ACCUMULATION OF CADMIUM BY DURUM WHEAT (*Triticum turgidum*): INFLUENCE OF SOLUTION CHEMISTRY AND ROOT MORPHOLOGY

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

EDWARD BERKELAAR

In partial fulfilment of requirements

for the degree of

Doctor of Philosophy

January, 2000

ABSTRACT

ACCUMULATION OF CADMIUM BY DURUM WHEAT (*Triticum turgidum*): INFLUENCE OF SOLUTION CHEMISTRY AND ROOT MORPHOLOGY

Edward Berkelaar
University of Guelph, 1999

Advisor:
Professor B. A. Hale

Plants can accumulate Cd which is found dissolved in soil solution, and these plants are the main source of Cd for humans. Cd is a bioaccumulating, non-essential metal which can be toxic to mammals. It is important to understand how solution chemistry and root morphology influence the amount of Cd accumulated by plants.

'Arcola' roots contained higher Cd concentrations ($\mu g\ Cd \cdot g^{-1}$ dry weight) than 'Kyle' roots after 0 to 200 minutes. Compared with root systems of 'Kyle' seedlings, 'Arcola' seedlings had a greater surface area, more root tips, and greater ratios of surface area:root dry weight and number of root tips:root dry weight. These morphological differences were consistent with observed cultivar differences in root Cd concentration.

According to the Free Ion Model, the uptake of Cd by roots should be related to the solution Cd^{2+} concentration. In 'Kyle' and 'Arcola', the solution Cd^{2+} concentration significantly underestimated bioavailability of Cd to roots, as measured by Cd accumulation, when complexed forms of Cd, such as $CdCitrate^{-}$, $CdEDTA^{2-}$, or $CdSO_4^0{}_{(aq)}$ were present. Enhanced accumulation of Cd presumably occurred due to accumulation of Cd-complexes, and/or due to enhanced diffusion of Cd to the root surface. Diffusion rates were similar to uptake rates, so diffusion could have been the rate limiting step in Cd

accumulation, a failure in one of the assumptions of the FIM.

When exposed for longer durations (0 to 72 hrs), root Cd concentrations of 'Kyle' and 'Arcola' seedlings were not significantly different from one another, but Cd concentrations in 'Arcola' shoots were significantly less than in 'Kyle' shoots, indicating that Cd was more mobile in 'Kyle' than 'Arcola' seedlings. This observation is consistent with previously reported differences in grain accumulation of Cd by these two cultivars.

The results presented are important to those people wishing to regulate soil chemistry for the protection of foodstuffs, and those people wishing to use plants to phytoremediate contaminated soil, since it provides valuable information about how Cd speciation in soil solution and root morphology influence the amount of Cd found in plant tissue.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Bev Hale, for her wonderful advice and support during the past four years. I am thankful for the chance to pursue this project, and for the opportunity to present this work at numerous conferences.

I would also like to thank my advisory committee, Dr. Les Evans, Dr. Larry Peterson, and Dr. Wilfried Rauser, for their guidance and helpful suggestions during the course of my PhD program and especially for their help in the final preparation of the thesis.

I appreciate the effort of the examining committee, Dr. Ward Chesworth (chair), Dr. Bev Hale, Dr. Michael McLaughlin (external examiner) Dr. Gary Parkin, and Dr. Wilfried Rauser as they reviewed the thesis.

Thanks to Ms. Leslie Mitchell and Mr. Larry Pyear for their technical assistance maintaining plant material and aiding with various experiments; to Dr. Peter Campbell and his lab (INRS-Eau, Université du Québec) for their MINEQL$^+$ and solution chemistry advice; to Mr. Peter Smith and Mr. Will Gower for their help with the GF-AAS and Cd analysis; to Dr. Klironomos for the use of his WinRhizo package, and to William Sears for his assistance with the statistical analysis.

I am grateful for the close friends I've made while a graduate student at the University of Guelph. Debbie, Demos, Richard, and Tom: I have enjoyed, and will miss, the stimulating discussions and encouragement you have provided over the years. Thank you!

