apatite were detected at the angle of 29.46° and 32.04° in PR4 (Fig 4d). The peaks at 31.92° and 32.28° originated from hydroxyapatite were provided in PR5 (Fig 4e). In addition to hydroxyapatite (HA), the presence of fluorapatite (F) and Apatite (A) was also observed at the angles of 33.08° and 25.92° in the XRD pattern of PR5 (Fig 4e). Meanwhile, carbonate fluorapatite (CFA) was also detected at the angle of 10.8 ° in PR5 (Fig 4e). The angles of 31.84°, 29.48°, 25.86°, and 32.96° are observed for hydroxylapatite, carbonate fluoapatite, and fluoapatite in PR6 (Fig 4f).

2.4.5 The mineralogical composition and Cd content of phosphate rocks

PR3 and PR4 showed that carbonate fluorapatite was more prominent than other phosphate rocks (Table 4). In Fig 4 c and d, the carbonate fluorapatite mineral was also found for PR3 and PR4. The current study confirmed that high Cd content phosphate rocks sample of PR3 and PR4 was concerned with dominant minerals component of carbonate fluorapatite. This suggested that Cd might be exchanged to Ca and/or occluded in carbonate fluorapatite during its formation by sedimentation of phosphate rock. The most prominent mineral of carbonate fluorapatite and high Cd concentration were found in PR3 and PR4 than other PRs. Results presented high Cd content as the major element in the studied rocks, related mainly with the major component mineral of carbonate fluorapatite in PR3 and PR4 than other PRs (Table 4).

The environment might be contaminated by Cd from PRs through dry milling of PRs (air pollution), dry and wet deposition of mining and milling dust (soil pollution), increased anthropogenic input of Cd in soils by fertilizer (water pollution), food contamination by Cd (toxic to human). Cadmium in PRs pollutes the environment by accumulation in air, soil, water bodies and food chain. Continuous use of PRs sources causes the Cd to accumulate in the environment which then reach up to permissible limits posing health problem to human life, because Cd can lead to poisoning at higher concentrations.

2.5 Discussion

Phosphate rocks were mainly composed of CaO, P_2O_5, SiO_2, Al_2O_3, SO_3 and Fe_2O_3. The results from this study showed a good agreement with the literature (Cevik et al. 2010). The global range of Cd concentration in the other studies as given in Table 3 is 0.035 - 243 mg kg^{-1} while the average concentrations determined in the present study lies over the global range of Cd in phosphate rocks. The results revealed that the main source of Cd in the produced phosphate fertilizer was due to the impurities in the phosphate rock. Also a potential Cd accumulation in soil is expected in the long term due to the high application rate of phosphate fertilizers. Results of this study showed that Cd was abundant in PR4 and in varying amount present in other phosphate rocks. Oxidation is considered to be one of the main processes, which release Cd from marine sediments (Hunt 1982; Rosenthal and Lam 1995). The concentration of Cd of PR increased in the following observed sequences: PR5 < PR1 < PR2 < PR6 < PR3 < PR4. A detailed chemical characterization of rock was required to be checked and accounts for Cd concentration because the quantification of Cd relative to nutrient value of rock could help in designing and improving chemical process used for production in industry. The Cd concentration is reflected in the textural and compositional characteristics of the PR, as well as in the degree of amalgamation of the PR strata. In order to evaluate the potential effects on human health and on the ecosystems it is important to carry out detailed studies on mineral composition, Cd concentration and chemical characteristic in different phosphate rocks. The presence of high Cd in the studied PR3, PR4 and PR6 samples was a major concern because of high toxicity of Cd. Cd is classified as a Class 1 human carcinogen (Group B1) by the

International Agency for Research on Cancer (IARC, 1993) and the World Health Organization literature (WHO, 1992). The results suggest that PR3, PR4 and PR6 can exert detrimental effects on human health and on the environment. Cd in PR pollutes environment by accumulation in air, soil, water bodies, food chain, etc. In the case of PR samples, the following species were observed: fluorapatie ($Ca_5F(PO_4)_3$), apatite ($Ca_5(PO_4)_3(OH)$, hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_{2,}$ carbonate hydroxyapatitie $Ca_5(PO_4,CO_3)_3(OH)$, carbonate fluorapatite ($Ca_5(PO_4,CO_3)_3F$, and quartz (SiO_2). This composition is consistent with those published by other authors who have studied phosphate rocks of sedimentary origin from different places (Rutherford 1994; Gnandi and Tobschall 2003). Also, chemical and mineral characteristics are investigated in the phosphate rock samples. The results show that all PR are calcium and phosphate rich compounds, mainly of fluorapatite, hydroxyl apatite, carbonate fluorapatite and apatite. There is a possible risk of contaminants accumulating in soils and crops due to Cd impurities in fertilizers and soil, which needs to be addressed. Cd is of special concern in the phosphate industry. Cd concentration is one of the most important factors in deciding the quality of phosphate resources, which does not have any standard permissible limit, because the maximum allowable concentration depends on soil characteristics, irrigation water quality, crop type, etc (Aydin et al. 2010). However, European countries implemented a limit of 10 mg kg^{-1} of Cd on phosphate rock imports (Roseli et al. 2009).

2.6 Conclusion

PR samples of different countries were analyzed for chemical, mineral composition and Cd concentration using the techniques of X-ray fluorescence, X-ray diffraction and inductively coupled plasma mass spectrometry. The Cd concentrations in PRs of this study in increasing order of mean value (mg kg^{-1}) were: of the origin from PR5 < PR1 < PR2 < PR6 < PR3 < PR4. The concentrations of Cd determined in PR3 and PR4, investigated in the present study, were comparably higher than those determined for other PRs. Like other PRs elsewhere in the world, they were highly enriched in Cd. The result revealed that hazardous metals Cd content of the phosphate rock was lower in PR1, PR2 and PR5 than in samples imported from other countries and were within the safety limits. To avoid Cd contamination in soil after long-term application of P fertilizer, which has high Cd content, it was better to use low Cd concentration PR in making P fertilizer. As currently there exists no commercial means of removing these Cd completely during fertilizer manufacturing process, only way to reduce Cd in produced fertilizer is to use PR with lower Cd concentration. Removal of Cd from the phosphate fertilizer during the manufacturing process is possible, however, the available technology may not be economically viable at current economic fertilizer prices. Restricting phosphate production to low- Cd phosphate rock or removing Cd during processing will increase the cost of fertilizer and may restrict the amount of phosphate fertilizer available for trade. This could have important implications for food production particularly in developing nations.

CHAPTER 3. CADMIUM AND MINERAL CHARACTERISTICS OF DIFFERENT PHOSPHATE FERTILIZERS

3.1 Abstract

Mineral and cadmium concentration of phosphate fertilizers (PFs), which are important for crop production, were investigated and those of PF of China, Japan and Myanmar were compared using X-ray diffraction (XRD) and inductively coupled plasma mass spectrometry (ICP-MS). Elemental analysis results of PF samples showed that they were mainly composed of fluorapatite ($Ca_5(PO_4)_3F$) and carbonatehydroxyapatite ($Ca_{10}(PO_4,CO_3)_6(OH)_2$). The Cd concentration levels in all PF samples, which result in the Cd input source of crop production ranged from 0.04 to 4.01 mg kg^{-1}, lower than those of the literature so far published. Cd concentration, hazardous for human health, of PF6 (Japan) was lowest among the samples of this study. Total phosphorus concentration in all PFs is 12 to 50% on the average. PF is a source of cadmium pollution of soil, water, food chain etc. Therefore, the information of mineral and Cd concentration of the PFs is required prior to its use. This study also confirmed that PF2 and PF5 exhibited higher Cd content and prominent mineral component of carbonate fluorapatite suggesting that Cd might be exchanged to Ca and/or occluded in carbonate fluorapatite during its formation by sedimentation.

3.2 Introduction

Since the 1950s, the application of plant nutrients, including phosphate fertilizers (PF), has increased substantially (Tisdale et al., 1985). Each year, more than 30 million metric tons of phosphate nutrients are consumed worldwide; with more than 99% coming from phosphate rocks (IFA, 2005). Those products have helped farmers increase crop production and have made culture possible in nutrient-deficient lands. Phosphate fertilizers are widely used to correct phosphorus deficiencies in agricultural soils but the concentrations of cadmium in these fertilizers, often greater than 10 mg kg^{-1} (Mortvedt 1987), can dramatically increase soil cadmium concentrations. Several studies have found a linear relationship between the concentration of cadmium in the soil and the quantity of superphosphate fertilizer applied (Rothbaum et al, 1986; Mortvedt, 1981). Phosphate fertilizers contain traces of cadmium that can be accumulated in soil with repeated application of phosphate fertilizer. Phosphate fertilizer application is a significant contributor of trace element, especially for Cd accumulation in cropland soils (McLaughlin et al., 1996). Cadmium is potentially hazardous pollutant in the environment and is highly toxic to plants, micro- organisms, animals and human beings even at a very low concentration (Flick et al., 1971). It can cause adverse animal and human health impacts at high levels or at lower levels if exposure occurs over a prolonged period. To prevent soil from cadmium contamination of phosphate fertilizer, it is primordial to remove cadmium from phosphate ore. The determination of cadmium (Cd) in fertilizers is of major interest, as this element can cause growth problems in plants, and also affect animals and humans. The object of this study is to investigate cadmium concentration and mineral composition of phosphate fertilizers from different countries and to elucidate the relationship between the Cd content and carbonate fluorapatite among different phosphate fertilizers.

3.3 Material and Methods

3.3.1 Sample collection and preparation

Samples were collected from China 1(PF1), China 2 (PF2), China 3 (PF3), Japan 1 (PF4), Japan 2 (PF5), Japan 3 (PF6) and Myanmar (PR7). The sample was crushed, ground to powder, homogenized and kept in an oven at 110 ºC for 1 day to remove moisture if any. The 7 samples of PFs were analyzed in order to estimate mineral composition, the concentration of Cd by XRD and ICP-MS.

3.3.2 Scanning electronic microscopy (SEM)

The morphological structure of phosphate fertilizers was obtained by using scanning electron microscopy. Samples were mounted on plate and taken photos by Keyence VE 9800.

3.3.3 Mineral analysis

Quality control was assured by the use of four replicates analyses performed on all samples, the use of reagent blanks, standard reference and internal control samples were also analyzed in order to monitor analytical accuracy and precision. All the samples were also analyzed for their mineral content by XRD (Rigaku, Ultima IV). This Xray differaction analysis was same procedure as (2.3.6).

3.3.4 Digestion procedure for Cd

Seven phosphate fertilizers were digested by using the same procedure as the analysis mentioned above (2.3.4).

3.3.5 Cd analysis

This Cd analysis was done same procedure as the analysis mentioned above (2.3.5).

3.4 Results

3.4.1 Cd content in phosphate fertilizers

The average concentrations of Cd in the PFs in the world were given in (Table 7). Cd concentration is from 0.04 to 4.01 mg kg^{-1}. PF5 showed the highest Cd content of 4.01 mg kg^{-1} compared with other PFs. PF6 had the lowest Cd content (0.04 mg kg^{-1}). Also PF2 had a notable concentration (3.17

mg kg^{-1}). Likewise a significant concentration (3.11 and 2.37 mg kg^{-1}) were found in PF4 and PF7. Cd concentration of 1.64 and 1.46 mg kg^{-1} was found in PF1 and PF3. Cd is of special concern in the phosphate industry. Comparative studies were performed to check Cd concentration in the samples under study and those measured worldwide were given in (Table 6 and Table 7). Cd concentration is one of the most important factors on deciding the quality of phosphate resources. The global range of Cd concentrations as given in (Table 7) are 0.2 - 345 mg kg^{-1} while the average concentration determined in the present study lies within the global range of Cd in phosphate fertilizer.

Table 6. Cd content and dominant minerals in different phosphate fertilizers

Fertilizers	Cd content (mg kg^{-1})	Intensity of peak at 2 θ: 29 degree (CPS)	Dominant Minerals
PF1 (China 1)	1.69	6.08	Carbonate hydroxyapatite
PF2 (China 2)	3.17	106	Apatite, Carbonate fluorapatite
PF3 (China 3)	1.46	20.3	Hydroxyapatite
PF4 (Japan 1)	3.11	68.3	Hydroxyapatite, Carbonate fluorapatite
PF5 (Japan 2)	4.01	21.6	Carbonate flurapatite, Hydroxyapatite
PF6 (Japan 3)	0.04	43	Hydroxyapatite
PF7 (Myanmar)	2.37	216	Apatite, Carbonate hydroxyapatite Carbonate fluorapatite

Table 7. Cd content of fertilizer according to different sources

Fertilizers	Cd con. (mg kg^{-1}DW)	Reference
Calcium superphosphate	15 - 90	(1)
Single superphosphate	3.6 - 35.7	(2,3)
Monoammonium phosphate	6 - 94.5	(3)
Diammonium phosphate	6.5 - 75	(3)
Potassium chloride	<1.0	(3)
Potassium sulphate	<1.0	(3)
Ammonium sulphate	<1.0	(3)
Diammonium phosphate	2 -153	(4,5)
Triple superphosphate	174	(6,7)
P fertilizer	2 - 81	(8)
Phosphate fertilizers	0.2 - 345	(9)
NPK fertilizer	6.5 - 27	(10)

Sources: (1) Sparrow et al. 1993, (2) Iretskaya et al. 1998, (3) Mclaughlin et al. 1995,(4) Mortvedt, 1981, (5) Mortvedt and Giordano, 1977, (6) Reuss et al. 1978, (7) Mulla et al. 1980, (8) Jaakkola, 1977 (9) Allow and Steninnes 1999 (10) He and Singh 1995.

3.4.2 Scanning electron microscopy analysis

The surfaces of the phosphate fertilizers from different countries were examined by scanning electron microscope (SEM). Micrographs at the magnification of 1000 are shown in (Plate 5). The results showed that the samples were different shape among the different phosphate fertilizers with the diameter of less than 10 micro m except PF6 (200 μm) at the magnification of 50. Comparison of these micrographs indicated that the surface appearance of the phosphate fertilizers was different.

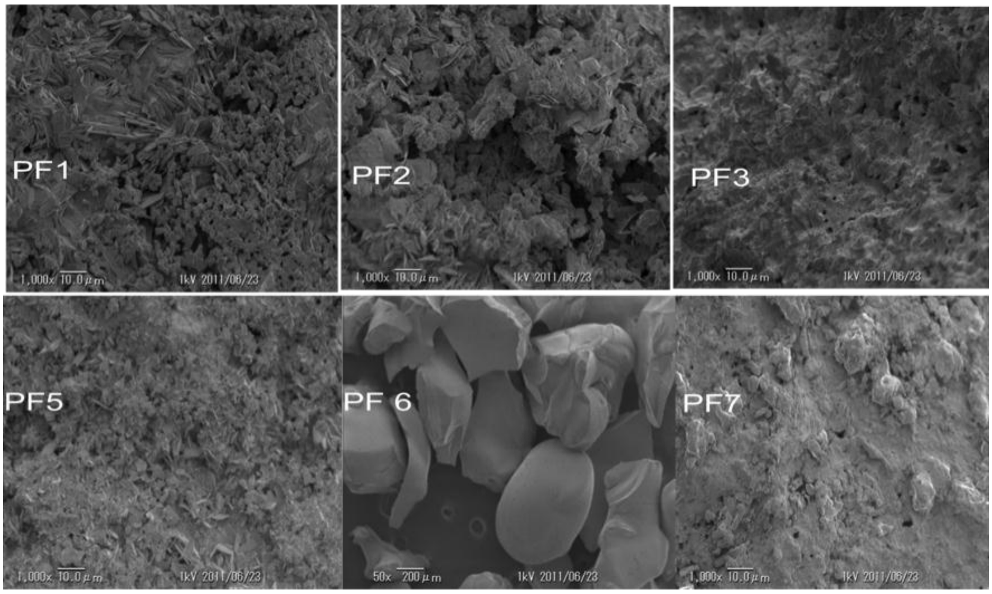
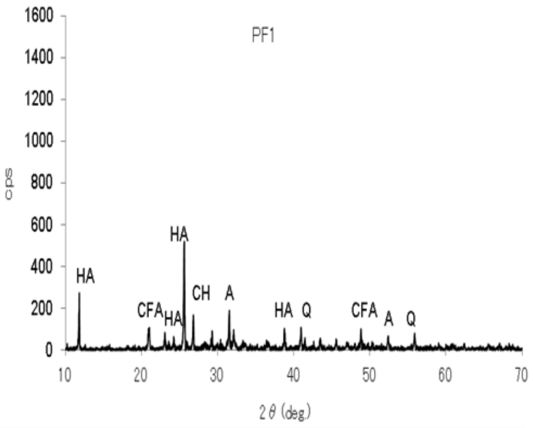
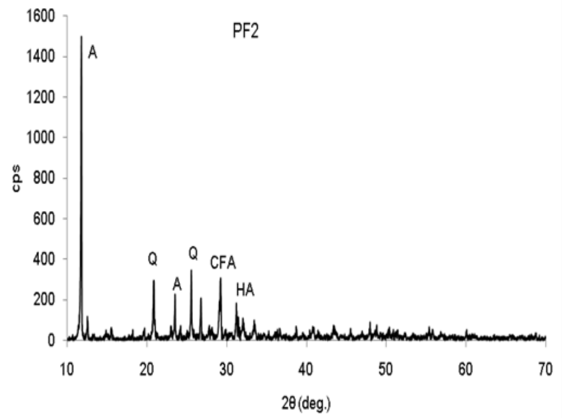
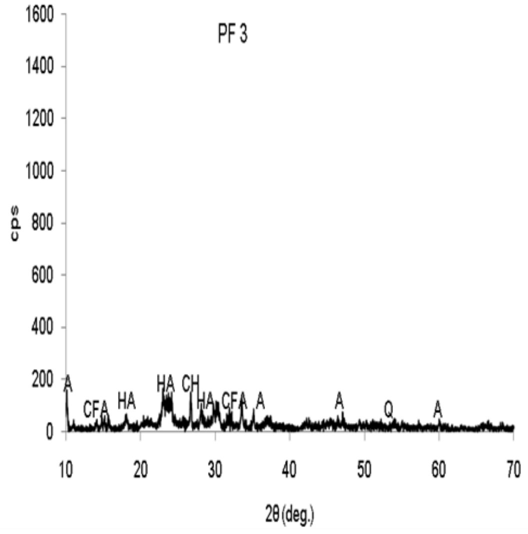


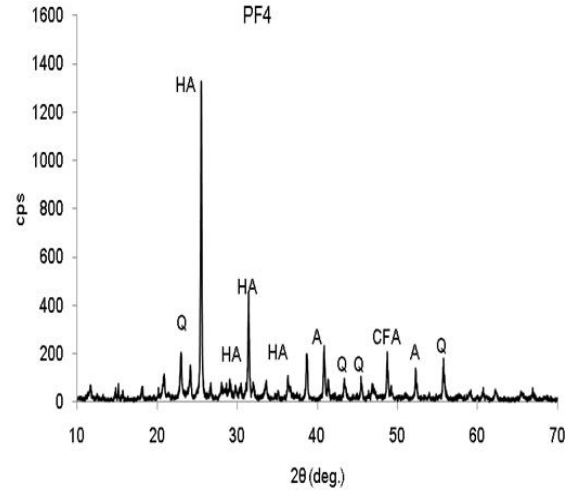
Plate 5. SEM (X1000) micrograph of the phosphate fertilizers from different countries

China 1 (PF1), China 2 (PF2), China 3 (PF3), Japan 2 (PF5), Japan 3 (PF6), Myanmar (PF7)


PF1
cps
1600
1400
1200
1000
800
600
400
200
0
HA
CFA
HA
HA
CH
A
HA
Q
CFA
A
Q
10
20
30
40
50
60
70
2θ (deg.)


PF2
cps
1600
1400
1200
1000
800
600
400
200
0
A
Q
A
Q
CFA
HA
10
20
30
40
50
60
70
2θ(deg.)


PF 3
cps
1600
1400
1200
1000
800
600
400
200
0
A
CFA
HA
HA
CH
HA
CFA
A
A
Q
A
10
20
30
40
50
60
70
2θ(deg.)


PF4
cps
1600
1400
1200
1000
800
600
400
200
0
HA
Q
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CFA
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Q
10
20
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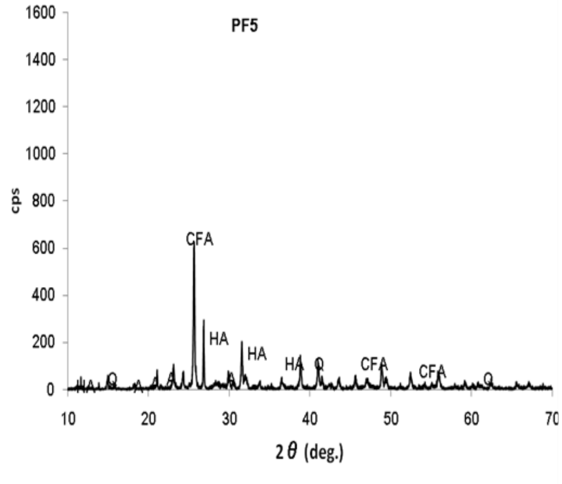
70
2θ(deg.)


PF5
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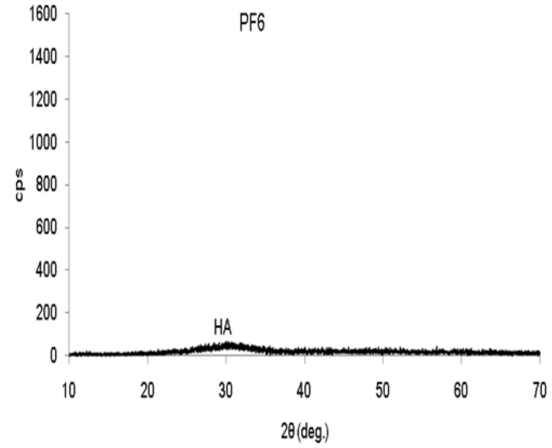
2θ (deg.)


PF6
cps
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HA
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70
2θ(deg.)

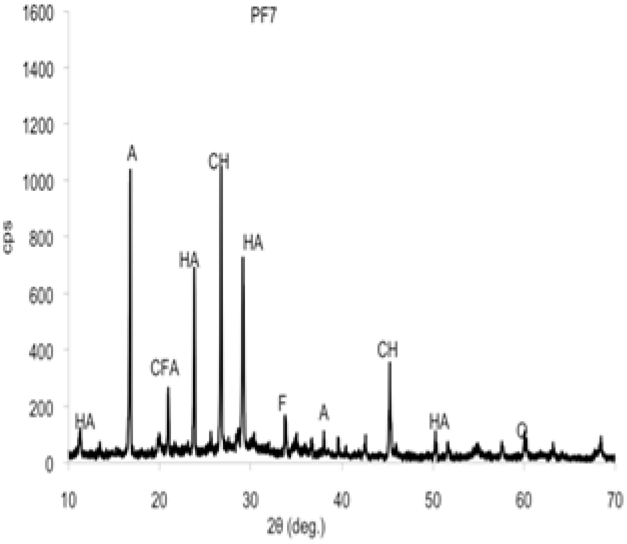


Figure 5. X-ray diffraction diagram of phosphate fertilizers from different countries

A=Apatite, CFA=Carbonate fluoapatite, CH=Carbonatehydroxylapatite, F=Fluoapatite, HA=Hydroxyapatite, Q=Quartz, PF1, PF2, PF3 (China), PF4, PF5, PF6 (Japan), PF7 (Myanmar)

4.3 The mineral composition of phosphate fertilizers analyzed by X-ray diffraction

The 2θ angles of 11.84°, 25. 64°, and 31.56°, hydroxyapatite were detected for in PF1. The presence of apatite was observed at the angles of 11.76° in PF2). The presence of carbonate fluorapatite and apatite, was observed at the angles of 10.08°, 23°, 26.72° and 30.12° in PF3. The presence of hydroxyapatite was observed at the angles of 25.5° and 31.42° in PF4. The presence of apatite and carbonate fluorapatite was observed at the angles of 25.68° and 26.88° in PF5. The peaks at 31.58° and 38.84° originated from apatite and hydroxyapatite were provided in PF5. In addition to apatite and hydroxyapatite (HA), the presence of quartz (Q) and carbonate fluorapatite (CFA) were also observed at the angles of 41°, 48.9° 55 and.86 ° in the XRD pattern of PF5. The presence of hydroxyapatite, was observed at the angles of 30° in PF6. The angles of 16.78°, 23.8°, 26.76°, 45.22° and 29.14° were observed for apatite, carbonate hydoxyapatite, hydroxyapatite in PF7. Meanwhile, carbonate fluorapatite (CFA) was also detected at the angle of 20.94° in PF7 (Fig 5).

