CROP MANAGEMENT IMPACTS ON MYCORRHIZAL COLONIZATION AND CADMIUM AVAILABILITY IN AGRICULTURAL CROPS

BY

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ABSTRACT

Akhter, Fardausi M. M.Sc., The University of Manitoba, September 2008. <u>Crop management impacts on mycorrhizal colonization and cadmium availability in agricultural crops.</u> Major Professor: Dr. Mario Tenuta.

Cadmium is a naturally occurring element in mineral soils. It may accumulate in crops to levels that are of concern in human diets. The study aimed at determining the effect of selected crop management practices on above ground cadmium concentration and accumulation in durum wheat and flax. Management practices included tillage (conventional and reduced), crop sequence (flax-durum wheat and canola-durum wheat) and phosphorus fertilization (low and high level). The approach used was to determine single and interactive effects of these management practices on cadmium concentration and accumulation in the above ground tissues and whether arbuscular mycorrhizal fungal colonization of roots had a role.

Among crop management practices studied, tillage and high level of P fertilizer containing Cd as impurities increased above ground tissue Cd concentration the most. In the case of high Cd loading to soil through P fertilizer with high concentration of Cd, Cd concentration and accumulation of flax and durum wheat tissue was simply related to DTPA extractable Cd in soil. Cd concentration was higher in above ground flax tissues compared to durum wheat substantiating flax as high Cd accumulating crop. Conventional tillage and canola as a proceeding crop reduced arbuscular mycorrhizal fungal colonization in durum wheat although only the former was related to increased Cd concentration of durum wheat. Phosphorus fertilizer reduced arbuscular mycorrhizal fungal colonization in durum wheat but not in flax. Thus, a clear effect of arbuscular mycorrhizal colonization on Cd uptake was not evident in this field study. However, multiple-linear regression analysis revealed that root mycorrhizal colonization variables (either percent arbuscular root colonization, arbuscular colonization density, or mycorrhizal Cost:Benefit) to be negatively associated with Cd concentration of above ground tissue for both durum wheat and flax. This indicated potential association of mycorrhizal fungi in restricting Cd transfer to above ground tissues.

In conclusion, above ground Cd concentration and accumulation most clearly increased with addition rates of Cd laden phosphorus fertilizer. Conventional tillage was the other management practice examined that increased Cd concentration and accumulation; however, the effect was small. Mycorrhizal colonization of roots was reduced with canola as a previous crop, conventional tillage and increasing P addition. Relation of mycorrhizal colonization to Cd concentration and uptake was weak. The study indicates basis to detail the relationship between mycorrhizal colonization to Cd uptake of crops under controlled environment conditions where confounding field conditions are reduced and the impact of absence and presence of colonization can be determined.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

1.1 Cadmium in Soils and Plants

Cadmium (Cd) is a non-essential trace element to plants, animals and human. This element has attracted much attention in soil science and plant nutrition, because (i) it is toxic to humans at concentrations lower than those lethal to plants, (ii) it is more mobile and bioavailable than many other metals, and (iii) its effects on humans are chronic (McLaughlin and Singh, 1999). The two main causes of Cd in soils are geological parent materials and inputs from extraneous sources, which for the most part are anthropogenic in origin. Soils derived from Cd rich parent materials have concentrations up to 24 mg total Cd kg^{-1}(Alloway and Steinnes, 1999). Anthropogenic means of increased Cd in agricultural soils include atmospheric deposition from industrial sources, fertilizers, manures and municipal sewage wastes. These anthropogenic sources may exceed the release of this metal from natural sources by two-fold or more (Nriagu and Pacyna, 1988). In urban and industrial areas, most soil Cd comes from atmospheric deposition. A long-term experiment conducted in the UK clearly showed gradual increase in soil Cd over time with farmyard manure and fertilizer application (McLaughlin and Singh, 1999). In many agricultural systems, the main sources of Cd inputs to soils are fertilizers, soil amendments, manures and sewage biosolids (Lugon-Moulina et al., 2006; Manciulea and

Ramsey, 2006; McLaughlin et al., 1996), which are also slowly increasing with time by applying either mineral or organic fertilizers (Alloway and Steinnes, 1999).