Lastly, I dedicate this thesis to my parents (Jannie and Peter), my sisters (Wendy and Julie) and Dawn. Thank you for your constant encouragement and support.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ANOVA: analysis of variance

'Arcola': cultivar of durum wheat (*Triticum turgidum*); lower grain accumulator of Cd

$CaCitrate^-$: calcium citrate; a soluble complexed form of calcium

CEC: cation exchange capacity

Cd: cadmium, a non-biologically essential metal, mass = 112.41 $g \cdot mole^{-1}$

Cd^{2+}: free ionic form of cadmium

$CdCitrate^-$: cadmium citrate; a soluble complexed form of cadmium

$CdEDTA^{2-}$: cadmium EDTA; a soluble complexed form of cadmium

$(CdL_n)^{2-nz}$ or CdL_n^0: complexed form of Cd

$CdSO_{4\,(aq)}^0$: cadmium sulphate; a soluble complexed form of cadmium

EDTA: ethylenediamine tetraacetic acid; $C_{12}H_{16}O_8N_2$, mass = 292.25 $g \cdot mole^{-1}$

FIM (or FIAM): Free Ion Model (or Free Ion Activity Model); model used to predict the effect of dissolved metals on living organisms which presumes effect of a dissolved metal can be related to the free ionic form of the metal

GF-AAS: graphite furnace atomic absorption spectrometer

HEDTA: N-(2-hydroxyethyl)-ethylenediamine triacetic acid; $C_{10}H_{18}N_2O_7$, mass = 278.26 $g \cdot mole^{-1}$

HDPE: high density polyethylene

HMW: high molecular weight

'Kyle': cultivar of durum wheat (*Triticum turgidum*); higher grain accumulator of Cd

L^{z-} or L: a ligand; can combine with metals to form metal-ligand complexes

LMW: low molecular weight

$MgCitrate^-$: magnesium citrate; a soluble complexed form of magnesium

$MINEQL^+$: a chemical equilibrium program for personal computers; Version 3.0

NIST: National Institute of Standards and Technology

NTA: nitrilotriacetic acid

SAS: statistical analysis program for personal computers, Version 6.12

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Cadmium: Characteristics and Uses

Lead, mercury and cadmium are non-essential, metallic elements sometimes called heavy metals because of their relatively high densities (greater than approximately 5 $g \cdot cm^{-3}$). The term "heavy metals" often includes lighter metals such as aluminum and metalloids such as arsenic and antimony (Lester, 1987) because "heavy" has become synonymous with toxic. Cadmium (Cd; atomic number = 48, atomic weight = 112.411) is relatively rare; its estimated average natural abundance in the earth's crust is about 0.55 $g \cdot tonne^{-1}$ (ppm) (Cherian *et al.*, 1985). It was identified as an element quite recently, in 1817, and has only been used in significant amounts during the past half century. The concern over Cd is due to the fact that it can be absorbed from the soil by plants which are then consumed as food products, it bioaccumulates in mammalian tissues, and can be toxic to humans.

Refined Cd is used extensively in electroplating because of its resistance to corrosion, in various alloys which are noted for their great resistance to fatigue, and in many solders, including silver solder, because of its low melting point. Compounds of cadmium are, or have been, used in batteries, pigments (cadmium yellow and cadmium orange), antiseptics and fungicides, phosphors in both black and white and colour televisions, and additives in rubber and plastics. Both the production and disposal of these products can result in dispersion of Cd into the environment (Department of the Environment, 1980; Cherian *et al.*, 1985; Lester, 1987).

1.2 Cadmium in the Environment

Dispersion of Cd into the environment occurs as a result of both natural and anthropogenic events. Volcanic eruptions, forest fires, submarine activities and weathering of the earth's crust release Cd into the environment, predominantly into the atmosphere (Department of the Environment, 1980; Canadian Environmental Protection Act, 1994). While natural events do contribute to Cd dispersal in the environment, most of the Cd released is from anthropogenic sources. Because Cd is an impurity of non-ferrous ores such as zinc, lead and copper, release of Cd into the environment has occurred for as long as these metals have been refined, although release of Cd into the environment as a result of producing these metals has risen as production has increased. The burning of coal and, to a lesser extent, oil also releases Cd into the environment. The amount of Cd released into the environment has risen considerably over the past 50 years as refining of Zn has increased and more uses for Cd have been discovered, resulting in increased refining of Cd. In 1992, Canada produced 1963 tonnes of Cd, exporting 1580 tonnes and keeping 383 tonnes, while an additional 39.3 tonnes of refined Cd and Cd compounds were imported. (Canadian Environmental Protection Act, 1994). Data compiled by Environment Canada in 1994 indicate that, every year, 147 tonnes of Cd are released into the atmosphere, 12 tonnes into aquatic environments, and approximately 340 tonnes of Cd slag, sludges and solid wastes are disposed on land. Very little is known about the bioavailability or nature of the Cd disposed on land. The application of phosphatic rock fertilizers and sewage sludge containing Cd also results in the dispersion of Cd into the environment, and this may be important since it may increase levels of Cd

on agricultural soils, and may potentially have an impact on the concentration of Cd in food products (McLaughlin *et al.*, 1996).

