# LONG TERM EFFECT OF FERTILIZER APPLICATION ON CADMIUM UPTAKE BY OAT (*Avena sativa*) PLANT

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# Long Term Effect of Fertilizer Application on Cadmium Uptake by Oat (*Avena sativa*) Plant


**Submitted by:**

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# ABSTRACT

Phosphate fertilizers contain varying amounts of Cd and other heavy metals as contaminants from phosphate rock (PR). Periodic applications of these fertilizers resulted in measurable accumulations of Cd in soils and in harvested crops. A long term field study for fourteen cropping seasons (1992-2006) was conducted on an experimental plot located at the Norwegian University of Life Sciences (Ås, Norway) to evaluate the effect of application of cadmium enriched phosphate fertilizer on soil solution dynamics of cadmium and Cd accumulation in Oat (*Avena sativa*). Treatments consisted of three NPK fertilizer sources containing 1, 90, 381 mg Cd $kg^{-1}$ P and supplying 0.03, 2.7, 11.43 g Cd $ha^{-1}yr^{-1}$. Surface soil samples (0-20 cm) were collected after harvesting of plant in 1992, 1995, and 2006. Plant samples were analyzed for 1992, 1995, 1997, 2002 and 2006. Soil samples were analyzed for total and extractable Cd, pH, dissolve organic carbon (DOC) and $PO_4^{3-}$. Analysis of plant samples was done for total concentration of Cd and plant uptake of Cd was calculated. A general trend of decrease in plant Cd with increased soil pH was observed throughout the experiment period except 1992 where plant Cd concentration did not significantly change with soil pH. Increasing the addition of Cd input through fertilizers only increased the Cd concentration in soil, but the Cd concentration in oat grain was not affected significantly. $NH_4NO_3^-$ extractable Cd in the soil increased with increasing rate of Cd through different sources but the concentration of extractable Cd in the soil decreased with increased pH throughout the experimental period except in 1995 where soil pH did not show any consistent effect on extractable Cd. Plant Cd did not show any significant correlation with extractable Cd in soil. Hence the concentration of Cd in oat grain was not significantly affected by Cd input through fertilizers.

*Keywords:* Cadmium; Cd uptake; extractable Cd; NPK fertilizers; oat; plant Cd; phosphate fertilizers; soil pH; soil solution

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# DECLARATION

I, Dipendra do hereby declare to Norwegian University of Life Science that, this Thesis is my original work and that it has never been submitted for a degree award in any other University.

Signature

Date…29 / 07 / 2013

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# LIST OF SYMBOLS AND ABREVIATIONS

| | |
|---|---|
| % | Percent |
| $^{\circ}C$ | Degree Celsius |
| P | Phosphorus |
| Cd | Cadmium |
| NPK | Nitrogen, Phosphorus and Potassium |
| $mgkg^{-1}$ | Milligram per Kilogram |
| g/ha | Gram per hectare |
| mg/l | Milligram per liter |
| mg/ha | Milligram per hectare |
| M | Molar concentration |
| Mm | Millimeter |
| M | Meter |
| $m^2$ | Meter Square |
| mM | MilliMolar |

# CHAPTER ONE

# INTRODUCTION

Since the middle of the last century, commercial fertilizers have played a critical role in increasing food and fiber production around the world. However these fertilizers may also contain some nonessential and harmful elements such as cadmium, lead and/or arsenic (Grant *et al.,* 2008). There is a concern about whether continuous use of such fertilizers over a long period of time may cause an accumulation of these harmful trace elements to high levels which ultimately increases risk to environmental and human health (Huang *et. al.*, 2004).

Cadmium (Cd) is a trace element that is naturally present in soils. It can also be introduced into soil through anthropogenic activities such as fertilization, irrigation, pesticide application, organic waste disposal and atmospheric deposition (Alloway and Steinnes, 1999; Sheppard *et al.*, 2009). Cadmium is listed as one of the 7$^{th}$ most hazardous substances that can cause potential threat to human health due to its known or suspected toxicity by Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of year 2011(ATSDR, 2011). Cadmium is a human carcinogenic element that affects human cardiovascular, neurological, renal, respiratory, gastrointestinal and reproductive functions (ATSDR, 2011).

Most of trace elements are persistent in soil because of their relative immobility in soils. However, Cd is known as more mobile and soluble than other trace elements contained in fertilizers (Chen *et al.*, 2004). Cadmium enters human body from environment through ingestion via food (especially plant based food) mechanism (Krishnamurti *et al.,* 1999; Vig *et al.*, 2003). Cadmium is present in plant materials due to its uptake from soil (McLaughlin & Singh, 1999). The availability of Cd in soil and its uptake by plant depend on several soil and plant factors.

Phosphorus (P) is an essential nutrient for crop production and growth. For soils deficient in P, application of organic or inorganic fertilizers is needed to achieve optimum crop yields. Although P fertilizers represent the major anthropogenic input of phosphorus to agricultural soils, both inorganic P fertilizers and organic P sources (sewage, sludge and manure) contain Cd (Sheppard *et al.,* 2009). In addition to direct inputs of Cd, phosphorus fertilizer can indirectly affect Cd accumulation in crops through its effects on soil chemistry, crop growth, and microbial interactions (Grant, 2011). When we apply phosphorus fertilizer to our cropland, we are also feeding trace element (mainly Cd) which after certain time lead to its accumulation in our cropland soil (Grant and Sheppard, 2008; Jiao *et al.,* 2012). Plants take up Cd with water and nutrients when grown in Cd contaminated soil (Akhter, 2012). The amount of Cd taken up by an individual plant depends on the amount of bioavailable Cd present in the soil (Sheppard *et al.,* 2007) and the physiological and morphological characteristics of the plant (Grant *et al.,* 1999).

Loganathan *et al.,* (1995) showed a clear relationship between the amount of phosphorus (P) fertilizer use and Cd accumulation in plants. Cadmium can be present in phosphate fertilizer at concentrations ranging from 0 to 300 $mgkg^{-1}$, depending on the provenance of the phosphate rock (Mortvedt *et al.,* 1981). In field studies in Sweden (Andersson and Siman, 1991) and in the Canadian prairies (Grant and Bailey, 1997), Cd concentration in grain and seeds of several crops consistently increased with increasing phosphorus (P) application. Therefore, the management of P fertilizer application, both in the short term and long term, can influence the potential accumulation of Cd in foods. Hence it is important to minimize Cd accumulation in agricultural soils.

For a single time application or few application, the amount of addition will be insignificant compared with total volume of receiving soil and its presence in soils cannot be easily 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 (Chen *et al.,* 2007). In long term field studies in USA and UK, no significant change in the Cd content of soils was found from the application of phosphorus fertilizer (Jones *et al.,* 2002). Researchers in Norway reported similar findings (Jeng and Singh, 1995). However, researchers in Australia and the U.K. reported that long-term applications of P fertilizers increased concentration of Cd in surface soil (Nicholson *et al.,* 1994; Loganathan *et al.,* 1995). Hence no any agreement on the cause of cadmium accumulation through long term phosphorus fertilization can be established. Increased Cd accumulation in oat, ryegrass, carrot, and lettuce was reported in response to the application of P or NPK fertilizer that contains relatively high Cd (He and Singh 1994a; Huang *et al.,* 2004).

To better understand the effect of phosphorus fertilizer on phytoavailability of Cd in soil-plant system, it is necessary to distinguish fertilizer's direct and indirect effect on the addition of Cd to the soil. Assessing the impact of phosphate fertilization on the accumulation of Cd in soils and its transfer to plants requires adequate knowledge of how various factors affect Cd phytoavailability. Soil properties that can influence Cd availability include pH, clay type, chloride content and the content of soil organic matter and Fe and Mn oxides (McLaughlin *et al.,* 2000). Of these soil properties, soil pH is often regarded as the most important factor (Hooda *et* *al.,* 1997; Grant *et al.,* 1999). Soil pH is one of the important factors regulating the available Cd in soil, hence the Cd concentration and uptake by plants.

Investigations of pH effect on the available Cd by plants are usually conducted by liming the soil to desired pH levels. In most cases, liming increased the Cd adsorption and reduced the Cd availability to plants in soils (Guttormsen *et al.,* 1995; Singh *et al.,* 1995). However, increasing soil pH does not always reduce Cd availability in soils and plant uptake, because this relationship depends on other factors such as soil characteristics, plant species and field conditions (He and Singh, 1993a). Singh *et al.,* (1995) found that the decrease in Cd concentration was not consistent at pH levels beyond 6.5. Besides the soil and plant factors, the field conditions can change the Cd availability to plants, due to a better plant root system induced by liming or environmental conditions (He and Singh, 1993b).

This study was undertaken at the experimental farm of the Norwegian University of Life Sciences Ås, located in south-eastern part of Norway. This area is one of the most productive Norwegian agriculture regions, and is characterised by their acid soils.

The objectives of the present study were to:

1. Investigate the effect of Cd contained in NPK fertilizer on total and extractable Cd in soils and in plants at different pH levels and

2. Evaluate the long term effect of Cd input through fertilizers on soil Cd accumulation, its extractability and plant uptake.

In order to achieve above objectives following hypothesis was made before conducting the experiment

1. Long term use of Cd enriched phosphorus increases total soil cadmium

2. Increase of total soil cadmium increases plant cadmium.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Brief Introduction

Changing scenario with economic development of the society to large scale urbanization and industrialization during the 20th century has noticeably increased the demand for food. Commercial fertilizers contributed to 50-75% in case of developing countries and to 30-40% in U.S.A to increase the food production (Singh, 1994). This extensive use of commercial fertilizers has a risk of harmful trace elements present in them which later get into soil-plant system (Heinegg *et. al.,* 2009). Trace metals also get accumulated due to anthropogenic emissions into environment via atmospheric deposition (Tu, 2000). Beside this, application of trace elements contaminated effluents for crop production (Wang and Qin, 2006), phosphate fertilizers (Basta *et al.,* 2001; Zhou, 2003) and many other agrochemicals may contaminate soil and water resources to the extent that adversely affects environment and human health (Ahsan *et al.,* 2007). A common feature of trace elements, despite of whether they are biologically essential or not, are that these might cause toxic effects at low concentrations (Adriano, 2001; Kabata-Pendias, 2001).

## 2.2 Phosphorus Fertilizer

The commercial fertilizer had played a major role to fulfill increasing demand of world food production. It is estimated that the contribution of fertilizers to increased food production ranges as high as 50-75 % in some developing countries, and in U.S.A 30- 40% (Singh, 1994). Because of the more extended use of fertilizer and their great importance for crop production, more studies and attention also had been paid to understand and quantify the potentially toxic risk of the trace elements contained in them. These elements can get into the soil-plant system and enter into the foodstuffs. Concentrations of those potentially toxic hazardous elements are largely dependent on the raw materials used to produce them. In phosphate fertilizer the raw material used is known as phosphate rock. The Phosphate fertilizers are generally the major source of trace metals among all mineral fertilizers (Nziguheba and Smolders, 2008).

The types of raw material used for phosphate fertilizer is divided in two types, (i) the phosphate sedimentary rocks such as shales and pelitic types, accounts for 88% of total phosphate rock production, and is mainly found in North Africa, especially in countries such as Morocco and

Tunisia, and (ii) the igneous rocks like basalts, are mainly produced in Russia and South Africa. The first type is more abundant than the igneous one. The potentially hazardous metals are mainly Cadmium, Chromium, Mercury, Uranium and Vanadium, where Cd is considered more hazardous because of its higher solubility in soils and uptake by crops plants. The sedimentary phosphate rock has an average Cd content of 20.6 mg $kg^{-1}$, whereas igneous rock has an average of 1.5 mg $kg^{-1}$. The phosphate rock containing higher amount of Cd are located in Senegal, Togo, Tunisia and Morocco, while the lowest Cd is found in those from Russia, South Africa and Syria (Dylevskaia, 2002).

**Table 1:** Origin and concentrations of P and Cd in selected phosphate rocks (Grant, 2011).

| Phosphate rock | Origin | P (g/kg) | Cd (mg/kg) |
|---|---|---|---|
| **Gafsa** | Tunisia | 134 | 38 |
| **North Florida** | USA | 133 | 3 |
| **Jordanian** | Jordan | 134 | 4 |
| **North Carolina** | USA | 127 | 48 |
| **Sechura Desert** | Peru | 131 | 11 |
| **Mexican** | Mexico | 140 | 8 |
| **Nauru Island** | South Pacific | 156 | 100 |
| **Arad** | Israel | 141 | 12 |

In addition of phosphate rock types, there are other factors which influence the harmful impurities concentration in fertilizers, such as production technologies employed and type of fertilizer produced. The higher Cd contents are found in super phosphates, Single superphosphate (SSP) and Triple superphospahte (TSP), as in straight rock products. In NP fertilizer production the process used can cause great variation in Cd concentration in the final product. The thermal or wet process technology results in a low cadmium concentration, although it is not normally used as the costs of the process are excessively high.

### 2.3 Soil Cadmium

Cadmium (Cd) was discovered in 1817 by Strohmeyer of Germany, who isolated it from calamine (zinc carbonate). The name cadmium is derived from *cadmia*, the ancient Greek name for calamine (Nriagu, 1980). Cd is a non-essential, potentially toxic element for both plants and animals. It is highly mobile and bioavailable in the environment (McLaughlin and Singh, 1999).

### 2.3.1 Occurrence of Cadmium

In the environment cadmium containing ores are rare. The only important cadmium mineral is Greenockite (CdS), which mainly contains cadmium and sulphide and is associated with sphalerite (ZnS). They are always associated with Zn, on an average of Cd:Zn ratio of 1:350 (Hamedi, 1999). Therefore Cd element is produced mainly as a byproduct from mining, smelting and refining ores of Zn, and in lesser degree of lead and copper. Cadmium element is mainly used in batteries, especially in Cd-Zn batteries. The rest of applications are diversified for pigments, coatings and plating, and as stabilizers for plastics.

### 2.3.2 Cadmium in Soils and Plants

The major sources of Cd in soils are atmospheric emissions from mining and direct application of phosphate fertilizers, sewage, sludge, manure and composted municipal solid waste on agricultural soils, and accidental contamination from industrially contaminated land and mine waste dumps (Diskshith & Diwan, 2003). But among all sources, Cd containing phosphate fertilizers are a major source of anthropogenic Cd in agricultural systems (Sheppard *et al.,* 2007; Grant and Sheppard, 2008; Grant, 2011). These fertilizers may contain Cd as a contaminant at levels ranging from 0 mg/kg to as high as 340 mg/kg on a total dry weight basis (Alloway and Steinnes, 1999). Long term application of such fertilizers was reported to result in Cd accumulation in agricultural soils in Canada (Sheppard *et al.,* 2007), the United States (Mulla *et al.,* 1980), Australia (Williams and David, 1976), New Zealand (Roberts *et al.,* 1994), Britain (Nicholson *et al.,* 1994), Norway (Baerug and Singh, 1990), and Denmark (Christensen and Tjell, 1991).

In order to be biologically relevant, Cd must be bioavailable to plants. The term "bioavailability" has been defined as the extent to which a chemical can be absorbed by a living organism and reach the systemic circulation (Kelley *et al.,* 2002). Therefore, along with total Cd, the bioavailable fraction of Cd in the soil is also important in determining Cd toxicity to plants. Bioavailable forms of Cd in soil include free $Cd^{2+}$, $Cd^{2+}$ organic ligands ($Cd^{2+}$organic acids, $Cd^{2+}$humate), $Cd^{2+}$ inorganic ligands ($CdCl^{+}$, $CdOH^{+}$). The ability to release $Cd^{2+}$ from these complexes in the soil system depends on a number of factors including soil pH (Peijnenburg *et al.,* 2000), organic matter (Murray *et al.,* 2011), cation exchange capacity (Bolan *et al.,* 2003a, 2003b), presence of competing or complexing ions (Gao *et al.,* 2011), and crop management practices (Gao *et al.,* 2010).

### 2.3.3 Long Term Accumulation of Cadmium in Soils

Cadmium accumulation in agricultural soils due to the long-term use of phosphate fertilizers has been recognized in several studies from a number of countries (Singh 1991). Baerug and Singh (1990) for example showed that long-term use of commercial fertilisers increased the Cd concentration of soils in three regions of Norway. Semu and Singh (1996) studied the accumulation of heavy metals in soils and plants in Tanzania after the long-term use of fertilisers. They conclude that high total and extractable Cd concentrations in soil from the high fertilised sites compared to native sites was caused by the long term use of P fertilisers. Mulla *et al.,* (1980) showed that the concentration of Cd in a surface soil treated with broadcast phosphate fertiliser for 36 years averaged 1mg Cd $kg^{-1}$ as compared to 0.07 mg $kg^{-1}$ in the control. The concentration of Cd in the surface soil was highly correlated with the total soil P. Rothbaum *et al.,* (1986) investigated Cd accumulation on a permanent grassland site at Rothamsted, England which had received phosphate fertiliser for 100 years. Results indicated that there was an increase in total soil Cd concentration from 0.17 mg $kg^{-1}$ to 0.44 mg $kg^{-1}$ during this period. When atmospheric Cd inputs are removed, the annual rate of increase in soil Cd amounts to at least 4 g $ha^{-1}$ $y^{-1}$. Jones *et al.,* (2002) found fertilized soils had significantly lower levels of available and total metals than those of non-fertilized soils. The Rengen Grassland Experiment showed that after 65 years of fertilizer application, the soil was not contaminated with Cd (Hejcman *et al.,* 2009).

## 2.4 Cadmium Toxicity and Plant growth

Cadmium plays an important role for plant growth. The effect of Cd on plant growth parameters are generally studied by changing concentration of Cd in growth media, in soil or in irrigation water (Hussain, 2010). Germination of rice was slightly stimulated under low Cd concentration (0.01 to 1.50 mM Cd), while severely depressed under 2.0 mM Cd concentration. Cadmium concentration in roots and shoots increased with increasing Cd level. Differences among genotypes with respect to the effects of Cd on rice crop were remarkable in shoots rather than roots (Wu *et al.,* 2002).

Similarly number of pods per plant and pods per seed were reduced to 37% and 26% in mung bean when it was subjected to Cd concentration of 6, 9 and 12 $mgkg^{-1}$ in alkaline soil. However, 2.5 mg $kg^{-1}$ Cd had negligible effect (Wahid and Ghani, 2008). Uptake of Cd and its accumulation was found maximum during initial growth period of wheat which also interfered with uptake of $Zn^{2+}$ and $Mn^{2+}$ (Shukla *et al.*, 2003) and K, Zn, Ca and Fe (Vassilev, 2002) from growth medium.

Cadmium (Cd) is considered a non-nutrient element for almost every living biota with the exception of *Thalassiosira weissflogii*, a marine diatom that uses $Cd^{2+}$ as a substitute for $Zn^{2+}$ to maintain optimal growth rate when $Zn^{2+}$ is limiting (Lane *et al.*, 2005). In most environmental conditions, Cd comes in contact with roots first and then moves towards other organs. The visible symptoms of Cd toxicity in the roots include reduced root elongation (Dong *et al.*, 2007) and root browning. Once Cd moves from the root to the shoot, leaf chlorosis and leaf rolling are the first visible symptoms to appear in the aboveground organs.