The Cd concentration of Manitoba soil ranges from 0.1 to 7.9 mg kg^{-1} (Haluschak et al., 1998). The Cd content in Manitoba soil is highly related to texture. The average Cd content in coarse textured soil was 0.1 mg kg^{-1}, whereas it was four times higher in fine textured soil (Haluschak et al., 1998). The highest concentrations occured in soils derived directly from shale bedrock or those in which a large content of weathered shale particles comprise the soil parent material (Haluschak et al., 1998). Upon weathering, these shales release Cd^{2+} into solution. This ionic species is the form that Cd is taken up by plants, although concentration in solution will be low, because Cd^{2+} forms complexes and organic chelates in soil.

The cadmium cycle is fairly complex in agricultural soils (Figure 1.1). The accumulation of soil Cd in food crops is dependent on a number of physical, chemical and biological processes that control the solubility and form of Cd in the soil solution, especially in the rhizosphere (Welch and Norvell, 1999). There are a myriad of reactions and interactions of Cd with soil components, other nutrients, metals, plant, climate and management factors, which may affect Cd transfer through the food chain. For a sustainable Cd management strategy, it is important to control inputs of Cd to the soil as well as transfer to plants and into the food chain.

1.2 Mechanism of Cadmium Uptake and Translocation in Plants

1.2.1 Mechanisms of Cadmium Uptake

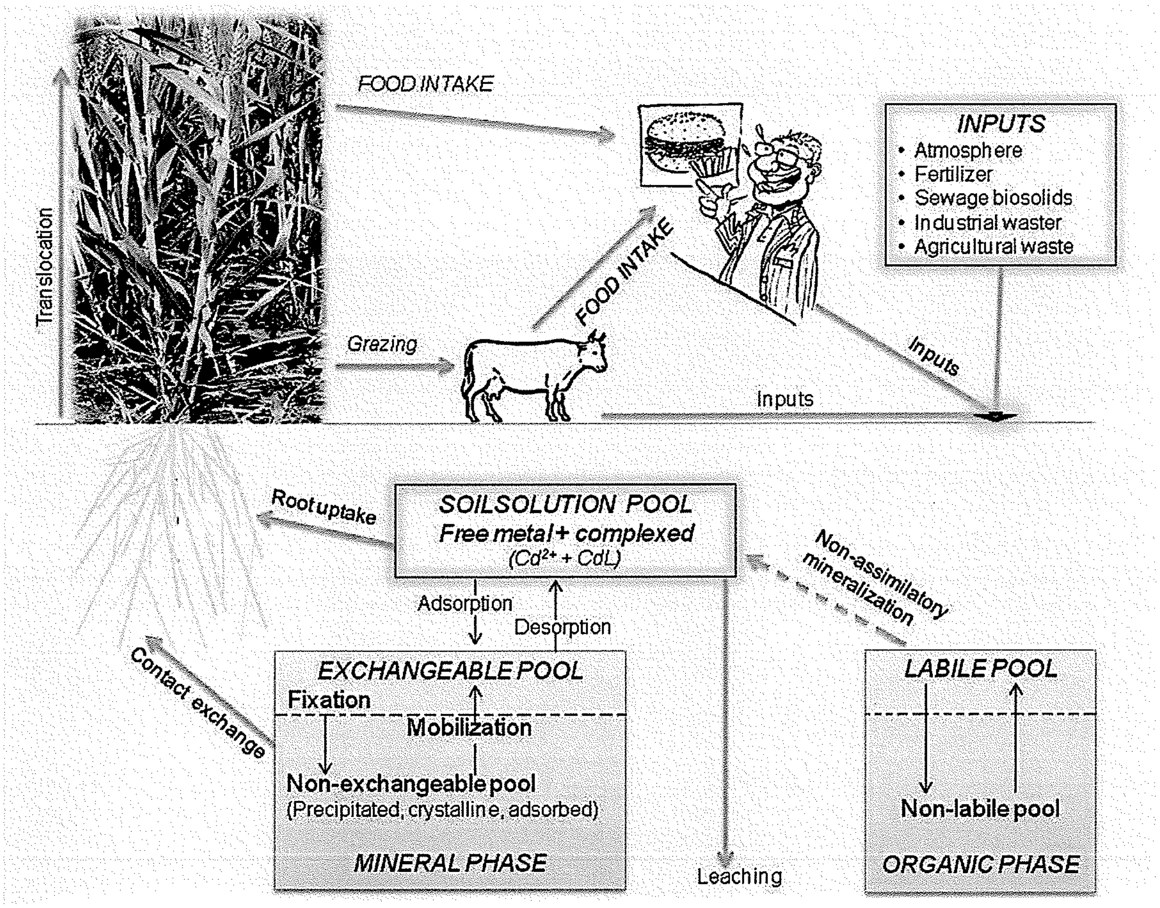

Figure 1.1 Fluxes of Cd in soils, plants and the food chain (reproduced from McLaughlin and Singh, 1999, with kind permission of Springer Science and Business Media)

Cd can be taken up by plants either by foliar or root absorption and then transfered to different plant parts (Liu et al., 2003; Cobb et al., 2000; Florijn and Van Beusichem, 1993). Root absorption is the dominant pathway for aqueous Cd uptake and accumulation to the plants. Other forms of Cd in the soil solution are in the forms of inorganic or organic complexes, and some of these complexes may participate directly in root uptake (Welch and Norvell, 1999).