Once in the environment, Cd does not break down, although its mobility, bioavailability and amount of time spent in different compartments within the environment (atmosphere, soil, water, or living tissue) are affected by various processes. Much of the Cd released into the atmosphere (i.e. from smelting) becomes oxidized to cadmium oxide (CdO), and is associated with fine aerosols, or dissolved in water vapour. Most Cd present in the atmosphere is removed by dry or wet deposition within four weeks, and usually within 1000 km of the source (Canadian Environmental Protection Act, 1994).

In aqueous media, Cd is often found in its free ionic form, Cd^{2+}, though several factors result in the removal of the free ion from solution. Organic particulates in the water serve as a surface for Cd^{2+} adsorption, where it may subsequently sediment out of the water. After deposition, Cd will either remain in the sediment or become redissolved once the organic particulates decay. Acidic water tends to have more Cd dissolved in it since Cd^{2+} is more soluble at lower pH and the adsorption of Cd to particulates tends to be inhibited at lower pH (Lester, 1987; Canadian Environmental Protection Act, 1994). In marine waters, Cd becomes more soluble as the salinity increases, although less is taken up by marine than freshwater organisms because of competition with Ca^{2+} (Canadian Environmental Protection Act, 1994).

In soils, Cd is found bound to soil particles, or dissolved in the soil solution, either in its free ionic form or complexed with soluble ligands. Cd is quite mobile and available in soils which have a low pH, a low percentage of organic matter and a low CEC (i.e.

sandy soils), while mobility is restricted in soils which have higher amounts of organic matter, clays and hydrous metal oxides (Canadian Environmental Protection Act, 1994). When soluble ligands (i.e. citric acid) are present in the soil solution, the formation of soluble Cd complexes may result. In this case, dissolved Cd^{2+} would be in equilibrium with both the soluble Cd complexes and Cd bound to soil particles. The bioavailability of these complexes to living organisms is not well understood. Microorganisms in soil also have an effect on the speciation of Cd. In one study determining the availability of Cd 38 days after $Cd(NO_3)_2$ was added to sterile and non-sterile acid sandy loam soil, researchers found that Cd in the non-sterilized soil was more mobile, since a significant amount of Cd existed as a hydrophilic organic complex, which was present as a result of the microbial activity in the non-sterilized soil (Chanmugathas and Bollag, 1988).

The average natural abundance of Cd in the earth's crust is estimated at 0.55 $g \cdot tonne^{-1}$ (ppm) (Cherian *et al.*, 1985). Amounts in soil vary considerably from region to region, due to both natural factors and anthropogenic activities. The mean Cd levels from several studies on soils from rural, urban and agricultural soils from across Canada were in the range of 0.56 to 1.1 $mg \cdot kg^{-1}$ (ppm) on a dry weight basis, although considerably higher levels were reported in the immediate vicinity of sources of Cd, such as copper or zinc smelters (Canadian Environmental Protection Act, 1994). Within a few metres of one smelter, levels were as high as 151 $mg \cdot kg^{-1}$, but increased levels (approximately 5 $mg \cdot kg^{-1}$) were still noted over 40 km away. Data from studies comparing the Cd concentration in soils amended with Cd contaminated sludge with the Cd concentration in non-amended soils demonstrated that the average levels of Cd in soils treated with sludge were slightly

higher (0.68 $mg \cdot kg^{-1}$) than non-treated soils (<0.5 $mg \cdot kg^{-1}$) (Canadian Environmental Protection Act, 1994). In a survey of the distribution of Cd in soils across 850 000 km^2 of the Canadian prairies, Cd levels were in the range of <0.2-3.8 $mg \cdot kg^{-1}$ with a mean of 0.28 $mg \cdot kg^{-1}$ (Garrett, 1994). Most of the variability (96%) was noted at scales <20x20 km, indicating the high variability of Cd levels in soils from nearby sampling sites. Levels of Cd in soils of Essex County were 0.38 $mg \cdot kg^{-1}$, which is comparable to levels in prairie soils (Weis and Barclay, 1985).