3.5 Discussion

The global range of Cd concentration in the other studies as given in Table 3.2 is 0.2 – 345 mg kg^{-1} while the average concentrations determined in the present study lies within the global range of Cd in phosphate fertilizers. The results reveal that the main source of Cd in the produced phosphate fertilizer is due to the impurities in the phosphate rock. Cd accumulation in soil is one of anthropogenic source of the high application rate of phosphate fertilizers. Results of this study show that Cd is abundant in PF5 and in varying amount present in other phosphate fertilizers. Oxidation is considered to be one of the main processes, which release Cd from marine sediments (Hunt 1982; Rosenthal and Lam 1995). The concentration of Cd of PFs decreased in the following observed sequences: PF5 > PF2 > PF4 > PF7 > PF1 > PF3 > PF6. A detailed chemical characterization of fertilizer is required to be checked and accounts for Cd concentration because the quantification of Cd relative to input of Cd in agricultural soil. Phosphorus fertilizers contain Cd at widely varying concentrations related to the source of phosphate rock and the manufacturing process because the rock phosphate raw materials are the sole source of the fertilizer cadmium. The results suggest that phosphate fertilizer of this study could not exert detrimental effects on human health and on the environment because of low Cd concentration compared to other studies. The intensity of the peak from fluorapatite is highest near 33° in some samples. In the case of PFs samples, the following species were observed: fluorapatie ($Ca_5F(PO_4)_3$), apatite ($Ca_5(PO_4)_3(OH)$, hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$, carbonate hydroxyapatitie $Ca_5(PO_4,CO_3)_3(OH)$, carbonate fluorapatite ($Ca_5(PO_4,CO_3)_3F$, and quartz (SiO_2). This composition is consistent with those published by other authors who have studied phosphate rocks of sedimentary origin from different places (Gnandi and Tobschall 2003). The current study confirmed that high Cd content phosphate fertilizers sample of PF2 and PF5 was concerned with dominant minerals component of carbonate fluorapatite (Table 6).

3.6 Conclusion

PFs samples of different countries were analyzed for mineral composition, Cd concentration using the techniques of X-ray diffraction and inductively coupled plasma mass spectrometry. The Cd concentrations in PF of this study in decreasing order of mean value (mg kg^{-1}) are: of the origin from PF5 > PF2 > PF4 > PF7 > PF1 > PF3 > PF6. The concentrations of Cd determined investigated in the present study are comparably lower than those determined for other PFs of other studies. Like other PFs elsewhere in the world, they are highly enriched in Cd. The result revealed that hazardous metals Cd content of the phosphate fertilizer was lower in PF6 of fused magnesium phosphate from Japan than in samples imported from other countries and were within the safety limits. To avoid Cd contamination in soil after long-term application of P fertilizer, it is better to use PF with low Cd concentration. This could have important implications for agricultural practice of food production.

CHAPTER 4. APPLICATION OF DIFFERENT PHOSPHATE FERTILIZERS ON KOMATSUNA GROWN ON MELANUDAND AND HAPLAQUEPT

4.1 Abstract

Phosphorus fertilizers are essential to obtain high productivity, however, they contain cadmium that can contaminate the soil and threaten the health of animals and humans. The present study focused the determination of Cd concentrations in different phosphorus fertilizers as well as the absorption of cadmium by Komatsuna grown on Melanudand and Haplaquept amended with such fertilizers. The experiment was set up as a completely randomized design in a factorial scheme. The factors were five phosphorus sources PF1 (China 1), PF2 (China 2), PF3 (China 3), PF4 (Japan 1), and PF7 (Myanmar 1) applied at 120 kg ha^{-1} of P_2O_5. Cd availability to Komatsuna was altered as a result of fertilizer application. On the other hand, Cd uptake was significantly increased with addition of the tested fertilizers compared to control (without fertilizer treatment). Such an increase was a function of the fertilizer types. The highest Cd contents in Komatsuna reached 0.034 and 1.24 mg kg^{-1} by (China 1) application whereas the lowest one resulted 0.029 and 1.08 mg kg^{-1} from (Myanmar) treatment of Melanudand and Haplaquept. It was included that total Cd concentrations in soils were the main controls on Cd contents in plants. All the phosphorus sources tested increased Cd concentration in Komatsuna except PF7.

4.2 Introduction

Trace metals, such as Cd, may accumulate in the soil, be taken up by plants and be passed on in the food chain (Friberg et al., 1992; Nicholson and Jones, 1994). To access any potential problems posed by increases in soil Cd levels, information is needed on how much of the Cd applied in phosphate fertilizer is retained in the soil, how much of this is available to plants, any differences in accumulation behavior between soil and fertilizer types. This study assesses accumulation of Cd in Melanudand and Haplaquept by comparing soil samples applied by different phosphate fertilizers. Phosphorus is a key nutrient in crop production, along with nitrogen and potassium. However, a possible negative effect of phosphate fertilizers is the contamination of cultivated lands by trace metals such as Cd, Cu, Mn, Ni, Pb and Zn naturally present in the phosphate rocks used to manufacture the fertilizers. Soil contamination by Cd originating from phosphate fertilizers has become a concern in several countries. Thus, much research has been conducted to evaluate the presence of such metals in these amendments (McBride & Spiers, 2001; Loganathan et al., 2003). The Cd concentration in phosphate fertilizers is dependent on the type of rock phosphate used as raw material. Due to the relatively low concentrations of Cd and Pb in fertilizers, many researchers (Camargo et al., 2000; Prochnow et al., 2001) have recommended their use without drawbacks regarding environmental contamination. However, little information is available either on the uptake of Cd by Komatsuna and Cd concentration in soils fertilized with different phosphate fertilizers or on the long-term accumulation of Cd in soils. Cadmium concentration in maize amended with phosphate fertilizers was significantly affected not only by P sources but also by their localization in the soil (Prochnow et al., 2001). The application of fertilizers could increase the heavy metal bioavailability in soils due to the chemical alterations they provoked in the system (Tu et al., 2002). Taking into account that phosphate fertilizers can be an important via of cadmium to the food chain, this work aimed to determine the Cd concentration in different phosphate fertilizers, as well as the uptake of Cd by Komatsuna grown on Melanudand and Haplaquept amended with them.

4.3 Materials and Methods

The soil used in the experiment was Melanudand and Haplaquept collected from the 0-20 cm depth in Saiwai cho, Fuchu campus and Fuchu Hommachi, Tokyo University of Agriculture and Technology, air dried, crushed and sieved through a 2 mm sieve. Soil samples placed in pots received different kinds of five phosphate fertilizers: China (PF1), China (PF2), China (PF3), Japan (PF4), and Myanmar (PF7). The Phosphate (equivalent to rates of 120 kg ha^{-1} of P_2O_5) was incorporated into each soil sample in the pots. Ten seeds of Komatsuna seed were used in each pot. After germination, only four plants per pot were left. The treatments were arranged in a 2 x 6 factorial scheme, set in a completely randomized block design, with five replications. The cultivation period is Feb 19th to May 8th, 2010.

4.3.1 Soil sampling and analysis

We collected soil samples per pot. Samples to a depth of 15 cm was air-dried for 14 days, crushed, and passed through a 2-mm sieve, then roots and other plant fragments were removed before chemical analysis. Soil pH (1:2.5 w/v soil to water ratio) was measured with an M7 pH meter (Horiba, Kyoto, Japan). Total N (TN) was determined with an MT-700 CN Coder (Yanaco, Kyoto, Japan). The available P (Bray No. 2) was determined by the Bray No. 2 extract test (Bray and Kurtz 1945), and phosphate was quantified by the ascorbic acid color development method (Murphy and Riley 1962) and was measured with a BioSpec–1600, UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) at 880 nm. Cation exchange capacity and exchangeable bases were determined by the method of Schollenberger and Simon (1945) using a Z-5010 polarized Zeeman atomic absorption spectrophotometer (Hitachi, Tokyo, Japan). Exchangeable NH_4-N (available N) was extracted with 2 mol L^{-1} KCl and analyzed by colorimetry using the indophenol-blue method (Ashidate 1994), and absorbance was read with a

Bio Spec-1600 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) at 635 nm.

For the analysis of total Cd, we added 10 mL of concentrated HNO_3 to 1 g of air-dried ground soil in a 200-mL conical flask. The flask was heated gradually to 140°C, then 5 mL of concentrated $HClO_4$ was added. Heating continued until dense white smoke came from the samples, and when no more smoke was produced, the residues were washed using $HClO_4$ and the solution was transferred to a flask and evaporated completely. The dry residues were dissolved in 15 mL of 2 mol L^{-1} HNO_3 and filtered through a No. 5C filter paper (Advantec, Tokyo, Japan) and then transferred into 100-mL volumetric flasks. Total Cd in the filtrates was determined with a Z-5010 graphite-furnace atomic-absorption spectrophotometer (Hitachi, Tokyo, Japan) at a wavelength of 228.8 nm. Standards were prepared to calibrate the instrument by diluting stock laboratory solutions of each element in 0.3 mol L^{-1} nitric acid. The nitric acid solution was also analyzed to provide a calibration blank. The Cd analysis also required the preparation of a matrix modifier to be added to each sample before analysis; this required the addition of 5 μL of 2% $NH_4H_2PO_4$ solution and 5 μL of 0.4% $Mg(NO_3)_2$ solution to a 20-μL aliquot of the sample or a lab standard.

Acid-extractable Cd was determined with the use of 0.1 mol L^{-1} HCl. A 5-g subsample of the air-dried soil was shaken on an end-over-end shaker for 1 h with 25 mL of 0.1 mol L^{-1} HCl. Cd in the filtrate was then determined with the Z-5010 spectrophotometer. All reagents were of analytical grade (Wako Pure Chemical Industries, Osaka, Japan). For quality control of plant tissue (see section 2.2) and soil analysis, standard reference materials from the Japan Society for Analytical Chemistry (JSAC) were used. The results of laboratory analysis were compared with the certified values for the standard reference materials: spinach leaves (NIST No. 1570a) and soil (JSAC No. 0411). The total soil Cd value in JSAC No. 0411 was 0.22 ± 0.01 mg Cd kg^{-1}, versus a certified value of 0.27 ± 0.02 mg Cd kg^{-1}.

4.3.2 Plant sampling and analysis

The komatsuna plants were harvested from each pot. The plants were then separated into leaves and roots, and washed thoroughly with tap water to remove soil and then rinsed with deionized water. They were then oven-dried at 70 ^oC for 48 h, and the total dry weight (DW) of leaves was recorded as the dry matter yield. The growth of the plants in each treatment was evaluated by measuring the plant height, number of leaves, the wet and dry weights of leaves.

Oven-dried plant samples were ground in an MM 301 mixer mills (Retsch, Haan, Germany) before chemical analysis. After digestion in a 750-W microwave oven, the Cd content of the leaves was determined with a Series II inductively coupled plasma mass spectrometer (Thermo Scientific, Winsford, U.K). Plant samples (0.2 g) were combined with 8 mL of concentrated HNO_3, 1 mL of 35% H_2O_2, and 1 mL of Milli Q water in a Teflon digestion container, and heated for 20 min at 170 watts followed by 10 min at 100 watts. The temperature inside the digestion container was set at 90 ± 5 °C. Afterwards, the containers were allowed to cool down beside a fan for 15 to 20 min. The heating procedure was repeated twice to obtain complete digestion and a clear solution. We then transferred 10-mL aliquots of the solutions into 100-mL plastic volumetric flasks, diluted the solution to 100 mL with 1% trace-element-grade HNO_3, filtered it through a membrane filter (mixed cellulose ester, 0.45-μm pore size; Advantec, Tokyo, Japan) to remove any particles, and stored it in capped plastic vials at 5°C until analysis could be completed. Values were compared with certified spinach leaf samples provided by the National Institute of Standards and Technology (NIST No. 1570a). The value for the spinach standard sample was 2.54 ± 0.08, compared with the NIST certified value of 2.89 ± 0.07 mg Cd kg^{-1}. The certified standard samples were included at every stage of analysis to maintain quality control.

4.3.3 Statistical analysis

Analysis of variance was performed using the JMP 8.01 software (SAS Institute 2008). Significant differences between treatment means were identified using Tukey's (HSD) test ($P < 0.05$).

Table 8. Contents of phosphate and cadmium in each fertilizer of this study

Fertilizer	Source	Total Cd (mg kg^{-1})	Citric acid soluble Cd (mg kg^{-1})	Water soluble Cd (mg kg^{-1})	P_2O_5 %
PF1	China	1.64	0.87	0.71	12
PF2	China	3.17	1.43	1.01	16
PF3	China	1.46	1.25	1.1	46
PF4	Japan	3.11	1.18	1.06	18
PF7	Myanmar	2.37	1.28	0.04	50

Table 9. Application of phosphate fertilizer and input of Cd in each treatment

Treatment	Level of application (kg P_2O_5 ha^{-1})	Input of Cd from fertilizer (g ha^{-1})
Control	0	0
PF1	120	1.6
PF2	120	2.4
PF3	120	0.36
PF4	120	2.2
PF7	120	0.56

Plate 6. Komatsuna leaves after harvest

Plate 7. Komatsuna in pots at two weeks after sowing

4.4 Results

4.4.1 Effect of phosphate fertilizers application on Cd concentration in soil

The phosphate fertilizer presented different Cd concentrations (Table 8). The PF3 presented the lowest total Cd concentration among the tested fertilizers. PF1 showed the lowest citric acid soluble Cd. However, The PF7 fertilizer contained the lowest waster soluble Cd than other PFs. The amounts of Cd added to the soil in the fertilizer application ranged from 0.36 to 2.4 g ha^{-1}(Table 9). The amounts of total soil Cd increased by 0.18 to 0.22 and 2.34 to 2.83 mg kg^{-1} in Melanudand and Haplaquept (Fig 6). The amounts of total soil Cd increased slightly in Melanudand but not significantly with increasing fertilizer rate because of the low input of Cd in the phosphate fertilizer. The amounts of citric acid-extractable soil Cd increased by 0.028 to 0.034 mg kg^{-1}and 0.37 to 0.40 mg kg^{-1} at control and phosphate application in Melanudand and Haplaquept (Fig 7). The 0.1 mol L^{-1} HCl acid-extractable Cd increased by 0.08 to 0.013 and 1.23 to 1.53 in Melanudand and Haplaquept, which may include a soil Cd fraction that is available to the komatsuna plants (Fig 8). The water-soluble Cd increased by 0.001 to 0.0012 and 0.0028 to 0.0034 in Melanudand and Haplaquept. Water soluble Cd, which may include a soil, Cd fraction that is available to the komatsuna plants (Fig 9).

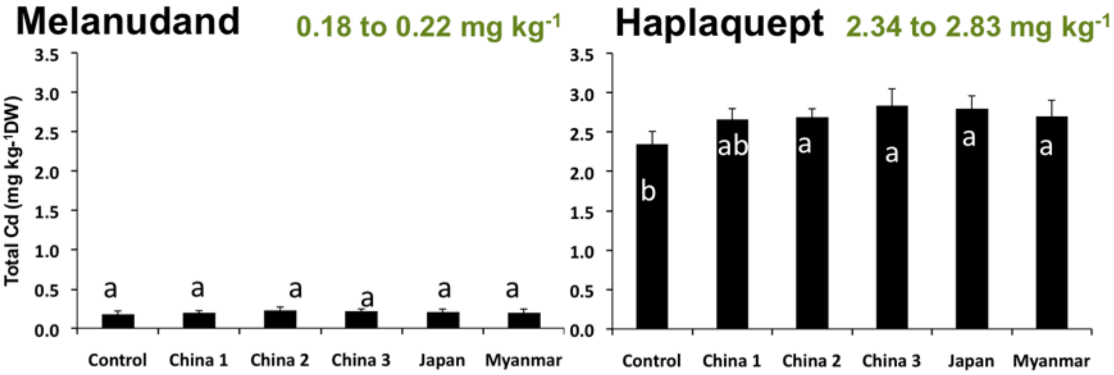


Figure 6. Effect of different phosphate fertilizers on Total Cd in soils

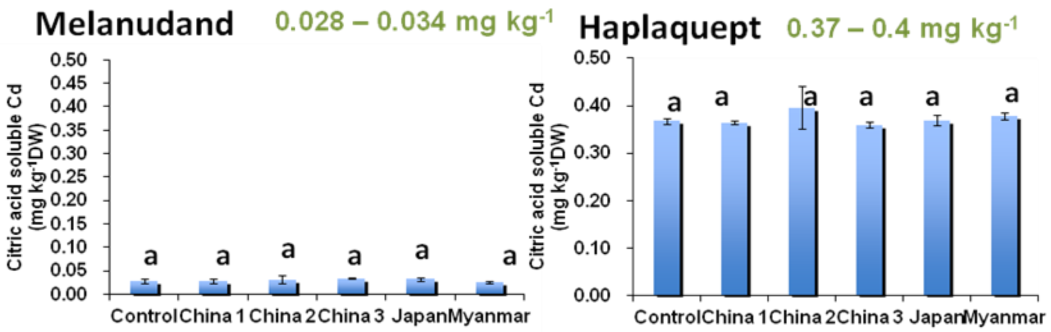


Figure 7. Effect of different phosphate fertilizers on citric acid soluble Cd in soils

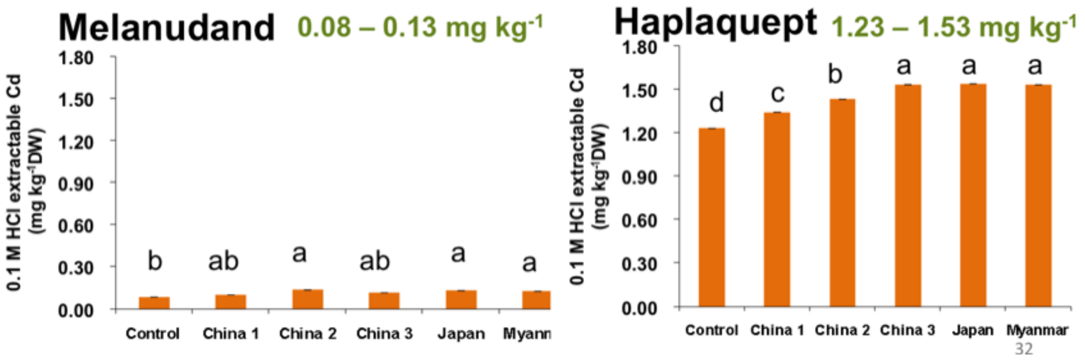


Figure 8. Effect of different phosphate fertilizers on HCL acid extractable Cd in soils

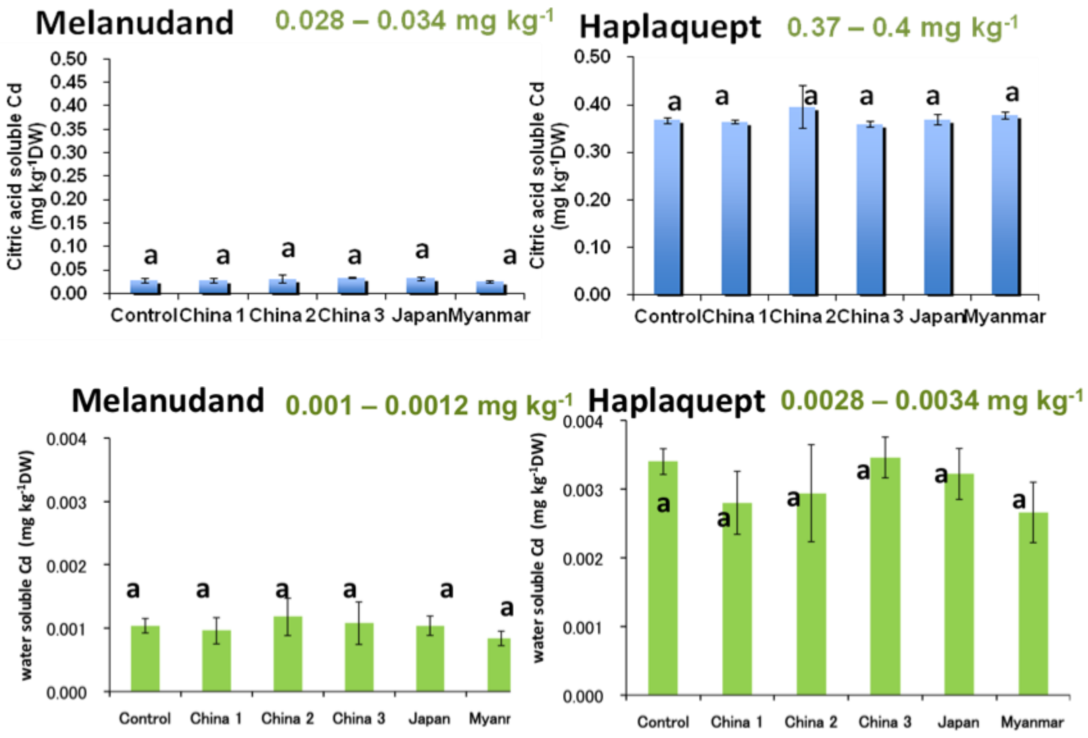


Figure 9. Effect of different phosphate fertilizers on water soluble Cd in soils

4.4.2 Effect of phosphate fertilizer application on yield of dry Komatsuna leaves

The yield of dry komatsuna leaves increased with phosphate application than control especially in PF7 Myanmar fertilizer treatment in Melanudand and Haplaquept. However, a reduction in leaf yield occurred at the PF1 China 1 fertilizer application treatment. However, none of the differences were significant among the four fertilizers of china 1,2, 3 and Japan. There was considerable variation in dry leaf weight in both Melanudand and Haplaquept of the presence and the absence of phosphate application. There was no significant difference in yield between the control plot and the phosphate plots in either soil except PF7 Myanmar (Fig 10).

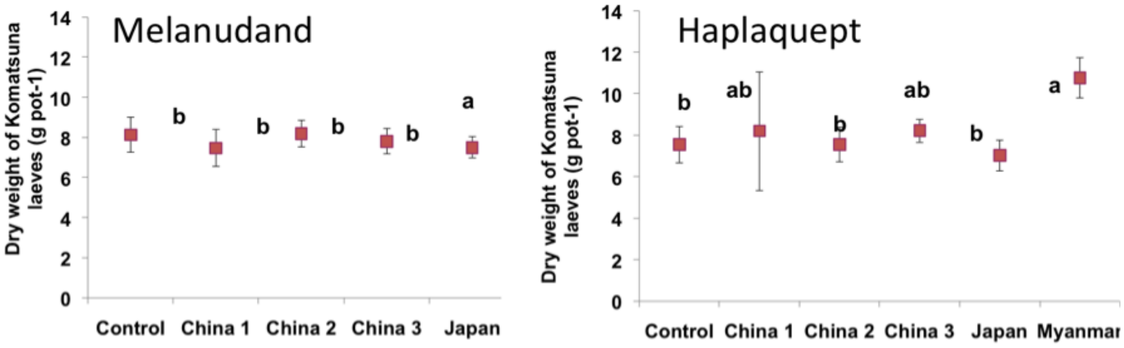


Figure 10. Effect of different phosphate fertilizers on dry weight of Komatsuna leaves

4.4.3 Effect of phosphate fertilizer application on Cd concentration and uptake by Komatsuna leaves

The effects of different fertilizer application on the Cd concentration in dry leaves are shown in Figure 11. We observed a significant difference between Melanudand and Haplaquept in the concentrations of Cd in dry Komatsuna leaves. We also observed a significant different in the mean Cd concentration in the leaves with phosphate application compared to control; the Cd content increased from 0.029 mg kg^{-1} to 0.034 mg kg^{-1} grown on Melanudand and from 1.08 to 1.24 mg kg^{-1} grown on Haplaquept (Figure 11).