## 2.5 Bioavailability of Cadmium

The bioavailability of metals like cadmium in soil depends on their solution concentration which in turn is dependent on the soil processes like cation exchange capacity, specific adsorption, soil moisture percentage, precipitation, biological influence and complexation (Basta *et al.*, 2005; Carrillo-Gonzalez *et al.*, 2006). Many of these factors vary seasonally and temporally, and are interrelated and also inhibit prediction of cadmium bioavailability. So changing one factor may affect several others. The differential response of species and varieties to environmental changes also contributes to differences in uptake of cadmium from soils. Table 2 summarizes some of the factors which affect plant Cd concentrations.

### 2.5.1 Soil pH

Soil solution pH is widely considered as the major soil factor controlling plant uptake of Cd from soils and their mobility due to its effect on surface charge properties of the solid phase and hydrolysis of metal cations (Hong *et al.*, 2008). The effect of pH on Cd availability in soils is a function of the effect of pH on retention of Cd by soil surfaces. There have been numerous studies involving glasshouse and field trial experiments showing the effect pH has on plant uptake. Andersson and Nilsson (1974) studied the effect of pH on Cd uptake by fodder rapeseed and found that an increase in soil pH from lime application decreased Cd concentration in this crop. Similarly Han and Lee (1996) showed Cd uptake in radish was significantly decreased by liming.

Guttormsen *et al.*, (1995) examined the effect of soil pH on Cd uptake by cabbage and carrot over 3 years in a field experiment. Cadmium concentrations in cabbage and carrot were 23 and

46 % higher at pH 5.5 than at pH 6.5. Increasing the soil pH increases the deprotonation of carboxyl and hydroxyl groups; hence negative charge density in the soil solution is greater at lower pH. Therefore the $Cd^{2+}$ ion has more chance to bond with soil colloids, thus increase the absorption by soil, decreasing the availability by the crop (He and Singh, 1993).

Table 2: Factors affecting cadmium uptake by plant from soil (*adapted from* Chaney and Hornick 1978).

| **Factors** | **Effect on Cadmium uptake by plants** |
|---|---|
| **Soil Factors** | |
| **1. pH** | Uptake increase as pH decreases |
| **2. Soil salinity** | Uptake increases with salinity |
| **3. Amount of Cadmium** | Uptake increases with increase in concentration |
| **4. Micronutrients** | e.g. zinc deficiency increases uptake |
| **5. Macronutrients** | May increase or decrease |
| **6. Temperature** | High temperature increases uptake |
| **Crop Factors** | |
| **1. Species and cultivars** | Leafy veg>root veg>cereals>fruits |
| **2. Plant tissue** | Leaf>grain, fruit and edible root |
| **3. Leaf age** | Older>younger |
| **4. Metal interaction** | Presence of zinc reduces uptake of Cd |

### 2.5.2 Total Cadmium and Divalent Cations

Total soil Cd concentrations are also equally important like soil pH which finally affects plant Cd uptake. Generally, higher the soil Cd concentration, the greater will be the plant Cd concentration (Akahane *et al.*, 2010). When $Ca^{2+}$ is present in soils, especially in calcareous ones, this cation inhibit the Cd sorption by soil, due to the competitive effects between $Ca^{2+}$ and $Cd^{2+}$. This effect is because both cations have similar ionic radius (Thakur *et al.*, 2006). Therefore it exist a high affinity of calcite ($CaCO_3$) surface for Cd, which might displace Cd from the sorption sites in the soil and increase its concentration in the soil solution (Kashem and Singh, 2002). Cadmium in that solution is in $CdCO_3$ form, which has a solubility product constant less than the solubility product constant of $CaCO_3$, which make logical that $Cd^{2+}$ will substitute by $Ca^{2+.}$

### 2.5.3 Soil Salinity and Inorganic Ligands

Bingham *et al.,* (1986) studied the potential of chloride and sulphate salinity to affect uptake of Cd in Swiss chard (*Beta vulgaris*). It was found that a significant increase in the soluble Cd concentration in the soil, lead to an elevated leaf Cd concentration as a result of the application of chloride salts in combination with $CdNO_3$, however sulphate had little effect. McLaughlin *et al.,* (1994) showed that soil salinity was a major factor associated with high tuber Cd concentrations in a range of potato crops irrigated with high chloride waters in South Australia.

The addition of P as phosphate to solution appears to enhance metal retention by soil. Whether the P is added to the same solution as the metal ions, this effect enhanced the immobilization of Cd in soils, thereby decreasing its phytoextractability (Hong *et al.,* 2008). There are two ways for immobilization of $Cd^{2+}$. One of those is through the precipitation of $Cd^{2+}$ as cadmium orthophosphate $Cd_3(PO_4)_2$, whereas the other one is the $Cd^{2+}$ adsorption, induced by phosphate. As is reported in Thakur *et al.,* (2006), $Cd^{2+}$ and phosphate may influence the sorption of each other, either by competing for the sorption sites or by influencing the surface charge potential.

### 2.5.4 Micronutrients

Another important soil factor controlling plant Cd concentration is soil micronutrient status mainly Zn. Studies have demonstrated no effect, synergistic, and antagonistic relationship between soils Zn and plant Cd concentration. Haghiri (1974) for example found additions of Zn to soil increased Cd concentrations of soybean shoots. McLaughlin *et al.,* (1993) report a reduction in the Cd content of potato tubers when Zn is applied to a Zn adequate soil. Choudhary *et al.,* (1994) found that Zn applied to soil decreased Cd concentration in durum wheat grain (*Triticum turgidum*), however foliar applications of Zn had no effect on grain Cd concentration. Oliver *et al.,* (1997) showed that foliar application of Zn can reduce Cd concentration in wheat grain although the amount of Zn applied would have to be higher than current recommended concentrations applied to ameliorate Zn deficiency in wheat crops. It appears that Zn may play an integral role in Cd availability for a number of crops. However its success in reducing Cd uptake appears dependent on soil Zn status and method of application.

### 2.5.5 Macronutrients

Fertiliser additions to soil can increase Cd concentration in the soil solution, even if the fertiliser contains no Cd, through the effect of fertiliser application on soil pH and ionic strength of the soil solution (Naidu *et al.*, 1994). In addition, Mitchell *et al.*, (1997) in their study confirmed that N fertilisers could increase the solubility of Cd and increase its availability to plants. Grant *et al.*, (1996) showed that additions of $NH_4NO_3$ fertiliser increased Cd concentrations in barley as well as increased crop yield and this increase in Cd accumulation could be a result of increased root interception and enhanced mass flow.

Although potassium has little effect on Cd availability, the associated sulphate or chloride anions are important. In a study by Sparrow *et al.*, (1994), they found that four out of six potato crops fertilized with KCl accumulated greater Cd concentrations than those fertilised with $K_2SO_4$ fertiliser. Similarly, Grant *et al.*, (1996) showed that the application of KCl fertiliser tended to increase Cd concentrations in malting barley. They indicated that the chloride component of KCl fertiliser can form complexes with Cd which could increase the mobility of Cd in the soil system and its phytoavailability. In contrast, McLaughlin *et al.*, (1995) found no effect on potato Cd concentration when KCl and $K_2SO_4$ fertiliser application were compared.

### 2.5.6 Soil Temperature

There is some evidence to suggest that soil temperature can affect plant uptake of Cd. Haghiri (1974) showed that with an increase in soil temperature from 15.5°C - 32.2°C, there was an increase in Cd accumulation in soybean shoots from 5.16 to 9.47 mg $kg^{-1}$. Chang *et al.*, (1987) investigating long term sludge application of Cd and Zn accumulation in Swiss chard and radish, found that environmental conditions including soil temperature influenced Cd accumulation in plant tissue. Hooda and Alloway (1993) showed that Cd uptake by ryegrass from soils amended by Cd salts and sludge application was significantly higher in pots in the warm (25°C) environment than those from the cool (1°C) environment.

## 2.6 Effect of Phosphorus on Plant Cadmium Uptake

Addition of P fertilizer in soil also influenced availability of Cd through its effects on soil pH, ionic strength and plant growth. To explain whether increase in the Cd concentration was due to contamination of fertilizer or due to P effect on Cd, some researchers used reagent grade fertilizer. However, no significant difference was observed between the Cd concentrations in durum wheat with commercial fertilizer compared with reagent grade P fertilizers (Choudhary *et al.*, 1994). Under controlled conditions, Choudhary *et al.*, (1994) applied reagent grade mono-ammonium phosphate to wheat which increased Cd concentration. Similarly, in a pot study, reagent grade triple superphosphate and mono-ammonium phosphate both increased Cd concentration in durum wheat grain (Jiao *et al.,* 2004). Concentration of Cd in durum wheat at several locations across the Canada was increased by application of P fertilizer. This increase was unrelated to the concentration of Cd present in the fertilizer (Grant *et al.,* 2002).

Zinc might inhibit Cd translocation from roots to shoots. Zhao *et al.,* (2005) reported the effect of Zn (0, 1, 5, 10 mg $kg^{-1}$ soil) and P (0, 10, 50, 100 mg $kg^{-1}$ soil) application on growth and Cd accumulations in shoots and roots of winter wheat seedlings. Phosphorus and Zn showed complex interactions in their uptake. Cadmium in shoots decreased significantly with increasing Zn except at P addition of 10 mg $kg^{-1}$. In contrast, root Cd concentration increased significantly except at Zn addition of 5 mg $kg^{-1}$. Cadmium concentrations increased in shoots but decreased in roots with increasing P supply.

Mann *et al.,* (2002) observed significant increases in Cd concentrations of fertilized soils relative to unfertilized soils, but the magnitude of such changes varied widely between studies. McGrath and Tunney (2010) found an increase in soil Cd of slightly more than 0.05 mg $kg^{-1}$ after a total application of 930 kg P $ha^{-1}$ over 31 years in a grazing trial. Analysis of stored sample of the fertilizer used in 1999 contained 38.6 mg $kg^{-1}$ Cd. Sometimes, no clear cut accumulation trend was noted in fields receiving long-term P fertilizer applications. Jeng and Singh (1995) showed that the soil Cd content was only slightly increased after 70 years of phosphate fertilizer application. The annual increases of Cd in fertilized soils varied from 0.04 to 0.12% of total soil Cd contents depending on the source of fertilizer. Richards *et al.,* (1998) examined Cd accumulations in soils that received annual phosphorus applications of 0, 22.5, 45 and 90 kg $P_2O_5$ $ha^{-1}$. They found no evidence that Cd was accumulating in soils, after 29 years of crop production.

The accumulation apparently not only depends on the concentration of Cd in the fertilizer, but the application rate, the plant removal and losses from the system which in turn are affected by the soil chemical, biological and environmental factors (Grant and Sheppard, 2008). The inconsistent observations in the literature indicate an importance of regional scale assessment with uncertainty analysis.

### 2.7 Long Term Effects of Phosphate Fertilizer on Soil and Plant Cadmium Content

Phosphorus fertilizer is one of the major contaminant of Cd and Zn in agricultural soil. Long term effect of P fertilizer due to its contamination is reviewed by Grant and Sheppard (2008) in detail. Briefly a positive significant relationship between total P and total Cd in soil was recorded in Saudi Arabia (Saltali *et al.,* 2004), United States (Mulla *et al*, 1980), Europe (Nicholson *et al.,* 1994) and Australia (Williams and David, 1976) as a result of long term additions of P fertilizers.

Reports are available where soil Cd increase was not observed due to application of low cadmium P fertilizer, < 5 mg $kg^{-1}$ (Richards *et al.,* 1998) but monitoring of their long term impact is necessary. Level of fertilizer contamination may lead to contrary results in determining Cd or Zn accumulation in soil and plants. Therefore, further research is needed in multi-cropping area where usually high rates of fertilizers are applied to crops.

Crop Cd concentration is strongly affected by soil Cd concentration (Adams *et al.,* 2004) hence long-term increase of Cd in soil resulting from P application can lead to higher crop Cd accumulation (Kashem and Singh, 2002; Brennan and Bolland, 2004; Grant *et al.,* 2011). For example, in field studies conducted on soils across the Canadian Prairies, the concentration of Cd in durum wheat grain increased with both the application rate and Cd concentration of P fertilizers, and reflected the total input of Cd to the soils over time (Figure 1).

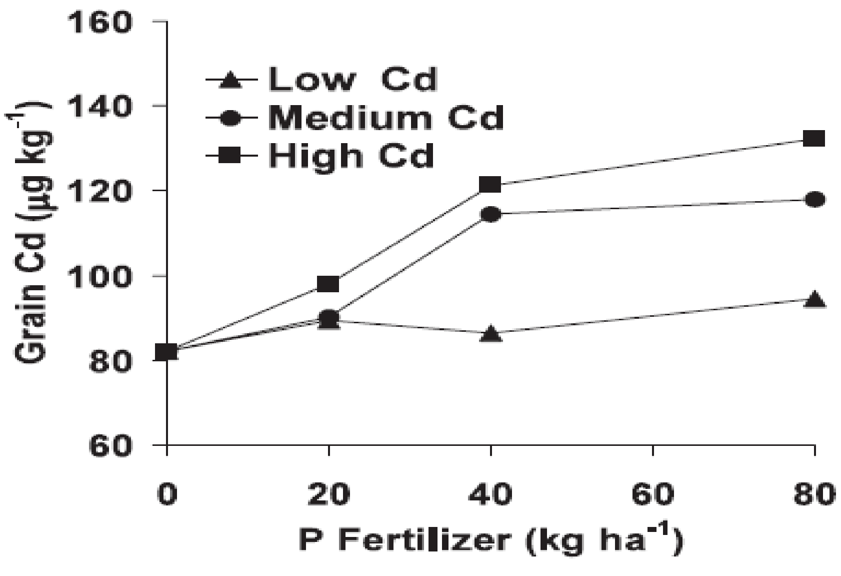


**Figure 1:** Effect of seven years of application of P fertilizers in Cd concentration of wheat with low (0.38), medium (71) and high (211) $mgkg^{-1}$Cd (adapted from Grant *et al.*, 2011).

Many of the factors that affect soil bioavailability of trace element are influenced by long term application of P fertilizers (Grant and Sheppard, 2008). Cakmak *et al.,* (2010) reported 40 year application of P fertilizers (MAP at rate of 26, 39, and 52 kg P $ha^{-1}yr^{-1}$) significantly decreased pH and increased CEC and clay content of the soil. Thus, phosphate fertilizer applications not only increase Cd concentration of soils but also may change their chemical speciation and thus bioavailability.

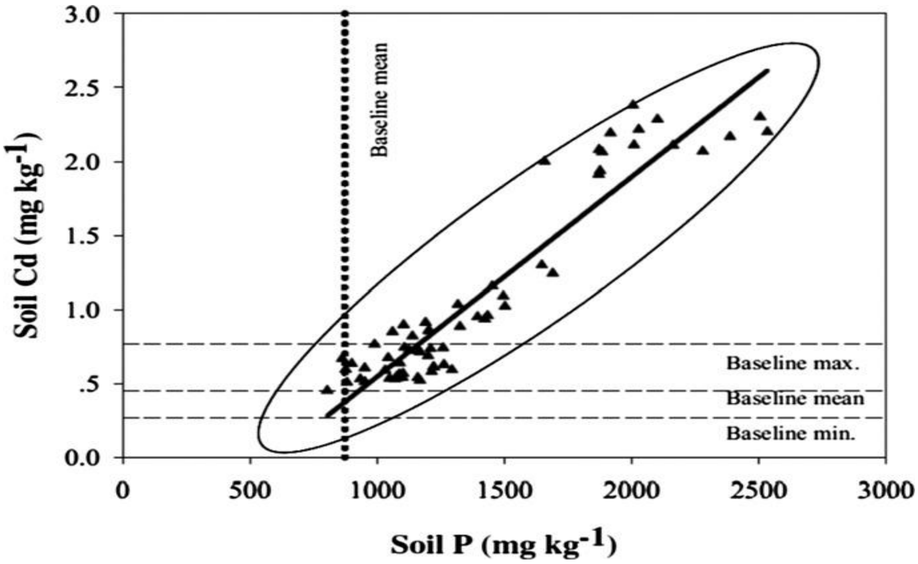


**Figure 2:** Soil P *vs.* Cd contents of cropland soils in a vegetable growing region in California (data based on Chen *et al.*, 2008).

For Cd, their bioavailability and mobility in soils may be attenuated with the addition of phosphates (Tu *et al.,* 2000; Knox *et al.,* 2006). Tu *et al.,* (2000) found that phosphorus fertilizers altered the distribution of Cd in soils. Munksgaard and Lottermoser (2011) found among various types of phosphate fertilizers, potassium orthophosphate fertilizer was the most effective amendment for Cd stabilization in mining impacted soils in Australia.

The immobilization of Cd by P depends greatly on the level of Cd in the soil and the level of P applied (Basta *et al.,* 2001; Hong *et al.,* 2008). In addition to soil P content, the bioavailability of Cd in soils is known to be affected by many other factors (Grant and Sheppard, 2008). As with the Cd, Zn may also accumulate in the soil over time with repeated applications of P fertilizers (Lambert *et al.,* 2007). Presence of Zn in P fertilizer may reduce the Cd accumulation in crops as Zn and Cd are chemically similar and may compete for binding sites in the soil system and for uptake and translocation within the plant (Zhao *et al.,* 2005). Therefore, changes of Cd bioavailability in soils due to P fertilizer application are limited in most cases.

## 2.8 Plant Uptake

Increasing soil cadmium concentration will commonly be associated with their increased accumulation in crops (Adams *et al.*, 2004). Cadmium uptake by plants can be influenced by the type of P fertilizer applied (McLaughlin *et al.*, 1995). Huang *et al.,* (2004), Jones *et al.,* (2002) found fertilized soils had significantly lower levels of available and total metals than those of non-fertilized soils. The Rengen Grassland Experiment showed that after 65 years of fertilizer application, the soil was not contaminated with Cd (Hejcman *et al.,* 2009). In a two year experiment it was shown that lettuce plants fertilized with phosphate rock absorbed less Cd in the first year than the second year, yet lettuce fertilized with TSP absorbed more Cd in the first year than the second year.