There are a number of root processes that increase the solubility of Cd in the rhizosphere that may also have large effects on the amount of Cd diffusing to root surfaces. These root processes include root-cell efflux of H^+ ions, release of respired carbon dioxide, root efflux of organic acid and reductants (e.g. phenolic compounds like lignin, flavonoids etc.), the uptake of macro- and micronutrient cations and anions and Cl^- (Welch and Norvell, 1999). These processes affect Cd availability and absorption to varying degrees depending on plant species, genotype and environmental conditions.

1.2.2 Intra-cellular Homeostasis: Complexation, Compartmentation and Efflux

The fate of Cd^{2+} after influx across the root-cell plasma membrane is complex. Cd^{2+} is highly reactive and preferentially binds to sulphhydryl ligand groups rather than to other ligands (e.g. carboxyl, hydroxyl, amino-N etc.). As a result, Cd^{2+} competes with other sulphur binding metals such as Zn, Ni and Cu for active functional binding sites in essential cytosolic metabolites of plants (e.g. glutathione and other sulphhydryl-containing proteins). In the cytosol, a set of low molecular weight cysteine-rich polypeptide (phytochelatins or cadystins) form complexes with Cd^{2+} (Rauser, 1995). Wu et al. (1995) reported that Cd^{2+} phytochelatins can be transported into vacuoles directly and transfer their Cd to higher molecular weight metallothionein-type proteins (>10,000 daltons) for storage of Cd in inactive forms in the vacuole. Phytometallophores (e.g. nicotianamine, mugineic acid) could also play a role in controlling Cd^{2+} activity in cytosol and intra- and intercellular symplastic transport of Cd^{2+}(Welch and Norvell, 1999), however to my knowledge there is no published reports in support of this assumption. Organic acids (e.g., citric acid) are also reported to complex Cd^{2+} in the cytosol (Senden et al., 1995). Cd^{2+} can also be transported into cell organelles via a membrane transport protein Cd^{2+}/H^+ antiporter (Salt and Wagner, 1993). This protein exchanges H^+ for Cd^{2+} during Cd^{2+} transport into the vacuole. Among all the Cd complexers, glutathione (GSH) is the most important which can be exposed to non-growth inhibiting levels of Cd whereas Cd(II)-phytochelatins are only important at higher Cd exposures (> 5 μM Cd) (Vögeli-Lange and Wagner, 1996).