The Cd concentration in Komatsuna fresh weight grown in Melanudand was under the limit of CODEX, however, that grown in Haplaquept was over the limit of limit of CODEX allowable maximum level (0.2 mg kg^{-1} FW) (Figure 12). On average, the Cd uptake by komatsuna increased from 0.2 to 0.3 and 8.1 to 9.9 μg pot^{-1} in Melanudand and Haplaquept (Fig 13). The amount of Cd added through the fertilizer was low compared with the amount already present in the soil to a depth of 15 cm (2.34 mg kg^{-1}) or (2340 g ha^{-1}) because Cd in the fertilizer may be more available to the plants than soil Cd, which is more likely to be bound to various complexes in non-exchangeable form. The concentration differences among the different phosphate fertilizer were significant in Haplaquept but not in Melanudand, possibly because of different soil organic matter content, pH, CEC, clay content.

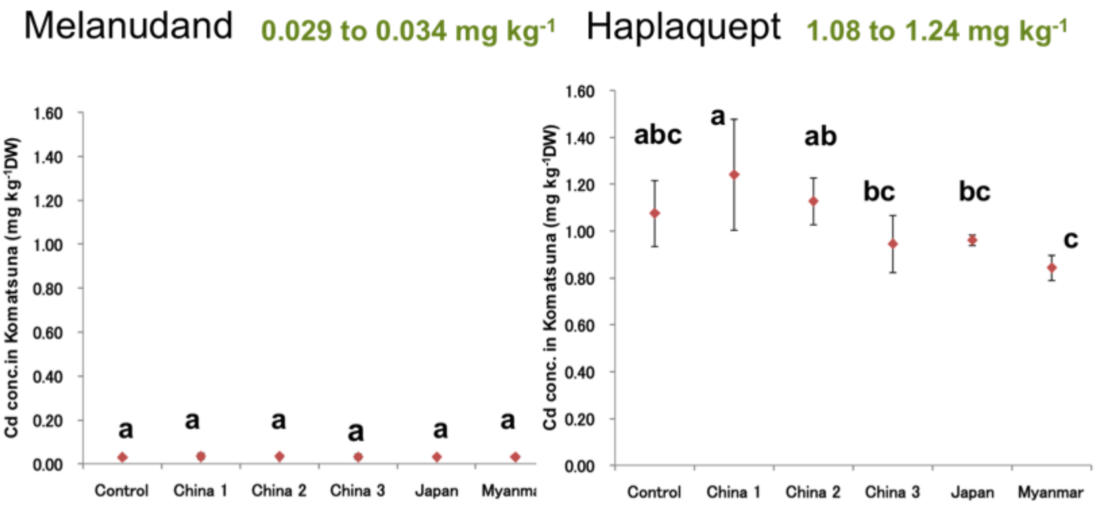


Figure 11. Effect of different phosphate fertilizers on Cd concentration in Komatsuna dry leaves grown on Melanudand and Haplaquept

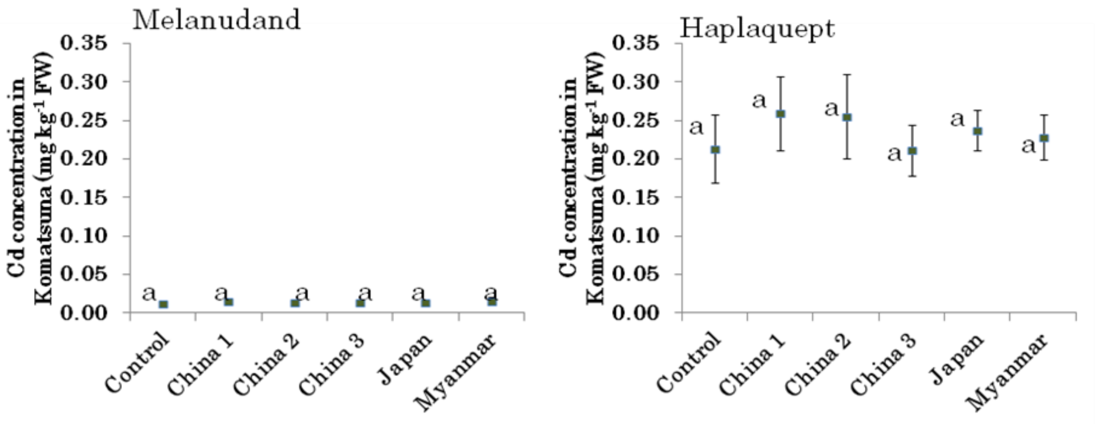


Figure 12. Effect of different phosphate fertilizers on Cd concentration in Komatsuna

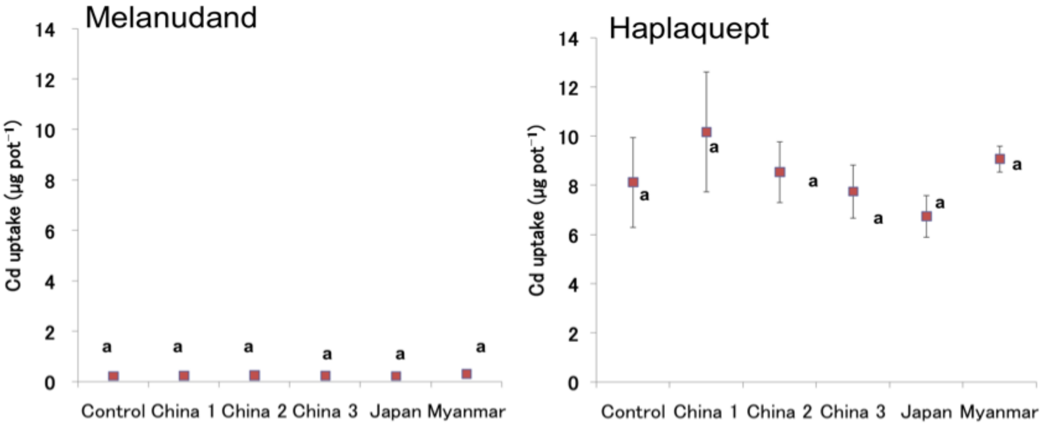


Figure 13. Effect of different phosphate fertilizers on Cd uptake by Komatsuna dry leaves grown on Melanudad and Haplaquept

Cadmium concentrations in crops generally follow the trend for soils, but on average, accumulate less than in soils. Cadmium concentrations in different phosphate fertilizers of this study superphosphate are lower than the concentrations obtained in other studies (Prochnow et al., 2001; McBride & Spiers, 2001). This result agrees with other statements that Brazilian rock phosphates present low heavy metal concentrations (Camargo et al., 2000) and that such concentrations vary for phosphates obtained from different regions. The PF3 (China 3) presented the lowest Cd concentration as shown in Fig. 11. There was no clear relation between Cd concentrations in fertilizers and the concentration of Cd in Komatsuna. This can be attributed to the solubility of the different sources. The lowest Cd concentrations were observed in plants fertilized with PF7. The Cd concentrations in plants were also higher for PF1 and PF2 than other PFs in both Melanudand and Haplaquept. This corroborates to the effect of source solubility on Cd uptake. The PF1 presented the lower Cd concentration among the tested fertilizers. Nevertheless, this fertilizer provided the larger Cd concentration in Komatsuna (Fig 11). This indicates that the Cd chemical form in this fertilizer was probably decisive in determining the Cd solubility and plant uptake. Other authors also found increases in Cd uptake by plants as a consequence of phosphate fertilizer applications (Mulla et al., 1980; Mortvedt, 1987; Prochnow et al., 2001). Therefore, these results indicate that phosphate fertilizers can be an important via for Cd entry in the food chain. The Cd translocation to the aboveground parts of the plants observed in here suggests that more studies are needed, owing to concerns regarding Cd accumulation at the highest levels of the food chain. Cd concentration in plants grown on Melanudand and Haplaquept fertilized with PF1 was significantly higher (Fig 9). The effect on Cd uptake for Komatsuna treated with PF7 was due to PF7's lower water solubility and lower Cd content and higher dry Komatsuna dry leaves as compared to other PF. In spite of the high solubility of the triple superphosphate, the Cd concentration in plants grown on soil amended with this fertilizer was higher in Haplaquept as compared to Melanudand. This can be due to the low adsorption of Cd on specific adsorption sites in the soil (Alloway, 1990) and to the low cation exchange capacity of the soil (Haplaquept) as show in Table 12. The low water solubility of PF7 as compared to other PFs is the most probable reason for such result. Cadmium mobility in the soil and uptake into plants is a complex function of the quantity of cadmium bound to soil and organic matter and the proportion in the soil solution (and hence available for uptake). The relationship between soil solution and uptake into plants is itself dependent on the soil condition, texture, type, pH, mineral content and various crop factors. Soil factors that increase the uptake of cadmium by plants are: low pH; high salinity; high total cadmium concentrations; low organic matter content; low cation exchange capacity; low clay; low iron and zinc; and high temperature. The most important of these factors is the pH level of the soil. The availability of cadmium to plants also depends on the pH of the rhizosphere and the ability of roots to acidify their surroundings in order to solubilise elements for enhancing uptake.

CHAPTER 5. INFLUENCE OF DIFFERENT RATE OF CALCIUM SUPERPHOSPHATE AND FUSED MAGNESIUM PHOSPHATE FERTILIZER APPLICATION ON CADMIUM UPTAKE BY KOMATSUNA GROWN ON MELANUDAND AND HAPLAQUEPT

5.1 Abstract

Cadmium (Cd) accumulation in soils and crops can be mitigated by reducing phosphate fertilizer application. A pot experiment was conducted on Japanese Melanudand and Haplaquept to investigate the influence of calcium superphosphate and fused magnesium phosphate on acid-extractable and total soil Cd on Komatsuna (*Brassica rapa* var. *perviridis*) growth and Cd uptake. The phosphate application produced total soil Cd ranging from 2.11 to 2.24 mg kg$^{–1}$ and 0.1 mol L^{-1} HCl extractable Cd ranging from 1.36 to 1.41 mg kg$^{–1}$ in Haplaquept and ranging from 0.48 to 0.52 mg kg^{-1} and 0.1 mol L^{-1} HCl extractable Cd ranging from 0.06 to 0.08 mg kg^{-1} in Mealnudand. Cd input reached 5.6 g ha$^{–1}$ and 0.048 g ha$^{–1}$at 240 kg ha$^{–1}$ of calcium super phosphate and fused magnesium phosphate. Phosphate fertilizers affected dry-matter yield of leaves to different degrees in each fertilizer. Compared with the control (no fertilizer), 240 kg ha$^{–1}$ calcium superphosphate and fused magnesium phosphate increased little Cd concentration in dry leaves and there was no significant difference.

5.2 Introduction

Komatsuna (*Brassica rapa* var. *perviridis*), also known as Japanese mustard spinach, is a popular leafy vegetable grown in Japan, Taiwan, and Korea. The leaves are usually stir-fried, pickled, boiled, or added to soups, or are used fresh in salads. The name "Komatsuna" originated from its place of origin, Komatsugawa, in the Edogawa ward of Tokyo. In Japan, the second-highest production of Komatsuna comes from the Tokyo region, and the Fuchu alluvial plain is one of the major production regions near Tokyo.

Currently, 95 cultivars of Komatsuna are registered in Japan according to Japan Seed Trade Association personal communication. One of the most popular cultivars in the Tokyo region is Nakamachi.

Cadmium (Cd), a potentially hazardous heavy metal, is present at varying levels in soils and water used for crop production. Soil Cd tends to increase as a result of continuous long-term application of phosphate fertilizers through its presence as a contaminant, and could increase the risk of Cd accumulation in plants. Compared with most nutrients, Cd has a relatively low mobility, so fertilizer applications can result in Cd accumulation near the soil surface, in the major plant rooting zone (Hamon et al. 1998; Loganathan and Hedley 1997). From a medical perspective, Cd is considered to be a dangerous element in fresh vegetables. Cd toxicity especially affects humans because of our longevity and the accumulation in our organs of Cd in food (Tudoreanu and Phillips 2004). Elevated Cd levels can cause kidney damage, and even low levels of dietary Cd have been linked with renal dysfunction, liver damage, lung edema, anemia, and hypertension (Basta et al. 1998). Other diseases associated with Cd exposure are pulmonary emphysema and *itai-itai* (“ouch-ouch”) disease (Yeung and Hsu 2005), which results from painful bone demineralization (osteoporosis) when Cd replaces calcium in the bones. Cadmium poisoning has occurred worldwide, and caused more than 100 deaths in Japan from 1922 to 1965 (Yeung and Hsu 2005). Therefore, it is desirable to reduce the Cd concentration in the human diet. In April 2006, the maximum allowable level in leafy vegetables was lowered to 0.2 mg kg^{-1} fresh weight (FW) by the Codex Committee on Food Additives and Contaminants (CCFAC) of the FAO and WHO. Metal availability to plants in the soil, rather than the total metal concentration, is the primary concern, because the available metal concentration is a better indicator of the amount available for plant uptake. However, many studies have determined both parameters.

Cd is present at varying levels as a contaminant in phosphate fertilizers. One environmental source of Cd is the phosphate ore used in the production of these fertilizers. The main source of this ore is phosphate rock of sedimentary origin. Commercial fertilizers have played a critical role in increasing vegetable production around the world for many decades, but many phosphate and trace element fertilizers contain elevated quantities of such heavy metals as Cd, lead (Pb), and arsenic (As) (Mortvedt 1985,1996; Raven and Leoppert 1997).

Soluble Cd becomes a major problem because high levels of Cd in many agricultural fields have resulted from long-term use of phosphate fertilizers, sewage sludge applications, and irrigation with wastewater (Sharma and Agrawal 2005; Irtelli and Navari-Izzo 2006). There are concerns about whether this will cause an accumulation of metals to dangerous levels, thereby increasing the environmental and human health risks. Cadmium input into agricultural soils in phosphate fertilizers varies with the fertilizer's Cd content, application rate, and application frequency. Bœrug and Singh (1990) reported that long-term application of phosphate fertilizers in Norway increased the total soil Cd concentration, although not the plant Cd concentration. Mortvedt (1984, 1987) showed that long-term application of triple superphosphate fertilizer did not significantly increase the plant Cd concentration, and concluded that the plant availability of the Cd in this fertilizer was low despite high application rates.

Plant response to the Cd in phosphate fertilizer therefore appears to depend on the plant genotype, the Cd concentration in the fertilizer, the rate and method of fertilizer application, the soil type, and climatic conditions. Cd uptake is a dynamic process. As a plant takes up Cd, the equilibrium Cd concentration in the soil solution is maintained by the input of Cd from particulate-bound species. Thus, the Cd concentration in the soil solution is also controlled by the nature and amount of particulate-bound Cd species in the soil.

Among plant species, the Cd concentration is generally higher in leafy species such as spinach, Komatsuna and in root crops such as carrots than in grain crops. The major objective of our study was to evaluate the influence of different rate of calcium super phosphate and fused magnesium phosphate fertilizers application on Cd uptake by komatsuna grown on Melanudand and Haplaquept.

5.3 Materials and Methods

The study was carried out to investigate the cadmium concentration in soil, Komatsuna and Komatsuna Cd uptake as affected by the application of different rate of calcium superphosphate and fused magnesium phosphate fertilizers into two different soils. For pot experiment, two types of soils (Melanudand and Haplaquept) were collected from Saiwai Cho, Fuchu campus and Fuchu Hommachi, TUAT respectively. The (2 x 2 x 4) factorial arrangement was randomly assigned in Completely Randomized Design with three. Treatments are two types of fertilizers containing different Cd conc. and control (without fertilizer). The concentrations of Cd and P_2O_5 in calcium superphosphate and fused magnesium phosphate fertilizers were 4.01 mg kg^{-1}, 17% and 0.04 mg kg^{-1}, 20 % respectively, each kind of fertilizer was applied at the rate of 60, 120 and 240 kg P_2O_5 ha^{-1}. All treatments were replicated in three times. Before sowing of Komastuna, fertilizers in accordance with respective rates were applied and incorporated into the soil at about 15 cm depth in pot experiments. All the pots received the same amounts of basal fertilizers. N and K fertilizers were applied by hand at the recommended rates for the Tokyo region to prevent N and K from limiting growth: Nitrogen at 70 kg N ha^{-1} as $(NH_4)_2SO_4$ (21% N and 0.01 mg Cd kg^{-1}) and K at 50 kg K_2O ha^{-1} as KCl (60% K_2O and 0.04 mg Cd kg^{-1}). Ten seeds of Komatsuna were sown in each pot, which were thinned to six plants after germination. The cultivation period was April 25th to June 19th, 2009.

5.3.1 Soil sampling and analysis

The procedure was same as above 4.3.1.

5.3.2 Plant sampling and analysis

The procedure was same as above 4.3.2.

5.3.3 Statistical analysis

Analysis of variance was performed using the JMP 8.01 software (SAS Institute 2008). Significant differences between treatment means were identified using Tukey's (HSD) test ($P < 0.05$).

Treatments of this experiment

S1 = Melanudand,

S2 = Haplaquept,

F1 = Calcium superphosphate,

F2 = Fused magnesium phosphate,

T1 = 0 kg ha^{-1},

T2 = 60 kg ha^{-1}

T3 = 120 kg ha^{-1}

T4 = 240 kg ha^{-1} 2 x 2 x 4 x 3 = 48 pots for With Komatsuna cultivation

Plate 8. Komatsuna in pots two months after sowing

5.4 Results

5.4.1 Effect of different rate of calcium superphosphate and fused magnesium phosphate application on Cd concentration in soil

The contents of P_2O_5 and Cd were shown in Table 10. The amount of Cd added through these P_2O_5 sources were also shown in Table 11. Physical and chemical properties of soils used in this study were shown in Table 12.

Table 10. Concentration of Cd and P_2O_5 in calcium superphosphate and fused magnesium phosphate

Fertilizer	Name	Source	Total Cd (mg kg^{-1})	P_2O_5 %
PF5	Calcium superphosphate	Japan	4.01	17
PF6	Fused magnesium phosphate	Japan	0.04	20

Table 11. Level of phosphate application and input of Cd in each treatment

Treatments	Level of application ($kg\ P_2O_5\ ha^{-1}$)	Input of Cd from fertilizer ($g\ ha^{-1}$)
Control	0	0.000
Calcium superphosphate	60	1.400
Calcium superphosphate	120	2.800
Calcium superphosphate	240	5.600
Fused magnesium phosphate	60	0.012
Fused magnesium phosphate	120	0.024
Fused magnesium phosphate	240	0.048

Table 12. Physical and chemical properties of soils used in this study

Parameter	Melanudand	Haplaquept
pH (1:2.5 in water)	6.3	5.5
Total N ($g\ kg^{-1}$)	8.5	3.6
Total C ($g\ kg^{-1}$)	97	37
Exchangeable NH_4-N ($mg\ kg^{-1}$)	98	57
Available P_2O_5 (Bray No. 2 P_2O_5; $mg\ kg^{-1}$)	51	52
Cation exchange capacity ($cmol^+\ kg^{-1}$)	28.5	19.8
Exchangeable cations ($cmol^+\ kg^{-1}$)		
K	1.1	0.42
Ca	16	9.4
Mg	6.4	4.3
Na	0.043	0.037
Soil texture class name	Sandy clay loam	Sandy loam
Particle size distribution (%)		
Clay	30	16
Silt	24	33
Sand	46	51
Total Cd ($mg\ kg^{-1}$)	0.41	2.02
0.1 mol L–1 HCl–extractable Cd ($mg\ kg^{-1}$)	0.08	1.2

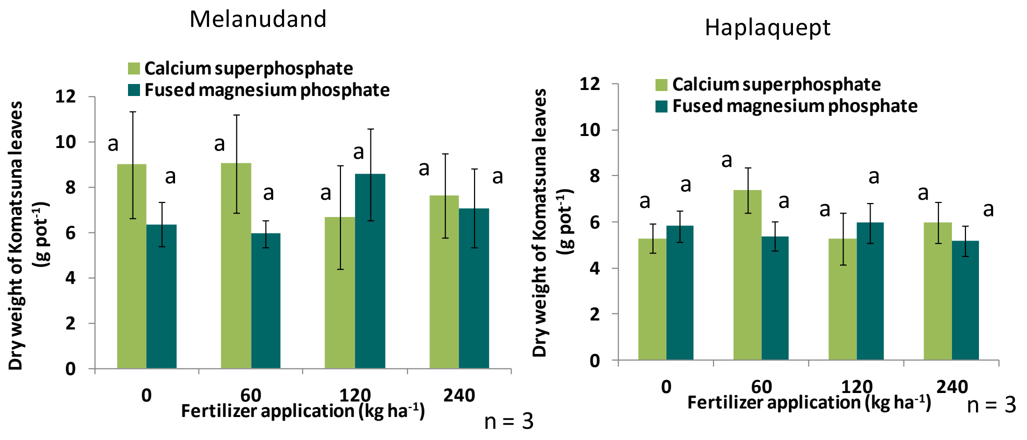
Soil properties of Melanudand and Haplaquept before the fertilizer applications were pH 5.5 and 6.3, TN 3.6 and 8.5 g kg^{-1}, exchangeable NH_4-N 57 and 98 mg kg^{-1}, available phosphate (Bray No. 2) 52 and 51 mg P_2O_5 kg^{-1}, CEC 28.5 and 19.8 cmolb (+) kg^{-1}. The soil had a moderate cation exchange capacity of 19.8 cmol (+) kg^{-1} in Haplaquept. The higher CEC value was found in Melanudand. The amounts of total soil Cd increased by 0.10 mg kg^{-1} and by 0.13 mg kg^{-1} at the highest level of phosphate application in Melanudand and Haplaquept (Table 12). The amounts of total soil Cd increased slightly but not significantly with increasing fertilizer rate because of the low input of Cd in the phosphate fertilizer. The amounts of 0.1 mol L^{-1} HCl acid-extractable soil Cd increased by 0.01 mg kg^{-1} and 0.06 mg kg^{-1} at the highest level of phosphate application in Melanudand and Haplaquept (Table 13). The acid-extractable Cd may include a soil Cd fraction that is available to the komatsuna plants. The amounts of Cd added to the soil in the fertilizer application ranged from 1.42 to 5.68 g ha^{-1} and 0.012 to 0.048 g ha^{-1} by application of 60 to 240 kg P_2O_5 ha^{-1} of calcium superphosphate and fused magnesium phosphate fertilizer application (Table 11).

5.4.2 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on the yield of dry komatsuna leaves

The yield of dry komatsuna leaves decreased with increasing phosphate application. However, none of the differences were significant. Calcium superphosphate application gave higher dry matter yield of Komatsuna leaves. There was considerable variation in dry leaf weight in both the presence and the absence of phosphate application. There was no significant difference in yield between the control plot and the phosphate plots in either soil (Fig 14).