The evidence for P fertilizer application affecting plant uptake of trace elements however were not entirely unequivocal. Mulla *et al.,* (1980) found that the Cd contents of barley harvested from the soil media receiving varying P treatments were not significantly different from those of the control treatment, although at the surface 15 cm depth the Cd levels of treated soils were elevated by as much as 14 times over the control soil. He and Singh (1994) compared the Cd uptake by oats, ryegrass, carrot and spinach grown on sandy and loamy soils that were amended with Cd salt, low Cd NPK fertilizer, high Cd NPK fertilizer, or rock phosphate at comparable amounts of Cd input. The plants grown with the high Cd NPK fertilizer accumulated the most Cd. In the sandy soil, the Cd uptake by oats and ryegrass fertilized by the high Cd NPK fertilizer was two times more than those grown in control soil. For the phosphate rock treatment, the Cd contents in the plant tissue were not significantly different from those in plants grown on the control soil. In a follow-up, Singh and Myhr (1998) examined Cd uptake by barley in a field experiment and found that the bioavailability of phosphate rock borne Cd was significantly less than that of the NPK fertilizers and inorganic $CdCl_2$ salts borne Cd. Despite the fact that extractable Cd concentrations in soils fertilized with of phosphate rock increased in proportion with the amounts applied, the plant uptake of Cd did not change significantly. In a long-term field investigation, Hamon *et al.,* (1998) showed that the Cd contents of field grown wheat increased as corresponding amounts of superphosphate applied increased. Huang *et al.,* (2004) reported that less than 1% of the Cd added through fertilizer application accumulated in the lettuce biomass.

## 2.9 Extractant for Determining Plant Available Cadmium in Soils

The usefulness of a soil extractant to predict the plant available fraction of soil Cd depends on its ability to simulate soil solution conditions during the extraction e.g. pH, ionic strength. The majority of studies examining the relationship between plant uptake of Cd from soils and the Cd in soil extracts have been carried out with Cd added to soils, either as soluble inorganic salts

(Han and Lee, 1996) or using soil contaminated by sewage sludge application (Jing and Logan 1992). There are numerous laboratory methods that have been proposed and evaluated to measure Cd phytoavailability in soils (Table 3). These soil extractants can be broadly grouped in the following categories:

1. Weak salts (e.g. $CaCl_2$, $NH_4Cl$);

2. Chelating agents (e.g. EDTA, DTPA);

3. Weak acids ($CH_3COOH$);

4. Dilute solutions of strong acids (e.g. HCI, $HNO_3$).

The efficiency and predictability of a given extractant can be strongly influenced by soil and plant factors, and as a consequence there is no universal extractant for Cd. Symeonides and McRae (1977) compared seven extractants, and found the highest significant relationship between Cd concentration in radish and unbuffered 1M $NH_4NO_3$ solution. Similar conclusions were drawn by He and Singh (1994) and Guttormsen *et al.,* (1995) who showed that 1M $NH_4NO_3$ was sensitive to soil pH. However, Krishnamurti *et al.,* (1995 ) showed data that in comparison with 6 other existing soil test methods for bioavailable Cd, extraction of the soil with unbuffered 1 M $NH_4C1$ provided the best measure of Cd uptake for durum wheat. Andrewes *et al.,* (1996) comparing 6 different extractants to predict plant available Cd on soils that had accumulated Cd under "normal" New Zealand agricultural practices found that 0.01 M $CaCl_2$ to be the best indicator of plant available Cd. Andrewes *et al.,* (1996) argued that $CaCl_2$ is sensitive to the soil factors which are known to contribute to plant uptake of Cd e.g. pH, organic matter and P fertilizer application. Similarly, Whitten and Ritchie (1991) found 0.01 M $CaCl_2$ as an excellent predictor of the Cd status in clover *(Trifolium subterraneum*) for Australian soils. These authors state that $CaCl_2$ extracts at the ionic strength of the soil solution and is more appropriate than acidic or buffer redox extractants because the pH of $CaCl_2$ extracts is similar to that in the soil. Chelating agents such as EDTA (ethylene diamine tetraacetic acid) and DTPA (diethylene triamine pentaacetic acid) have also been used to predict of Cd phytoavailability. Jackson and Alloway (1991) suggest EDTA could predict Cd concentration in lettuce and cabbage, although stronger relationships were found using $CaCl_2$.

**Table 3:** Chemical reagents used for cadmium extraction by different workers (*adapted from* Gray, 1998).

| Extractant | Soil/Solution ratio | Time of extract (hrs) |
|---|---|---|
| **0.01 M $CaCl_2$** | 1:5 | 16 |
| **0.1M $CaCl_2$** | 1.2.5 | Not given |
| **0.05 M $CaCl_2$** | 1:6 | 16 |
| **1M $NH_4NO_3$** | 1:10 | 1 |
| **1M $NH_4Cl$** | 1:6 | 16 |
| **1M $NH_4OAc$** | 1:10 | 0.5 |
| **0.05 M EDTA** | 1:6 | 1 |
| **0.05 M $Ca(NO_3)_2$** | 1:10 | 16 |
| **1N HCL** | 1:2 | 1 |
| **0.5N $CH_3COOH$ (pH 7.0)** | 1:10 | 0.5 |
| **AB-DTPA (pH 7.6)** | 1:2 | 0.25 |

# CHAPTER THREE

# MATERIAL AND METHODS

## 3.1 Field Experiment

A long term fertility experiment was initiated in 1992 at the Experimental Farm of department of plant and environment Sciences, Norwegian University of Life Sciences. The experiment was conducted on a loam soil (39% sand, 40% silt and 21% clay), classified as Fluventic Humaquept (Soil Survey Staff, 2010). Before conducting the experiment the soil contained 2.4% organic carbon, 0.19 mg Cd $kg^{-1}$ and 82 mg extractable P $kg^{-1}$. Before starting this experiment, the field was used for liming experiment with different pH levels over a long period of time. The cadmium (Cd) experiment was imposed on this liming experiment.

The experiment was designed as a factorial randomized block design with 24 plots (two replicates and twelve treatments). Each block represents a replicate consisting of three Cd levels i.e 0.03, 2.7, 11.43 g $ha^{-1}$ $y^{-1,}$ and four pH levels i.e. A=5.0, B=5.3, C=5.6 and D=6.0. Due to small differences in pH level of 5.3 and 5.6, only 3 pH levels, A C and D, were selected. Each plot was 4.5 m × 10 m = 45 $m^2$ (Figure 3), with the harvesting area of 2×8=16$m^2$. The surface soil samples (0-20 cm) for this study were collected from three years 1992, 1995 and 2006 while those of grain samples from five years 1992, 1995, 1997, 2002 and 2006.

Before conducting the experiment, the plots were treated with lime (dolomite 31.5 %) to achieve four pH levels. Except control plot (treatment A) all other treatments received 2 Mg lime $ha^{-1}$. Phosphorus fertilizers were applied at the rate of 30 kg $ha^{-1}$ and three different types of fertilizers with different Cd concentration were used. The first fertilizer (NPK with 17%, N 5% P, and 13%K) contained 1 mg Cd $kg^{-1}$ P, and supplied about 0.03 g Cd $ha^{-1}$ $yr^{-1}$. Similarly the second fertilizer (15% N, 15%P, 15%K) contained 90 mg Cd $kg^{-1}$ P and supplied 2.7 g Cd $ha^{-1}$ $yr^{-1}$, and the third fertilizer (15% N, 15% P, 15% K), contained about 381 mg Cd $kg^{-1}$ P and supplied 11.43 g Cd $ha^{-1}$ $yr^{-1}$.

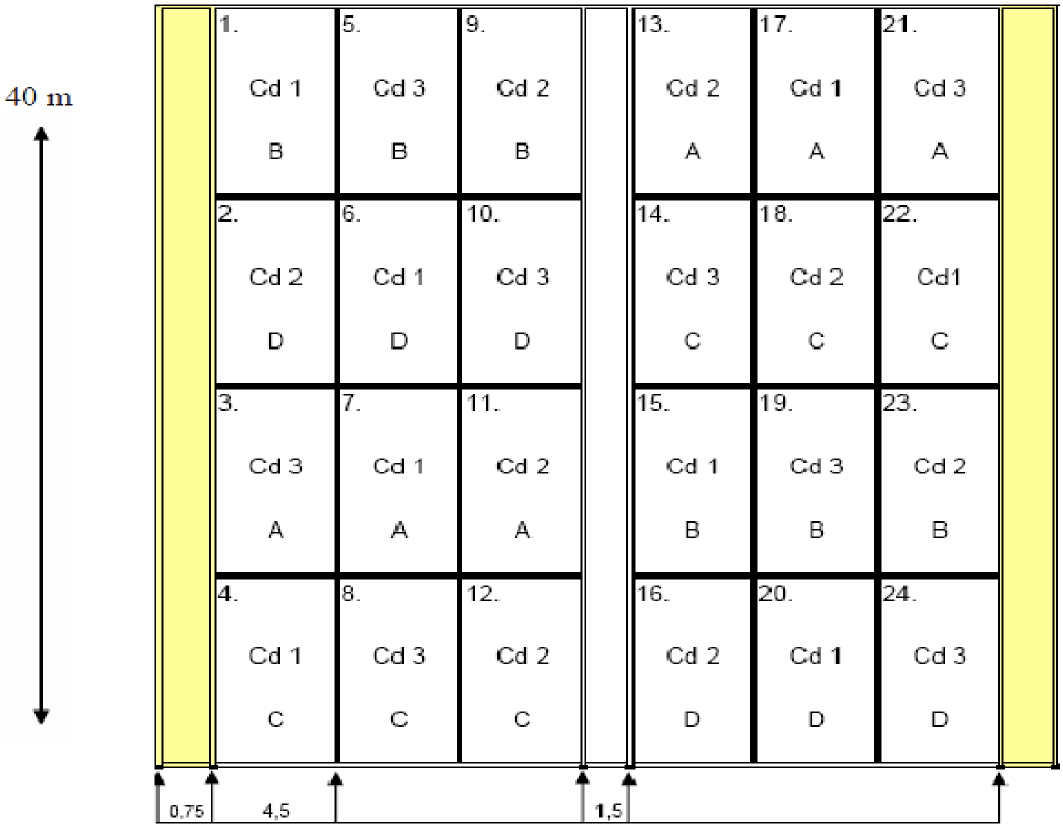


**Figure 3:** Experimental lay out.

During the initial phase of the experiment it was planned to have a crop rotation of wheat, barley and oat, but due to failure of grain production by barley and wheat in control plots only oat was grown as a test crop for most of the years. All the fertilizers were applied as basal dose before planting of crop. Soil samples were collected after harvesting of the crop. In some of the years (e.g.1998, 2000, 2005), NPK fertilizer applied contained slightly different levels of Cd and specially at Cd level 3, where the original fertilizer was exhausted and replaced by another fertilizer.

### 3.2 Collection of Soils

In each of the plot 6-8 subsamples were collected from surface layer of 0-20 with soil auger, which were made into a composite sample. During soil sample collection a grid pattern with Zig-Zag line was followed so that it represents the entire plot. This stratified random method increases the precision for the field as well as increases the accuracy of soil tests. This composite sample was then air dried ground and passed through a 2 mm sieve prior to analysis.

## 3.3 Analysis

### 3.3.1 Analysis of Soil Samples

During the analysis of soil samples, total and extractable Cd, cation exchange capacity, dissolved organic carbon and soil pH were determined as described below.

#### A) Total and Extractable Cd

For total Cd analysis 1g of soil sample was placed into a Teflon tube with 5 ml of freshly sub boiled ultra pure nitric acid, and the digestion was performed with an Ultraclave. The start pressure was 50 bars and the Ultraclave temperature was 250ºC for about 25 minutes. In addition to total Cd, the same soil samples were also extracted with 30 ml of 0.01M $NH_4NO_3$, having soil solution ratio of 1:10. The $NH_4NO_3$ solution was made by adding 2 g of 0.01M $NH_4NO_3$, together with 2 litres of deionised water. This solution was mixed with 3 g of soil, and then shaken overnight, and the next day it was centrifuged for 30 min. The suspension was passed through a Blue Ribon (diameter 125 mm) filter, and kept overnight at room temperature. The concentration of Cd in the digested and extracted solutions was determined by ICP-MS (Perkin Elmer Sciex Elan 6000) using Rh (Rhodium) as internal standard. Hence now we use the term extractable Cd for $NH_4NO_3$ extractable Cd.

#### B) Dissolve Organic Carbon, $Cl^-$, $SO4^{2-,}$ $PO_4^{3-}$

For the determination of dissolved organic carbon (DOC), $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$ ions soil samples were extracted with 30 ml of 0.01 M $KNO_3$, having a soil solution ratio of 1:10. The $KNO_3$ solution was made by adding 2 gm of 0.01 M $KNO_3$, together with 2 litres of de-ionised water. After this the solution was mixed with 3 gm of soil and then shaken overnight and was centrifuged for 30 min the next day. Finally the soil suspension was passed through a blue ribon filter (diameter 125 mm), and kept overnight at room temperature. The concentration of DOC was determined by a Shimadzu TOC-V analyser with Pt-catalyzed combustion and that of $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$ ions by Ion Chromatography.

#### C) Soil pH

Soil pH was measured by glass electrode (Orion pH-instrument model SA 720 with combination pH electrode) and pH was measured at a soil-water ratio of 1:2.5 w/v (Mc Lean, E.O., 1982).

### D) Soil Cation Exchange

1M ammonium acetate at neutral pH was used to determine soil cation exchange capacity (H+, $Ca^{2+}$, $K^{+}$, $Mg^{2+}$, and $Na^{+}$). First of all, 3 gm of soil was taken in 100 ml of bottle and suspension was made by adding 20 ml of $NH_4AC$ and after this it was shaken on an end over end shaker over night. On the next day the suspension was passed through a Blue Ribon (Diameter 125 mm) filter, and made to 25 ml with $NH_4AC$ (Schollenbergen *et al.*, 1945). The extracted solution was analysed for cations by ICP-OES method.

### 3.3.2 Analysis of Plant Samples

The plant samples were subjected to determine total dry matter determination and the ananlysis of grain Cd concentration. To determine dry matter yield, grain samples were oven dried at 105ºC. The grain samples were digested by dry ashing the plant samples at 450ºC with treatment of 1:2 concentrated HCL: $HNO_3$ mixture. The heating and acid digestion step was done twice and the collected residue was then dissolved in 5 ml of 1:1 $HNO_3$ solution and finally diluted to 50 ml by double distilled water prior to analysis of Cd by Inductively coupled plasma (ICP) method (Almås, 2001). The total grain cadmium uptake was calculated by multiplying the Cd concentration in plant with dry matter yields from each plot of the field experiment.

### 3.3.3 Statistical Analysis

The data were statistically evaluated by using Minitab15.0 program. The utilized models included linear regression, correlation and analysis of variance. An F-test was performed to determine if the variances of the two mean values were significantly different. If the variances of the mean values were found to have significant difference, then an independent t-test for unequal variances was performed. If the variances of the mean values were found to have no significant difference, then an independent T-test for equal variances was performed. Analysis of Variance (ANOVA) was used to test for differences in the average cadmium uptake between three cadmium concentration levels and three pH levels.

# CHAPTER FOUR

# RESULTS

## 4.1 Initial Soil Characteristics (Year 1992)

Long term field experiment was conducted at the experimental farm of the Department of Plant and Environmental Sciences, Norwegian university of life sciences (UMB), Ås from 1992 to 2006. Soil of the experiment was loam with 39% sand, 40% silt and 21% clay. This soil was classified as Fluventic Humaquept (Soil Survey, 2010). The soil contained 2.4% organic carbon, 0.17 $mgkg^{-1}$ total cadmium and 82$mgkg^{-1}$ extractable phosphorus.

During the whole experimental period pH was tried to be maintained at the same defined level. However, these pH values changed continuously due to climatic factors as well as due to occasional (to maintain soil pH levels) liming over 14 years (Table 4). The long term experiment with phosphorus fertilization affects the dissolved organic carbon content in soils. In 2006 the values of DOC were significantly lower compared with those in 1992 due to the intensive use of the crop without any crop rotation. Due to continuous phosphorus fertilization, small changes in plant available phosphorus, total soil Cd concentration, $Ca^{+2}$, $Mg^{+2}$, $Na^{+}$, $K^{+}$ were noticed in 2006 as compared to year 1992 (Table 4).

**Table 4:** General properties of soil retrieved at 1992 and 2006.

| Parameters | 1992 | 2006 |
|---|---|---|
| **pH** | 4.8, 5.4, 5.9 | 5.0, 5.6, 6.1 |
| **Dissolved Organic C (mg/l)** | 110 | 39 |
| **Plant available P (mg/kg)** | 82 | 91 |
| **Total Cd (mg/kg)** | 0.17, 0.19, 0.17 | 0.18, 0.21, 0.22 |
| **$Ca^{+2}$(mg/kg)** | 4.9 | 7.3 |
| **$Mg^{+2}$(mg/kg)** | 0.62 | 0.87 |
| **$Na^{+}$(mg/kg)** | 0.05 | 0.09 |
| **$K^{+}$(mg/kg)** | 0.3 | 0.5 |

## 4.2 Year 1992

### 4.2.1 Soil Cadmium Concentration

NPK fertilizers with three different cadmium concentration levels were added at three rates equivalent to 0.03, 2.70 and 11.43 g $ha^{-1}y^{-1}$. Cadmium was added in NPK fertilizers to maintain three levels of cadmium. In this year total soil Cd concentration varies significantly ($p=0.002$) with Cd input without consistent trend of increase or decrease in soil Cd concentration (Table 5).

**Table 5:** Effect of Cd input on total cadmium and extractable Cd in 1992.

| Cd Input (g/ha) | Total Cd (mg/kg) | Extractable Cd (mg/kg) |
|---|---|---|
| **0.03** | 0.174±0.004 | 0.007±0.005 |
| **2.7** | 0.197±0.003 | 0.007±0.005 |
| **11.43** | 0.174±0.004 | 0.007±0.005 |
| **$LSD_{0.05}$** | 10.94 | NS |

***NS: No significant differences.***

Similarly no significant relationship occurs in extractable Cd with Cd input (Table 5, Figure 4).

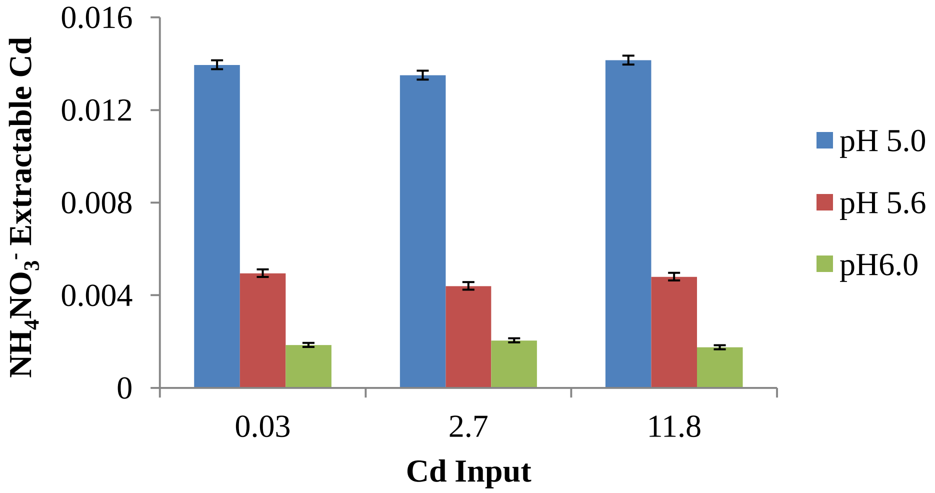


**Figure 4:** Effect of soil pH and Cd input on extractable Cd (1992).

The extractability of soil Cd was significantly decreased by increasing soil pH ($p<0.05$) (Figure 5).