1.3 Effects on Human Health

The cause for concern about non-essential metals, such as Cd, Hg, or Pb, in the environment is their effect on human health. Cd is absorbed into the body through the respiratory (most common route of industrial exposure) and digestive tracts. Approximately 20-60 % of the Cd from inhaled Cd-containing aerosols is absorbed into the bloodstream (Cherian *et al.*, 1985). In the general population, the primary source of Cd is from food products.

Absorption of Cd from the digestive tract is a passive process, with approximately 5-7% of the ingested Cd being absorbed, though this is strongly dependent on a number of factors, including the nutritional status (especially Ca and Fe levels), and age of the individual (Cherian *et al.*, 1985; Lester, 1987). Once in the body, Cd is circulated in the blood and deposited mainly in the liver and kidneys, which usually contain roughly half of the body's Cd, although occupationally exposed individuals also have a significant amount of Cd in their lungs. The digestive tract, bone, heart, pancreas and testes also contain Cd

upon exposure (Cherian *et al.*, 1985; Lester, 1987). Cd is long lived in humans, with the biological half-life estimated to be over 10 years (10-40 years for the kidney and 5-10 years for the liver) (Cherian *et al.*, 1985; Lester, 1987).

Toxic effects of heavy metals can be either acute or chronic. Acute response to Cd is rare, and generally results from either occupational exposure to CdO fumes or massive ingestion of Cd contaminated food. Symptoms due to occupational exposure occur 4-10 hours after exposure, and include dyspnea (difficulty breathing), coughing, chest pain and sometimes a burning sensation in the chest. Flu-like symptoms may also occur, with chills, fever and muscular pain in the back and limbs, as well as acute pulmonary edema (swelling of the lungs due to fluid) if the dose was high enough. Depending on the severity of the dose, the duration of symptoms may either lessen after one week, or result in death. Acute symptoms resulting from ingestion of Cd contaminated food include vomiting, abdominal pains, salivation and choking attacks. While short term exposures to high concentrations of Cd are very harmful, they are rare, and of greater importance to human health are the effects of exposure to low concentrations over a long period of time.

Chronic exposure to Cd affects the kidneys, where Cd tends to concentrate in the body. Renal damage, characterized by tubular proteinuria (increased excretion of low-molecular-weight proteins in the urine, due to reduced absorption of these proteins by the proximal tubules of the kidney) may occur once Cd concentrations in the kidney reach a certain level. The production of active vitamin D, which mediates calcium uptake by the kidneys, is reduced due to Cd induced renal damage, and the result is osteomalacia (weakening of bones). An extreme example of this was reported in Japan (Itai-Itai

disease) in 1955 and was due to Cd poisoning of the Jinzu River resulting from a faulty wastewater-treatment system in the Kamioka mine (Lester, 1987). Over a thirty year period, local residents accumulated high levels of Cd in their bodies by drinking the river water and using it to irrigate their rice paddies.

1.4 Cadmium in Food Products

While more serious cases of Cd toxicity result from occupational exposure, the average person receives most of his or her Cd from the food they eat or from smoking; both sources result from plant accumulation of Cd from soils. The amount of Cd in the diet depends on several factors, including the amount of Cd in the soils in which the plants were grown, the amount absorbed by the plant from the soil (which depends on what was bioavailable to the plant), the proportion of the absorbed Cd transported within the plant to the part of the plant consumed, and the amount of that plant part consumed. Levels of Cd in foods vary considerably, from 3 to 50 $\mu g \cdot kg^{-1}$ (ppb) on a fresh weight basis (Table 1.1). The World Health Organisation (WHO) has set 60 to 70 $\mu g \cdot day^{-1}$ as the maximum tolerable intake for an adult, and the CODEX Alimentarius Commission of the Food and Agriculture Organisation of the United Nations and World Health Organisation (FAO/WHO) has proposed 0.1 $mg \cdot kg^{-1}$ as a maximum limit for Cd in grain and oilseeds destined for export (WHO, 1989).

Table 1.1: Concentrations of Cd in various foods on a fresh weight basis (Wagner, 1993).