Table 13. Effect of different rate of calcium superphosphate and fused magnesium phosphate on Cd concentration in soil

Treatment **Soils**	**Fertilizers**	**Rate ($kg\ ha^{-1}$)**	**Soil pH**	**Cd concentration ($mg\ kg^{-1}$)** **0.1 mol L^{-1} HCl extractable**	**Total**
Melanudand	Calcium superphosphate	0	6.09 ± 0.16 abc	0.08 ± 0.01 ab	0.43 ± 0.03 b
		60	6.15 ± 0.10 abc	0.08 ± 0.03 ab	0.45 ± 0.06 b
		120	6.05 ± 0.05 abc	0.09 ± 0.04 a	0.48 ± 0.05 ab
		240	5.95 ± 0.01 abc	0.07 ± 0.01 ab	0.54 ± 0.03 a
	Fused magnesium phosphate	0	6.07 ± 0.10 abc	0.08 ± 0.01 ab	0.48 ± 0.03 b
		60	6.28 ± 0.03 abc	0.05 ± 0.01 b	0.51 ± 0.02 ab
		120	6.42 ± 0.06 a	0.06 ± 0.01 b	0.49 ± 0.02 ab
		240	6.24 ± 0.14 abc	0.06 ± 0.01 b	0.52 ± 0.03 a
Haplaquept	Calcium superphosphate	0	5.88 ± 0.31 bc	1.36 ± 0.21 ab	2.11 ± 0.14 a
		60	6.02 ± 0.06 abc	1.32 ± 0.16 ab	2.19 ± 0.09 a
		120	6.02 ± 0.06 abc	1.33 ± 0.12 ab	2.22 ± 0.15 a
		240	5.97 ± 0.14 abc	1.41 ± 0.17 a	2.24 ± 0.04 a
	Fused magnesium phosphate	0	5.79 ± 0.42 c	1.36 ± 0.21 ab	2.11 ± 0.14 a
		60	6.22 ± 0.21 abc	1.29 ± 0.19 bc	2.18 ± 0.10 a
		120	6.29 ± 0.05 abc	1.18 ± 0.22 c	2.16 ± 0.14 a
		240	6.32 ± 0.13 ab	1.32 ± 0.20 ab	2.22 ± 0.15 a
Significant level of treatment					
Soils			ns	***	***
Fertilizers			*	ns	ns
Rate			***	ns	ns
Soil x fertilizer x Rates			**	***	***



JMP Stat -Tukey’s test at 0.05 level

Figure 14. Effect of different rates of calcium superphosphate and fused magnesium phosphate on dry weight of Komatsuna leaves

5.4.3 Effect of different rate of calcium superphosphate and fused magnesium phosphate on Cd concentration and uptake by Komatsuna dry leaves

The effects of different application rates and kinds of phosphate on the Cd concentration in dry leaves are shown in Figure 14. We observed a significant difference between Melanudand and Haplaquept in the concentrations of Cd in dry weight of Komatsuna leaves. We also observed a significant increase in the mean Cd concentration in the leaves with increasing phosphate application grown in Haplaquept; the Cd content increased from 0.19 mg kg^{-1} to 0.23 mg kg^{-1} grown on Melanudand and decreased from 1.52 to 1.28 mg kg^{-1} grown on Haplaquept (Fig 15). The amount of Cd added through the fertilizer was low compared with the amount already present in the soil to a depth of 15 cm (2.02 mg kg^{-1}) or (2020 g ha^{-1}) because Cd in the fertilizer may be more available to the plants than soil Cd, which is more likely to be bound to various complexes in non-exchangeable form. The concentration differences among the application rates were significant in Hapaquept but not in Melanudand, possibly because of different soil organic matter content, pH, CEC, clay content. The Cd concentrations in the leaves of all treatments (FW basis) were generally below CCFAC's allowable maximum level (0.2 mg kg^{-1} FW). There was also no significant difference in Cd uptake by Komatsuna dry leaves (Fig 13). However, the higher Cd uptake was occurred in calcium superphosphate fertilizer applied treatment in both of soils. The higher uptake of Cd was found in Haplaquept than Melanudand because of different Cd concentration in original soil.

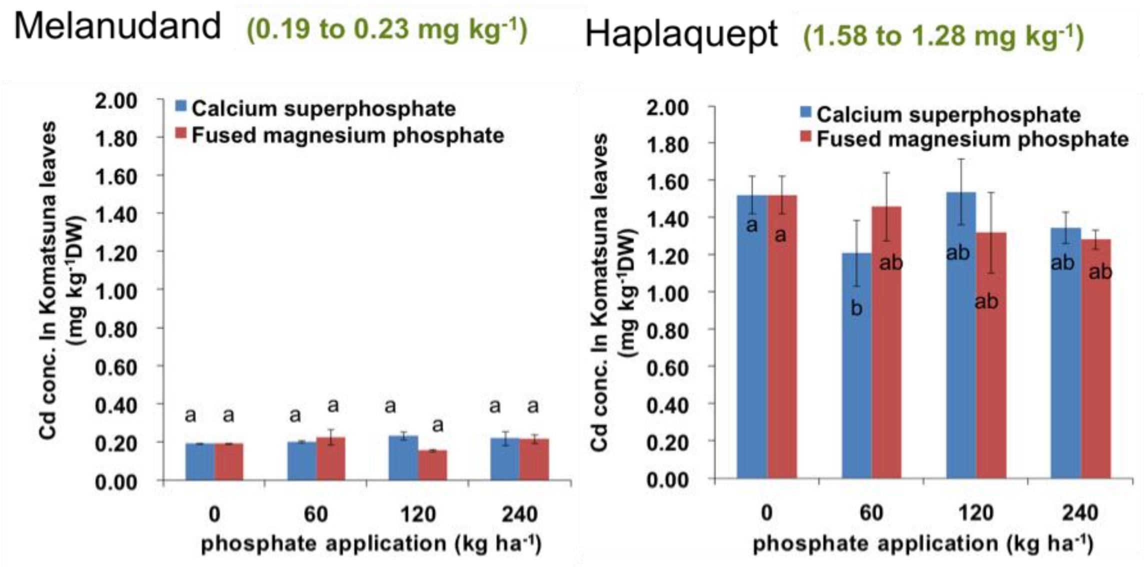


Figure 15. Effect of different rates of calcium superphosphate and fused magnesium phosphate on Cd concentration in dry Komatsuna leaves

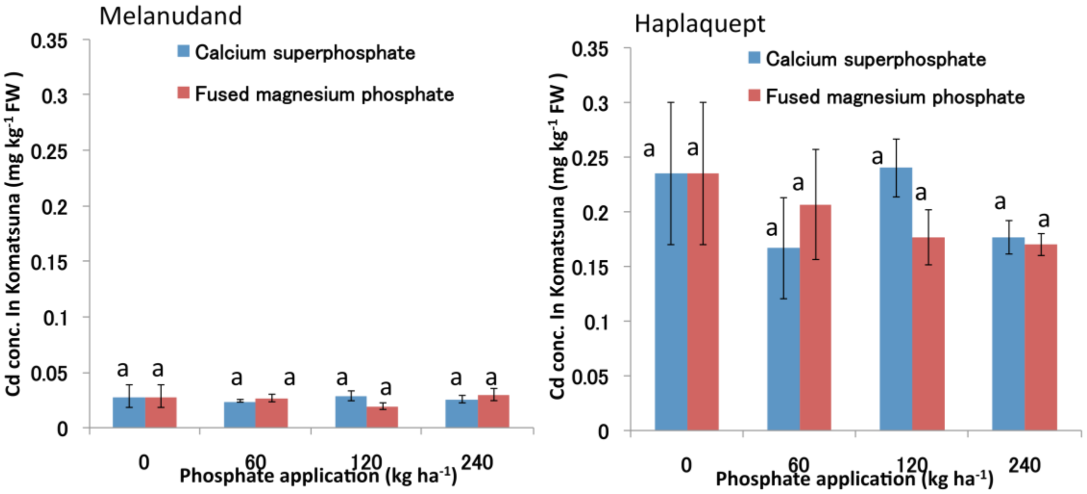
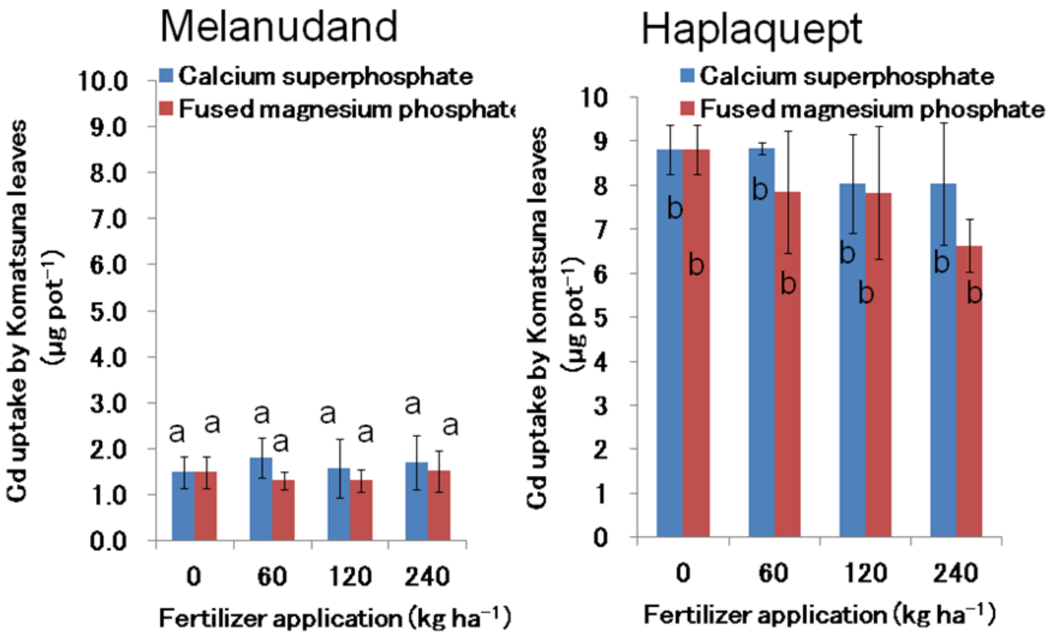


Figure 16. Effect of different rates of calcium superphosphate and fued magnesium phosphate on Cd concentration in Komatsuna fresh leaves



n = 3 JMP stat Tukey's test at 0.05 level n = 3

Figure 17. Effect of different rates of calcium superphosphate and fued magnesium phosphate on Cd uptake by dry Komatsuna leaves

5.5 Discussion

5.5.1 Effect of different rate of calcium superphosphate and fused magnesium phosphate application on Cd concentration in soil

Soil is contaminated with Cd in many agricultural soils, and food ingestion is a major pathway for entry of this Cd into the human body. Minimizing the concentrations of Cd in agricultural products is therefore desirable to reduce the health risks from this contamination. Repeated applications of P fertilizers increased the soil solution concentration of both Cd and Zn in field and laboratory experiments (Lambert et al. 2007). Long-term use of phosphate fertilizer that contains high level of Cd may lead to accumulation Cd in the soil, and this may eventually increase plant Cd uptake. In addition, Cd in fertilizer may be more bioavailable than that in the soil, since it is less likely to be bound to organic matter and other complexes that may make soil Cd unavailable to plant. Both the input and plant uptake of Cd tend to be small relative to the pool of Cd present in the soil (Christensen and Huang 1999). In many long-term field studies, an increase in soil Cd as a result of phosphate fertilizer application was associated with increased plant Cd concentrations, indicating that Cd accumulation in the soil led to higher phytoavailability of Cd (Williams and David 1976; Mulla et al. 1980; Jones et al. 1987; Jones and Johnston 1989; Kashem and Singh 2002). In a long-term experiment involving the application of triple superphosphate, Mulla et al. (1980) showed that the total Cd concentration in surface layers of a soil that received broadcast phosphate fertilizer for 36 years increased from 0.07 to 1.00 mg kg^{-1}. Cd concentrations in barley grains and leaves grown in this soil were not elevated above those grown in the control soils, but those in Swiss chard grown in a greenhouse in the same soil were significantly higher than those in the control (Mulla et al. 1980).

5.5.2 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on the yield of dry komatsuna leaves

Phosphate application slightly increased yield of fresh and dry weight of Komatsuna leaves. The effect of phosphate application on yield of Komatsuna was rather different in two soils. Higher level of application caused a large biomass reduction of Komatsuna. There was no relationship between phosphate application and yield of fresh and dry Komatsuna in Melanudand and Haplaquept. The height of Komatsuna was higher in Melanudan than in Haplaquept because of good fertility in Melanudand.

5.5.3 Effect of different rates of calcium superphosphate and fused magnesium phosphate on Cd concentration and uptake by Komatsuna dry leaves

The effect of phosphate fertilizer on Cd accumulation in komatsuna has rarely been studied. Continuous use of phosphate fertilizer increased the Cd content in cultivated soils (Alloway 1990). The amount of Cd added to the soils by fertilizer application was generally much greater than its total removal by harvested crops (He & Singh 1994a). In accordance with the observations of Singh (1990), we observed a net accumulation of Cd in soils. The application of Cd-containing NPK fertilizers, and especially P fertilizers, increased the Cd concentration in all crops studied by He and Singh (1994a), and the highest Cd concentration in these crops occurred when high-Cd fertilizer was used. The Cd concentration in durum wheat at several locations across the Canadian prairies increased in the year of application of P fertilizer, but the increase was unrelated to the Cd concentration in the fertilizer (Grant et al. 2002).

Increasing Cd concentration in grain crops with increasing Cd content in the commercial fertilizers that were applied has also been reported

(Andersson and Hahlin 1981; He and Singh 1994a). Jaakola (1979) reported increased Cd uptake by vegetable crops with increasing application rates of Cd-containing mineral fertilizer. Cd in superphosphate is water-soluble and is associated with both the phosphate and sulfate contents of the fertilizer. Williams and David (1973, 1976) found that Cd in superphosphate appeared to be as readily available to plants as Cd in $CdCl_2$. Cd uptake by plants depended strongly on the soil Cd content (Lund et al. 1981; Alloway and Morgan 1985). For these reasons, the use of Cd-containing fertilizers increased Cd uptake by plants. Growth chamber studies also showed that the application of phosphate fertilizer containing Cd increased the Cd concentration in a range of crops (Singh 1990; He and Singh 1995; Grant et al. 1999; Jiao et al. 2004). Increased soil pH also increases the adsorption of Cd by soils and thus reduces its extractability (Andersson and Siman 1991; He and Singh 1994a; Guttormsen et al. 1995; Christensen 1984; Kuo et al. 1985; King 1988). Since the soil in the present study had a pH of 6.3 in Melanudand, the phytoavailability of the Cd may have been high. This Cd availability differed in Haplaquept because of approximately 0.9 unit decrease in Haplaquept compare with Melanudand. A large body of evidence suggests that continuous use of phosphate fertilizers increases the Cd concentration in soils and plants (Williams and David 1976; Mortvedt et al. 1981; Jones and Johnston 1989; Ervio et al. 1990; He and Singh 1993).

Although Cd appears to be accumulating slightly in the soil of our study site, our analyses showed non-significant increases in Cd concentrations in plant tissues. Similar results were reported (Isermann 1982). The reduction in the availability of Cd from triple superphosphate to lettuce could be related to high P availability, which decreases Cd uptake by plants (Street et al. 1978) either by interfering with Cd translocation from the roots to aboveground plant parts (Williams and David 1976) or by promoting the capability of the soil or its constituents to adsorb Cd (Bolan and Duraisamy 2003; McGowen et al. 2001). The application of phosphate fertilizer did not affect Cd uptake by komatsuna in a previous study (Hattori et al. 2002). Plant uptake of both native and exogenous Cd generally decreased with increasing soil pH (Tables 13). Applications of very high rates of P, above those normally used for crop production, can immobilize Cd by means of P-induced adsorption of Cd^{2+} and by precipitation of Cd (Bolan and Duraisamy 2003; McGowen et al. 2001). This may explain the decreased Cd concentration at the highest P level (Table 13). Time also affects Cd availability. As a result of long-term chemical processes, the bioavailability of metals in soils can decrease over time, with little or no reduction in the total metal concentration (Lock and Janssen 2003). One of the most important parameters is probably pH, since this influences the Cd transformation process. The total Cd accumulated in the aboveground biomass of lettuce was 4.75 ± 0.28 g ha^{-1} in limed soil and 5.97 ± 0.36 g ha^{-1} in unlimed soil after 2 years (Huang et al. 2004). The Cd concentration in leaf tissues of vegetables averaged 2.0 mg kg^{-1} dry weight at Mukuvisi site and 1.1 mg kg^{-1} dry weight at Pension site (Mapanda et al. 2007). Komatsuna has a high ability to take up Cd during the 2 to 3 weeks after sowing (Takahashi et al. 2009).

There have been reports (Jaakkola 1977; Smilde and van Luit 1983; Mortvedt 1984, 1987) of no significant increase in Cd concentrations in plants due to the application of phosphate fertilizers, even after long-term use of such fertilizers. This may be because the phytoavailability of Cd added to soils through fertilizer application depends on the soil properties, the form of the Cd in the fertilizer, and the ability of the plants to absorb Cd from the soil. The concentration of Cd in komatsuna that was grown in soil (1.6 mg kg^{-1} of acid-extractable Cd and soil pH 5.6) was 0.20 mg kg^{-1} (Kikuchi et al. 2005), which is comparable to our results and equal to the maximum allowable level for leafy vegetables.

CHAPTER 6. INFLUENCE OF DIFFERENT RATE OF CALCIUM SUPERPHOSPHATE AND FUSED MAGNESIUM PHOSPHATE FERTILIZER APPLICATION ON CADMIUM UPTAKE BY SPINACH

6.1 Abstract

Cadmium is a common impurity in phosphate fertilizers and may contribute to soil Cd accumulation. Changes in total and bioavailable Cd burdens to agricultural soils and the potential for spinach Cd accumulation resulting from fertilizer input were investigated. The potential risks originating from cadmium accumulations in cropland soils through the fertilizer applications have been public concern. To determine whether the use of calcium superphosphate and fused magnesium phosphate fertilizer application resulted in measurable cadmium accumulation in soils and spinach harvested, soil and spinach samples were collected from treatments of pot experiment at Japan. Soil samples after extraction with 0.1 mol L^{-1} HCl and digestion HNO_3, $HClO_4$ and spinach samples after digestion with HNO_3, H_2O_2 and M.Q. water were analyzed for Cd. Cadmium based on fertilizer input and crop removal was worked out. Neither the total nor the 0.1 mol L^{-1} HCl extractable Cd in the soil was significantly affected by Cd added through fertilizer, though a tendency of higher Cd in soils from the pots receiving higher amounts of fertilizer was observed. The same trend was also observed for the Cd concentration in spinach. Although Cd tended to accumulate as a result of P fertilization, the rate of increase was slow. The increase in the total Cd content of fertilized pots varied from 0.03 to 0.15 mg kg^{-1} indicating that it may take very long time, depending upon the fertilizer input, to accumulate Cd equivalent to that currently present in the soil.

6.2 Introduction

Potential cadmium (Cd) uptake by food crops from applied phosphate

(P) fertilizers has become an important environmental issue because of the potential health hazards to human life from consuming foods that may contain a significant amount of Cd. The amount being added in a single application may be insignificant compared with the volume of receiving soil, its presence in soils may not be readily detectable by the routine field sampling and measurement protocols, but repeated application may lead to a gradual buildup of these elements in agricultural soils over time.

Cadmium (Cd) is a toxic trace pollutant for humans, animals and plants, which enters the environment mainly from industrial processes and phosphate fertilizer application, and is readily transferred to the food chain (Wagner, 1993). Cadmium from phosphate fertilizers poses a potentially serious threat to soil quality and, through the food chain, to human health. Cadmium is a naturally occurring, non-essential heavy metal, which is present at low concentrations in air, water, and soils that have been accumulating through the use of fertilizers derived from phosphate rock. Cadmium naturally occurs in phosphate rock, from which phosphate fertilizer is made. Superphosphate fertilizers contain not only major elements necessary for plant nutrient and growth, but also trace metal impurities such as Cd, Pb, or Hg, (Frieberg et al, 1992; Singh 1994; Nicholson and Jones, 1994; Zhang and Shan 2001). It is therefore an important anthropogenic source of soil contamination with heavy metals (Nriagu, 1990). The soil is an important sink for cadmium, lead, mercury, chromium and other heavy metals (Alloway, 1996) The uptake of these heavy metals by plants especially leafy vegetables is an avenue of their entry into the human food chain with harmful effects on health. The major hazard to human health of cadmium is its chronic accumulation in the kidneys where it causes dysfunction if the concentration in the kidney cortex exceeds 200 mg/kg fresh weight (Alloway, 1996). Typical health problems resulting from excessive body burden of Cd include: renal dysfunction, liver damage, lung edema, anemia, hypertension (Basta, et al. 1998).

Uptake of Cd in plants depends on the concentration of Cd in the soil solution and the rate of transfer from the solid phase into the soil solution for replenishment of the Cd taken up by the plant roots. The replenishment of the Cd from solid phase to soil solution may be increased by fertilizer application. He and Singh (1994) found that a NPK fertilizer containing a high concentration Cd (417 mg Cd kg^{-1} P) significantly increased the Cd concentration in rye grass, oat, carrot and lettuce compared to control. No such increase was found at the lower concentration of Cd (100 mg Cd kg^{-1} P) in the NPK applied. Evidently, these contradictory results were caused by a variety of factors, such as soil properties, Cd concentration in the fertilizer applied, rates of application and plant species. Application of Cd containing fertilizers may not be appreciably increase the plant Cd concentration at present, but low annual application my result in elevated Cd concentration in the cultivated layer, especially where high Cd fertilizers are used.

Both input and removal of Cd tend to be small relative to the pool of Cd present in the soil (Christensen and Huang 1999). However, if input exceeds removal, Cd will accumulate in the soil over time. Agricultural soils generally receive most of their Cd pollution from the atmosphere, fertilizers, pesticides, and manure (Nriagu, 1990; McLaughlin *et al*., 1996). Among the anthropogenic sources, phosphate fertilizers have been considered as the primary source of Cd that may contaminate soils (Fergusson, 1990). However, the risk of Cd toxicity to the soil ecosystem or of movement of Cd into the human diet will also depend on the bioavailability of the Cd present in the soil. Very few studies have considered Cd movement resulting from the long-term application of phosphate fertilizers. The accumulation of Cd in edible parts of crop plants is increasingly becoming a public health issue in many nations (Jin et al. 2002). Soil contamination by Cd is due to both natural and anthropogenic sources. The objective of this study is to identify the effect of different rate of calcium superphosphate and fused magnesium phosphate on Cd uptake by spinach grown on Melanudand and Haplaquept.

6.3 Materials and Methods

The pot experiment was conducted at Hommachi Farm, Field Science Center for Education and Research of Tokyo University of Agriculture and Technology (TUAT) The farm is in Fuchu City, Tokyo (35º 39/N, 139º 28/E). Two types of soils (Melaundand and Haplaquept) were collected from Saiwai Cho, Fuchu campus and Fuchu Hommachi, receptively. The 2 x 2 x 3 factorial arrangement was made using Completely Randomized Design with 3 replications. The tested variety of spinach was Jiromaru by applying three levels of application of F1-calcium superphosphate and F2- fused magnesium phosphate fertilizers (T1-0, T2-140, T3-280 kg P_2O_5 ha^{-1}). The study period was from May 3^{trd} to July 3rd 2010.

6.3.1 Soil sampling and analysis

This procedure was same as above 5.3.1.

6.3.2 Plant sampling and analysis

This procedure was same as above 5.3.2.

6.3.3 Statistical analysis

This procedure was same as above 5.3.3.