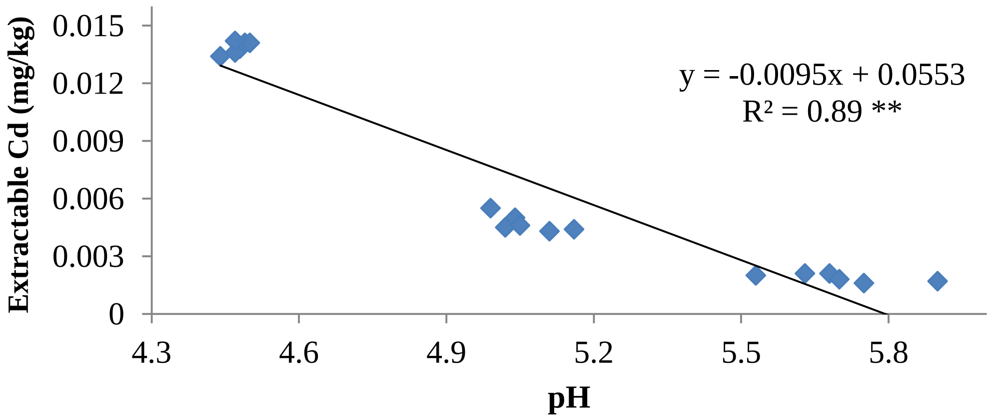


**Figure 5:** Effect of soil pH on $NH_4NO_3^-$ extractable Cd (1992).

### 4.2.2 Plant Cadmium Concentration

No significant relationship between Cd input and plant Cd concentration is observed (Table 6).

**Table 6:** Effect of Cd input on plant Cd in 1992.

| Input Cd (g/ha) | Plant Cd (mg/kg) |
|---|---|
| **0.03** | 0.115±0.015 |
| **2.7** | 0.098±0.032 |
| **11.43** | 0.101±0.026 |
| **$LSD_{0.05}$** | NS |

The relationship between pH and plant Cd concentration (Figure 6) shows a poor correlation between them. The reason behind this kind of weak relationship between pH and plant Cd could be due to small contribution of input Cd concentration through fertilizer in relation to Cd present in soil.

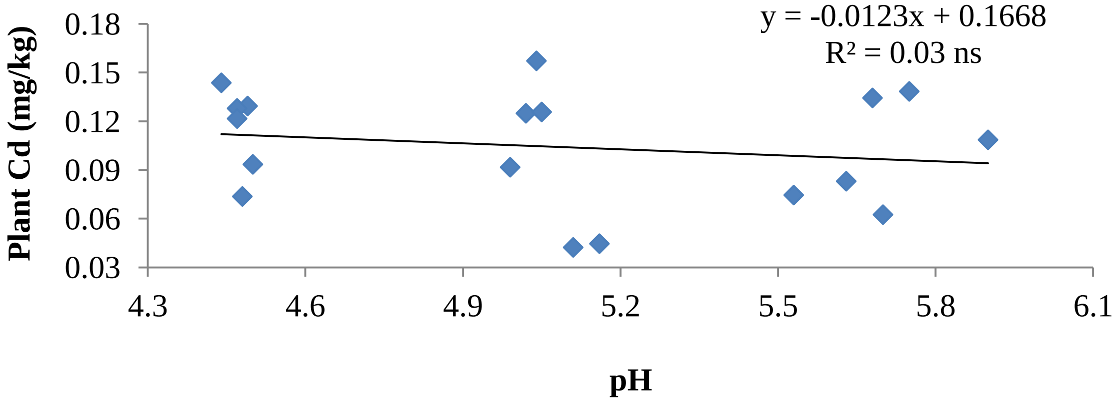


**Figure 6:** Effect of soil pH on plant Cd in 1992.

Plant Cd concentration also shows a poor correlation with extractable Cd (Figure 7). It may be due to low levels of extractable Cd in soil.

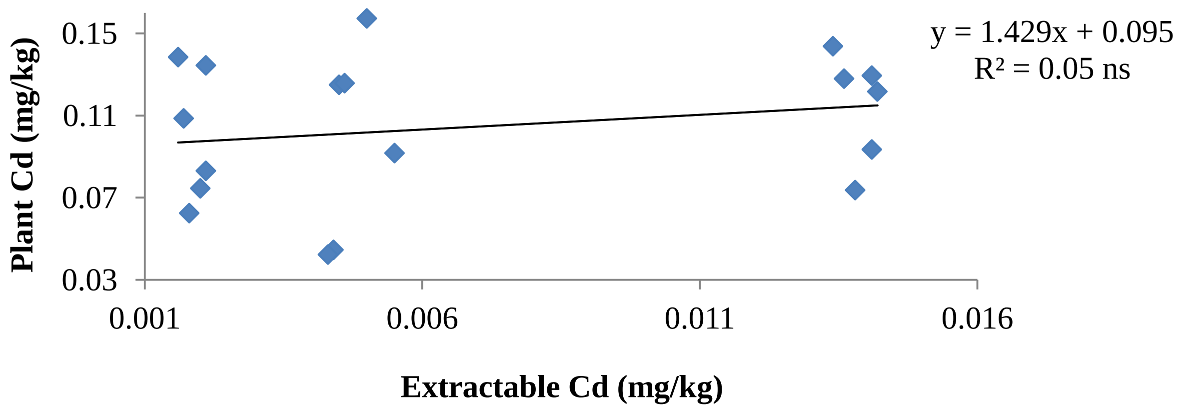


**Figure 7:** Correlation between plant Cd and extractable Cd of all soil samples in 1992.

### 4.2.3 Grain yield and Cadmium uptake

Neither grain yield nor uptake of Cd is significantly related with soil pH or with Cd input (Table 7 and Table 8). Increasing pH shows higher grain yield but the differences were not statistically significant (Table 8). This kind of inconsistent trend in yield and Cd uptake may be due to field condition rather than pH and Cd input effect.

**Table 7:** Effect of Cd input on grain yield and Cd uptake in 1992.

| Input Cd (g/ha) | Yield (kg plant /ha) | Uptake Cd (mg/ha) |
|---|---|---|
| **0.03** | 940±158 | 109±22 |
| **2.7** | 1035±237 | 106±36 |
| **11.43** | 1003±152 | 96±15 |
| **$LSD_{0.05}$** | NS | NS |

**Table 8:** Effect of soil pH on grain yield and Cd uptake in 1992.

| pH | Yield (kg plant /ha) | Uptake Cd (mg/ha) |
|---|---|---|
| **4.48** | 773±54 | 84±24 |
| **5.06** | 1178±137 | 115±15 |
| **5.70** | 1027±60 | 113±26 |
| **$LSD_{0.05}$** | NS | NS |

### 4.3 Year 2006

#### 4.3.1 Soil Cadmium Concentration

The relationship between Cd input and total cadmium concentration is presented in Table 9. Soil cadmium concentration as well as extractable cadmium concentration is not significantly affected by different Cd input through fertilizers.

**Table 9:** Effect of Cd input on total and extractable Cd (mean ± std.devn) in soil in 2006.

| Cd Input | Total Cd (mg/kg) | Extractable Cd (mg/kg) |
|---|---|---|
| **0.03** | 0.186±0.019 | 0.004±0.003 |
| **2.7** | 0.215±0.007 | 0.004±0.003 |
| **11.43** | 0.221±0.015 | 0.006±0.004 |
| **$LSD_{0.05}$** | **NS** | **NS** |

The $NH_4NO_3^-$extractable Cd slightly increased with increase in Cd input (Figure 8). There is a positive but non-significant relationship between extractable cadmium and Cd input (Table 9). Only at pH 5.0 and 6.0 a positive trend is observed for all concentration of Cd input through fertilizers. But at pH 5.6 the extractable Cd doesn't show a positive trend relationship with increasing Cd input. At pH 5, extractable Cd was significantly higher than two pH levels at all level of Cd input.

However at the same Cd input, soil pH values play a major role for changing extractable Cd concentration. That is increases in soil pH value significantly decreases ($p<0.05$) $NH_4NO_3^-$ extractable Cd (Figure 8, Table 10).

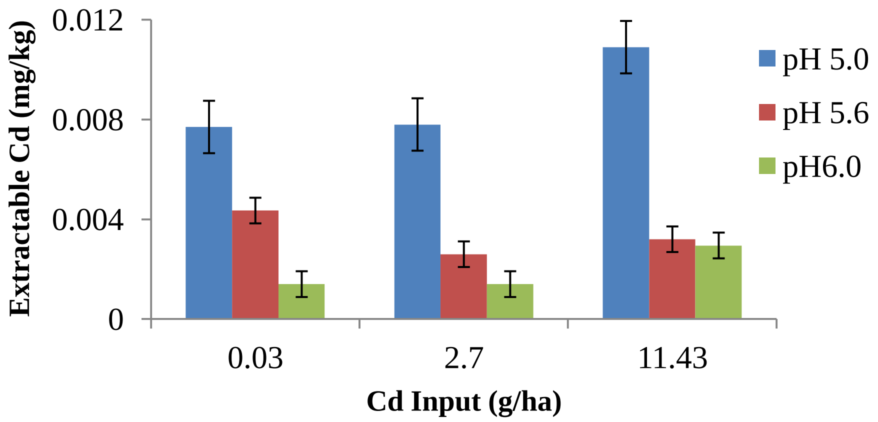


**Figure 8:** Effect of soil pH and Cd input on extractable Cd (2006).

**Table 10:** Effect of soil pH on extractable Cd in 2006.

| Soil pH | Extractable Cd (mg/kg) |
|---|---|
| **5.0** | 0.009±0.001 |
| **5.6** | 0.003±0.001 |
| **6.0** | 0.002±0.001 |

### 4.3.2 Plant Cadmium Concentration

Increase in Cd input through fertilizers from 0.03g/ha to 11.43 g/ha doesn’t significantly affect the Cd concentration in plant (Table 11). However, at 11.43g/ha Cd input plant Cd concentration is higher than the two other Cd inputs.

**Table 11:** Effect of Cd input on plant Cd in 2006.

| Input Cd (g/ha) | Plant Cd (mg/kg) |
|---|---|
| **0.03** | 0.025±0.009 |
| **2.7** | 0.025±0.009 |
| **11.43** | 0.029±0.011 |
| **$Lsd_{0.05}$** | NS |

Plant cadmium concentration varies significantly ($p < 0.05$) with soil pH. Increase in soil pH decreases cadmium concentration in plant (Figure 9).

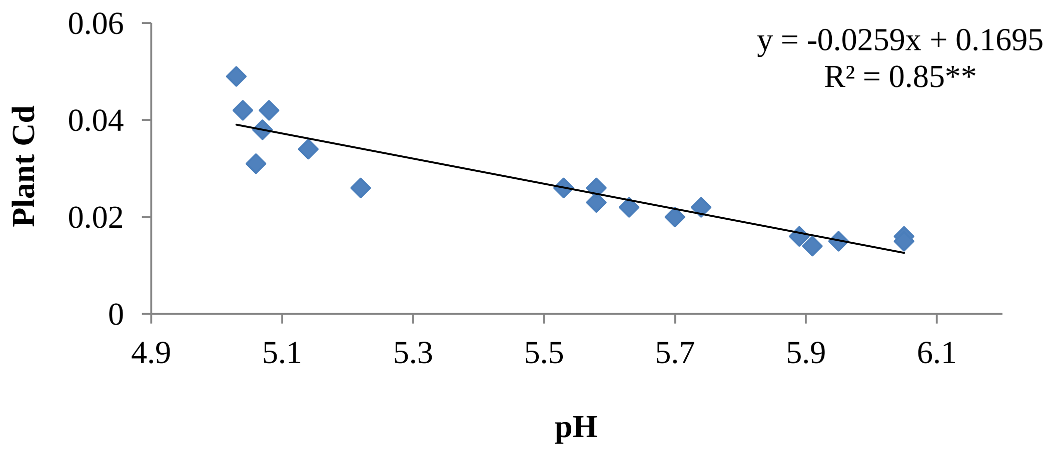


**Figure 9:** Effect of soil pH on plant Cd in 2006.

Figure 10 illustrates the comparison of plant cadmium concentration at different soil pH levels and Cd input. Increase in soil pH from 5.0 to 6.0 at all Cd level reduces plant Cd concentration. This rate of decrease is higher at the highest Cd input (11.43) than at two other levels of Cd input. So at higher level of Cd concentration (11.43), plant Cd concentration is highest even at low pH level (5.0).

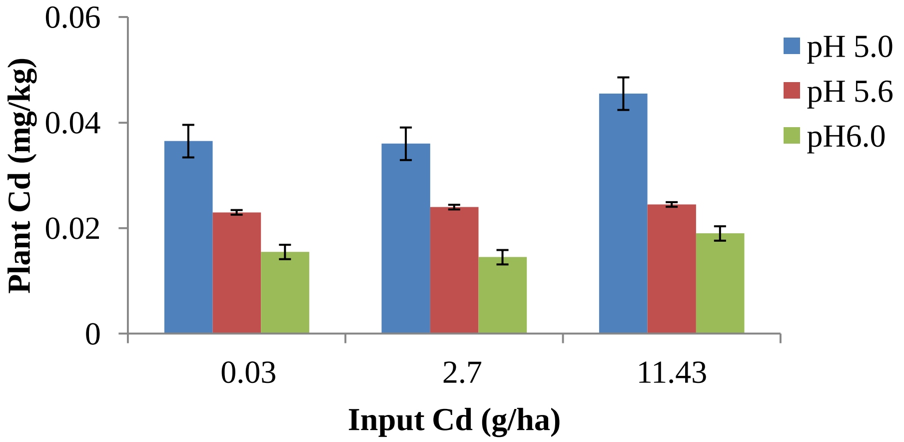


**Figure 10:** Effect of soil pH and Cd input on plant Cd (in 2006).

Soil samples were collected after harvest and analyzed for extractable Cd for comparing it with plant Cd. Plant cadmium concentration is positively correlated with extractable Cd ($R^2$=0.91 & p <0.05) (Figure 11). It may be due to solubility effect which is correlated well with plant uptake.

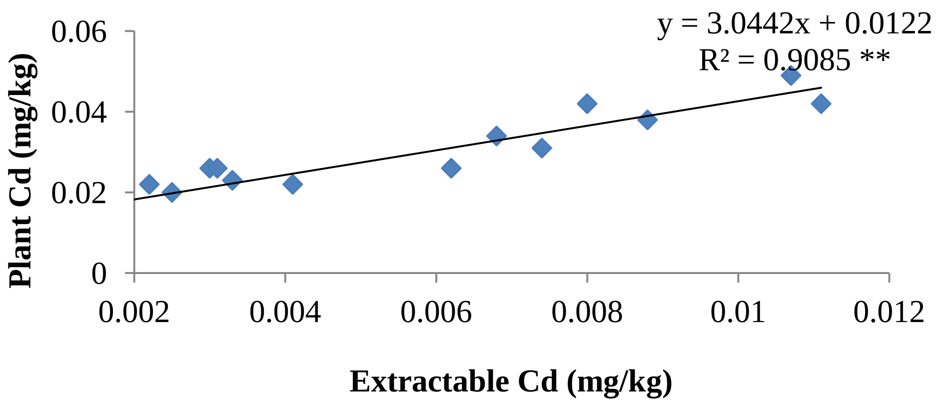


**Figure 11:** Correlation between plant Cd and extractable Cd of all soil samples in 2006.

### 4.3.3 Grain Yield and Cadmium Uptake

The total grain cadmium uptake is calculated by multiplying plant Cd concentration with dry matter yields from each plot of the field experiment. As NPK fertilizers were applied at the same rates (15 %N, 15% P and 15% K) to all plots, yield did not differ among different plots.

The crop yield was not significantly affected by changes in soil pH. However, plant Cd uptake decreases significantly with increase in soil pH level (Table 12). There is no significant relationship between grain yield and plant cadmium uptake with Cd input through fertilizers (Table 13).

**Table 12:** Effect of soil pH on grain yield and Cd uptake in 2006.

| pH | Yield (kg/ha) | Uptake Cd (mg/ha) |
|---|---|---|
| **5.0** | 1188±40 | 47±3.76 |
| **5.6** | 1247±33 | 30±0.45 |
| **6.0** | 1127±24 | 18±2.16 |
| **$LSD_{0.05}$** | NS | 26.85 |

**Table 13:** Effect of Cd input on grain yield and Cd uptake in 2006.

| Input Cd | Yield (kg plant /ha) | Uptake Cd (mg/ha) |
|---|---|---|
| **0.03** | 1186±69 | 30±11.09 |
| **2.7** | 1222±42 | 30±11.37 |
| **11.43** | 1154±38 | 34±13.06 |
| **$LSD_{0.05}$** | NS | NS |

So soil pH is the main factor affecting Cd uptake by plant. Furthermore, at the same level of Cd input, uptake of Cd is significantly higher at pH treatment 5.0 than at other pH levels. However, no significant difference in Cd uptake at pH 5.6 and 6.0 is observed (Figure 12).

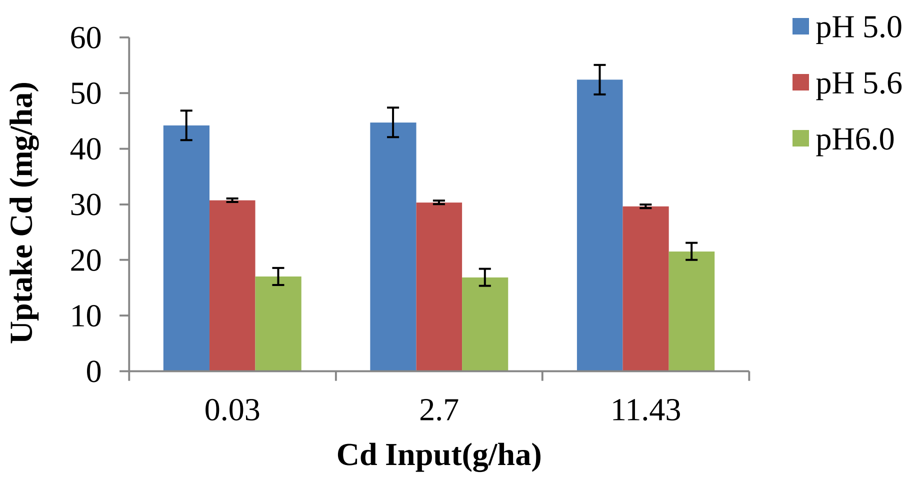


**Figure 12:** Effect of soil pH and Cd input on plant Cd uptake in 2006.

### 4.4 Year 1995, 1997 and 2002

#### 4.4.1 Extractable Cadmium Concentration

Comparing with 2006 and 1992 data an unexpected result between soil pH and extractable Cd was seen in 1995. There is no significant effect of soil pH on extractable Cd (Figure 13). The reason behind very weak correlation between soil pH and extractable Cd could be due to higher grain yield with greater absorption level which may modify effect of soil pH because greater proportion of soluble Cd was taken up by plants and thus leaving lower amount in the soil.