Food Type	Cd concentration on a fresh weight basis ($\mu g\ Cd \cdot kg^{-1}$ food)	amount of Cd consumed per day ($\mu g\ Cd \cdot day^{-1}$) *
grain and cereal	23.2	9.9
potatoes	48.0	8.5
leafy vegetables	40.5	2.2
legume vegetables	6.2	0.4
root vegetables	32.2	1.0
fruits	3.0	0.7
meat, fish and poultry	15.3	4.0
beverages	3.0	2.1

*determined be multiplying the concentration of Cd in each food type by the average amount of each food type consumed

1.5 Cadmium Accumulation by Plants; Influence of Bioavailability, Root Morphology, and Translocation

Non-essential metals such as Cd are not required nutrients by plants, but may be accumulated by different plant tissues to varying degrees. There is also considerable variability both between species and between different cultivars within a species in the amounts of Cd that will be accumulated by the plant. Some plant species only take up limited amounts of the metal from the soil, and are called 'excluders' (Baker, 1981; Taylor, 1987). Species which concentrate metals in their tissues are called accumulators, while other species, which have roughly similar concentrations of the metal in their tissue as in the soils are called 'indicator' species (Baker, 1981). In a survey of Cd levels in plants located in Essex County in southwestern Ontario, Cd levels in plants (on a dry weight basis) were found to be roughly equal to those found in soils (Weis and Barclay, 1985). Cadmium concentrations in corn and soybean ranged from 0.10 to 0.58 $mg \cdot kg^{-1}$, and were highest in roots, followed by stems, leaves, and seeds.

Plants accumulate Cd which is dissolved in soil solution, and soil characteristics such as pH, percent of organic matter, CEC, and the type and quantity of ligands dissolved in the soil solution affect accumulation by influencing the concentration and speciation of Cd in the soil solution. Soils with a higher pH, CEC, or soil organic matter content have a reduced proportion of dissolved Cd, since a greater proportion of Cd is bound to soil particles. In the soil solution which contains dissolved ligands (L^{z-}), dissolved Cd exists as the free ion (Cd^{2+}), or as one of several metal ligand complexes (CdL_n^{2-nz}), which are in

equilibrium with each other ($Cd^{2+} + L^{z-} \rightleftharpoons CdL_n^{2-nz}$). The actual proportion of the total dissolved Cd present as Cd^{2+} depends on the type and concentration of ligands dissolved in solution, as well as other factors, such as the concentration of inorganic ions, pH and temperature of the soil solution. The species of Cd in the soil solution (ionic Cd^{2+}, or bound to various organic or inorganic complexes; CdL_n^{2-nz}) is important since it influences the phytoavailability of Cd; Cd^{2+} is considered to be the most bioavailable form of Cd, although the bioavailability of CdL_n^{2-nz} is not known.

There is considerable evidence, for both aquatic organisms and higher plants, that accumulation or toxicity of dissolved metals such as Cd correlate best with the concentration of the free ion (Cd^{2+}) in solution, and not the total concentration of the dissolved metal (Campbell, 1995). This has led to the formation of the Free Ion Model, or Free Ion Activity Model (FIM or FIAM) to explain the effects of dissolved metals on organisms which are exposed to them (Morel and Hering, 1993; Parker and Pedler, 1997).

Physical factors may also influence uptake of metals by plants. Root morphology influences uptake of mineral elements: increased phosphorus concentration in plant tissue has been related to longer root hairs or different root length/shoot weight ratios (Föhse *et al.*, 1988). Bowen and Rovira (1971) demonstrated that the majority of phosphate and sulphate was accumulated by lateral roots of the seminal root system of 14 day old wheat seedlings, and suggested that varieties which produce more lateral roots may be better at utilizing phosphorus. In a study on root morphology of wheat genotypes differing in zinc efficiency (the ability to grow and yield better in Zn-deficient soil), it was observed that the Zn-efficient genotype tended to have longer and thinner roots than the Zn-inefficient

genotype (Dong *et al.*, 1995). Using a cadmium-selective microelectrode to measure Cd^{2+} flux along roots of *Thlaspi caerulescens* (a Zn/Cd hyperaccumulator), *Thlaspi arvense* (a related nonaccumulator) and *Triticum aestivum*, Piñeros *et al.* (1998) demonstrated that the flux of Cd^{2+} to the roots was greatest near the root tip, but occurred along the whole length of the root. This suggests that both the number of root tips in a root system, and the total surface area may influence the amount of Cd accumulated by a plant.