Table 14. Level of phosphate application and input of Cd in each treatment

Treatment	Level of application (kg P_2O_5 ha^{-1})	Input of Cd from fertilizer (g ha^{-1})
Control	0	0
Calcium superphosphate	140	3.3
Calcium superphosphate	280	6.6
Fused magnesium phosphate	140	0.028
Fused magnesium phosphate	280	0.056

Treatments of this experiment

S1 = Melanudand, S2 = Haplaquept,

F1 = Calcium superphosphate, F2 = Fused Magnesium phosphate,

Three replications for each treatment in Spinach cultivation,

36 pots of Spinach cultivation

Control = 0 kg ha^{-1} (without fertilizer),

level 1 =140 kg ha^{-1},

level 2 = 280 kg ha^{-1}

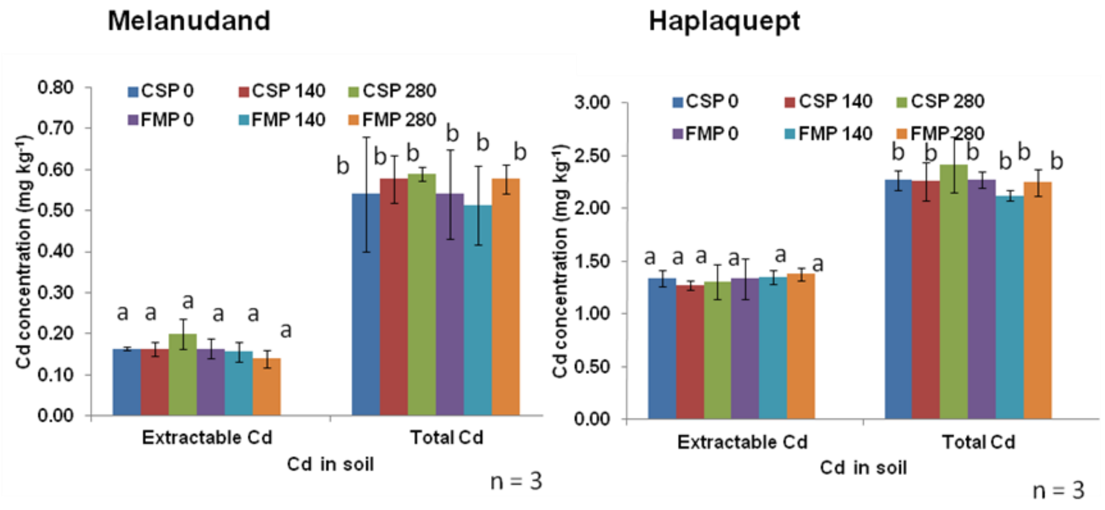
2 soils x 2 fertilizers x 3 treatments x 3 replications

Plate 9. Spinach in pot

6.4 Results

6.4.1 Effect of different rate of calcium superphosphate and fused magnesium phosphate application on Cd concentration in soil

The amounts of Cd added through these P_2O_5 sources were also shown in Table 14. The amounts of total soil Cd increased by 0.03 mg kg^{-1} and by 0.15 mg kg^{-1} at the highest level of phosphate application in Melanudand and Haplaquept (Fig 18). The amounts of total soil Cd increased slightly but not significantly with increasing fertilizer rate because of the low input of Cd in the phosphate fertilizer. The amounts of 0.1 mol L^{-1} HCl acid-extractable soil Cd increased by 0.03 mg kg^{-1} and 0.05 mg kg^{-1} at the highest level of phosphate application in Melanudand and Haplaquept (Fig 18). The 0.1 mol L^{-1} HCl acid-extractable Cd may include a soil Cd fraction that is available to the komatsuna plants. The amounts of Cd added to the soil in the fertilizer application ranged from 3.3 to 6.6 g ha^{-1} and 0.028 to 0.056 g ha^{-1} by application of 140 to 280 kg P_2O_5 ha^{-1} of calcium superphosphate and fused magnesium phosphate fertilizer application (Table 14).



JMP – stat Student t test at 0.05 level

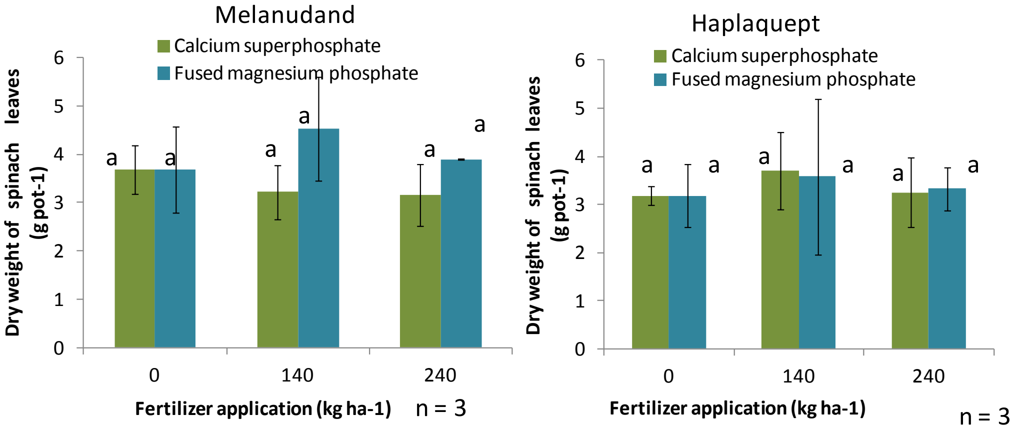
Figure 18. Effect of different rate of calcium superphosphate and fused magnesium phosphate application on Cd concentration in soil

CSP – calcium superphosphate and FMP – Fused magnesium phosphate

6.4.2 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on the yield of dry spinach leaves

The yield of dry spinach leaves decreased with increasing phosphate application. However, none of the differences were significant. Calcium superphosphate application gave higher dry matter yield of spinach leaves

3.18 to 3.70 g pot^{-1} grown on Haplaquept (Fig 19). However, fused magnesium phosphate application gave higher yield of spinach 3.68 to 4.53 g pot^{-1} grown on Melanudand. There was considerable variation in dry leaf weight in both the presence and the absence of phosphate application between two soils. There was no significant difference in yield between the control plot and the phosphate plots in either soil (Fig 19). The greater yield of spinach was occurred in Melanudand than Haplaquept.



JMP Stat -Tukey's test at 0.05 level

Figure 19. Effect of different rates of calcium superphosphate and fused magnesium phosphate on dry weight of spinach leaves

6.4.3 Effect of different rate of calcium superphosphate and fused magnesium phosphate application on Cd concentration and uptake by spinach dry leaves

The effects of different application rates and kinds of phosphate on the Cd concentration in dry leaves are shown in Figure 19. Significant difference between Melanudand and Haplaquept in the concentrations of Cd in dry weight of Komatsuna leaves. There was significant increase in the mean Cd concentration in the leaves with increasing phosphate application grown in Haplaquept; the Cd content increased from 0.74 mg kg^{-1} to 0.78 mg kg^{-1} grown on Melanudand and increased from 12.1 to 15.2 mg kg^{-1} grown on

Haplaquept (Fig 20). The concentration differences among the application rates were significant in Hapaquept but not in Melanudand, possibly because of different soil organic matter content, pH, CEC, clay content. The Cd concentrations in the leaves of all treatments (FW basis) grown in Haplaquept were generally over CCFAC's allowable maximum level (0.2 mg kg^{-1} FW) (Figure 21). There was also no significant difference in Cd uptake by spinach dry leaves. However, the higher Cd uptake was occurred in calcium superphosphate fertilizer applied treatment in Haplaquept and in fused magnesium phosphate fertilizer applied treatment in Melanudand. The higher uptake of Cd in spinach was also found in Haplaquept than Melanudand because of different Cd concentration in original soil (Fig 22).

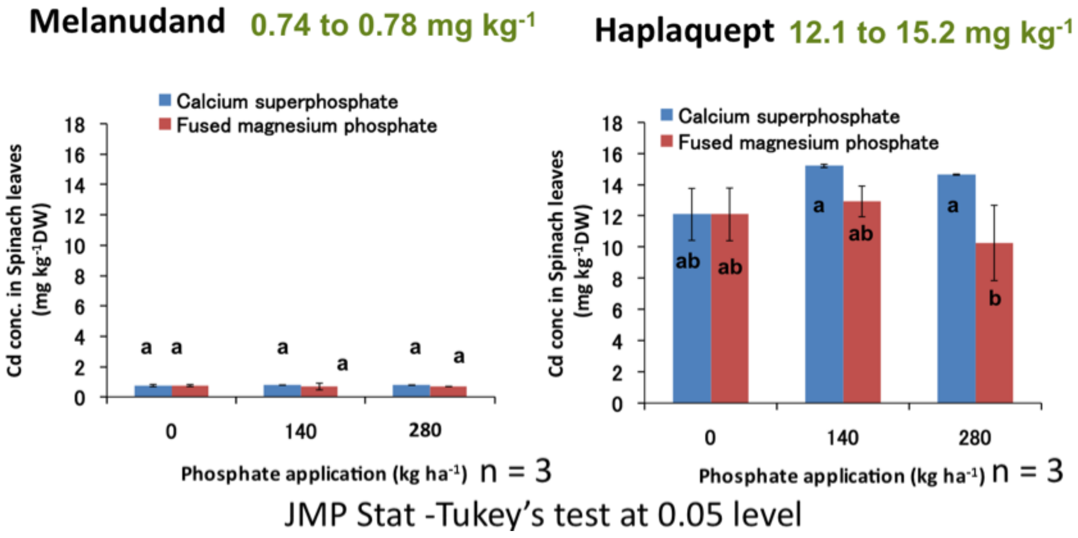


Figure 20. Effect of different rates of calcium superphosphate and fused magnesium phosphate on Cd concentration in dry spinach leaves

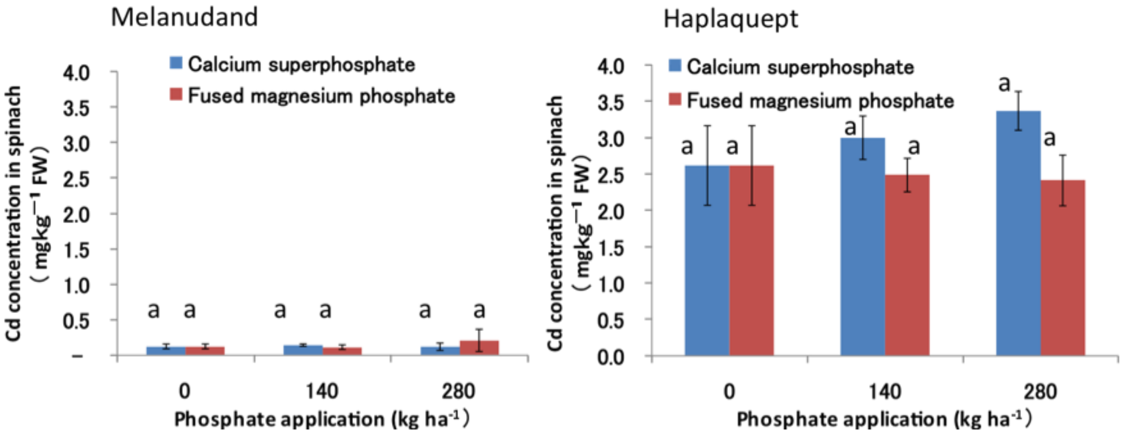


Figure 21. Effect of different rates of calcium superphosphate and fused magnesium phosphate on Cd concentration in fresh spinach leaves

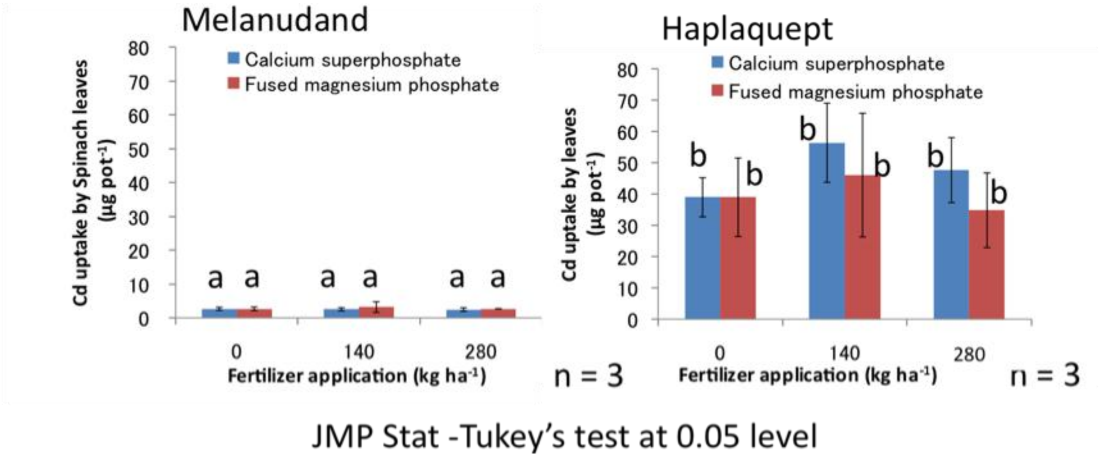


Figure 22. Effect of different rates of calcium superphosphate and fused magnesium phosphate on Cd uptake by spinach dry leaves

6.5 Discussion

6.5.1 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on Cd concentration in soil

Gray et al (2001) calculated that between 40 and 56% of Cd was non labile from soils containing Cd from phosphate fertilizer addition. Clearly, the proportion of non-labile Cd in soils varies depending upon the source of the

Cd, the soils physical and chemical characteristics and the length of time the Cd has been in contact with the soil. The phosphate fertilizer had substantially more Fe, Al and Mn oxides, which are soil components shown to be involved in Cd fixation (Gray et al., 1998). The amount of Cd added to the soils through different fertilizers in this study ranged from 3.3 to 6.6 g ha^{-1} from calcium superphosphate and 0.028 to 0.056 g ha^{-1} from fused magnesium phosphate application. Impurities in phosphate fertilizer increase total levels of Cd in soil. Hansen and Tjell (1982) reported that in Denmark, the use of phosphate fertilizers and additions through atmospheric precipitation are responsible for the largest inputs of Cd to soils. Cadmium in easily soluble fertilizers may increase easily soluble and plant available fractions in soils more than the total amounts, and low annual accumulations of Cd may finally build up undesired concentrations in the cultivated layer, especially where high-Cd fertilizers are applied.

6.5.2 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on the yield of dry spinach leaves

Melanudand was higher organice matter content. Yield of spinach grown in Melanudand was higher than that grown in Haplaquept. Phosphate application did not increase signicantly yield of Komatsuna.

6.5.3 Effect of different rates of calcium superphosphate and fused magnesium phosphate application on Cd concentration and uptake by spinach dry leaves

The Cd concentrations in spinach were in all cases much higher when it was grown in Haplaquept than when grown in Melanudand (Fig 20). This suggests that Cd uptake by plants was to a great extent dependent on the Cd content of the soils. Similar results have been reported by others (Lund et al.

1981) found that In a pot experiment with seven soils naturally high in Cd, the Cd concentration in Swisschard, radish and pepper increased as its concentration in the soils increased. Fertilizer management can impact on Cd concentration in crops. In long-term field studies, Andersson and Siman (1991) observed that Cd concentrations in seed consistently increased with increasing phosphate application, when the fertilizer contained 70 to 150 mg Cd kg^{-1} phosphate. They attributed the increase to the Cd contained in the phosphate fertilizer. Cadmium concentration of the grain increased as grain yield increased, indicating a large effect on total Cd accumulation in the grain. Plant species differ in their ability to absorb heavy metals from soils. Results of He and Singh (1994) showed that Cd concentrations in plants, at any level of Cd addition, differed considerably and were in the order spinach > carrots > ryegrass > oats. Whether the increase in soil Cd will occur as a result of application of Cd-containing fertilizers depends upon the balance between the amount of Cd added through fertilizations and the amount of Cd removed by harvested crops and by leaching. In the present study, although each pot of the soil received less amount of Cd from fused magnesium phosphate fertilizer application on volume basis than that from calcium superphosphate fertilizer application pot, the harvested crops removed much more Cd from the Haplaquept than from Melanudand. This was due to the higher plant yields in Melanudand as well as the higher Cd concentrations in plants grown in Haplaquept than grown in Melanudand. The amount of Cd added to the soils by fertilization in most cases was much greater than its total removal by the harvested crops. In accordance with the observations by Singh (1991), the calculation in this study also showed a net accumulation of Cd in soils. However, this net a ccumulation of Cd in Melanudand was more pronounced than that in Haplaquept because of the lower Cd recovery by the harvested crops in the Melanudand than in Haplaquept. Mortvedt (1982) suggested that the chemical form of Cd contained in water- soluble P fertilizers is $Cd(H_2PO_4)_2$, $CdHPO_4$, or a mixture of these salts, which would be predicted to form during the manufacture of these phosphate fertilizers. Thus, it could be assumed that Cd would not react further with phosphate in soil solution from the applied P fertilizers. The plant Cd uptake was not significantly difference by phosphate fertilizer treatments but there was little difference in the effect of higher rates of phosphate fertilizer application treatment (Fig 22).

The increase in Cd concentration in the spinach in the current study could be due to the Cd applied as a contaminant in the phosphate fertilizer. Rate of application of phosphate fertilizer had little effect on either dry leaves yield spinach or Cd concentration in the spinach leaves. The introduction of phosphate fertilizers with lower Cd concentrations is important if the rate of Cd accumulation in soils is to be reduced. In soils, most trace elements are not readily soluble and remain in solid phases. They may be adsorbed by clays and organic matter, may be associated with primary minerals, and may form inorganic precipitates. The behavior of trace elements in the soil–water–plant system is dependent on their chemical forms.

A tendency of slightly higher Cd concentration in plants and greater Cd removal by spinach in the pots receiving higher doses of phosphate fertilizer was seen (Fig 22). The Cd concentrations in Spinach were in all cases much higher when it was grown in Haplaquept than when grown in Melanudand. The total uptake of Cd by Spinach was calculated by multiplying the Cd concentration by dry matter yields for each pot. The effects of different levels and kinds of phosphate fertilizer on the Cd uptake were similar to those reported for plant Cd concentration. The uptake of spinach leaves increased up to the application 140 kg ha^{-1}. Then there was a sharp decline with further addition of both of fertilizers, owing to the decrease in dry weight of Spinach leaves. Application of phosphate decreased Cd uptake by 8% in Andisols and by 10% in Inceptisol at 280 kg ha^{-1} applied treatment.

CHAPTER 7. THE INFLUENCE OF PHOSPHATE FERTILIZER APPLICATION LEVELS AND CULTIVARS ON CADMIUM UPTAKE BY KOMATSUNA (*Brassica rapa* var. *perviridis*)

7.1 Abstract

Cadmium (Cd) is a common impurity in phosphate fertilizers and application of phosphate fertilizer may contribute to soil Cd accumulation. Changes in Cd burdens to the agricultural soils and the potential for plant Cd accumulation resulting from fertilizer input was investigated in this study. A field experiment was conducted on Haplaquept to investigate the influence of calcium superphosphate on extractable and total soil Cd and on growth and Cd uptake of different Komatsuna (*Brassica rapa* var. *perviridis*) cultivars. Four cultivars of Komatsuna were grown on the soils and harvested after 60 days. The superphosphate application increased total soil Cd from 2.51 to 2.75 mg kg$^{–1}$, 0.1 0.1 mol L^{-1} HCl extractable Cd from 1.48 to 1.55 mg kg$^{–1}$, 0.01 mol L^{-1} HCl HCl extractable Cd from 0.043 to 0.046 mg kg$^{–1}$ and water extractable Cd from 0.0057 to 0.0077 mg kg$^{–1}$. Cd input reached 5.68 g ha$^{–1}$ at a rate of 240 kg ha$^{–1}$ superphosphate fertilizer application. Superphosphate affected dry-matter yield of leaves to different degrees in each cultivar. 'Nakamachi' produced the highest yield in 2008 and 'Hamami No. 2' in 2009. Compared with the control (no phosphate fertilizer), application of superphosphate at a rate of 240 kg ha$^{–1}$ increased the Cd concentration in dry leaves by 0.14 mg kg$^{–1}$ in 'Maruha', 1.03 mg kg$^{–1}$ in 'Nakamachi', 0.63 mg kg$^{–1}$ in 'SC8-007' in 2008, and 0.19 mg kg$^{–1}$ in Maruha', 0.17 mg kg$^{–1}$ in 'Hamami No. 2', while it decreased by 0.27 mg kg$^{–1}$ in 'Nakamachi' in 2009. Field experiments in two years demonstrated that applications of different levels of calcium superphosphate did not influence Cd concentration in soil and Komatsuna significantly. However, there was significant difference in Cd concentration of fresh and dry Komatsuna leaves among four cultivars in 2008 and 2009. The highest Cd concentration was found in Nakamachi cultivar

(2.14 mg kg^{-1} in 2008 and 1.91 mg kg^{-1} in 2009). The lowest Cd concentration was observed in Maruha cultivar (1.51 mg kg^{-1} DW) in 2008 and in Hamami No.2 cultivar (1.56 mg kg^{-1} DW) in 2009. A decreasing trend of the Cd concentration was found in Nakamachi followed by SC8-007, Hamami No. 2 and Maruha successively. It is necessary to consider a low uptake cultivar for growing in a Cd polluted soil. In these two years results, Maruha cultivar was the low Cd uptake cultivar compared to the others.

7.2 Introduction

Komatsuna (*Brassica rapa* var. *perviridis*) is a popular leafy vegetable grown in Japan. The name "Komatsuna" originated from its place of origin, Komatsugawa, in the Edogawa ward of Tokyo (Kamei 1985). In Japan, the second-highest production of Komatsuna comes from the Tokyo region (MAFF 2009), Fuchu city in Tokyo with an alluvial plain is one of the major production regions of Komatsuna. Currently, 95 cultivars of Komatsuna are registered in Japan according to Japan Seed Trade Association (personal communication). The most popular cultivars in the Tokyo region are 'Nakamachi', 'SC8-007', 'Maruha', and 'Hamami No. 2'.

Cd, a potentially hazardous heavy metal, is present at varying levels in soils and water used for crop production. Cd poisoning has occurred worldwide, and caused more than 100 deaths in Japan from 1922 to 1965 (Yeung and Hsu 2005). Therefore, it is desirable to reduce the Cd concentration in the human diet. In April 2006, the maximum allowable level in leafy vegetables was lowered to 0.2 mg kg^{-1} fresh weight (FW) by the Codex Committee on Food Additives and Contaminants (CCFAC) of the FAO and WHO. The rice (*Oryza sativa* L.) was the main contributor to Japanese people (37.2 %) of dietary exposure to cadmium, followed by the "vegetable and seaweed" (16.6 %), "sea food" (16.1%), "potatoes and cereals" (12.9%), and "others" (17.2 %) (Committee for food security, 2009).

Cd is present at varying levels as a contaminant in phosphate fertilizers. One environmental source of Cd is the phosphate ore used in the production of these fertilizers. The main source of this ore is phosphate rock of sedimentary origin. Soluble Cd becomes a major problem because high levels of Cd in many agricultural fields have resulted from long-term use of phosphate fertilizers, sewage sludge applications, and irrigation with wastewater (Sharma and Agrawal 2005; Irtelli and Navari-Izzo 2006). There are concerns about whether this will cause an accumulation of metals to dangerous levels, thereby increasing the environmental and human health risks. Cd input into agricultural soils through phosphate fertilizers varies with the fertilizer's Cd content, application rate, and application frequency. Plant response to the Cd in phosphate fertilizer therefore appears to depend on the plant genotype, the Cd concentration in the fertilizer, the rate and method of fertilizer application, the soil type, and climatic conditions (Jackson and Alloway, 1991; Tiller et al., 1997). Cd uptake is a dynamic process. As a plant takes up Cd, the equilibrium Cd concentration in the soil solution is maintained by the input of Cd from particulate-bound species. Thus, the Cd concentration in the soil solution is also controlled by the nature and amount of particulate-bound Cd species in the soil.