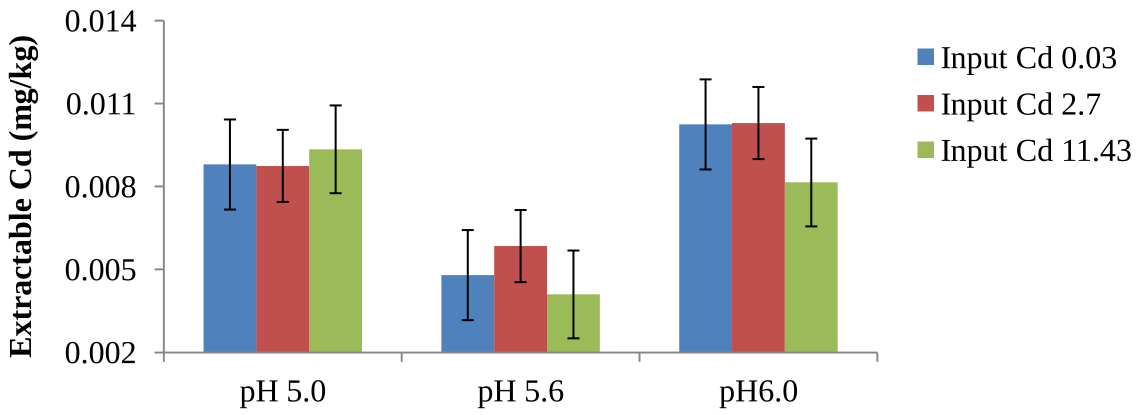


**Figure 13:** Effect of soil pH and Cd input on extractable Cd (1995).

#### 4.4.2 Plant Cadmium Concentration

Increase in concentration of Cd input through fertilizers has significant positive relationship with plant Cd concentration in year 1997 and 2002. However, year 1995 has non-significant negative correlation between plant Cd concentration and Cd input through fertilizers (Figure 14).

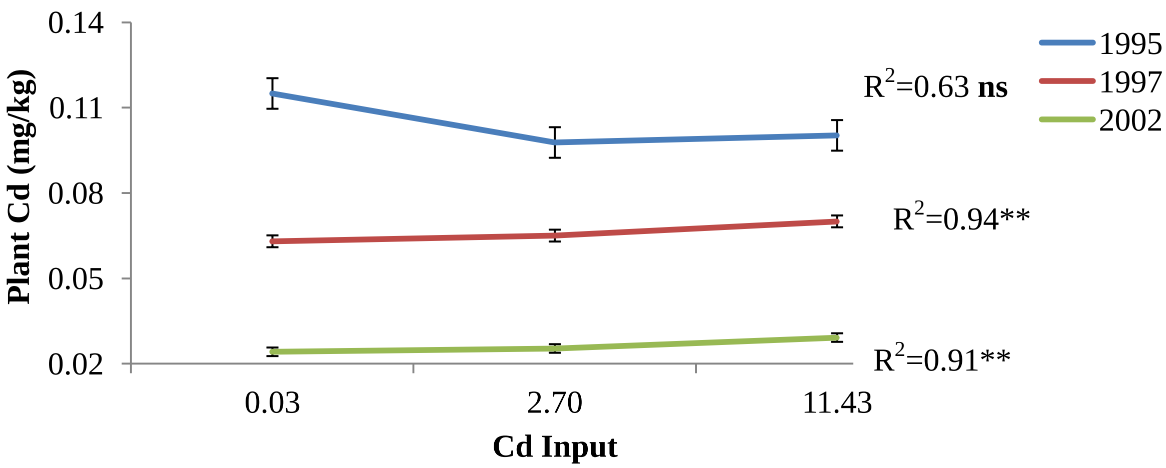


**Figure 14:** Effect of Cd input on plant Cd concentration in year 1995, 1997 and 2002.

In 1995, only two pH values i.e. pH 5.6 and pH 6.0 were studied. There is significant relationship ($p<0.05$) between soil pH level and plant Cd concentration (Figure 15). Years 1997 and 2002 show strong negative correlation between soil pH level and plant Cd concentration.

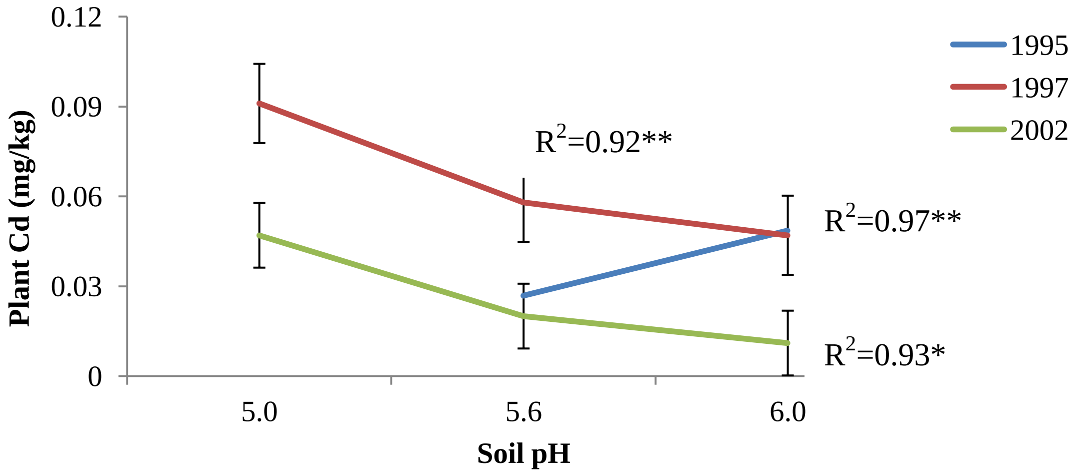


**Figure 15:** Effect of soil pH on plant Cd concentration for the year 1995. 1997 and 2002.

### 4.4.3 Grain yield and Cadmium uptake

The total cadmium uptake is the function of dry matter yields and plant Cd concentration. As same amount of fertilizers were applied in all experimental plots yield cannot be expected to increase. However, in the year 1995 due to unusual weather conditions, the grain yield was higher than the rest of years. And there is also significant difference between plant yield and soil pH value as yield increased with increase in soil pH level (Table 14).

**Table 14:** Effect of soil pH on plant yield and Cd uptake (1995).

| pH | Yield (kg plant /ha) | Uptake Cd (mg/ha) |
|---|---|---|
| **5.6** | 4628±209 | 124±11 |
| **6.0** | 4973±14 | 241±26 |
| **$LSD_{0.05}$** | 5.06 | NS |

But with Cd input, yield doesn't show any significant relationship (Figure 16). With increase in Cd input concentration from 0.03 to 2.7 yields get significantly increased for year 1995 and 2002 and further increase of Cd input from 2.7 to 11.43 decreases yield of plant. Hence there doesn't exist any significant relationship between input Cd and yield for year 1995 and 2002. However, for year 1997 a positive correlation between input Cd and yield occurs as shown in figure 16.

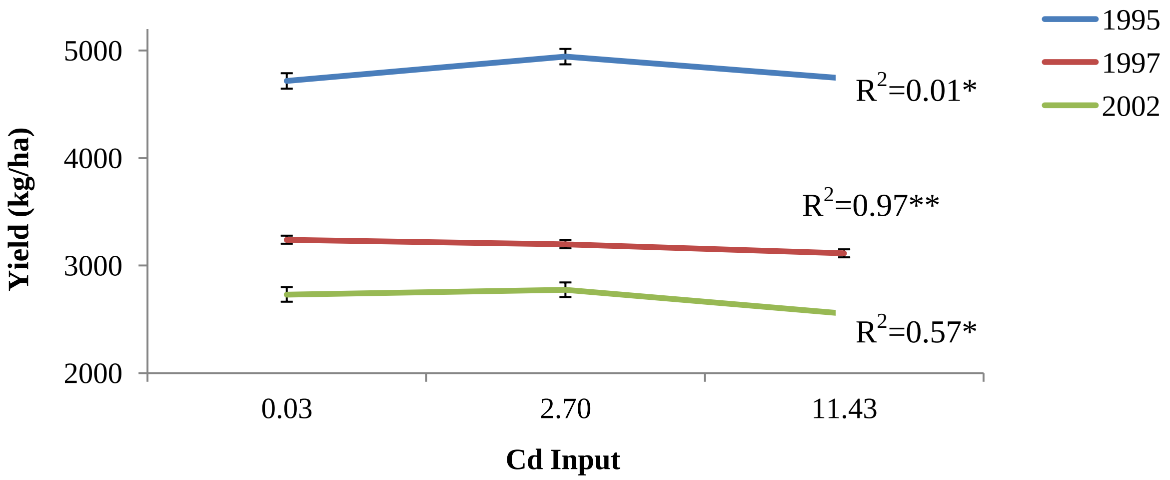


**Figure 16:** Effect of Cd input on yield of plant for year 1995, 1997 and 2002.

In year 1995, there exist a good correlation between soil pH and plant Cd uptake (Figure 17). It means soil pH does affect Cd uptake by plant.

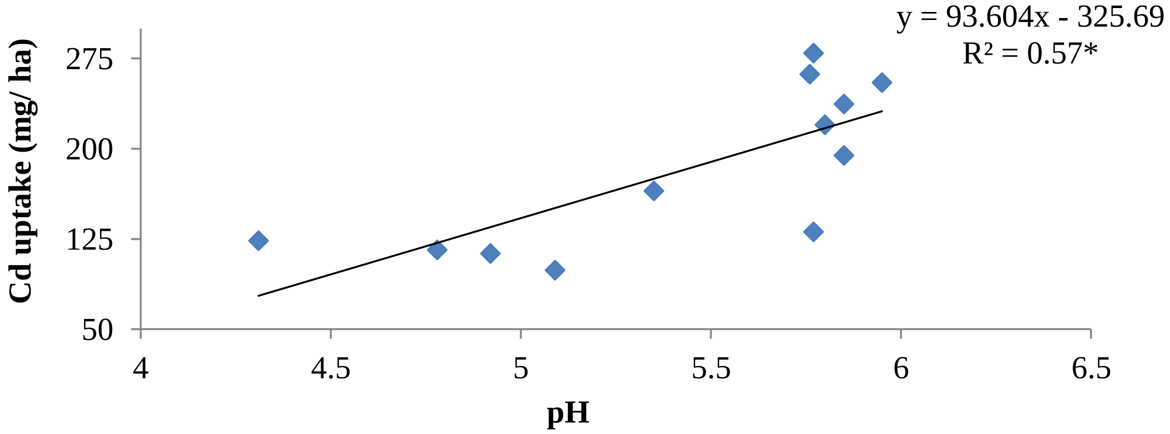


**Figure 17:** Effect of soil pH on Cd uptake by plant in 1995.

Furthermore, Cd uptake by plant was affected by crop yield (Figure 18). Therefore a weak positive correlation ($R^2$= 0.34) between these two variables does exist even though it was not found to be significant.

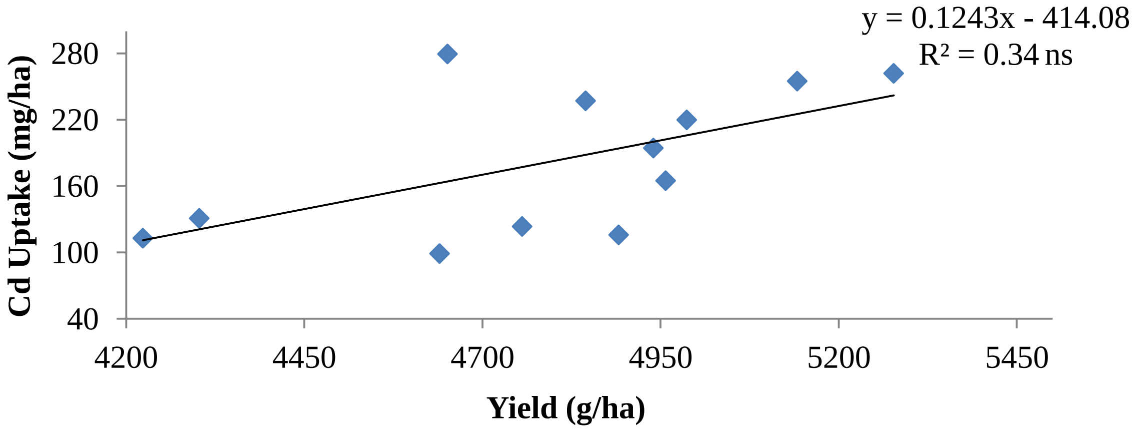


**Figure 18:** Effect of plant yield on plant Cd uptake in 1995.

Compare to 1992 and 2006, in 1995 two factors play major roles to control Cd absorption and plant Cd concentration. These two factors are grain yield and soil pH value. In these two controlling factors soil pH has more strong effect ($R^2$= 0.57) than plant yield ($R^2$= 0.34). However in year 1992 and 2006, only soil pH is major factor affecting absorption of plant Cd and total Cd concentration in plant.

## 4.5 Long term effect

The same three doses of Cd concentration (0.03, 2.7 and 11.43 g/ha) were applied through P fertilizer during 14 year of long term experiment. The changes in total Cd, extractable Cd, plant Cd, yield and uptake are described in this section.

### 4.5.1 Total Cd

Total cadmium concentration of soil varies differently during experimental years for each Cd treatment. For cadmium level 11.43, an increase in total soil Cd concentration was observed throughout experimental period. However, increment is more pronounced after year 1995. For 2.7 Cd input soil Cd decreased in 1995 but increased in 2006. However for cadmium level 0.03 soil cadmium increased consistely despite at lower rate than the higher Cd input levels.

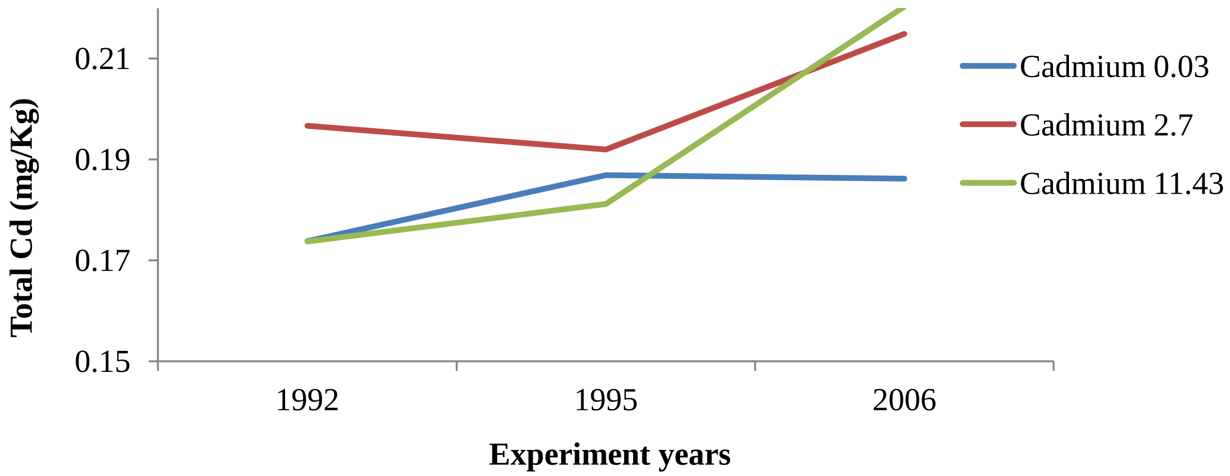


**Figure 19:** Long term effect of Cd input through fertilizers on total soil Cd concentration.

### 4.5.2 Extractable Cd

The data on extractable Cd is available only for the year 1992, 1995 and 2006. The year 1992 was the starting year and hence there is no significant difference in extractable Cd concentration. Higher correlation between Cd input and extractable Cd is observed for Cd level of 11.43 ($R^2$=0.57) and least ($R^2$=0.37) for Cd level 2.7 (Figure 20).

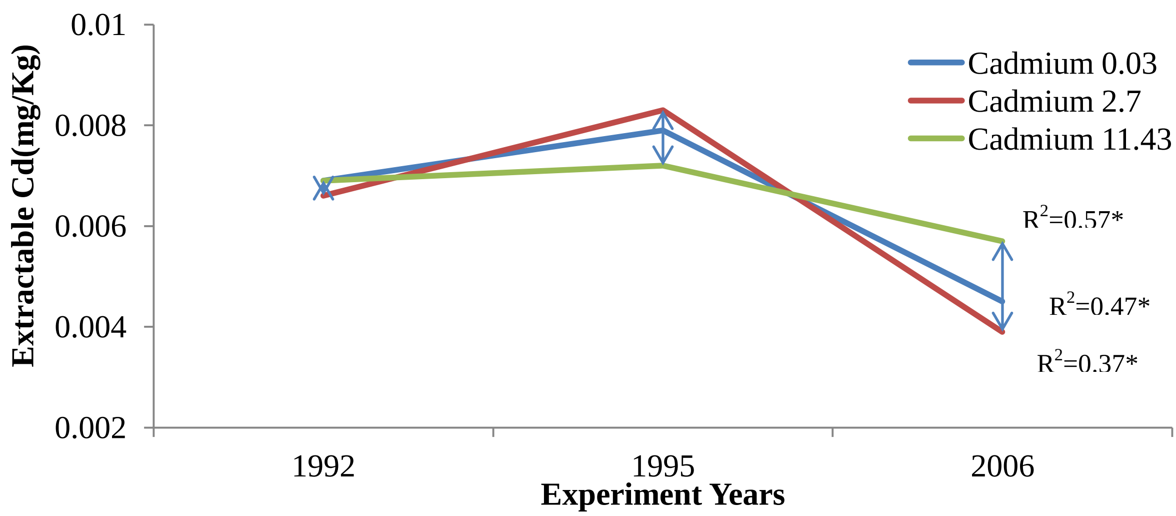


**Figure 20:** Long term effect of Cd input on extractable Cd concentration.

### 4.5.3 Plant Cd

Cadmium concentration in oat crop decreases during the period of 1992-1995 and then increases from 1995-1997, being highest in 1997 and then it decreases until 2006 (Figure 21). The differences in Cd among the years were significant ($p < 0.05$).