A portion of the Cd accumulated by root tissue, which is influenced by soil characteristics, solution chemistry, root physiology and possibly root morphology, is the Cd which is ultimately available for translocation to shoot tissue and those tissues (leaves, seeds) which are harvested and consumed by humans. While there is inter and intraspecific variation in Cd accumulation by root tissue, there is also considerable variation in root to shoot translocation of Cd. Plants typically have higher concentrations of Cd in roots than in stems and leaves, with even lower concentrations of Cd found in fruits, grains or seeds (Coughtrey and Martin, 1978; Jastrow and Koeppe, 1980; Kubota *et al.*, 1992). 'Kyle' and 'Arcola', two cultivars of durum wheat (*Triticum turgidum*) differ in their shoot Cd concentrations when grown under similar conditions (Chan, 1996). It seems that some species (or cultivars) differ in how mobile Cd is within the plant once it is accumulated by root tissue.

1.6 Phytotoxicity and the Fate of Cadmium in Plant Tissue

Tissue concentrations of non-essential metals such as Cd will increase with no adverse effect on plant function until the concentration in plant tissue reaches toxic levels,

and plant growth begins to decline. Growth of plants over a range of essential metal (i.e. zinc) concentrations in tissue will increase as concentrations of the metal become adequate and then decrease as levels become toxic.

As a non-essential metal, Cd has no known function in plants. Its electron configuration is similar to Zn, however, and both lose two electrons to form Cd^{2+} and Zn^{2+} in solution; this is the mechanism of Cd toxicity in both plants and animals, including humans. Cd^{2+} has the ability to replace Zn^{2+} in certain metalloenzymes (enzymes which require a specific metal to attain some property which is lacking without the metal), which interferes with the enzyme's activity. Zn occurs in a wide range of enzymes including alcohol dehydrogenase and enzymes involved in protein metabolism (Sharpe, 1992), which helps explain the extreme toxicity of Cd.

Plants can either detoxify Cd in plant tissue (tolerance), or they can exclude Cd from the symplast (exclusion). The fate of Cd^{2+} once it is in the symplast has been studied extensively. There is strong evidence to suggest that the presence of Cd^{2+} in the symplast activates an enzyme responsible for the synthesis of non-protein polypeptides with repeating (γ-Glu-Cys) units which have the ability to chelate Cd^{2+}. Five families of γ-Glu-Cys peptides (also called cadystin, class III metallothioneins, or phytochelatins) have been discovered so far. All five of these classes have the chemical structure $(\gamma\text{-Glu-Cys})_n\text{-X}$; where n=2 to 7 (depending on the organism, and level of Cd exposure), and X=Gly (true phytochelatins), Glu, β-Ala, Ser, or nothing at all, depending on the class of γ-Glu-Cys peptides (Rauser, 1995). Phytochelatins ($(\gamma\text{-Glu-Cys})_n\text{-Gly}$) are synthesised by the transfer of the γ-Glu-Cys dipeptide from glutathione (γ-Glu-Cys-Gly) to either a

receptor glutathione molecule, or a growing phytochelatin chain ($(\gamma\text{-Glu-Cys})_n\text{-Gly}$ + $(\gamma\text{-Glu-Cys}) \rightarrow (\gamma\text{-Glu-Cys})_{n+1}\text{-Gly}$) (Grill *et al.*, 1989). The enzyme responsible for the transfer has been named γ-glutamylcysteine dipeptidyl transpeptidase (or phytochelatin synthase), and requires metals to become activated. Cd^{2+} is the most efficient activator of the enzyme. Other metals such as Ag^{+}, Bi^{3+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Au^{+}, Ni^{2+}, and Co^{2+} are less efficient activators of the enzyme, and therefore do not result in the same size increase in phytochelatin levels as seen with Cd^{2+} exposure (Grill *et al.*, 1989; Ahner and Morel, 1995). The reasons that other metals are not as efficient at inducing phytochelatin synthesis may be that plants have other methods of chelating these toxic metals. Significant amounts of cellular Ag^{+} and Zn^{2+} ions, for example, may be bound to membranes (Ahner and Morel, 1995). The enzyme is constitutive, and is self regulated in the sense that the product of the reaction (phytochelatins) which it catalyses chelates the metal (Cd^{2+}) which activates the enzyme (Grill *et al.*, 1989). Little is known about the role of phytochelatins, and whether their production is induced, by exposure to the low concentrations of Cd typically found in agricultural soils.