Vegetables contribute to essential diet components through vitamins, iron, calcium and other nutrients. Unfortunately, vegetables can contain undesirable compounds such as heavy metals. There is a growing concern to potential risk of Cd. Among vegetable crops, leafy vegetables are most vulnerable to heavy metal pollution (Mapanda et al. 2007; Wang et al. 2007). Phosphate fertilizer is considered to be the main source of Cd in agricultural soil because it contains Cd as a contaminant (Kongshaug et al. 1992, Williams and David 1973). As far as we could determine, there are no papers available which evaluate the effects of different levels of P fertilizer on Cd concentration in Komatsuna. Cultivar differences in the concentration of Cd have been well documented in many crop species, including rice (*Oryza* *sativa* L.) (liu et al. 2003; Arao et al. 2003), wheat (*Triticum aestivum* L.) (Zhang et al. 2000, 2002), barley (*Hordeum vulgare* L.) (Chen et al. 2007), maize (*Zea mays* L.) (Kurz 1999) and peanut (*Arachis hypogaea* L.) (McLaughlin 2000). However, limited information is available on Cd concentration of Komatsuna among different cultivars. The objectives of this study are to examine the effects of different levels of calcium superphosphate on Cd uptake by four cultivars of Komatsuna, and thus to evaluate the Cd uptake of main Komatsuna cultivars planted in Tokyo area.

7.3 Materials and Methods

7.3.1 Study site

The study field was established in 2008 and 2009 at the Hommachi Farm, Field Science Center for Education and Research, Tokyo University of Agriculture and Technology (35º39’N, 139º28’E). Four levels of calcium superphosphate fertilizer application {0, 60, 120, and 240 kg phosphorus pentaoxide (P_2O_5) ha^{-1}} were applied based on the recommended application level for the Tokyo region (60 kg P_2O_5 ha^{-1}). These treatments were applied by hand in a randomized block design with three replicates. The concentrations of total, 0.1 mol L^{-1} HCl extractable, 0.01 mol L^{-1} HCl extractable, water extractable Cd and P_2O_5 in the fertilizer were 4.01, 1.49, 0.93, 0.79 mg kg^{-1} and 17% respectively, which resulted in a Cd input of 1.42, 2.84, and 5.68 g ha^{-1} from the 60,120, and 240 kg P_2O_5 ha^{-1} applications. Nitrogen (N) and potassium (K) fertilizers were applied by hand to all treatments at the recommended rates for the Tokyo region to prevent N and K from limiting growth: Nitrogen at 70 kg N ha^{-1} as ammounium suphate $(NH_4)_2SO_4$ (21% N and 0.01 mg Cd kg^{-1}) and K at 50 kg K_2O ha^{-1} as potassium chloride (KCl) (60% K_2O and 0.04 mg Cd kg^{-1}). We used four Komatsuna cultivars that are commonly grown in the study area: 'Maruha', 'Nakamachi', and 'SC8-007' in 2008 and 'Maruha', 'Nakamachi', and 'Hamami

No. 2' (because of shortage of SC8-007) in 2009. Each of the 12 plots was 1 m × 1 m, with five rows per plot at a spacing of 20 cm × 10 cm between plants, for a total of 50 plants per plot. The two border rows were not used for sampling and analysis to avoid edge effects. Only one cultivar was grown in each row of each plot. The soil is classified as Haplaquept with a clay loam texture (International Soil Science Society). Komatsuna was grown from 5 July to 15 August in 2008 and from 9 November to 5 February in 2010.

7.3.2 Soil sampling and analysis

The procedure was same as above 4.3.1.

7.3.3 Plant sampling and analysis

Komatsuna cultivars were harvested at two months after planting from each plot and fresh weights were determined. Five plants in the middle of three rows of each cultivar in each treatment were harvested. The procedure was same as above 4.3.2.

7.3.4 Statistical analysis

Analysis of variance was performed using the JMP 8.01 software (SAS Institute 2008). Significant differences between treatment and cultivar means were identified using Tukey's (HSD) test ($P < 0.05$); significant differences between fertilizer application means were identified using Dunnett's test ($P < 0.05$).

7.4 Results

7.4.1 Effect of phosphate fertilizer application on Cd concentration in soil

The properties of the soil samples are presented in (Table 15). The amounts of total soil Cd increased by 0.05 mg kg^{-1} and by 0.18 mg kg^{-1} at the highest level of phosphate application in 2008 and 2009 (Table 16). The amounts of total soil Cd increased slightly whether there was a significant correlation between fertilizer rates and soil Cd contents with increasing fertilizer rate because of the low input of Cd in the phosphate fertilizer. There was no significant difference on amounts of 0.1 mol L^{-1} HCl acid-extractable soil Cd increased by 0.05 mg kg^{-1} and 0.02 mg kg^{-1} at the highest level of phosphate application in 2008 and 2009 (Table 16). The amounts of 0.01 mol L^{-1} HCl acid-extractable soil Cd also increased slightly with increasing fertilizer rate in both years, but the difference was not significant. There was increasing trend in water extractable Cd in soil from 0.0057 to 0.0077 mg kg^{-1} at 240 kg ha^{-1} calcium superphosphate phosphate fertilizer application compared to control. There was no significant difference in water extractable and 0.01 mol L^{-1} HCl extractable Cd in soil for both of years among the different levels of phosphate fertilizers inspite of increase of soil pH (Table 16). The amounts of Cd added to the soil in the fertilizer application ranged from 1.42 to 5.68 g ha^{-1} (0.0014 to 0.0056 mg kg^{-1}).

7.4.2 Effect of phosphate fertilizer application on the yield of fresh and dry Komatsuna leaves

In 2008, the yield of fresh and dry Komatsuna leaves increased with increasing phosphate application in 'Maruha' (Table 17). However, a reduction in leaf yield occurred in 'Nakamachi' and 'SC8-007' at the highest application rate. The plant height of Komatsuna was in the range of 29 to 41 cm. The numbers of leaves were 9 to 12 leaves. However, there was no significant difference among the treatments.

In 2009, leaf dry weight increased continuously with increasing application rate up to an application rate of 120 kg ha^{-1}. However, leaf dry weight in Maruha decreased at 120 kg application and Hamami showed no changes in 2009 (Table 17). There was no significant difference in the yield of fresh and dry Komatsuna leaves, even with the excess amount of phosphate fertilizer application in the short term. The plant height of Komatsuna in 2009 was in the range of 12 to 15 cm. The numbers of leaves were 9 to 13 leaves. However, there was no significant difference among the treatments.

7.4.3 Effect of cultivars on yield of fresh and dry Komatsuna leaves

There was a significant difference in the plant height, yield of fresh and dry Komatsuna leaves among the cultivars in 2008, however there was no significant difference in 2009. The highest yield was observed in Nakamachi cultivar in 2008 while Hamami No.2 in 2009 (Table 18). The lowest yield was observed in SC8-007 cultivar in 2008 and in Maruha cultivar in 2009 (Table 18). The decreasing trend on Komatsuna yield among cultivars was Nakamachi followed by Maruha and SC8-007 successively in 2008. However the decreasing trend of Komatsuna yield in the year 2009 was found as Hamami No. 2 followed by Nakamachi, which in turn followed by the variety Maruha. The highest plant height was observed in Maruha cultivar in 2008. There was no significant difference in the plant height among cultivars in 2009. The number of leaves was also not different among the cultivars in both of years.

7.4.4 Effect of phosphate fertilizer application on Cd concentration in fresh and dry Komatsuna leaves

The effects of phosphate fertilizer levels on the Cd concentration in fresh and dry leaves of Komatsuna cultivars are shown in Table 17. The Cd concentration of Komatsuna was not significantly different even with the excess amount of phosphate-fertilizer application. There was a significant increase in the mean Cd concentration in the leaves with increasing phosphate application in 2008 but not in 2009. Across all Komatsuna cultivars, the Cd content increased significantly from 1.44 mg kg^{-1} to 2.04 mg kg^{-1} in 2008 while 1.70 to only 1.73 mg kg^{-1} in 2009 (Table 18). On the average, the Cd uptake by Komatsuna amounted to 0.28%, 0.40%, 0.42%, and 0.44% of the total soil Cd in 2008 and to 0.29%, 0.32%, 0.35%, and 0.26% of the total soil Cd in 2009 for fertilizer rates of 0, 60, 120, and 240 kg P_2O_5 ha^{-1}, respectively. However, the Cd concentrations in the leaves of all cultivars (FW basis) were generally below CCFAC's allowable maximum level (0.2 mg kg^{-1} FW) except for 'Nakamachi' and 'SC8-007' in 2008.

7.4.5 Effect of cultivars on Cd concentration in fresh and dry Komatsuna leaves

There was significant difference in Cd concentration of fresh and dry Komatsuna leaves among four cultivars in 2008 and 2009. The highest Cd concentration was found in Nakamachi cultivar (2.14 mg kg^{-1} DW and 1.91 mg kg^{-1} DW) in 2008 and 2009. The lowest Cd concentration was observed in Maruha cultivar (1.51 mg kg^{-1} DW) in 2008 and in Hamami No.2 cultivar (1.56 mg kg^{-1} DW) in 2009. The decreasing trend on Cd concentration among four cultivars was Nakamachi followed by SC8-007 and Maruha in 2008 and by Maruha and then Hamami No. 2 serially in 2009.

7.5 Discussion

7.5.1 Effect of phosphate fertilizer application on Cd concentration in soil

In accordance with the observations of Singh (1990) the application of a Cd- containing NPK fertilizer (40 mg Cd kg $^{-1}$) at the rate of 30 mg P kg $^{-l}$ soil did not increase Cd concentrations in oat (*Avena sativa* L.) or rape (*Brassica napus* L.), but application of the same fertilizer at a higher rate (90 mg P kg $^{-1}$) increased the Cd concentrations in both crops, and, Singh (1991) observed a net accumulation of Cd in soils. Repeated applications of P fertilizers increased the soil solution concentration of both Cd and Zn in field and laboratory experiments (Lambert et al. 2007). The current study confirmed an earlier finding that long-term use of phosphate fertilizer that contains high level of Cd may lead to accumulation of Cd in the soil, and this may eventually increase plant Cd uptake (Mortvedt et al. 1981; Jones and Johnston 1989; Ervio et al. 1990; He and Singh 1993), since this study showed that the increase in total, 0.1 mol L^{-1} HCL, 0.01 mol L^{-1} HCl and water extractable Cd concentration in soil was observed after application of phosphate fertilizer (Table 16). There was no significant difference in total, 0.1 mol L^{-1} HCl extractable, 0.01 mol L^{-1} HCl extractable, water extractable Cd in soil among the treatments. However, there was increasing trend in water extractable Cd in soil with fertilizer application treatment than control.

In many long-term field studies, an increase in soil Cd as a result of phosphate fertilizer application was associated with increased plant Cd concentrations, indicating that accumulation of Cd in the soil led to higher phytoavailability of Cd (Williams and David 1976; Mulla et al. 1980; Jones et al. 1987; Jones and Johnston 1989; Kashem and Singh 2002). One of the most important parameters is probably pH, since this influences the Cd transformation process. Increased soil pH also increases the adsorption of Cd by soils and thus reduces its extractability (Kuo et al. 1985; King 1988;

Guttormsen et al. 1995). Since the soil in the present study had a pH of 5.5 in 2008, the phytoavailability of the Cd may be high. This Cd availability differed in 2009 because of an increase in pH of approximately 0.6 unit compared with the pH in 2008.

7.5.2 Effect of phosphate fertilizer application on the yield of fresh and dry Komatsuna leaves

In our finding, phosphate application typically increased yield of fresh and dry weight of Komatsuna leaves. The effect of phosphate application on yield of Komatsuna was rather species-specific. Higher level of application caused a large biomass reduction of Komatsuna. There was no relationship between calcium superphosphate application and yield of fresh and dry Komatsuna. The height of Komatsuna was higher in 2008 than in 2009 because of the effect of cold weather.

7.5.3 Effect of cultivars on yield of Komatsuna leaves

As shown in Table 18, Komatsuna leaves yield differed significantly among cultivars in 2008. The maximum yield of Komatsuna leaves was obtained in Nakamachi cultivar. In 2009, Hamami No.2 cultivars showed the highest yield of Komatsuna leaves. Hamami No.2 and Nakamachi cultivars had better growth than other two cultivars in both of year.

7.5.4 Effect of phosphate fertilizer application on Cd concentration in fresh and dry Komatsuna leaves

The application of Cd-containing NPK fertilizers, and especially P fertilizers, increased the Cd concentration in all crops studied by He and Singh (1994a), and the highest Cd concentration in these crops occurred when high-Cd fertilizer was used (Kashem and Singh 2002). The Cd concentration in durum wheat (*Triticum durum* L.) at several locations across the Canadian prairies increased in the year of application of P fertilizer, but the increase was unrelated to the Cd concentration in the fertilizer (Grant et al. 2002). Increasing Cd concentration in grain crops with increasing Cd content in the commercial fertilizers applied has also been reported (Andersson and Hahlin 1981; He and Singh 1994). The Cd concentration in Komatsuna of this study increased with application of phosphate fertilizer (Table 18). Jaakola (1979) reported increased Cd uptake by vegetable crops with increasing application rates of Cd-containing mineral fertilizer. Both the input and plant uptake of Cd tend to be small relative to the pool of Cd present in the soil (Christensen and Huang 1999). In this study, water extractable Cd in calcium superphosphate fertilizer was 0.79 mg kg^{-1}. Cd in superphosphate is water-soluble and is associated with both the phosphate and sulfate contents of the fertilizer, since Williams and David (1973, 1976) reported that Cd in superphosphate appeared to be as readily available to plants as Cd^{2+} in cadmium chloride ($CdCl_2$).

Growth chamber studies also showed that the application of phosphate fertilizer containing Cd increased the Cd concentration in a range of crops (He and Singh 1995; Grant et al. 1999; Jiao et al. 2004). Our finding demonstrated that the increase of Cd concentration has not been observed in Komatsuna when phosphate fertilizer was applied (Table 18). It appears that Komatsuna cultivars took up Cd mostly from the fertilizers because Cd in fertilizer is more available to plants than Cd in soil which is more likely to be bound to various complexes in non-exchangeable form (Xie and Mackenzic, 1988).

Our analyses showed non-significant increases in Cd concentrations in plant tissues. Similar results have been reported by Isermann (1982); Smilde and van Luit (1983); Williams and David (1976). Our results are in line with other studies that the Cd concentration in P fertilizers also did not influence Cd concentration of potato (*Solanum tuberosum* L.) in the yearof application under field conditions (Sparrow et al. 1993; Mclaughlin et al. 1995). The reduction in the availability of Cd from triple superphosphate to lettuce

(*Lactuca sativa* L.) could be related to high P availability, which decreases Cd uptake by plants (Street et al. 1978) either by interfering with Cd translocation from the roots to aboveground plant parts (Williams and David 1976) or by promoting the capability of the soil or its constituents to adsorb Cd (Bolan and Duraisamy 2003; McGowen et al. 2001). The application of phosphate fertilizer did not affect Cd uptake by Komatsuna in a previous study (Hattori et al. 2002). Plant uptake of both native and exogenous Cd generally decreased with increasing soil pH (Tables 16 and 17) and which varied widely (the range of variation shown in Table 16).

Komatsuna has a high ability to take up Cd during the 2 to 3 weeks after sowing (Takahashi et al. 2009). Many reports have found no significant increase in Cd concentrations in plants due to the application of phosphate fertilizers, even after long-term use of such fertilizers (Jaakkola 1977; Smilde and van Luit 1983; Mortvedt 1984, 1987). This may be because the phytoavailability of Cd added to soils through fertilizer application depends on the soil properties, the form of the Cd in the fertilizer, and the ability of the plants to absorb Cd from the soil. The concentration of Cd in Komatsuna that was grown in soil (1.6 mg kg^{-1} of acid-extractable Cd and soil pH 5.6) was 0.20 mg kg^{-1} (Kikuchi et al. 2005), which is comparable to our results and equal to the maximum allowable level for leafy vegetables.

In 2008, the Cd concentration in fresh leaves exceeded this maximum level in 'Nakamachi' and 'SC8-007', possibly due to high water absorption by Komatsuna during the summer (Table 18).

7.5.5 Effect of cultivars on Cd concentration in fresh and dry Komatsuna leaves

In the present study, Cd concentration in Komatsuna leaves differed significantly among cultivars in 2008 and 2009. The highest Cd concentration in Komatsuna leaves was obtained in Nakamachi cultivar in both years. The lowest Cd concentration in Komatsuna leaves was obtained in Maruha cultivar in 2008 and Hamami No.2 cultivar in 2009. Maruha and Hamami No.2 were considered better cultivars to grow in Cd contaminated soil because of lower Cd accumulation than the other two cultivars in both years.

7.6 Conclusions

The following can be concluded based on the water, 0.01 mol L^{-1} HCl, 0.1 mol L^{-1} HCl extractable and total soil Cd concentrations and the Cd content in Komatsuna leaves. The application of different fertilizer levels, up to four times the standard level, resulted in no significant increases in Cd concentration in soil and plant in the two years.

The Komatsuna cultivars differed significantly in their Cd uptake. The total Cd uptake decreased in the order of 'Nakamachi' > 'SC8-007' > 'Maruha' = 'Hamami No. 2'. 'Nakamachi' showed the highest Cd uptake in both years. It is necessary to consider a low uptake cultivar for growing in a Cd polluted soil. In these results, from both years, Maruha cultivar was the lowest Cd uptake cultivar compared to the others.

Plate 10. Komatsuna in field at two months after sowing in field

Table 15. Some phyical and chemical properties of soil

Parameter	**Value**
pH (1:2.5 in water)	5.5
Total N (g kg^{-1})	3.6
Total C (g kg^{-1})	37.0
Exchangeable NH_4-N (mg kg^{-1})	57.0
Available P_2O_5 (Bray No. 2 P_2O_5; mg kg^{-1})	52.0
Cation exchange capacity ($cmol^+$ kg^{-1})	19.8
Exchangeable cations ($cmol^+$ kg^{-1})	
K	0.42
Ca	9.4
Mg	4.3
Na	0.037
Particle size distribution (%)	
Clay	16.0
Silt	33.0
Sand	51.0
Total Cd (mg kg^{-1})	2.5
0.1 mol L^{-1} HCl–extractable Cd (mg kg^{-1})	1.5
Soil classification	Haplaquept

Table 16. Effects of superphosphate fertilizer application on soil pH, extractable and total Cd contents in 2008 and 2009

Superphosphate application (kg ha^{-1})	Soil pH	Cd concentration in soil (mg kg^{-1})			
		water extractable	0.01 mol L^{-1}–HCl extractable	0.1 mol L^{-1}–HCl extractable	Total
2008 (5 July to 15 August)					
0 (Control)	5.43 ± 0.20	0.0057 ± 0.0020	0.043 ± 0.0003	1.48 ± 0.02	2.51 ± 0.14
60	5.50 ± 0.30	0.0065 ± 0.0023	0.040 ± 0.0003	1.48 ± 0.01	2.55 ± 0.18
120	5.42 ± 0.14	0.0065 ± 0.0014	0.045 ± 0.005	1.51 ± 0.02	2.47 ± 0.11
240	5.43 ± 0.30	0.0069 ± 0.0045	0.044 ± 0.001	1.53 ± 0.07	2.56 ± 0.11
2009 (9 November to 9 February)					
0 (Control)	6.11 ± 0.06	0.0057 ± 0.0015	0.045 ± 0.011	1.53 ± 0.09	2.57 ± 0.12
60	6.19 ± 0.22	0.0059 ± 0.0076	0.044 ± 0.001	1.48 ± 0.03	2.59 ± 0.15
120	6.09 ± 0.13	0.0057 ± 0.0007	0.046 ± 0.005	1.53 ± 0.07	2.56 ± 0.09
240	6.18 ± 0.05	0.0077 ± 0.0021	0.044 ± 0.002	1.55 ± 0.06	2.75 ± 0.12
Significance level of phosphate treatment (Prob > *F*)					
2008	ns	ns	ns	ns	ns
2009	ns	ns	ns	ns	ns

Notes: Data are expressed as mean ± standard deviation of three replicates. “ns” is not significant.

Dunnett’s test ($P < 0.05$) for comparison of the control with the three levels of application.

Table 17. Effect of superphosphate application on leaf yield and the concentration of Cd in komatsuna leaves in 2008 and 2009

Cultivar	**Phosphate level (kg ha^{-1})**	**Leaf yield (kg m^{-2} DW)**	**Leaf yield (kg m^{-2} FW)**	**Leaf Cd concentration (mg kg^{-1} DW)**	**Leaf Cd concentration (mg kg^{-1} FW)**
2008 (5 July to 15 August)					
'Maruha'	0	0.13 ± 0.03	1.16 ±0.10	1.45 ± 0.04	0.16 ± 0.02
	60	0.11 ± 0.02	0.95 ± 0.21	1.44 ± 0.46	0.17 ± 0.05
	120	0.19 ± 0.04	1.62 ± 0.46	1.58 ± 0.20	0.19 ± 0.02
	240	0.23 ± 0.02	1.92 ± 0.19	1.59 ± 0.23	0.18 ± 0.02
'Nakamachi'	0	0.21 ± 0.05	1.73 ± 0.33	1.42 ± 0.44	0.17 ± 0.05
	60	0.21 ± 0.02	1.80 ± 0.34	2.45 ± 0.09	0.28 ± 0.03
	120	0.17 ± 0.01	1.38 ± 0.23	2.26 ± 0.59	0.27 ± 0.04
	240	0.17 ± 0.03	1.28 ± 0.31	2.45 ± 0.13	0.32 ± 0.03
'SC8-007'	0	0.14 ± 0.03	1.14 ± 0.37	1.46 ± 0.25	0.18 ± 0.04
	60	0.17 ± 0.03	1.25 ± 0.04	1.84 ± 0.65	0.25 ± 0.08
	120	0.14 ± 0.01	1.04 ± 0.09	2.07 ± 0.59	0.27 ± 0.06
	240	0.14 ± 0.02	1.09 ± 0.19	2.09 ± 0.66	0.27 ± 0.10
2009 (9 November to 9 February)					
'Maruha'	0	0.14 ± 0.03	1.40 ± 0.50	1.64 ± 0.36	0.14 ± 0.03
	60	0.18 ± 0.03	1.25 ± 0.75	1.32 ± 0.18	0.12 ± 0.02
	120	0.14 ± 0.03	1.49 ± 0.18	1.82 ± 0.18	0.16 ± 0.03
	240	0.11 ± 0.02	1.46 ± 0.11	1.83 ± 0.24	0.17 ± 0.02
'Nakamachi'	0	0.13 ± 0.01	1.60 ± 0.93	1.93 ± 0.08	0.14 ± 0.02
	60	0.16 ± 0.04	2.47 ± 0.89	1.94 ± 0.20	0.14 ± 0.02
	120	0.18 ±0.001	1.91 ± 0.26	2.10 ± 0.23	0.16 ± 0.01
	240	0.13 ± 0.03	1.34 ± 0.71	1.66 ± 0.50	0.14 ± 0.04
'Hamami No. 2'	0	0.13 ± 0.04	1.52 ± 0.80	1.53 ± 0.16	0.12 ± 0.03
	60	0.14 ± 0.05	2.04 ± 0.75	1.47 ± 0.13	0.12 ± 0.02
	120	0.14 ± 0.03	2.00 ± 0.01	1.54 ± 0.06	0.13 ± 0.02
	240	0.14 ± 0.02	1.18 ± 0.62	1.70 ± 0.20	0.17 ± 0.03

Significance level of treatment (Prob > *F*)				
Cultivar (2008)	*	*	**	**
Phosphate level (2008)	ns	ns	ns	*
Cultivar × phosphate level (2008)	**	**	*	**
Cultivar (2009)	ns	ns	**	ns
Phosphate level (2009)	ns	ns	ns	ns
Cultivar × phosphate level (2009)	ns	ns	*	ns

Notes. Data are expressed as mean ± standard deviation of three replicates.

ns is not significant, *Significant at $P < 0.05$, **Significant at $P < 0.01$.