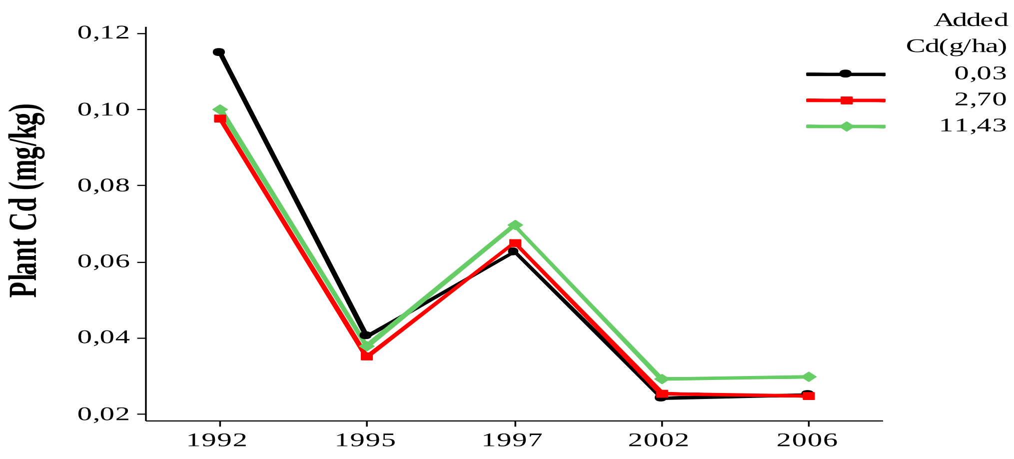


**Figure 21:** Effect of Cd input on Plant Cd during the long term experimental years.

In general plant Cd concentration decreases with increase in Cd input with minor exceptions (Table 15). Except for the year 2002, all the other years show non-significant relationship of plant Cd with Cd input.

**Table 15:** Relationship between plant Cd and Cd input with respect to year.

| **Input Cd (g/ha)** | **Plant Cd (mg/kg)** | | | | |
|---|---|---|---|---|---|
| | **1992** | **1995** | **1997** | **2002** | **2006** |
| **0.03** | 0.12±0.015 | 0.04±0.014 | 0.06±0.016 | 0.03±0.013 | 0.03±0.009 |
| **2.7** | 0.09±0.032 | 0.03±0.007 | 0.05±0.02 | 0.03±0.014 | 0.02±0.009 |
| **11.43** | 0.09±0.026 | 0.03±0.011 | 0.07±0.02 | 0.03±0.018 | 0.03±0.011 |
| **P Value** | *0.0698(ns)* | *>0.10(ns)* | *0.0453(ns)* | *0.0012* | *>0.10(ns)* |

Increase in soil pH decreases Cd concentration in plant. Lowest plant Cd concentration is observed in 2002 with pH 6 and it can be due to greater split of Cd into biomass tissues than the rest of the years. Compare with other years, 1997 and 2002 show significant relationship between plant Cd and change in soil pH. Hence we may conclude that soil pH plays a major role in controlling plant Cd than Cd input during the long term experimental period.

**Table 16:** Long term relationship between plant Cd and soil pH level.

| pH level | Plant Cd (mg/kg) | | | | |
|---|---|---|---|---|---|
| | **1992** | **1995** | **1997** | **2002** | **2006** |
| **5.0** | 0.10±0.028 | Na | 0.09±0.005 | 0.05±0.005 | 0.04±0.004 |
| **5.6** | 0.09±0.026 | 0.03±0.001 | 0.06±0.004 | 0.02±0.001 | 0.02±0.001 |
| **6.0** | 0.09±0.023 | 0.05±0.005 | 0.05±0.001 | 0.01±0.001 | 0.02±0.002 |
| **P Value** | *0.069(ns)* | *>0.10(ns)* | *0.006* | *0.001* | *>0.10(ns)* |

### 4.5.4 Grain Yield

Grain yield increases from year 1992 to 1995 and then it decreases significantly from 1995 to 2006. As shown in Figure 22, there is no significant difference in yield of plant during initial and final period of experiment or with level of Cd input

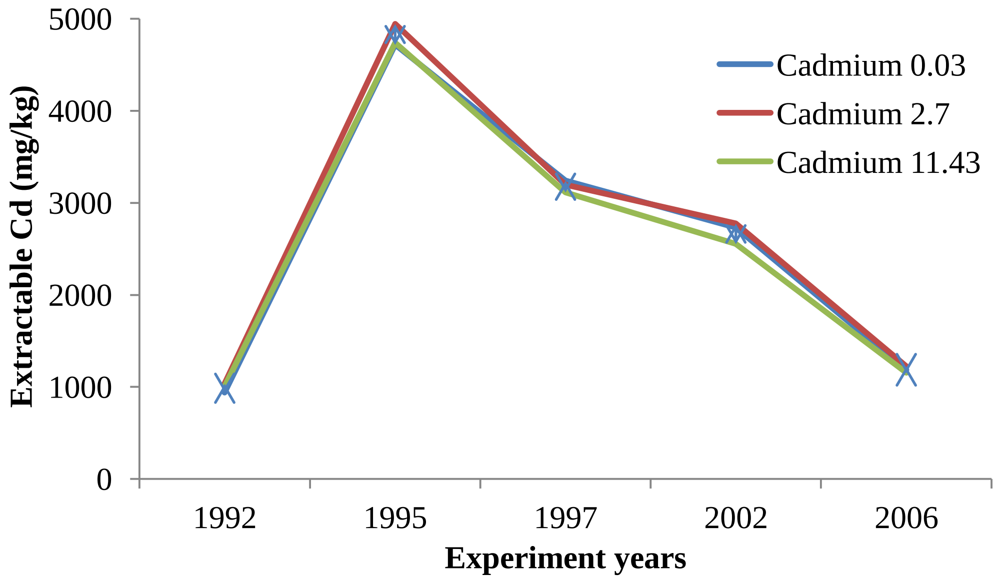


**Figure 22:** Long term relationship between yield and Cd input.

Intermediate pH (5.6) gives higher grain yield than the lower or higher pH levels for all the experimental years and the yield differences among pH levels are statistically significant for all the years (Table 17).

**Table 17:** Long term effect of pH on grain yield of plant.

| pH level | Grain yield kg/ha | | | | |
|---|---|---|---|---|---|
| | **1992** | **1995** | **1997** | **2002** | **2006** |
| **5.0** | 773±54 | na | 2972±333 | 2614±58 | 1188±40 |
| **5.6** | 1178±137 | 4628±209 | 3383±206 | 2855±109 | 1247±33 |
| **6.0** | 1027±60 | 4973±13 | 3046±76 | 2589±219 | 1127±24 |
| **P Value** | *P<0.01*** | *P<0.01*** | *P<0.01*** | *P<0.01*** | *P<0.01*** |

### 4.5.5 Yield-Uptake relationship

Figure 23, demonstrates yield and Cd uptake relationship. Uptake by plant is directly related to yield of plant and higher yield means higher Cd uptake by plant. A significant effect ($p<0.05$) of Cd uptake has been observed as a result of long-term fertilization. However, this effect is not clear in 1997 and 2006 being the year's highest and lowest crop yields respectively. Good correlation between yield and uptake was noticed in year 1995 with $R^2$=0.64 and lowest in year 2006 ($R^2$=0.087), 1997 ($R^2$=0.021) and 2002 ($R^2$=0.023).

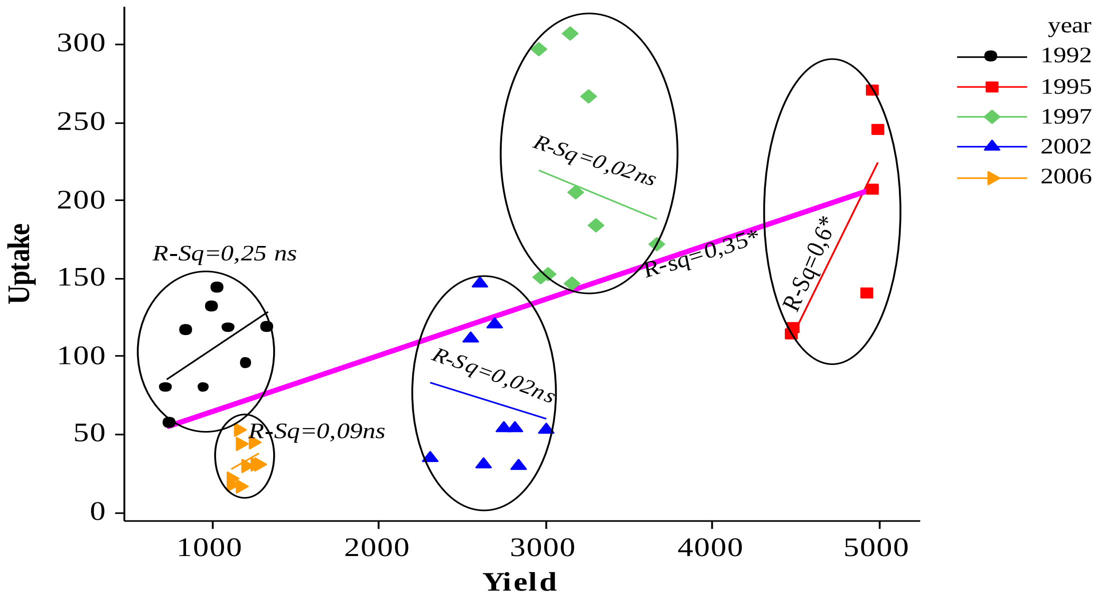


**Figure 23:** Long term relationship between Cd uptake and grain yield for all the soil samples collected after harvesting.

## 4.6 Correlation Matrix

Correlation matrix among different plant parameters is presented in Table 18. The correlation matrix showed that initial field pH has a positive and significant correlation with initial extractable Cd (r = 0.959**) and final extractable Cd (r = 0.87**). Similarly pH of 2006 has positive significant correlation with yield in 2006 (r = 0.97**). At the beginning of experimental period yield has positive correlation with total soil Cd content (r = 0.75**). However yield in 2006 is independent of total soil Cd in 2006 (r = -0.12**) but is dependent on initial soil Cd content (r = 0.88**). Also Cd uptake in 2006 is strongly correlated with extractable Cd in 2006 (r = 0.95**), plant Cd 2006 (r = 0.97**) and with total soil Cd 2006 (r = 0.65*). There seems to have a strong negative correlation of yield in 2006 with extractable Cd 1992 (r =-0.852**), extractable Cd 2006 (r = -0.97**), initial soil pH (r = -0.97**) and plant Cd in 2006 (r = -0.87**). Also uptake of Cd in 2006 has strong negative correlation with pH in 2006(r = -0.95**), yield in 2006 (r = -0.85**) and with initial uptake of soil Cd (r = -0.97**). The above results indicated that oat plant yield was dependent on pH and initial soil Cd concentration. However it is independent on added Cd concentration for particular year and also uptake of Cd is dependent on extractable Cd present in soil and with pH of soil but is independent of initial plant Cd concentration.

**Table 18:** Correlation matrix among different parameters as influenced by treatments.

| Pearson Correlations | *Ex. Cd 1992* | *Ex. Cd 2006* | *pH 1992* | *pH 2006* | *Plant Cd 1992* | *Plant Cd 2006* | *Total Cd 1992* | *Total Cd 2006* | *Yield 1992* | *Yield 2006* | *Uptake 1992* | *Uptake 2006* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Extract. Cd 1992** | 1 | | | | | | | | | | | |
| **Extract. Cd 2006** | 0.703 | 1 | | | | | | | | | | |
| **pH1992** | 0.959 | 0.87 | 1 | | | | | | | | | |
| **pH 2006** | -0.696 | -0.96 | -0.87 | 1 | | | | | | | | |
| **Plant Cd 1992** | 0.655 | -0.07 | 0.41 | 0.09 | 1 | | | | | | | |
| **Plant Cd 2006** | 0.478 | 0.96 | 0.71 | -0.96 | -0.35 | 1 | | | | | | |
| **Total Cd 1992** | -0.998 | -0.74 | -0.97 | 0.74 | -0.61 | -0.53 | 1 | | | | | |
| **Total Cd 2006** | -0.419 | 0.35 | -0.15 | -0.36 | -0.96 | 0.59 | 0.37 | 1 | | | | |
| **Yield 1992** | -0.791 | -0.12 | -0.59 | 0.12 | -0.98 | 0.16 | 0.75 | 0.89 | 1 | | | |
| **Yield 2006** | -0.852 | -0.97 | -0.97 | 0.97 | -0.16 | -0.87 | 0.88 | -0.12 | 0.35 | 1 | | |
| **Uptake 1992** | -0.232 | -0.85 | -0.5 | 0.86 | 0.583 | -0.97 | 0.29 | -0.79 | -0.41 | 0.71 | 1 | |
| **Uptake 2006** | 0.451 | 0.95 | 0.69 | -0.95 | -0.38 | 0.97 | -0.5 | 0.62 | 0.19 | -0.85 | -0.97 | 1 |

# CHAPTER FIVE

# DISCUSSION

## 5.1 Soil Cadmium Concentration

For most of the year's total soil cadmium concentration as well extractable cadmium concentration was not affected by different levels of Cd input. It may be due to relatively minor contribution of Cd input (0.03-11.43 g/ha) in comparison with Cd already present in the soil. Other factor that may reduce Cd bioavailability is fixation of Cd in soil pools that are inaccessible to plants. Similar kind of result was also reported by Jeng and Singh (1995) during the long term experimental period from 1963 to 199,where only minor changes in Cd content of soil samples from the treated plots with organic and inorganic fertilizers was noticed. Grant *et al.,* (1992) also did not find in their experiment any major change in Cd in durum wheat grain. The results reported by Sato *et al.,* (2010), Tan (2008) and Meers *et al.,* (2007) also support this finding. However, in a greenhouse experiment, He and Singh (1994a) reported that increasing the Cd input from 2,7 μg Cd $kg^{-1}$ soil to 12,5 μg Cd $kg^{-1}$ soil through P fertilizers resulted in an increase in total Cd in a loam soil from 18,7 μg Cd $pot^{-1}$ soil to 87,0 μg Cd $pot^{-1}$ soil.

The positive and non-significant relationship between $NH_4NO_3^-$extractable Cd and Cd input through fertilizers was also reported by Jeng and Singh (1995), Singh and Myhr (1997), Lambert *et al.,* 2007, and Grant and Sheppard (2008). Similar lack of relationship was also reported by Singh *et al.,* (1997) and He and Singh (1994b).

But the case is not always true when interacting effects of Cd input and soil pH are taken into consideration, as the effects may be into different directions. Increase in soil pH means increase in deprotonation of carboxyl and hydroxyl groups. Therefore negative charge density in the soil solution is greater at low pH than at high pH. The Cd ions are positively charged and thus more chances of binding with soil colloids thus increase the absorption by soil, decreasing the availability by the crop. Such results are reported by several authors (He and Singh, 1994b; Singh *et al.,* 1995; Guttormsen *et al.,* 1995; He and Singh, 1994b; Gao and Grant, 2012; Jiao *et al.,* 2004).

Hence we can justify soil pH as the most important factor regulating the amount of Cd available to plants in soil solution. This result is supported by Wang *et al.,* (2006) where they found significant increase of extractable Cd with decrease in soil pH for plant *Thalspi caerulescens*.

Similar trend between pH and extractable Cd was also observed in earlier investigations (Singh *et al.,* 1995; Guttormsen. *et al.,* 1995; He and Singh, 1994b). However, Whitten and Ritchie (1991) reported an increment of extractable Cd with increasing soil pH in an experiment with clover plant.

### 5.2 Plant Cadmium Concentration

Increase in Cd input through fertilizers from 0,03g/ha to 11,43g/ha did not show significantly relationship with Cd concentration in plant. However, slight increase of plant Cd concentration was noticed for most of the experimental year. Similar kind of finding was noticed by Gao and Grant (2012), Grant *et al.,* (2009), Grant and Shephard (2008), Singh and Kashem (2002) and Gao *et al.,* (2010). Similarly, within the same range of Cd concentrations as used in this experiment, Guttormsen *et al.,* (1995) reported significantly increased plant Cd concentration with increase in cadmium input through fertilizers. Similar kind of positive relation was noticed by Perriguey *et al.,* (2007) in their experiment with maize plant where they found increment in root Cd concentration according to increment of Cd in nutrient solution. The reason behind this could be due to sorption/desorption phenomenon at soil colloid. The phytoavailability of Cd in soil depends upon the concentration of Cd in soil solution and the ability of the solid phase to replenish Cd. Low concentrations of Cd in soil solution is probably controlled like other trace metals by sorption/desorption phenomena and increase of Cd concentration in soil solution increases plant cadmium concentration.

The total Cd accumulation is a measure of total soil Cd and accumulated P and support the assumption of a common origin for these elements, that is, as constituents of phosphate fertilizers. Simultaneous accumulation of Cd and phosphate fertilizers has too been observed in Gerritse *et al.,* (1998) and Wong (1985).

Increase in soil pH decreases plant cadmium concentration. These results are in accordance with those reported for extractable Cd and is supported by He and Singh (1994a), where increases in soil pH significantly decreases Cd concentrations in ryegrass, carrot and spinach in loam and sandy soil, except for oat grain in sandy soil. Guttormsen *et al.,* (1995); Singh and Myhr (1997) also report similar kind of findings in their experiments. Similar kind of linear relationship between plant Cd concentration and soil pH was also observed by Mench *et al.,* (1997) in their experiment of five soil series on wheat plant. Ten folds decrease in plant Cd concentration with liming has also been noticed by Ciecko *et al.,* (2001) in their experiment on *Triticale* and oil rapeseed. Mench *et al.,* (1997), Ciecko *et al.,* (2001) too support this finding in their separate experiment with wheat and rapeseed.

Soil pH is a major factor influencing Cd solubility and mobility in soils. Cd Solubility and ion activity decreases with increasing pH that finally increases Cd sorption. Therefore, the soil distribution coefficient ($K_d$), calculated as the ratio between the Cd concentration in the soil and the liquid phase, increases with increasing pH, while phytoavailability correspondingly decreases. Similar kind of findings was suggested by Holm *et al.,* (1995); Sauve *et al.,* (2000); Rieuwerts *et al.,* (2006), Gonzalez *et al.,* (2006) and Naidu *et al.,* (1994).

Moreover, interacting treatments, where both pH as well as Cd input is taken under consideration, shows greater decrease in plant Cd concentration with increase in pH and Cd input. It is due to the interacting effect where pH controls the solubility which is correlated well with plant uptake. Oliver *et al.,* (1995) find increase in pH range of soil from 4 to 6 after combine treatment of lime and sulfur decreases grain Cd concentrations from 0% to 83%. Under controlled conditions, Choudhary *et al.,* (1994) applied reagent grade mono-ammonium phosphate to wheat which increased Cd with decrease in soil pH level. It is due to solubility and ion activity which decreases with increase in pH that finally increases sorption of Cd. Effect of soil pH on Cd sorption too depends on Cd loading, the nature of soil constituents and the Cd sorption capacity of soils. This result is in consistent with finding of Haldar and Mandal (1981) on rice plant.

However, for year 1992 no relationship between soil pH, input Cd and plant Cd concentration could be assigned to poor growing condition and poor grain yield (Table 6, Figure 6). This can partly be explained by the failure of wheat and barley in the crop rotation at the lowest pH treatment and hence oat sown. The poor yields, low and untimely rains are other factors. Strong positive correlation between plant Cd and extractable Cd is due to the same effect of soil pH in both plant Cd and extractable Cd concentration. It is because of increase of sorption of soil Cd by increase in soil pH that reduces its extractability. Hence strong correlation was seen between plant Cd and extractable Cd.