1.2.3 Cadmium Transport

The entry of Cd into edible portions of crops involves transfer from roots to shoots. The transfer starts as a membrane transport process in parenchyma cells that adjoin xylem vessels within the endodermis of roots (Welch and Norvell, 1999). Here, xylem elements load Cd from symplastic pools in parenchyma cells. The chemical composition of xylem sap consists of a pH ranging from 5.0-6.0 with low concentrations of organic compounds, such as sugars, peptides and proteins. Cd can move in xylem sap as an inorganic cation (Cd^{2+}) as well as in complexes with organic molecules. Cd prefers to form bonds with sulphhydryl ligand groups, it also binds to N and O ligand groups. Cysteine and other sulphhydryl containing compounds (e.g., phytochelatins, glutathione) and various organic compounds, such as citrate and amino acids in xylem sap, can be important in transporting Cd from root to shoot (Welch and Norvell, 1999). Within the xylem, Cd moves with the transpiration stream and eventually unloaded from the xylem sap into leaf mesophyll cell apoplasmic spaces. After this, Cd must cross a plasma membrane to again enter the symplasm. In order to enter seed or grains, Cd crosses a companion cell plasma membrane adjacent to a sieve tube element to enter the phloem sap for transport. This means of transport is important because there are no mature xylem in tissues that can provide mineral elements directly to developing seeds and grains within a reproductive organ.

The chemical composition of phloem sap consists of a pH ranging from 7.0 - 8.0 with high concentrations of organic ligands (e.g. organic acids, amino acids, sugars, peptides and proteins). The redox potential of phloem sap is low and provides stability to sulphhydryl- containing ligands, which are the carriers of Cd. The Cd complexes in phloem sap may include phytometallophores, such as nicotianamine and the class 3 metallothioneins, the phytochelatins, as well as glutathione, cysteine and other sulphhydryl-containing molecules (Welch and Norvell, 1999). However, to my knowledge, there is no direct evidence for any specific Cd complexes in phloem sap. Once xylem transfer of Cd at phloem unloading sites within a reproductive organ occurs, Cd transports across a plasma membrane of cells associated with the developing embryonic tissues of the developing seed or grain begins (Welch, 1995). Therefore, Cd crosses numerous cell membrane barriers to enter edible plant organs, especially seeds and grains.

1.3 Factors Affecting Cadmium Availability to Plants

Cd availability to plants is affected by crop genetics and Cd activity in soil solution (Grant et al., 2008; Ozkutlu, et al., 2007; Dunbar et al., 2003; Ozturk, et al., 2003; Grant et al., 1999). Cd concentration in plant edible parts can be reduced by manipulating crop selection and crop and soil management practices (Grant et al., 2008). Cd concentration in soil solution is affected by plant growth, soil pH, cation-exchange capacity, rhizosphere chemistry, microbial activity, soil organic matter, ionic strength of the soil solution, competing or complexing ions, total and plant available Cd content of the soil (Grant and Sheppard, 2008; Carrillo-González et al., 2006; Bolan, et al., 2003; McBride, 2002; Peijnenburg et al., 2000; Grant et al., 1999). Important crop management practices which may impact on Cd concentration in the soil solution by affecting soil chemical properties or direct addition of Cd to the soil, or on crop growth and rooting distribution, are discussed below.

1.3.1 Phosphate Fertilizer Application

Most Cd in nature occurs as an atomic substitution for Zn in minerals (Mineral Information Institute, 2007). Only a few relatively pure Cd minerals are known (e.g. cadmium sulphide, CdS). In addition, Cd can occur as an impurity in phosphate minerals (Mineral Information Institute, 2007). Some natural phosphate ores may contain several hundred parts per million (ppm) of Cd (Mineral Information Institute, 2007), and so phosphate fertilizers manufactured from these ores may contain high concentrations of cadmium (Alloway and Steinnes, 1999). Phosphate fertilizers can contain Cd as a contaminant at levels varying from trace amount to as much as 300 mg Cd kg^{-1} of dry product (Alloway and Steinnes, 1999; Grant and Sheppard, 2008). All soils receiving these fertilizers will have an input of Cd, but how much will depend on the source of rock phosphate used for its manufacture and the amounts applied (Grant and Sheppard, 2008; McLaughlin et al., 1996). Phosphate fertilization can influence Cd phytoavailability directly through addition of Cd as an impurity or indirectly through immediate and long-term effects on soil characteristics (Grant and Sheppard, 2008; Lambert et al., 2007;