Significance for cultivar differences is based on Tukey's (HSD) test.

Significance for phosphate application is based on Dunnett's test.

Significance for cultivar × level of application is based on Tukey's (HSD) test.

DW, dry weight; FW, fresh weight.

Table 18. Mean yields and concentrations of Cd in Komatsuna leaves as function of cultivar and level of phosphate fertilizer application in 2008 and 2009

Cultivar	Yield ($kg\ m^{-2}$ DW)	Yield ($kg\ m^{-2}$ FW)	Cd concentration ($mg\ kg^{-1}$ DW)	Cd concentration ($mg\ kg^{-1}$ FW)
2008 (5 July to 15 August)				
'Maruha'	0.17 ± 0.05 ab	1.41 ± 0.46 ab	1.51 ± 0.24 b	0.18 ± 0.03 b
'Nakamachi'	0.19 ± 0.03 a	1.55 ± 0.35 a	2.14 ± 0.55 a	0.26 ± 0.07 a
'SC8-007'	0.15 ± 0.03 b	1.13 ± 0.20 b	1.87 ± 0.55 ab	0.24 ± 0.07 a
Phosphate ($kg\ ha^{-1}$)				
0 (Control)	0.16 ± 0.05 a	1.34 ± 0.39 a	1.44 ± 0.25 a	0.17 ± 0.02 a
60	0.16 ± 0.05 a	1.34 ± 0.42 a	1.91 ± 0.59 b	0.24 ± 0.02 b
120	0.17 ± 0.03 a	1.35 ± 0.36 a	1.96 ± 0.53 b	0.25 ± 0.02 b
240	0.18 ± 0.04 a	1.43 ± 0.43 a	2.04 ± 0.51 b	0.26 ± 0.02 b
2009 (9 November to 9 February)				
'Maruha'	0.13 ± 0.03 a	1.40 ± 0.62 a	1.66 ± 0.30 ab	0.14 ± 0.02 ab
'Nakamachi'	0.14 ± 0.03 a	1.83 ± 0.77 a	1.91 ± 0.31 a	0.15 ± 0.02 a
'Hamami No. 2'	0.15 ± 0.03 a	1.68 ± 0.66 a	1.56 ± 0.15 b	0.13 ± 0.03 b
Phosphate ($kg\ ha^{-1}$)				
0 (Control)	0.13 ± 0.03 a	1.51± 0.67 a	1.70 ± 0.27 a	0.14 ± 0.02 a
60	0.16 ± 0.04 a	1.92 ± 0.88 a	1.58 ± 0.32 a	0.13 ± 0.02 a
120	0.15 ± 0.03 a	1.80 ± 0.29 a	1.82 ± 0.29 a	0.16 ± 0.03 a
240	0.13 ± 0.03 a	1.32 ± 0.75 a	1.73 ± 0.30 a	0.15 ± 0.02 a

Means within a column for the cultivar and level of phosphate fertilizer application followed by the same letter do not differ significantly (Tukey's (HSD) test for cultivar, Dunnett's test for phosphate application, both $P < 0.05$). DW, dry weight; FW, fresh weight.

CHAPTER 8. DISCUSSION AND CONCLUSION

8.1 Discussion

Sedimentary rock contains high conc. of Cd (Hurst, 1989). Carbonate fluoapatite (CFA) is the principle mineral of sedimentary phosphate rock (Matthews and Nathan 1997). Prominent mineral of CFA and high Cd conc. were found in PR3, PR4, PF2 and PF5, suggesting that Cd might be exchanged to Ca and or occluded during its formation in CFA by sedimentation. Cadmium from phosphate fertilizers poses a potentially serious threat to soil quality and, through the food chain, to human health. The results of this study suggest that the response of Komatsuna and spinach depended more on original Cd content of soil than on the amount of Cd added in the different phosphate fertilizers. It is well know that, unlike Cd from industrial wastes and biosolids that generally contain very high Cd content and are applied at very high rates (Mg ha^{-1}), Cd accumulation in soil and Cd uptake from annually applied phosphate fertilizers at normal rate (kg ha^{-1}) could be an environmental issue only after long-term application of phosphate fertilizers (Loganthan et al., 1995). Short-term phosphate application should not result in a serious Cd accumulation in soils and its subsequent Cd uptake by Komatsuna and spinach, unless phosphate fertilizers contain unusually high Cd content. Since it is difficult to conduct a long-term pot experiment on Cd from annual phosphate application at normal rates, we tried to shorten the time frame by one-time application of high phosphate rates at 120 and 240 kg ha^{-1} for Komatsuna and 280 kg ha^{-1} for spinach. Mortvedt (1982) suggested that the chemical form of Cd contained water soluble phosphate fertilizers is $Cd(H_2PO_4)_2$, $CdHPO_4$, or a mixture of these salts, which would be predicted to form during the manufacture of these P fertilizers. Thus, it could be assumed that Cd would not react further with P in soil solution from the applied phosphate fertilizers. It should be pointed out that potential Cd toxicity to human health from Cd-contaminated food crops is based on Cd concentration in edible parts of crops rather than total Cd uptake by crops. For example, the maximum permissible Cd concentration in leafy vegetable generally adopted by Codex committee is 0.2 mg Cd kg^{-1} fresh weight. A low Cd concentration associated with application of Cd-containing PF7 fertilizer indicate a high total Cd uptake because of higher dry matter yield of Komatsuna. Therefore, to compare plant Cd concentration associated with different phosphate fertilizer treatments, we should also consider their Komatsuna and spinach yields. In the present study, all of the phosphate fertilizer treatments produced about the same crop yield of Komatsuna and spinach, and thus a direct comparison of Cd concentration in Komatsuna and spinach can be made. In other words, Cd uptake and Cd concentration are equally related to Cd availability from the different phosphate fertilizer sources and soils. However, Melanudand produced higher yield of Komatsuna and spinach than Haplaquept. Differences in Cd concentration in the Komatsuna and spinach seem to imply that different types of vegetables have different response to Cd accumulation. In spite of the mechanisms involved in the Cd uptake by roots (non-metabolic or metabolic), plants are know to respond to the amounts of readily mobile type of metals in soils (Liu et al. 2006; Tokalioglu and Kartal 2006). It should be noted that the pH value, as well as the percentage of clay content determines the solubility of Cd in the soil and their availability for uptake by Komatsuna and spinach. Low soil pH may induce Cd to be easily soluble and have ion exchange comparable to high pH. Lower soil pH was not found to be in saiwaicho area where also the clay content was higher in Melanudand (30%) than in Haplaquept (16%). The Komatsuna and spinach grown in Haplaquept appear to have higher concentration of Cd than those cultivated in Melanudand almost neutral respectively. Therefore Komatsuna and spinach grown in Melanudand are safe as they are less contaminated than those grown in Haplaquept. Cd concentration of Komatsuna and spinach showed large variations even at the same treatment and soil. This is probably due to the different plant uptake. Since Cd entered into soils, Komatsuna and and spinach began to absorb Cd through the metabolism of root systems. Once Cd came into the root cells, some of them were stored in the roots and others were transported to adjacent cells by conveying of tracheas along with the transpiration transferred upward and accumulated in leaves. The results of Komatsuna and spinach pot experiments suggested that uptake of Cd by plants may also be influenced by the amount of phosphate applied. The effect of different phosphate fertilizer on the cadmium content of the plant material differed substantially between Melanudand and Haplaquept. On the acutely low cadmium polluted soil, Melanudand the cadmium content of the Komatsuna and spinach decreased with increase in phosphate fertilizers at all levels of application, whereas on the more Cd polluted soil, Haplaquept level of phosphate application had only a small effect on the cadmium content of the Komatsuna and spinach, even at the the highest levels of application which were toxic. The uptake of Cd has been influenced both by factors tending to increase the soil Cd level and by factors tending to increase the solubility and availability of the soil Cd. The concentration of Cd in komatsuna and spinach increased Cd applied through different phosphate fertilizers but the increase was not found to be significant. He found that Cd addition at the rates ranging from 0.3 to 1.2 $ha^{-1}y^{-1}$ did not appear to increase the Cd concentration in plants in nine long-term soil fertility experiments in the United States of America. Although the rate of Cd addition in some treatments of the current experiment was higher than that in the experiment of Mortvedt (1987), it did not increase significantly the Cd concentration in plants of Komatsuna and spinach. One of the reasons for little or no increase in Cd uptake with increased Cd addition through different phosphate fertilizers may be that the contribution of added Cd (0.012 – 5.6 g ha^{-1}) in relation to that already present in the 0-15 cm soil depth (2260 g ha^{-1}) is rather small. Eriksson (1988) found that the concentration of Cd in rapeseed was higher in the clay soil than in the sand, when no Cd was added to the soils. But the Cd concentration in rapeseed was much higher in the sand than in the clay soil when 1 or 5 mg Cd kg^{-1} soil was added. Similar results were also obtained by Land et al. (1981) in swiss chard and radish crops. This implies that at high rates of Cd addition, soil properties controlled by the Cd present in the soil. Continuous use of phosphate fertilizer can also lead to the increase in the Cd content in cultivated soils (Alloway 1990). Jinadasa et al. (1997) found in a survey of Cd concentrations in vegetable crops that Cd concentration were generally poorly correlated with soil properties.

8.2 Conclusion

The Cd concentration levels in all PRs and PFs ranged from 0.15 to 507 and 0.04 to 4.01 mg kg^{-1}. Cd contents were significantly correlated with intensity of CFA in PRs and PFs. Effect was not significant among the different Cd contents in phosphate fertilizer on Cd concentration in Komatsuna. The highest Cd contents in Komatsuna reached 0.034 and 1.24 mg kg^{-1} by China 1 fertilizer application whereas the lowest are resulted 0.029 mg kg^{-1} and 1.08 mg kg^{-1} from Myanmar fertilizer application treatments of Melanudand and Haplaquept under the CODEX limit of 0.2 mg kg^{-1} FW. The concentration of Cd in Komatsuna was 10 times lower than that in spinach when grown in Cd polluted soil (Haplaquept) and 4 times in unpolluted soil (Melanudand). Cd concentration in Komatsuna was under the limit of CODEX. However, spinach was over the limit of Codex (0.2 mg kg^{-1} FW). It was recommended to grow the cultivars of cultivars of Maruha and Hamami No.2 Komatsuna in Cd-contaminated soil because of lower Cd accumulation. It was better to keep recommended rate of phosphate fertilizer and to select low Cd uptake cultivar to control Cd contamination in soils and crops.

The concentration of Cd in phosphate rock decreases as follows: PR5 < PR1 < PR2 < PR6 < PR3 < PR4. The concentration of Cd in fertilizers decreases as follows; PF4 < PF3 < PF1 < PF7 < PF6 < PF2 < PF5. The level of contamination of Melanudand with Cd was found to be not high. The levels of Cd accumulated by Komatsuna and spinach grown on Haplaquept were higher than those grown on Melanudand. The concentration of Cd in crops decreases as follows: Spinach > Komatsuna. The concentration of Cd in soil decreases as follows: Haplaquept > Melanudand. The concentration of Cd in Komatsuna and spinach decreases as follows: calcium superphosphate applied treatment > fused magnesium phosphate applied treatment. In the present studies, although addition of phosphate to the soil generally increased cadmium uptake by the Komatsuna and spinach, the effect of phosphate application on the cadmium content of the plant varied from soil to soil. On acutely high Cd polluted soil (Haplaquept), increasing additions of phosphate generally led to increase in the cadmium content of the plant than Melanudand. This study highlights the role of clay size fractions in Melanudand in the retention of Cd. This could result in less availability of Cd and less danger for Cd uptake by Komatsuna and spinach. However, it is also important to take into consideration phosphate fertilizers application practice because these would also influence the Cd uptake by plants. In case of Cd polluted soil (Haplaquept), it is recommended to plant Komatsuna because it showed less accumulation of Cd in it leaf portion compared with spinach. On acutely low Cd polluted Melanudand, increasing additions of phosphate generally led to decreases in the cadmium content of Komatsuna and spinach, probably largely because of substantial increases in Komatsuna and spinach yield, but on high Cd polluted Haplaquept, addition of phosphate increased the cadmium content of the plants as well as increasing the yield of Komatsuna and spinach. It would seem that level of available soil Cd may affect the Cd content of Komatsuna and spinach. Application of phosphate fertilizer increased Cd concentration and accumulation in Komatsuna and spinach leaves, with the effect being greater in Haplaquept than in Melanudand, possibly due to lower cation exchange capacity, lower pH, higher Cd concentration in soils. The total cadmium added to the soils and some key soil properties were better estimators of cadmium availability than an integrative parameter like water-extractable cadmium or even the calculated free Cd species. pH, clay and organic matter contents were the most indicative of the Komatsuna and spinach Cd. The concentrations of 0.1 mol L^{-1} HCl acid-extractable and total soil Cd increased with increasing fertilizer application, but not significantly. Concentrations of Cd in spinach exceeded CCFAC's allowable limit of 0.2 mg kg^{-1} FW. No consistent increases in plant Cd concentration were observed both soils. To mitigate the impacts of soil Cd contamination on plants, farmers should not exceed the recommended level of phosphate application for the Tokyo region (60 kg P_2O_5 ha^{-1}) for komatsuna.

8.3 Final Conclusions and Recommendation

Cd concentrtion in food product may be controlled by reducing Cd input in phosphate fertilizers, using Cd unpolluted soil and selecting low Cd uptake cultivar of crop as shown in Figure 23. For controlling Cd contamination in crops, soil of unpolluted soil as Melanudand, low Cd content fertilizer as fused magnesium phosphate and low Cd uptake crop as Komatsuna are better to be selected in cultivation.

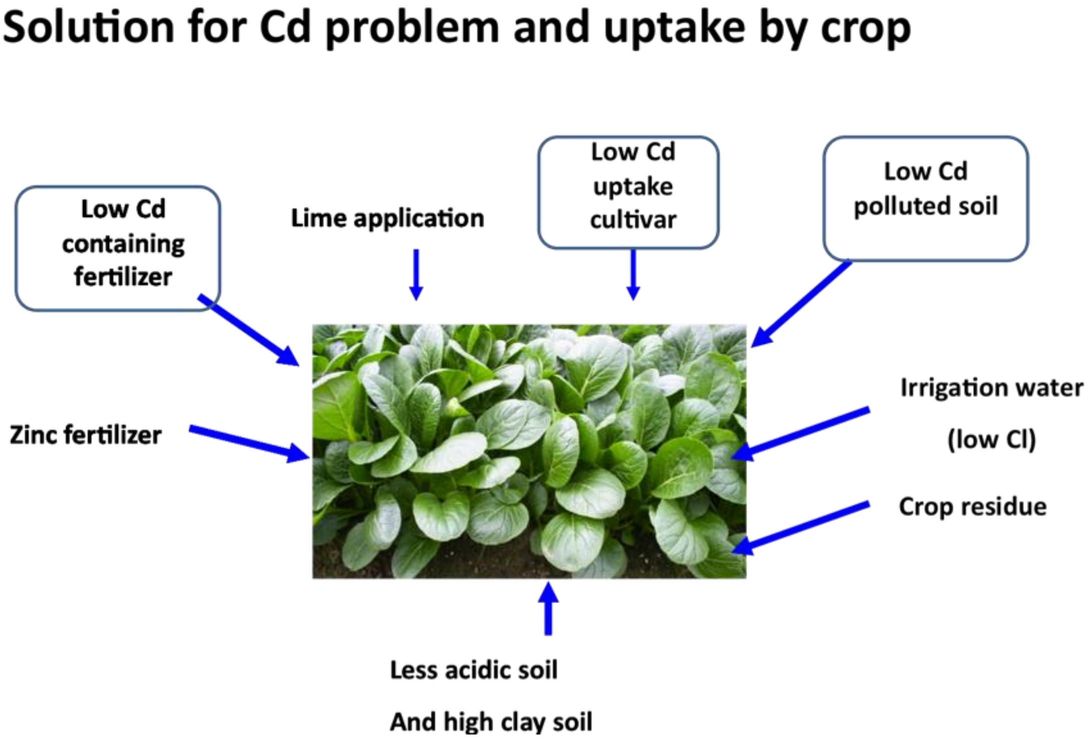


Figure 23. Factors for controlling Cd concentration in soil and crop

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APPENDICES

Appendix 1. Cd conc. in Melaundand by different fertilizer application treatment

Melanudand	**Total Cd mg kg^{-1}**	**Citric acid soluble Cd mg kg^{-1}**	**0.1 M HCl extractable Cd mg kg^{-1}**	**water soluble Cd mg kg^{-1}**
Control	0.21	0.035	0.092	0.0011
Control	0.16	0.023	0.096	0.0010
Control	0.22	0.027	0.072	0.0012
Control	0.14	0.028	0.080	0.0009
Control	0.20	0.031	0.084	0.0010
Average	**0.19**	**0.029**	**0.085**	**0.0010**
China 1	0.22	0.037	0.094	0.0008
China 1	0.17	0.027	0.102	0.0013
China 1	0.20	0.025	0.114	0.0008
China 1	0.18	0.026	0.096	0.0009
China 1	0.22	0.024	0.094	0.0010
Average	**0.20**	**0.028**	**0.100**	**0.0010**
China 2	0.23	0.024	0.122	0.0008
China 2	0.20	0.025	0.134	0.0016
China 2	0.30	0.028	0.134	0.0011
China 2	0.22	0.043	0.155	0.0011
China 2	0.20	0.037	0.131	0.0013
Average	**0.23**	**0.032**	**0.135**	**0.0012**
China 3	0.22	0.033	0.098	0.0016
China 3	0.25	0.033	0.122	0.0012
China 3	0.24	0.036	0.105	0.0008
China 3	0.19	0.031	0.1097	0.0008
China 3	0.21	0.037	0.157	0.0010
Average	**0.22**	**0.034**	**0.116**	**0.0011**
Japan	0.18	0.031	0.120	0.0009
Japan	0.23	0.036	0.100	0.0010
Japan	0.26	0.037	0.172	0.0010
Japan	0.20	0.027	0.109	0.0010
Japan	0.17	0.029	0.152	0.0013
Average	**0.21**	**0.032**	**0.131**	**0.0011**
Myanmar	0.23	0.024	0.147	0.0007
Myanmar	0.21	0.024	0.129	0.0008
Myanmar	0.25	0.027	0.104	0.0008
Myanmar	0.16	0.025	0.127	0.0009
Myanmar	0.14	0.027	0.121	0.0010
Average	**0.20**	**0.025**	**0.126**	**0.0009**

Appendix 2. Cd concentration in Haplaquept by different phosphate fertilizers treatments

Haplaquept	Total Cd mg kg^{-1}	Citric acid soluble Cd mg kg^{-1}	0.1M HCl extractable Cd mg kg^{-1}	water soluble Cd mg kg^{-1}
Control	2.24	0.37	1.25	0.0032
Control	2.19	0.36	1.27	0.0033
Control	2.28	0.37	1.25	0.0033
Control	2.46	0.36	1.14	0.0036
Control	2.57	0.38	1.24	0.0036
Average	**2.35**	**0.37**	**1.23**	**0.0034**
China 1	2.56	0.36	1.33	0.0035
China 1	2.60	0.37	1.36	0.0023
China 1	2.58	0.37	1.35	0.0029
China 1	2.71	0.37	1.33	0.0025
China 1	2.88	0.36	1.34	0.0028
Average	**2.66**	**0.36**	**1.34**	**0.0028**
China 2	2.54	0.38	1.43	0.0039
China 2	2.70	0.38	1.44	0.0034
China 2	2.73	0.48	1.42	0.0028
China 2	2.83	0.38	1.43	0.0024
China 2	2.63	0.36	1.44	0.0022
AVERAGE	**2.68**	**0.40**	**1.43**	**0.0029**
China 3	2.79	0.35	1.51	0.0036
China 3	3.00	0.36	1.52	0.0038
China 3	3.10	0.36	1.52	0.0030
China 3	2.66	0.36	1.53	0.0035
China 3	2.60	0.37	1.58	0.0034
Average	**2.83**	**0.36**	**1.53**	**0.0035**
Japan	3.00	0.39	1.59	0.0031
Japan	2.63	0.36	1.54	0.0035
Japan	2.85	0.36	1.53	0.0037
Japan	2.63	0.36	1.50	0.0028
Japan	2.86	0.38	1.52	0.0030
Average	**2.80**	**0.37**	**1.54**	**0.0032**
Myanmar	2.67	0.37	1.51	0.0025
Myanmar	2.65	0.38	1.55	0.0034
Myanmar	3.03	0.39	1.58	0.0024
Myanmar	2.66	0.38	1.51	0.0027
Myanmar	2.46	0.38	1.50	0.0023
Average	**2.69**	**0.38**	**1.53**	**0.0027**

Appendix 3. Cd concentration in Komatsuna and dry weight of Komatsuna grown in Melanudand by different phosphate fertilizers application treatments

Melanudand	Cd in Komatsuna leaves mg kg^{-1}DW	Dry weight of Komatsuna leaves g pot^{-1}	Cd uptake by Komatsuna leaves mg pot^{-1}	Cd in Komatsuna leaves mg kg^{-1}FW
Control	0.028	8.94	0.00025	0.013
Control	0.021	9.15	0.00019	0.013
Control	0.038	7.78	0.00030	0.013
Control	0.035	7.08	0.00025	0.012
Control	0.024	7.80	0.00018	0.014
Average	**0.029**	**8.15**	**0.00024**	**0.01307**
China 1	0.019	7.22	0.00014	0.013
China 1	0.048	6.85	0.00033	0.018
China 1	0.047	7.54	0.00035	0.019
China 1	0.023	9.05	0.00021	0.011
China 1	0.034	6.80	0.00023	0.013
Average	**0.034**	**7.49**	**0.00026**	**0.01481**
China 2	0.039	7.55	0.00029	0.015
China 2	0.028	7.84	0.00022	0.013
China 2	0.026	9.20	0.00024	0.013
China 2	0.045	7.90	0.00036	0.017
China 2	0.031	8.54	0.00026	0.014
Average	**0.034**	**8.21**	**0.00028**	**0.01422**
China 3	0.041	8.41	0.00034	0.019
China 3	0.031	7.54	0.00023	0.012
China 3	0.044	6.92	0.00031	0.015
China 3	0.015	8.41	0.00013	0.010
China 3	0.027	7.87	0.00021	0.014
Average	**0.032**	**7.83**	**0.00025**	**0.01391**
Japan	0.035	6.78	0.00024	0.013
Japan	0.026	7.51	0.00019	0.015
Japan	0.041	7.69	0.00032	0.015
Japan	0.028	8.25	0.00023	0.011
Japan	0.025	7.39	0.00018	0.013
Average	**0.031**	**7.52**	**0.00023**	**0.01342**
Myanmar	0.033	10.61	0.00035	0.018
Myanmar	0.041	10.03	0.00042	0.022
Myanmar	0.024	9.71	0.00023	0.011
Myanmar	0.023	10.25	0.00023	0.014
Myanmar	0.032	12.27	0.00039	0.015
Average	**0.031**	**10.57**	**0.00032**	**0.01581**

Appendix 4. Cd concentration in Komatsuna and dry weight of Komatsuna grown in Haplaquept by different phosphate fertilizers application