This type of relationship was also explained by Guttormsen *et al.,* (1995). However, He and Singh (1994a) reported no significant relationship between oat and rye grass grown in a loamy soil in their green house experiment.

## 5.3 Grain Yield and Cadmium Uptake

Application of the same doses of fertilizers in each plot means we cannot expect any significant difference in yield of plant and therefore yield did not differ much in any of the year under study. On an average higher yield of plant was observed in treatment field with low input Cd level i.e 0.03 $gha^{-1}yr^{-1}$. Similar kinds of results were noticed by Zhang *et al.,* (2002) in their solution culture experiment with wheat genotypes. But in contrast to our findings relative contribution of soil and fertilizer Cd was described in some previous studies by Eriksson (1988) on rapeseed, Land *et al.,* (1981) in swiss chard and radish crops. 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 $Cdkg^{-1}$ soil was added. Similarly soil pH doesn't significantly affect crop yield. This implies that at high rates of Cd addition, soil properties controlled the Cd uptake by plants, but at low rates, the Cd uptake was controlled by the Cd present in the soil (Singh and Myhr, 1998).

## 5.4 Long Term Effect

### 5.4.1 Plant Cd Concentration

During the long term experiment, plant Cd concentration in year 2006 decreases significantly ($p<0.05$) than year 1992 with respect to Cd input. The reason behind this could be due to dependency of Cd uptake with both Cd concentration in plant and its yield which results in highest Cd uptake in 1995. Guttormsen *et al.,* (1995) reported increase of Cd concentration in carrot and cabbage crops in the succeeding years. The reason behind this could be due to reduction in the available pool of Cd by fixation and/or Cd removal by the previous crop. Similarly no relation was found between Cd introduced into the soil by phosphate and Cd present in potato tubers in experiment of McLaughlin *et al.,* (1994) and Tiller *et al.,* (1997). Similar kind of non significant relationship between soil Cd and plant Cd with respect to year is reported by Jeng and Singh (1995) where they conclude that elevated Cd concentration in the soil did not increase Cd concentration in plants.

Plant Cd concentration decreases with increase in soil pH treatment for all of the year except 1995. The reason behind this is due to greater solubility of Cd in phosphate fertilizer at lower soil pH level. This result is supported by Eriksson (1989); He and Singh (1994a) where they records decreased plant Cd level with increased pH levels. Wang *et al.,* (2006) observed significant decrease of plant Cd with increase in soil pH for both root and shoot of *Thalspi* *caerulescens*. Our results are supported by McLaughlin *et al.,* (1996) hypothesis (experimentally not proved) that increasing pH of soils have fairly low affinity for Cd and minimal changes in affinity with pH, so that the small increase in $Cd^{2+}$ retention by the soil surfaces due to increasing pH is rather offset by the increased $Ca^{2+}$ concentrations in soil solution competing with Cd for sorption sites and increasing solution $Cd^{2+}$. Many other researchers; Almås *et al.,* (2007); Gandois *et al.,* (2010); Groenenberg *et al.,* (2009); Sauve *et al.,* (2000) and Tipping *et al.,* (2003) showed soil pH and organic matter present in soil as the main factors controlling the solubility of Cd. However, our results are not in consistent with an earlier study (Hatch, *et. al.,* 1988) where concentration of cadmium taken up by lettuce, ryegrass and watercress increases by factor of 4, 8 and 10 with increasing pH of the soil from 5.0 to 7.0.

### 5.4.2 Grain Yield and Extractable Cd

Similar to plant Cd concentration, grain yield of plant doesn't vary significantly during the long term experimental period with respect to input Cd concentration as treatment effect. The rate of N, P, and K application was uniform in all the treatments during the entire experimental period and hence yield differences are not expected. Similarly grain yield decreases with each of the year for each pH treatment level. This decrease in grain yield over the year is due to environmental factor such as rain distribution during the growth period. So yield variation over the year cannot be attributed to the amount of Cd input and pH. Higher grain yield at high pH level could be due to sensitiveness of plant to soil pH. Although long-term use of Cd enriched P-fertilizers increased the total concentration of Cd in soil, the impact on wheat production was insignificant in soils maintained at more alkaline range of normal cultivated soil pH levels (Chaudhary *et al.,* 2011).

Soil pH is an important factor affecting Cd-extractability in soils owing to its effect on adsorption sites. In general, increase in pH increases the sorption of Cd by soils and thus reduces its extractability (Singh and Myhr, 1998). Cd loading, the nature of soil constituents and the Cd sorption capacity of soils are major factors that affect Cd sorption by changes in soil pH. Among other extractant used for determining extractability of Cd, $NH_4NO_3^-$ is known to extract mainly the exchangeable fraction of Cd and hence is more sensitive to change in soil pH (He and Singh, 1994b). In our study $NH_4NO_3^-$ extractable Cd decreased at the higher pH level. Even though the extractable Cd in the soil increased with increased rate of Cd input through fertilizers, plant uptake of Cd was affected to a very limited extent. This implies that only one parameter i.e. extractable Cd, showed an increasing trend with increasing rate of Cd application, but the second parameter, i.e. plant uptake of Cd, generally remained unchanged. Hence there exist poor and non-significant correlation between plant uptake and extractable Cd. This finding is in consistent with finding of Singh and Myhr, (1998) in their cadmium uptake experiment with NPK fertilizers and phosphate rock in central part of Norway for four cropping season of barley on silt loam soil with pH adjustment of 5.5 to 6.5. Similar kind of significant relation between plant Cd and extractable Cd was observed by He and Singh (1994b) on their pot experiments conducted with similar Cd sources through fertilizers. However, Sauerbeck and Styperck (1985) reported less satisfactory correlation between extractable Cd and plant Cd in field with experiments uncontaminated soils.

# CHAPTER SIX

# CONCLUSION

Phosphorus is an essential nutrient for crop production, and an adequate P supply is required for achieving optimum crop yields. Long-term field experiments with focus on Cd are of importance as heavy dose of it is considered toxic for plant growth and development. This is also a major concern of chemical fertilizer industry whose major objective is to reduce the concentration of toxic elements like Cd.

In this long term experiment the major objective was to assess effect of Cd input and soil pH on the availability of Cd to plant. The results showed that input Cd has no significant effect on either plant Cd or soil Cd. Hence we can conclude that plant Cd as well as soil Cd did not change over time at the rates of Cd added within the range used in this experiment.

However, soil pH shows negative effect on Cd uptake and its availability to oat plant throughout the experiment. Due to occasional (every 4$^{th}$ or 5$^{th}$ year) liming soil pH was increased over 14 years leading to decreased plant Cd uptake and soil Cd concentration. Beside soil pH, grain yield also affect Cd uptake by oat plant as highest Cd content was noted at highest grain yield.

However, slight increase in extractable Cd in soil was observed after continuous fertilization. Since there was no significant effect of the rate of soil Cd application on the yield and plant Cd uptake but long term use of Cd enriched P-fertilizers increase the total concentration of soil Cd. Even though soil Cd concentration increases with increase in Cd enriched phosphorus fertilizer but bioavailability of Cd actually depends on soil pH rather than Cd input through fertilizers. So long term application of Cd enriched phosphorus fertilizer doesn’t increase plant Cd and Cd uptake.

Therefore we can summarize that reduction of the Cd accumulation in the soil can be obtained by using phosphorus fertilizers containing low Cd concentration.

# REFERENCES


Adams, M. L., Zhao, F. J., McGrath, S. P., Nicholson, F. A. & Chambers, B. J. 2004. Predicting cadmium concentrations in wheat and barley grain using soil properties. *J. Environ. Qual*.,33, 532-541.

Adriano, D.C. 2001. Trace elements in terrestrial environments, *Springer-Verlag*, New York, Berlin.

Ahsan, N., Lee, D. G., Lee, S. H., Kang, K.Y., Lee, J. J., Kim, P. J., Yoon, H. S., Kim, J. S. & Lee, B. H. 2007. Excess copper induced physiological and proteomic changes in germinating rice seeds. *Chemosphere*, 67, 1182-1193.

Akahane, I., Makino, T. & Maejima, Y. 2010. Effects of nitrogen fertilizer pH and electrical conductivity on the solubility of cadmium in soil solution. *Pedologist*, 53, 101–107.

Akhter, Fardausi. 2012. "Cadmium Accumulation and Distribution in Lettuce and Barley". *University of Western Ontario - Electronic Thesis and Dissertation Repository*. Paper 756. http://ir.lib.uwo.ca/etd/756

Alloway, B. J. & Steinnes, E. 1999. Anthropogenic addition of cadmium to soils. *In* M.J. McLaughlin & B.R.Singh 1999. Cadmium in Soils and Plants, *Dordrecht: Kluwer Academic Publishers*, 97-123.

Almås, Å. R. , Lofts, S., Tipping, E. & Mulder, J. 2007. Solubility of major cations and Cu, Zn and Cd in soil extracts of some contaminated agricultural soils near a zinc smelter in Norway: modelling with a multisurface extension of Wham. *Eur J Soil Sci*, 58, 1074–1086

Almås, Å. R. 2001. Changes in tolerance of soil microbial communities to Zn and Cd contaminations. Preliminary results. *Bioavailability 2001*, Chemical bioavailability in the terrestrial environment. Int. Conference, South Australia, Adelaide.

Andersson, A, & Nilsson, K. O. 1974. Influence of lime and soil pH on cadmium availability to plants. *Ambio*, 3, 198-200.

Andersson, A. & Siman, G. 1991. Levels of cadmium and some other trace elements in soils and crops as influenced by lime and fertiliser level. *Acta Agric Scand*, 41, 3–11.

Andrewes, P., Town, R. M., Hedley, M. J., & Loganathan, P. 1996. Measurement of plant available cadmium in New Zealand soils. *Australian Journal of Soil Research*, 34, 441-452.

ATSDR, 2011. Priority lists of hazardous substances. Retrieved from http://www.atsdr.cdc.gov/SPL/index.html

Baerug, R. & Singh, B. R. 1990. Cadmium levels in soils and crops after long-term use of commercial fertilizers. *Nor J Agric Sci,* 4, 251-60.

Basta, N.T., Ryan, J. A. & Chaney, R. L. 2005. Trace element chemistry in residual-treated soils: key concepts and metal bioavailability *J. Environ. Qual,* 34, 49-63.

Basta, N. T., Gradwohl, R., Snethena, K. L. & Schrodera, J. L., 2001. Chemical immobilization of lead, zinc, and cadmium in Smelter-contaminated soils using Biosolids and rock phosphate. *J. Environ. Qual*, 30, 1222-1230.

Bingham, F. T., Page, A.L., & Strong, J. E., 1980. Yield and cadmium content of rice grain in relation to addition rates of cadmium, copper, nickel, and zinc with sewage sludge and liming. *Soil Science,* 130, 32-38.

Bolan, N. S., Adriano, D. C., Duraisamy, P., Mani, A. & Arulmozhiselvan, K. 2003a. Immobilization and phytoavailability of cadmium in variable charge soils. I. Effect of phosphate addition. *Plant and Soil,* 250, 83-94.

Bolan, N. S., Adriano, D., Mani, P. & Duraisamy, A. 2003b. Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of lime addition. *Plant and Soil,* 251, 187-198.

Brennan, R. F. & Bolland, M. D. A. 2004. Wheat and canola response to concentrations of phosphorus and cadmium in a sandy soil. *Aust. J. Exp. Agric,* 44, 1025-1029.

Cakmak, D., Saljnikov, E., Mrvic, V., Jakovljevic, M., Marjanovic, Z., Sikiric, B. & Maksimovic, S. 2010. Soil properties and trace elements contents following 40 Years of phosphate fertilization. *J. Environ. Qual,* 39, 541-547.

Carrillo-Gonzalez, R., Simunek, J., Sauve, S. & Adriano, D. 2006. Mechanisms and pathways of trace element mobility in soils. *Adv. Agron,* 91: 111-178.

Chaney, R. L. & Hornick, S.B. 1978. Accumulation and effects of cadmium on crops. *In* J.Cole and S. Hiscock (eds.) Cadmium 77: First International Cadmium Conference.

Chang, A. C., Page, A. L. & Warneke, J. E. 1987. Long-term sludge application on cadmium and zinc accumulation in swiss chard and radish. *Journal of Environmental Quality,* 16, 217-221.

Chaudhary, M., Mobbs, H., Almås, Å. & Singh, B. 2011. Assessing long-term changes in cadmium availability from Cd-enriched fertilizers at different pH by isotopic dilution. *Nutrient Cycling in Agroecosystems,* 91, 109-117.

Chen, L., Guo, Y., Yang, L., & Wang, Q. 2007. SEC-ICP-MS and ESI-MS/MS for analyzing in vitro and in vivo Cd-phytochelatin complexes in a Cd-hyperaccumulator Brassica chinensis. *J Anal At Spectrom,* 22: 1403-8.

Chen, W. P., Krage, N., Wu, L. S., Page, A. L. & Chang, A. C. 2008. Fertilizer application and heavy metal contents in vegetable production soils of California. *Water Air Soil Poll*, 190, 209-219.

Chen, Y., Wang, C., Wang, Z. & Huang, S. 2004. Assessment of the contamination and genotoxicity of soils irrigated with wastewater. *Plant and Soil*, 261, 189-196.

Choudhary, M., Bailey, L. D. & Grant, C. A. 1994. Effects of zinc on cadmium concentrations in the tissue of durum wheat. *Canadian Journal of Plant Science 74*, 549-552.

Christensen, T. H. & Tjell, J. C. 1991. Sustainable management of heavy metals in agriculture. Example: Cadmium. *In* Heavy Metals in the Environment Vol 1. Ed. J G Farmer, 40-49. CEP *Consultants*, Edinburgh.

Ciecko, Z., Wyszkowski, M., Krajewski, W. & Zabielska, J. 2001. Effect of organic matter and liming on the reduction of cadmium uptake from soil by triticale and spring oilseed rape. *Science of the Total Environment*, 281, 37-45.

Grant, C. A. 2011. Influence of phosphate fertilizer on cadmium in agricultural soils and crops. *Pedologist*, 143-55.

Diskshith, T. S. S. & Diwan, P. V. 2003. Industrial guide to chemical and drug safety. *Wiley*, New York.

Dong, J., Mao, W.H., Zhang, G. P., Wu, F., Cai, Y. 2007. Root excretion and plant tolerance to cadmium toxicity – a review. *Plant Soil Environ*, 53, 193–200.

Dylevskaia, N. 2002. Approaches to Limiting the content of environmentally harmful impurities in Phosphate Fertilizers. IFA.11, 24-27.

Eriksson, J.E. 1989. The influence of pH, soil type and time on adsorption and uptake by plants of Cd added to the soil. *Water Air Soil Pollut*, 48, 317–335.

Eriksson, J. E. 1988. The effect of clay, organic matter and time on adsorption and plant uptake of cadmium added to the soils. *Water Air Soil Pollut.* 40, 359–373.

Gandois, L., Probst, A. &Dumat, C. 2010. Modeling trace metal extractability and solubility in French forest soils by using soil properties. *Eur J Soil Sci*, 61, 271–286

Gao, X., Akhter, F., Tenuta, M., Flaten, D. N., Gawalko, E. J. & Grant, C. 2010. Mycorrhizal colonization and grain Cd concentration of field-grown durum wheat in response to tillage, preceding crop and phosphorus fertilization. *Journal of the Science of Food and Agriculture*, 90, 750-758. http://dx.doi.org/10.1002/jsfa.3878.

Gao, X. & Grant, C. A. 2012. Cadmium and Zinc Concentration in Grain of Durum Wheat in Relation to Phosphorus Fertilization, Crop Sequence and Tillage Management. *Applied and Environmental Soil Science*, 2012, 10.

Gao, X., Akhter, F., Tenuta, M., Flaten, D.N., Gawalko, E.J. & Grant, C.A. 2010. Mycorrhizal colonization and grain Cd concentration of field-grown durum wheat in response to tillage, preceding crop and phosphorus fertilization. *J. Sci. Fd. Agric,* 90, 750-758.

Gao, X., Tenuta, M., Flaten, D. N. & Grant, C. A. 2011. Cadmium concentration in flax colonized by mycorrhizal fungi depends on soil phosphorus and cadmium concentrations. *Communications in Soil Science andPlant Analysis,* 42, 1882-1897.

Gerritse, R. G., Wallbrink, P. J. & Murray, A. S. 1998. Accumulation of Phosphorus and Heavy Metals in the Swan Canning Estuary, Western Australia. *Estuarine, Coastal and Shelf Science,* 47, 165-179.

Grant, C. A. & Sheppard, S. C. 2008. Fertilizer impacts on cadmium availability in agricultural soils and crops. *Human and ecolog. risk assessment,* 14, 210-228.

Grant, C.A., Bailey, L. D. J. T., Harapiak, N. A. & Flore. 2002. Effect of phosphate source, rate and cadmium content and use of *Penicillium bilaii* on phosphorus, zinc and cadmium concentration in durum wheat grain. *J. Sci. Food Agric,* 82, 301-308.

Grant, C. A., Bailey, L. D. & Therrien, M. C. 1996. The effect of N, P and KCl fertilizers on grain yield and Cd concentration of malting barley. *Fert. Res,* 45, 153–161.

Grant, C.A., Monreal, M.A., Irvine, R.B., Mohr, R.M., McLaren, D.L. & Khakbazan, M. 2009. Crop response to current and previous season applications of phosphorus as affected by crop sequence and tillage, *Canadian Journal of Plant Science, 89,* 49-66.

Grant, C. A., Clarke, J. M., Duguid, S. & Chaney, R. L. 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. *Science Total Environ,* 390**,** 301-10.

Grant, C. A., & Sheppard, S. C. 2008. Fertilizer impacts on cadmium availability in agricultural soils and crops. *Human and Ecological Risk Assessment: An International Journal,* 14, 210-228.

Grant, C. A., Bailey, L. D., Mc Laughlin, M. J. & Singh, B. R. 1999. Management factors which influence cadmium concentrations in crop. *In* M. J. Mc Laughlin & B. R. Singh (Eds.), Cadmium in soils and plants. *Dordrecht: Kluwer Academic press.*

Grant, C.A., & Bailey, L.D. 1997. Effects of phosphorus and zinc fertiliser management on cadmium accumulation in flaxseed. *J. Sci. Fd. Agric,* 73, 307-314.

Gray, C. W., McLaren, R. G., Roberts, A. H. C., & Condron, L. M. 1998. Sorption and desorption of cadmium from some New Zealand soils: effect of pH and contact time. *Australian Journal of Soil Research,* 36, 199-216.

Groenenberg, J. E., Romkens, P. F. A. M., Comans, R. N. J., Luster, J., Pampura, T., Shotbolt, L., Tipping, E. & Vries, W. 2009. Transfer functions for solid-solution partitioning of cadmium, copper, nickel, lead and zinc in soils derivation of relationship for free metal ion activities and validation with independent data. *Eur J Soil Sci*, 61, 58–73.

Guttormsen, G., Singh, B. R. & Jeng, A. S. 1995. Cadmium concentration in vegetable crops grown in a sandy soil as affected by Cd levels in fertilizer and soil pH. *Fertilizer Res,* 41, 27–32.