Chelated Cd appears to exist as one of two classes of Cd-binding complexes, called low molecular weight (LMW) and high molecular weight (HMW) complexes based roughly on migration of the complexes in gel filtration chromatography (Rauser, 1995). LMW complexes appear to be made up of γ-Glu-Cys peptides plus chelated Cd, while HMW complexes appear to be groups of γ-Glu-Cys peptides, chelated Cd and S^{2-} (Rauser and Meuwly, 1995). There is evidence to suggest that Cd^{2+} is pumped into the vacuole by a Cd^{2+}/H^{+} antiport (Salt and Wagner, 1993), and phytochelatins (with or without chelated

Cd) are pumped into the vacuole by a MgATP driven pump (Salt and Rauser, 1995). Together, these observations provide evidence to suggest that Cd is sequestered in the vacuole. In a study on Cd exposed tobacco plants, virtually all of the Cd and Cd-binding peptides in leaves were found in the vacuoles of leaf cells (Vögeli-Lange and Wagner, 1990).

The responses discussed previously appear to be very efficient at protecting plants from toxic effects of Cd, although there is evidence to suggest that levels of phytochelatin production alone are not responsible for differential plant sensitivity to Cd. In populations of Cd-tolerant and Cd-sensitive *Silene vulgaris*, for example, differential sensitivity to Cd did not appear to result from different phytochelatin levels (de Knecht *et al.*, 1992; 1994). Tolerant plants had a lower rate of phytochelatin synthesis as well as a lower rate of synthesis of the longer chain phytochelatins, which are stronger chelators of Cd^{2+}. Roots of sensitive plants had more Cd in them, but only after one or three days of exposure; after 7 days of exposure to Cd, the concentrations of Cd in the roots of sensitive and tolerant plants were similar. The investigators did not attribute differential sensitivity to differential uptake of Cd^{2+} by the roots, since levels of Cd in the roots of tolerant plants had to be three times the concentration in sensitive plants to have a similar effect on root growth. The authors suggest that a possible reason for differential sensitivity may be the rate or efficiency of sequestering Cd in the vacuole.

Mechanisms of exclusion of Cd from the symplast have not been studied, although exclusion mechanisms for other metals, especially Al, have been demonstrated. Taylor (1987) suggests four possible mechanisms of excluding metals from the symplast. One

mechanism is accumulation of metals in the cell wall, thus reducing uptake of metals into the symplasm. This has been clearly demonstrated for Zn, and a correlation between Zn tolerance and accumulation of Zn by cell wall fractions has been demonstrated in sixteen populations of *Agrostis tenuis* (Turner and Marshall, 1972). A problem with this mechanism is the question of how much of the metal can actually be accumulated by the cell wall, suggesting that if the mechanism exists, it may not play a major role in excluding metals from the symplast.

A second mechanism of exclusion may be the formation of a redox barrier at the plasma membrane. In reduced substrates, plants have been observed to create an oxidized zone near their roots which metals must pass through. The solubility of both Fe and Mn are reduced when they are oxidized (from Fe^{2+} to Fe^{3+}, and from Mn^{2+} to Mn^{3+}), and these metals are therefore less available, and less toxic, to the plant if they are in their oxidized state. Unlike Fe and Mn, dissolved Cd exists as only one rather stable oxidation state, Cd^{2+}, so this mechanism would not act directly on Cd. Mobilization and availability of Cd are reduced by hydrous metal oxides in the soil (Canadian Environmental Protection Act, 1994), and oxidation of the rhizosphere may result in changes to other aspects of soil chemistry, which may in turn influence Cd availability to the root.

The formation of a pH barrier may be a third mechanism of exclusion, since the solubility, and therefore availability of many metals is pH dependent. For example, Al undergoes a substantial increase in solubility as the pH drops from 5.0 to 4.5, and the existence of a relationship between Al-tolerance and the ability to maintain a higher pH in the growth medium provides evidence for pH modification of the rhizosphere as a possible

mechanism of tolerance to Al. Differential pH in the growth medium was shown to be related to differences in the relative absorption of cations and anions. For example, cultivars which used NH_4^+, instead of NO_3^-, as their source of nitrogen had the lowest rhizosphere pH and were most affected by Al (Taylor and Foy, 1985). It should be noted that consistent correlations between differential plant-induced rhizosphere pH changes and differential tolerance to Al are lacking, so that other mechanisms of tolerance to Al must also be present. The solubility of Cd in soil and aqueous media is also dependant on pH, but there have been no reports yet indicating whether plants modify their rhizosphere such that Cd availability is reduced.