Haplaquept	Cd in Komatsuna leaves mg kg^{-1}DW	Dry weight of Komatsuna leaves g pot^{-1}	Cd uptake by Komatsuna leaves mg pot^{-1}	Cd in Komatsuna leaves mg kg^{-1}FW
Control	1.02	6.99	0.007	0.256
Control	0.85	6.61	0.006	0.193
Control	1.18	8.00	0.009	0.250
Control	1.15	8.82	0.010	0.149
Control	1.17	7.37	0.009	0.219
Average	**1.08**	**7.56**	**0.008**	**0.213**
China 1	1.16	7.30	0.008	0.186
China 1	1.17	6.29	0.007	0.294
China 1	1.01	13.26	0.013	0.247
China 1	1.64	6.88	0.011	0.263
China 1	1.22	7.29	0.009	0.308
Average	**1.24**	**8.20**	**0.010**	**0.260**
China 2	1.11	8.34	0.009	0.211
China 2	1.05	8.35	0.009	0.237
China 2	1.30	7.72	0.010	0.337
China 2	1.08	6.91	0.007	0.209
China 2	1.09	6.51	0.007	0.283
Average	**1.13**	**7.57**	**0.009**	**0.255**
China 3	1.05	8.28	0.009	0.178
China 3	1.02	7.37	0.008	0.241
China 3	1.01	8.91	0.009	0.201
China 3	0.76	8.41	0.006	0.187
China 3	0.88	8.14	0.007	0.252
Average	**0.94**	**8.22**	**0.008**	**0.212**
Japan	0.97	7.14	0.007	0.243
Japan	0.96	6.44	0.006	0.277
Japan	0.99	8.14	0.008	0.214
Japan	0.93	6.28	0.006	0.214
Japan	0.95	7.18	0.007	0.239
Average	**0.96**	**7.04**	**0.007**	**0.237**
Myanmar	0.87	9.61	0.008	0.269
Myanmar	0.88	10.00	0.009	0.240
Myanmar	0.80	11.84	0.009	0.222
Myanmar	0.89	10.87	0.010	0.221
Myanmar	0.77	11.58	0.009	0.189
Average	**0.84**	**10.78**	**0.009**	**0.228**

Appendix 5. Cd concentration in Melanudand by different levels of calcium superphosphate and fused magnesium phosphate application treatment

Melanudand	Total Cd mg kg^{-1}	0.1 M HCl extract Cd mg kg^{-1}
Control	0.42	0.07
Control	0.45	0.09
Control	0.42	0.08
Average	**0.43**	**0.08**
CSP 60	0.48	0.09
CSP 60	0.45	0.08
CSP 60	0.43	0.07
Average	**0.45**	**0.08**
CSP 120	0.46	0.09
CSP 120	0.49	0.10
CSP 120	0.48	0.09
Average	**0.48**	**0.09**
CSP 240	0.57	0.07
CSP 240	0.52	0.07
CSP 240	0.53	0.08
Average	**0.54**	**0.07**
Control	0.48	0.07
Control	0.50.	0.09
Control	0.49	0.07
Average	**0.48**	**0.08**
FMP 60	0.49	0.05
FMP 60	0.51	0.07
FMP 60	0.54	0.06
Average	**0.51**	**0.06**
FMP 120	0.49	0.07
FMP 120	0.48	0.06
FMP 120	0.51	0.06
Average	**0.49**	**0.06**
FMP 240	0.52	0.07
FMP 240	0.53	0.06
FMP 240	0.51	0.06
Average	**0.52**	**0.06**

Appendix 6. Cd concentration in Haplaquept by different levels of calcium superphosphate and fused magnesium phosphate application treatment

Haplaquept	Total Cd mg kg^{-1}	0.1 M HCl extractable Cd mg kg^{-1}
Control	2.13	1.37
Control	2.11	1.35
Control	2.09	1.36
Average	**2.11**	**1.36**
CSP 60	2.19	1.33
CSP 60	2.17	1.35
CSP 60	2.21	1.27
Average	**2.19**	**1.32**
CSP 120	2.24	1.28
CSP 120	2.16	1.29
CSP 120	2.26	1.42
Average	**2.22**	**1.33**
CSP 240	2.31	1.44
CSP 240	2.16	1.43
CSP 240	2.24	1.37
Average	**2.24**	**1.41**
Control	2.08	1.39
Control	2.15	1.36
Control	2.09	1.32
Average	**2.11**	**1.36**
FMP 60	2.16	1.28
FMP 60	2.08	1.32
FMP 60	2.29	1.26
Average	**2.18**	**1.29**
FMP 120	2.19	1.2
FMP 120	2.06	1.18
FMP 120	2.24	1.17
Average	**2.16**	**1.18**
FMP 240	2.26	1.36
FMP 240	2.21	1.34
FMP 240	2.18	1.25
Average	**2.22**	**1.32**

Appendix 7. Cd concentrion, Dry weight and Cd uptake of Komatsuna grown on Melanudand by different different levels of calcium superphosphate and fused magnesium phosphate application treatment

Melanudand	Cd in Komatsuna leaves mg kg^{-1}DW	Dry weight of Komatsuna leaves mg kg^{-1}	Cd uptake by Komatsuna leaves µg pot^{-1}	Cd in Komatsuna leaves mg kg^{-1}FW
Control	0.36	10.91	3.91	0.05
Control	0.34	6.37	2.18	0.04
Control	0.19	9.7	1.88	0.02
Average	**0.30**	**8.99**	**2.66**	**0.03**
CSP 60	0.20	10.74	2.10	0.02
CSP 60	0.21	9.78	2.02	0.03
CSP 60	0.20	6.57	1.31	0.02
Average	**0.20**	**9.03**	**1.81**	**0.02**
CSP 120	0.21	5.77	1.22	0.03
CSP 120	0.25	9.29	2.33	0.03
CSP 120	0.24	4.96	1.21	0.02
Average	**0.24**	**6.67**	**1.59**	**0.03**
CSP 240	0.21	5.47	1.14	0.03
CSP 240	0.19	8.53	1.66	0.02
CSP 240	0.26	8.88	2.31	0.03
Average	**0.22**	**7.63**	**1.70**	**0.03**
Control	0.19	5.43	1.05	0.02
Control	0.19	6.28	1.19	0.02
Control	0.19	7.37	1.42	0.02
Average	**0.19**	**6.36**	**1.22**	**0.02**
FMP 60	0.25	5.29	1.33	0.03
FMP 60	0.25	6.09	1.50	0.02
FMP 60	0.18	6.44	1.13	0.02
Average	**0.22**	**5.94**	**1.32**	**0.03**
FMP 120	0.16	6.94	1.12	0.02
FMP 120	0.16	7.89	1.23	0.02
FMP 120	0.15	10.84	1.60	0.02
Average	**0.16**	**8.56**	**1.32**	**0.02**
FMP 240	0.23	5.39	1.24	0.03
FMP 240	0.23	8.88	2.04	0.03
FMP 240	0.19	6.93	1.30	0.02
Average	**0.22**	**7.07**	**1.53**	**0.03**

Appendix 8. Cd concentration, Dry weight and Cd uptake of Komatsuna grown on Haplaquept by different levels of calcium superphosphate and fused magnesim phosphate application treatments

Haplaquept	Cd in Komatsuna leaves mg kg^{-1}DW	Dry weight of Komatsuna leaves mg kg^{-1}	Cd uptake by Komatsuna leaves µg pot^{-1}	Cd in Komatsuna leaves mg kg^{-1}FW
Control	1.64	4.56	7.47	0.18
Control	1.75	5.69	9.97	0.22
Control	1.47	5.61	8.27	0.19
Average	**1.62**	**5.29**	**8.57**	**0.20**
CSP 60	1.18	7.43	8.77	0.14
CSP 60	1.40	6.4	8.99	0.22
CSP 60	1.05	8.37	8.77	0.14
Average	**1.21**	**7.40**	**8.84**	**0.17**
CSP 120	1.70	5.06	8.58	0.21
CSP 120	1.57	4.29	6.74	0.26
CSP 120	1.35	6.5	8.78	0.25
Average	**1.54**	**5.28**	**8.03**	**0.24**
CSP 240	1.25	5.95	7.44	0.18
CSP 240	1.40	6.85	9.62	0.16
CSP 240	1.39	5.06	7.03	0.19
Average	**1.35**	**5.95**	**8.03**	**0.17**
Control	1.69	5.07	8.58	0.22
Control	1.54	6	9.24	0.36
Control	1.46	6.38	9.34	0.24
Average	**1.57**	**5.82**	**9.05**	**0.27**
FMP 60	1.66	5.24	8.71	0.20
FMP 60	1.42	6.05	8.59	0.26
FMP 60	1.30	4.82	6.25	0.16
Average	**1.46**	**5.37**	**7.85**	**0.21**
FMP 120	1.45	6.59	9.57	0.18
FMP 120	1.07	6.33	6.79	0.15
FMP 120	1.44	4.95	7.14	0.20
Average	**1.32**	**5.96**	**7.83**	**0.17**
FMP 240	1.27	5.45	6.92	0.18
FMP 240	1.34	4.45	5.94	0.17
FMP 240	1.24	5.65	7.02	0.16
Average	**1.28**	**5.18**	**6.63**	**0.17**

Appendix 9. Cd concentration in Melanudand by different levels of calcium superphosphate and fused magnesium phosphate application treatments

Melanudand	Total Cd mg kg^{-1}	0.1 M HCl extractable Cd mg kg^{-1}
Control	0.42	0.16
Control	0.69	0.16
Control	0.50	0.15
Average	**0.54**	**0.16**
CSP 140	0.51	0.18
CSP 140	0.61	0.15
CSP 140	0.61	0.17
Average	**0.58**	**0.16**
CSP 280	0.61	0.22
CSP 280	0.58	0.22
CSP 280	0.58	0.16
Average	**0.59**	**0.20**
Control	0.65	0.15
Control	0.43	0.19
Control	0.54	0.18
Average	**0.54**	**2.26**
FMP 140	0.43	0.13
FMP 140	0.49	0.18
FMP 140	0.62	0.16
Average	**0.51**	**0.16**
FMP 280	0.54	0.13
FMP 280	0.58	0.12
FMP 280	0.61	0.16
Average	**0.58**	**0.14**

Appendix 10. Cd concentrations in Haplaquept by different levels of calcium superphosphate and fused magnesium phosphate application treatments

Haplaquept	Total Cd mg kg^{-1}	0.1 M HCl extractable Cd mg kg^{-1}
Control	2.26	1.27
Control	2.37	1.42
Control	2.19	1.34
Average	**2.28**	**1.34**
CSP 140	2.06	1.30
CSP 140	2.30	1.29
CSP 140	2.41	1.23
Average	**2.26**	**1.27**
CSP 280	2.13	1.14
CSP 280	2.47	1.47
CSP 280	2.64	1.31
Average	**2.41**	**1.30**
Control	2.24	1.50
Control	2.20	1.38
Control	2.35	1.12
Average	**2.27**	**1.33**
FMP 140	2.10	1.36
FMP 140	2.18	1.28
FMP 140	2.09	1.41
Average	**2.12**	**1.35**
FMP 280	2.14	1.40
FMP 280	2.21	1.31
FMP 280	2.39	1.43
Average	**2.25**	**1.38**

Appendix 11. Cd concentration in spinach leaves, yield and Cd uptake of spinach grown in Melanudand by different levels of calcium superphosphate and fused magnesium phosphate application treatments

Melanudand	Cd in spinach leaves mg kg^{-1}DW	Dry weight of spinach leaves mg kg^{-1}	Cd uptake by spinach leaves µg pot^{-1}	Cd in Spinach leaves mg kg^{-1}FW
Control	0.90	3.54	3.17	0.19
Control	0.90	2.55	2.30	0.13
Control	0.77	3.22	2.49	0.13
Average	**0.86**	**3.10**	**2.65**	**0.15**
CSP 140	0.78	2.87	2.24	0.13
CSP 140	0.79	3.87	3.06	0.14
CSP 140	0.78	2.91	2.27	0.16
Average	**0.78**	**3.22**	**2.52**	**0.14**
CSP 280	0.78	2.94	2.29	0.14
CSP 280	0.77	2.65	2.04	0.16
CSP 280	0.79	3.87	3.04	0.06
Average	**0.78**	**3.15**	**2.46**	**0.12**
Control	0.60	3.48	2.10	0.10
Control	0.59	5.23	3.11	0.10
Control	0.71	4.07	2.90	0.10
Average	**0.64**	**4.26**	**2.70**	**0.10**
FMP 140	0.52	3.29	1.71	0.09
FMP 140	0.59	5.15	3.06	0.10
FMP 140	0.93	5.15	4.79	0.15
Average	**0.68**	**4.53**	**3.19**	**0.11**
FMP 280	0.69	3.9	2.70	0.12
FMP 280	0.68	3.9	2.65	0.12
FMP 280	0.69	3.89	2.70	0.39
Average	**0.69**	**3.90**	**2.68**	**0.21**

CSP- Calcium superphosphate

FMP- Fused magnesium phosphate

Appendix 12. Cd concentration in spinach leaves, yield and Cd uptake of spinach grown in Haplaquept by different levels of calcium superphosphate and fused magnesium phosphate application treatments

Haplaquept	**Cd in Spinach leaves** **mg kg^{-1}DW**	**Dry weight of spinach leaves** **mg kg^{-1}**	**Cd uptake by spinach leaves** **µg pot^{-1}**	**Cd in Spinach leaves** **mg kg^{-1}FW**
Control	10.76	2.59	27.9	1.987
Control	13.62	2.93	39.9	2.400
Control	10.72	2.89	31.0	2.874
Average	**11.70**	**2.80**	**32.9**	**2.420**
CSP 140	15.29	4.17	63.7	2.868
CSP 140	15.25	4.17	63.6	2.788
CSP 140	15.09	2.77	41.8	3.341
Average	**15.21**	**3.70**	**56.4**	**2.999**
CSP 280	14.61	4.08	59.6	3.081
CSP 280	14.68	2.89	42.4	3.415
CSP 280	14.66	2.79	40.9	3.609
Average	**14.65**	**3.25**	**47.7**	**3.368**
Control	10.86	3.65	39.6	2.078
Control	14.24	4.17	59.4	3.348
Control	12.54	2.88	36.1	3.016
Average	**12.55**	**3.57**	**45.0**	**2.814**
FMP 140	12.43	5.38	66.8	2.752
FMP 140	12.30	2.24	27.6	2.365
FMP 140	14.07	3.11	43.8	2.340
Average	**12.93**	**3.58**	**46.1**	**2.485**
FMP 280	11.74	3.75	44.0	2.741
FMP 280	11.59	3.37	39.1	2.047
FMP 280	7.48	2.85	21.3	2.446
Average	**12.43**	**3.32**	**34.8**	**2.411**

CSP- Calcium superphosphate

FMP- Fused magnesium phosphate

Appendix 13. Cd concentrations in soil by different levels of calcium superphosphate application treatments

Treatment Phosphate level (kg P_2O_5 kg ha^{-1})	Total Cd mg kg^{-1}	water extractable Cd mg kg^{-1}	0.1M HCl extractable Cd mg kg^{-1}	0.01M HCl extractable Cd mg kg^{-1}
5 July to 15 August 2008				
0	2.60	0.0035	1.46	0.43
0	2.61	0.0075	1.51	0.43
0	2.35	0.0061	1.48	0.44
Average	**2.52**	**0.0057**	**1.48**	**0.43**
60	2.54	0.0042	1.46	0.40
60	2.74	0.0088	1.49	0.40
60	2.38	0.0066	1.49	0.41
Average	**2.55**	**0.0065**	**1.48**	**0.40**
120	2.57	0.0050	1.54	0.39
120	2.48	0.0069	1.48	0.49
120	2.35	0.0078	1.51	0.49
Average	**2.47**	**0.0066**	**1.51**	**0.46**
240	2.55	0.0043	1.58	0.44
240	2.46	0.0121	1.45	0.42
240	2.68	0.0043	1.56	0.45
Average	**2.57**	**0.0069**	**1.53**	**0.44**
9 Nov 2009 to 9 Feb 2010				
0	2.64	0.0056	1.48	0.37
0	2.64	0.0073	1.48	0.42
0	2.43	0.0043	1.65	0.59
Average	**2.57**	**0.0057**	**1.53**	**0.46**
60	2.43	0.0050	1.45	0.44
60	2.73	0.0072	1.50	0.46
60	2.62	0.0057	1.49	0.45
Average	**2.59**	**0.0059**	**1.48**	**0.45**
120	2.52	0.0066	1.50	0.41
120	2.49	0.0053	1.61	0.49
120	2.68	0.0052	1.49	0.52
Average	**2.56**	**0.0057**	**1.53**	**0.47**
240	2.88	0.0098	1.53	0.48
240	2.76	0.0056	1.62	0.42
240	2.64	0.0078	1.50	0.44
Average	**2.76**	**0.0077**	**1.55**	**0.45**

Appendix 14. Cd concentration in Maruha Komatsuna and leaves yield of Maruha Komatsuna by different levels of calcium superphosphate application treatments

Treatments	Phosphate level (kg ha^{-1})	Leaves yield kg m^{-2}DW	Leaves yield kg m^{-2}FW	Leaves Cd concentration mg kg^{-1}DW	Leaves Cd concentration mg kg^{-1}FW
5 July to 15 August 2008					
Maruha	0	0.16	1.27	1.42	0.18
Maruha	0	0.12	1.12	1.43	0.15
Maruha	0	0.11	1.07	1.49	0.16
Average		**0.13**	**1.16**	**1.45**	**0.16**
Maruha	60	0.13	1.10	1.25	0.15
Maruha	60	0.09	0.71	1.97	0.23
Maruha	60	0.12	1.05	1.10	0.13
Average		**0.11**	**0.95**	**1.44**	**0.17**
Maruha	120	0.15	1.15	1.39	0.18
Maruha	120	0.22	1.64	1.56	0.21
Maruha	120	0.21	2.07	1.79	0.18
Average		**0.19**	**1.62**	**1.58**	**0.19**
Maruha	240	0.24	1.99	1.33	0.16
Maruha	240	0.20	1.72	1.68	0.20
Maruha	240	0.23	2.07	1.77	0.19
Average		**0.23**	**1.92**	**1.59**	**0.18**
9 Nov 2009 to 9 Feb 2010					
Maruha	0	0.13	1.08	1.23	0.11
Maruha	0	0.17	1.98	1.82	0.16
Maruha	0	0.12	1.13	1.89	0.16
Average		**0.14**	**1.40**	**1.64**	**0.14**
Maruha	60	0.16	0.68	1.53	0.15
Maruha	60	0.19	2.09	1.19	0.10
Maruha	60	0.18	0.97	1.24	0.12
Average		**0.18**	**1.25**	**1.32**	**0.12**
Maruha	120	0.13	1.57	1.74	0.14
Maruha	120	0.11	1.28	1.69	0.15
Maruha	120	0.16	1.61	2.03	0.19
Average		**0.14**	**1.49**	**1.82**	**0.16**
Maruha	240	0.12	2.65	1.83	0.17
Maruha	240	0.10	0.43	1.60	0.16
Maruha	240	0.11	1.30	2.07	0.19
Average		**0.11**	**1.46**	**1.83**	**0.17**

Appendix 15. Cd concentration in Nakamachi Komatsuna and leaves yield of Nakamachi komatsuna by different levels of calcium superphosphate application treatments

Treatments	Phosphate level (kg ha^{-1})	Leaves yield kg m^{-2}DW	Leaves yield kg m^{-2}FW	Leaves Cd concentration mg kg^{-1}DW	Leaves Cd concentration mg kg^{-1}FW
5 July to 15 August 2008					
Nakamachi	0	0.22	1.74	1.05	0.13
Nakamachi	0	0.24	2.06	1.90	0.23
Nakamachi	0	0.16	1.40	1.30	0.15
Average		**0.21**	**1.73**	**1.42**	**0.17**
Nakamachi	60	0.21	1.73	2.50	0.31
Nakamachi	60	0.22	2.17	2.51	0.25
Nakamachi	60	0.18	1.50	2.35	0.29
Average		**0.21**	**1.80**	**2.45**	**0.28**
Nakamachi	120	0.16	1.13	1.57	0.22
Nakamachi	120	0.17	1.46	2.61	0.29
Nakamachi	120	0.18	1.56	2.59	0.31
Average		**0.17**	**1.38**	**2.26**	**0.27**
Nakamachi	240	0.15	1.07	2.56	0.35
Nakamachi	240	0.16	1.13	2.47	0.33
Nakamachi	240	0.21	1.63	2.31	0.29
Average		**0.17**	**1.28**	**2.45**	**0.32**
9 Nov 2009 to 9 Feb 2010					
Nakamachi	0	0.11	0.58	2.02	0.15
Nakamachi	0	0.13	1.83	1.91	0.14
Nakamachi	0	0.15	2.39	1.86	0.13
Average		**0.13**	**1.60**	**1.93**	**0.14**
Nakamachi	60	0.16	2.87	2.13	0.14
Nakamachi	60	0.19	3.10	1.95	0.13
Nakamachi	60	0.14	1.44	1.73	0.15
Average		**0.16**	**2.47**	**1.94**	**0.14**
Nakamachi	120	0.17	1.76	2.30	0.15
Nakamachi	120	0.16	1.76	1.84	0.15
Nakamachi	120	0.20	2.21	2.17	0.17
Average		**0.18**	**1.91**	**2.10**	**0.16**
Nakamachi	240	0.15	2.07	1.53	0.14
Nakamachi	240	0.10	0.66	2.23	0.18
Nakamachi	240	0.13	1.29	1.23	0.11
Average		**0.13**	**1.34**	**1.66**	**0.14**

Appendix 16. Cd concentration and leaves yield of SC8-007 and Hamami No.2 Komatsuna by different levels of calcium superphospahte application treatments

Cultivar	Treatment Phosphate level (kg P_2O_5 ha^{-1})	leaves yield kg m^{-2} DW	leaves yield kg m^{-2} FW	leave Cd concentration mg kg^{-1} DW	leave Cd concentration mg kg^{-1} FW
5 July to 15 August 2008					
SC8-007	0	0.18	1.55	1.25	0.14
SC8-007	0	0.12	0.97	1.74	0.22
SC8-007	0	0.11	0.88	1.39	0.18
Average		0.14	1.14	1.46	0.18
SC8-007	60	0.15	1.23	1.85	0.22
SC8-007	60	0.21	1.30	1.18	0.19
SC8-007	60	0.17	1.23	2.48	0.34
Average		0.17	1.25	1.84	0.25
SC8-007	120	0.13	1.06	2.51	0.31
SC8-007	120	0.15	1.13	2.30	0.31
SC8-007	120	0.13	0.95	1.40	0.19
Average		0.14	1.04	2.07	0.27
SC8-007	240	0.12	0.87	2.01	0.28
SC8-007	240	0.14	1.24	1.48	0.16
SC8-007	240	0.16	1.17	2.79	0.37
Average		0.14	1.09	2.09	0.27
9 Nov 2009 to 9 Feb 2010					
Hamami No.2	0	0.12	0.72	1.61	0.13
Hamami No.2	0	0.15	2.32	1.34	0.09
Hamami No.2	0	0.13	1.53	1.64	0.14
Average		0.13	1.52	1.53	0.12
Hamami No.2	60	0.13	1.62	1.61	0.12
Hamami No.2	60	0.18	2.91	1.35	0.10
Hamami No.2	60	0.12	1.58	1.45	0.14
Average		0.14	2.04	1.47	0.12
Hamami No.2	120	0.15	2.01	1.58	0.15
Hamami No.2	120	0.14	2.00	1.46	0.12
Hamami No.2	120	0.14	1.99	1.58	0.13
Average		0.14	2.00	1.54	0.13
Hamami No.2	240	0.17	1.80	1.51	0.14
Hamami No.2	240	0.09	0.55	1.93	0.19
Hamami No.2	240	0.16	1.18	1.68	0.17
Average		0.14	1.18	1.70	0.17