Haghiri, F. 1974. Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc, and soil temperature. *Journal of Environmental Quality,* 3, 180-183.

Haldar, M. & Mandal, L. N. 1981. Effect of phosphorus and zinc on the growth and phosphorus, zinc, copper, iron and manganese nutrition of rice. *Plant Soil*, 59,415-425.

Hamedi, J. 1999. Accumulation and mobility of heavy metals in soils irrigated with sewage effluent in Haryana, India. *Department of Soil and Water Sciences,* Agricultural University of Norway.

Hamon, R. E., McLaughlin, M. J., Naidu, R. & Correll, R. 1998. Long-term changes in cadmium bioavailability in soil. *Environ. Sci. Techno,* 32, 3699-3703.

Han, D. H., & Lee, H. 1996. Effects of liming on uptake of lead and cadmium by *Raphanus sativa. Archives of Environmental Contamination and Toxicology,* 31, 488-493.

Haq, A. U., Bates, T. E., & Soon, Y. K. 1980. Comparison of extractants for plantavailable zinc, cadmium, nickel and copper in contaminated soils. *Soil Science Society of America Journal,* 44, 772-777.

Hatch, D. J., Jones, L. H. P., & Burau, R. G. 1988. The effect of pH on the uptake of cadmium by four plant species grown in flowing solution culture. *Plant and Soil, 105,* 121-126.

He, Q. B. & Singh, B. R. 1994a. Crop uptake of cadmium from phosphorus fertilisers, I. Yield and cadmium content. *Water Air Soil Pollut,* 74, 251–265.

He, Q. B. & Singh, B. R. 1994b. Crop uptake of Cadmium from phosphorus fertilisers, II. Relationship with extractable soil cadmium. *Water Air Soil Pollut,* 74, 267–280.

He, Q. B & Singh, B. R. 1993a. Effect of organic matter on the distribution, extractability and uptake of cadmium in soils. *Journal of soil science*. 44, 641-650

He, Q. B. & Singh, B. R. 1993b. Plant availability of Cd in soils. I. Extractability of Cd in newly- and long-term cultivated soils. *Acta Agr. Scand,* 43, 134-141.

Heinegg, A., Maragos, P., Mason, E., Rabinowicz, J., Straccini, G., & Walsh, H. 2000. Soil contamination and urban agriculture: A practical guide to soil contamination issues for individuals and groups. *McGill School of Environment,* McGill University. Retrieved from http://www.ruaf.org/sites/default/files/guide on soil contamination.pdf.

Hejcman, M., Szakova, J., Schellberg, J., Srek, P. & Tlustos, P. 2009. The Rengen Grassland

Experiment: soil contamination by trace elements after 65 years of Ca, N, P and K fertilizer application. *Nutr. Cycl. Agroecosys,* 83, 39-50.

Holm, P.E., Christensen, T.H., Tjell, J.C. & McGrath, S.P. 1995. Speciation of cadmium and zinc with application to soil solutions. *J. Environ. Qual*, 24, 183-190.

Hong, C.O., Lee, D.K. & Kim, P.J. 2008. Feasibility of phosphate fertilizer to immobilize cadmium in a field. *Chemosphere,* 70, 2009-2015.

Hooda, P. S., and Alloway, B. J. 1993. Effects of time and temperature on the bioavailability of Cd and Pb from sludge-amended soils. *Journal of Soil* Science 44, 97-110.

Hooda, P. S., McNulty, D., Alloway, B. J. & Aitken, M. N. 1997. Plant availability of heavy metals in soils previously amended with heavy. Applications of sewage sludge. *Journal of the Science of Food and Agriculture,* 73, 446– 454

Huang, B., Kuo, S. & Bembenek, R. 2004. Availability of cadmium in some phosphorus fertilizers to field-grown lettuce. *Water Air Soil Poll*, 158, 37-51.

Hussain, Amir. 2010. "Accumulation and partitioning of cadmium, zinc and copper in cereal and legume crops under city effluent irrigation and phosphorus application." University of agriculture, Faisalabad, Pakistan. Ph.D Thesis and Dissertation Repository. http://prr.hec.gov.pk/Thesis/840S.pdf

Jackson, A. P. & Alloway, B. 1991. The phytoavailability of cadmium to lettuces and cabbages in soils previously treated with sewage sludges. *Plant and Soil*, *132,* 179-186.

Jeng, A.S. & Singh, B.R. 1995. Cadmium status of soils and plants from a long-term fertility experiment in southeast Norway. *Plant Soil,* 175, 67-74.

Jiao, Y., Grant, C.A. & Bailey, L.D. 2004. Effects of phosphorus and zinc fertilizer on cadmium uptake and distribution in flax and durum wheat. *J. Sci. Fd. Agric,* 84, 777-785.

Jiao, W., Chen, W., Chang, A.C. & Page, A.L., 2012. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: a review.

Environmental Pollution 168, 44e53.

Jing, J., & Logan, T. J. 1992. Effect of sewage sludge cadmium concentration on chemical extractability and plant uptake. *Journal of Environmental Quality* 21, 73-81.

Jones, C. A., Jacobsen, J. & Lorbeer, S. 2002. Metal concentrations in three Montana soils following 20 years of fertilization and cropping. *Commun. Soil Sci. Plant Anal*, 33, 01-1414.

Kabata-Pendias, A. 2001. Trace elements in soils and plants. *CRS press,* New York, USA, 123-164.

Kashem, M. A. & Singh, B. R. 2002. The effect of fertilizer additions on the solubility and plant-availability of Cd, Ni and Zn in soil. Nutr. Cycl. *Agroecosys*, 62, 287-296.

Krishnamurti, G.S.R., Huang, P.M. & Kozak, L.M. 1999. Sorption and desorption kinetics of cadmium from soils: influence of phosphate. *Soil Sci*, 164, 888–98.

Kelley, M. E., Brauning, S. E., Schoof, R. A. & Ruby, W. V. 2002. Assessing oral bioavailability of metals in soil. *Columbus, OH, Battelle Press.*

Knox, A. S., Kaplan, D. I. & Paller, M. H. 2006. Phosphate sources and their suitability for remediation of contaminated soils. *Sci. Total Environ*, 357, 271-279.

Kuo, S., Huang, B. & Bembenek, R. 2004. The availability to lettuce of Zn and Cd in Zn fertilizer. *Soil Sci*, 169, 363-373.

Lambert, R., Grant, C. & Sauve, S. 2007. Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. *Sci. Total Environ*, 378, 293-305.

Land, L. J., Betty, E. E., Page, A. L., Elliott, R. T. 1981. Occurrence of naturally high cadmium levels in soils and its accumulation by vegetation. *J. Environ. Qual.* 10, 551–556.

Lane, T. W., Saito, M. A., George, G. N., Pickering, I. J., Prince, R. C. & Morell, F. M. M. 2005. A cadmium enzyme from a marine diatom. *Nature*, 435, 42.

Loganathan, P., Mackay, A.D., Lee, J. & Hedley, M.J. 1995. Cadmium distribution in hill pastures as influenced by 20 years of phosphate fertilizer application and sheep grazing. *Aust. J. Soil Res*, 33, 859-871.

Mann, S. S., Rate, A. W. & Gilkes, R. J., 2002. Cadmium accumulation in agricultural soils in Western Australia. *Water Ai Soil Poll*, 141, 281-297.

McGrath, D. & Tunney, H., 2010. Accumulation of cadmium, fluorine, magnesium, and zinc in soil after application of phosphate fertilizer for 31 years in a grazing trial. *J. Plant Nutr*, Soil Sci. 173, 548-553.

Mc Lean, E.O. 1982. Soil pH and lime requirement. *In*: Page *et a.,*. Methods of soil analysis. Part 2. Chemical and Microbial Properties. 2nd ed. *Agronomy no.9*, Madison, Wisconsin, USA, 199-224.

McLaughlin, M. J., Hamon, R. E., McLaren, R. G., Speir, T.W. & Rogers, S. L. 2000. Review: a bioavailability based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Aust. J. Soil Res*, 38, 1037-1086.

McLaughlin, M. J. & Singh, B. R. 1999. Cadmium in Soils and Plants. *Dordrecht, The Netherlands: Kluwer Academic Publisher*

McLaughlin, M.J., Tiller, K.G., Naidu, R. & Stevens, D.P. 1996. Review: the behaviour and environmental impact of contaminants in fertilizers. *Aust. J. Soil Res,* 34, 1–54.

McLaughlin, M. J., Palmer, L.T., Tiller, K. G., Beech, T. A., & Smart, M. K. l994. Increased soil salinity causes elevated cadmium concentrations in field-grown potato tubers. *Journal of Environmental Quality* 23, 1013-1018.

McLaughlin, M. J., Maier, N. A., Freeman, K., Tiller, K. G., Williams, C. M. J., & Smart, M. K 1995. Effect of potassic and phosphatic fertiliser type, phosphatic fertiliser Cd content and additions of zinc on cadmium uptake by commercial potato crops. *Fertiliser Research,* 40, 63-70.

Meers, E., Laing, G. D., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F. M. G. & Verloo, M. G. 2007. Comparison of cadmium extractability from soils by commonly used single extraction protocols. *Geoderma*,141, 247–25.

Mench. M., Morel, J. L., Guckert, A., & Guillet, B. 1988. Metal binding with root exudates of low molecular weight. *J Soil Sci,* 39: 521-27.

Mench, H., Baize, D. & Mocguot, B. 1997. Cadmium availability to wheat in five soil series from the Y onne district, Burgundy, France. *Environmental Pollution* 95, 93-103.

Mitchell, L. G., Grant, C. A., &Racz, G. 1. 1997. Phytoavailability of cadmium in soils treated with nitrogen fertilisers. *In* Proceedings of Fourth International Conference on the Biogeochemistry of Trace Metals, 131-132. San Francisco, 23 - 27 June 1997.

Mortvedt, J. J., Mays, D. A. & Osborn, G. 1981. Uptake by wheat of cadmium and other heavy metal contaminants in phosphate fertilizers. *J Environ Qual,* 10, 193–197.

Mulla, D. J., Page, A. L. & Ganje, T. J. 1980. Cadmium accumulation and bioavailability in soils from long-term fertilization. *J Environ Qual*, 9: 408-12.

Munksgaard, N.C., Lottermoser, B.G., 2011. Fertilizer amendment of mining impacted soils from Broken hill, Australia: fixation or release of contaminants? *Water Air Soil Poll,* 215, 373-397.

Murray, H., Pinchin, T. A. & Macfie, S. M. 2011. Compost application affects metal uptake in plants grown in urban garden soils and potential human health risk. *Journal of Soils and Sediments,* 11, 815-829. http://dx.doi.org/10.1007/s11368-011-0359-y

Naidu, R., Bolan, N. S., Kookana, R. S. & Tiller, K. G., 1994. Ionic-strength and pH effects on the sorption of cadmium and the surface charge of soils. *European Journal of Soil Science,* 45, 419-429.

Nicholson, F. A., Jones, K. C. & Johnston, A. E. 1994. Effect of phosphate fertilizers and atmospheric deposition on long-term changes in the cadmium content of soils and crops. *Environ. Sci. Technol,* 28, 2170-2175.

Nriagu, J. O. & Pacyna, J. M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature,* 333, 134-139.

Nziguheba, G. & Smolders, E. 2008. Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. *Sci. Total Environ,* 390, 53-57.

Oliver, D. P., Wilhelm, N. S., McFarlane, D., Tiller, K. G. & Cozens, G. D. 1997. Effect of soil foliar applications of zinc on cadmium concentration in wheat grain. *Australian Journal of Experimental Agriculture* 37, 677-681.

Oliver, D.P., Gartrell, J.W., Tiller, K.G., Correll, R., Cozen, G.D., Youngberg, B.L. 1995. Differential response of Australian wheat cultivars to cadmium concentration in wheat grain. *Aust. J. Agri. Res*, 46, 873–886.

Peijnenburg, W., Baerselman, R., Groot, A.de, Jager, T., Leenders, D., Posthuma, L. & Veen, R. V. 2000. Quantification of metal bioavailability for lettuce (*Lactuca sativa* L.) in field soils. *Archives of Environmental Contamination and Toxicology,* 39, 420-430.

Perriguey, J., Sterckeman, T. & Morel, J. L. 2008. Effect of rhizosphere and plant-related factors on the cadmium uptake by maize. *Environmental and Experimental Botany,* 63, 333-341.

Richards, I. R., Clayton, C. J. U. & Reeve, A. J. K. 1998. Effects of long-term fertilizer phosphorus application on soil and crop phosphorus and cadmium contents. *J. Agric. Sci,* 131, 187-195.

Rieuwerts, J. S., Ashmore, M. R., Farago, M. E. & Thornton, I. 2006. The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils. Sci. *Total Environ*. 366, 864-875.

Roberts, A. H. C., Longhurst, R. D & Brown, M. W. 1994. Cadmium status of soils, plants and grazing animals in New Zealand. *New Zealand Journal Agriculture Research,*37: 119-29. http://dx.doi.org/10.1007/s002440010123

Rothbaum, H. P., Goguel, R. L., Johnston, A. E. & Mattingly, G. E. G. 1986. Cadmium accumulation in soils from long-continued applications of superphosphate. *Journal of Soil Science* 37,99-107.

Saltali, K., Sari, H., Mendil, D. & Altin, S. 2004. Cadmium and phosphorus accumulates in soil under intensive cultivation in Turkey. *Acta Agriculturae Scandinavica*, Section B- *Plant Soil Science,* 54, 267-272.

Sauerbeck, D. R. & Styperck, B. 1985. Evaluation of chemical methods for assessing the Cd and Zn availability from different soils and sources. *In:* Leschber, R., Davis, R.D., L'Hermite, P. Eds. Chemical Methods for Assessing Bio-Available Metals in Sludges and Soils. *Elsevier*, Amsterdam, 49–67.

Sato, A., Takeda, H., Oyanagi, W., Nishihara, E. & Murakami, M. 2010. Reduction of cadmium uptake in spinach (Spinacia oleracea L.) by soil amendment with animal waste compost.

*Journal of Hazardous Materials,* 181, 298-304.

Sauve, S., Norvell, W. A., McBride, M. & Hendershot, W. 2000. Speciation and complexation of cadmium in extracted soil solutions. *Environ. Sci. Technol.* 34, 291-296

Schollenbergen, C. J. & Simon, R. H. 1945. Determinative exchange capacity and exchangeable bases in soil ammonium acetate method. *Soil Science,* 59, 13-24.

Semu, E. & Singh, B. R. 1996. Accumulation of heavy metals in soils and plants after long-term use of fertilizers and fungicides in Tanzania. *Fertiliser Research* 44, 241- 248.

Sheppard S.C., Grant C.A., Sheppard M.I., Jong R. & Long J. 2009. Risk indicator for agricultural inputs of trace elements to Canadian soils. *J Environ Qual*, 38, 919-932.

Sheppard, M. I., Sheppard, S. C. & Grant, C. A. 2007. Solid/liquid partition coefficients to model trace element critical loads for agricultural soils in Canada. *Can J Soil Sci*, 87, 189-01.

Shukla, U. C., Singh, J., Joshi, P.C. & Kakkar, P. 2003. Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat seedlings. *Biolog. Trace Element Res.* 92, 257-273.

Singh, B.R. 1994.Trace element availability to plants in agricultural soils, with emphasis in fertilizer inputs. *Environmental Reviews,* 2, 133-146.

Singh, B. R. 1991. Unwanted components of commercial fertilizers and their agricultural effects. *Proceedings of Fertiliser Society London*, Dec, 2-28.

Singh, B. R. & Myhr, K. 1998. Cadmium uptake by barley as affected by Cd sources and pH levels. *Geoderma*, 84, 185-194.

Singh, B. R., Narwal, R. P., Jeng, A. S. & Almås, Å. 1995. Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. *Commum.soil science. Plant analysis,* 26, 2123-2142.

Soil Survey Staff. 2010. Keys to Soil Taxonomy, 11$^{th}$ ed. USDA. *Natural Resources Conservation Service*, Washington, DC.

Sparrow, L. A, Salardini, A. A. & Johnstone, J. 1994. Field studies of cadmium in potatoes III. Responses of c.v Russet Burbank to sources of banded potassium. *Australian Journal of Soil Research*, 45, 243-249.

Symeonides, C. & McRae, S.G. 1977. The assessment of plant-available cadmium in soils. *J. Environ. Qua,.* 6, 120–123.

Thakur, S. K., Tomar, N. K & Pandeya, S. B., 2006. Influence of phosphate on cadmium sorption by calcium carbonate. *Geoderma,* 130, 240-249.

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M. R., Lofts, S., Hill, M. T. R., Fargo, M. E. &

Thornton, I. 2003. The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. *Environ Pollut,* 125,213–225.

Tu, C., Zheng, C.R & Chen, H.M. 2000. Effect of applying chemical fertilizer on forms of lead and cadmium in red soil. *Chemosphere,* 41, 133-138.

Vassilev, A. 2002. Physiological and agroecological aspects of cadmium interaction with barley plants: An overview. *J. Central European Agric,* 4, 65-74.

Vig, K., Megharaj, M., Sethunathan, N. & Naidu, R. 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil. *Advances in Environmental Research,* 8, 121-135.

Wahid, A. & Ghani, A. 2008. Vareital differences in mungbean (Vigna radiata) for growth, yield, toxicity symptom and cadmium accumulation. *Annals of Appl. Biol.* 152, 59-69.

Wang, X. S. & Qin. 2006. Spatial distribution of metals in urban top soils of xuzhou (China): controlling factors and environmental implications. *Environ Geol,* 49, 905-914.

Wang, G., Su, M.Y., Chen, Y.H., Lin, F.F., Luo, D. & Gao, S.F. 2006. Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. *Environmental Pollution,* 144, 127-135.

Whitten, M. G. & Ritchie, G. S. P. 1991. Calcium chloride extractable cadmium as an estimate of cadmium uptake by subterranean clover. *Australian Journal of Soil Research,* 29, 215-221.

Williams, C. H. & David, D. J. 1976. The Accumulation in Soil of Cadmium Residues from Phosphate Fertilizers and their Effect on the Cadmium Content of Plants. *Soil Science*, 121, 86-93.

Wu, Z. N. I., Xio, Y., & Xin-xian, L., 2002. Differences of cadmium absorption and accumulation in selected vegetable crops. *Journal of Environmental Science,* 14, 399-405.

Zhang, G., Fukami, M. & Sekimoto, H. 2002. Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Research,* 77, 93-98.

Zhao, Z. Q., Zhu ,Y. G., Smith, S. E. & Smith, F. A. 2005. Cadmium uptake by winter wheat seedlings in response to interactions between phosphorus and zinc supply in soils. *J. Plant Nutr*, 28, 1569-1580.

Zhou, Q. 2003. Interaction between heavy metals and nitrogen fertilizer applied to soil vegetable systems. Bull. Environ. *Contam. Toxicol.* 71, 338-344