A fourth method of exclusion is the exudation of molecules which either chelate the metal in question by making it unavailable for uptake, or compete with the sites on the root where the metal is transported across the membrane. Exudation of chelates has been observed in response to deficiencies in Fe and Zn, where the chelates served to mobilize deficient essential nutrients (Taylor, 1987; Zhang *et al.*, 1991). Exudation of both citric and malic acid from the roots of various species has been observed in response to Al stress, and for both snapbeans (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.), tolerant cultivars tended to secrete more exudates than sensitive cultivars (Miyasaka *et al.*, 1991; Delhaize *et al.*, 1993; Basu *et al.*, 1994b). The protective effect of exudates was demonstrated in an earlier experiment with carrot cell suspension cultures, which demonstrated that when medium conditioned with Al-tolerant carrot cells was used to grow Al-sensitive carrot cells, their sensitivity to Al stress decreased (Ojima and Ohira, 1985). Four organic acids were discovered in the medium from Al-tolerant cells, one of

which was citric acid. When citric acid was added to unconditioned medium, it was found to reduce toxicity of sensitive carrot cells to Al. More recently, exudation of polypeptides in response to Al was observed in several cultivars of wheat (*Triticum aestivum* L.) (Basu *et al.*, 1994a). Cultivars which were more tolerant to Al had increased exudation of polypeptides in general, but also had increased exudation of specific polypeptides with stronger association with Al, suggesting a role in tolerance to Al. Exudates from cultivars of durum wheat (*Triticum turgidum*) have been identified in sterile nutrient solutions (Cieslinski *et al.*, 1997), though the influence of these exudates on speciation of Cd in solution, or on bioavailability of Cd is not known.

1.7 Research Objectives

The concentration of Cd in the environment has been increasing during recent decades due to anthropogenic activities such as smelting. There is considerable interest in amending agricultural soils with sewage sludge, a rich source of organic matter, but addition of sludge may increase the amount of Cd in the soils in which agricultural crops are grown since these sludges often contain metals such as Cd. Different species of plants (or cultivars of the same species) growing under similar conditions and exposure to Cd often accumulate different amounts of Cd in their tissues (Baker and Walker, 1990; Jalil *et al.*, 1994). An understanding of how Cd moves from soil into the plant and to those plant parts which are harvested and then consumed, and why there are differences among species or cultivars of the same species is very important, since Cd is a non-essential metal which can bioaccumulate in tissues over the lifetime on an individual. It is important,

therefore, to limit the daily consumption of Cd. There may also be economic impacts to producing grains or oilseeds which contain relatively high concentrations of Cd, since the CODEX Alimentarius Commission of the Food and Agriculture Organisation of the United Nations and World Health Organisation (FAO/WHO) has proposed 0.1 $mg \cdot kg^{-1}$ as a maximum limit for Cd in grain and oilseeds destined for export (WHO, 1989). Grain of durum wheat grown on the Canadian prairies often contains a Cd concentration which exceeds this limit. Phytoremediation of metal contaminated soils can take advantage of enhanced understanding of which forms of Cd are bioavailable, and how these influence mechanisms of accumulation and translocation of Cd. In this field, it is desirable not only to have plants with a high rate of accumulation of Cd from the soil, but also a high rate of translocation to shoots, which could then be easily harvested and disposed.

There were two major objectives to the research carried out and presented in this thesis. The first objective was to determine how the bioavailability of dissolved Cd was influenced by altering exposure solution chemistry by adding compounds (both natural and synthetic, organic and inorganic) which formed soluble complexes with Cd (CdL_n^{2-nz}) or by altering concentrations of Ca^{2+} and Mg^{2+}, which might compete with Cd^{2+} for uptake (Chapters 2, 3 and 4). The second objective was to characterize cultivar differences which may be responsible for the observed differences in grain accumulation of Cd by two cultivars of durum wheat, 'Kyle' and 'Arcola'. The goals were to determine if differences in Cd accumulation by root tissue of these cultivars could be related to observed differences in root morphology (Chapter 5), and to determine if differences in grain accumulation were reflected by differences in root or shoot translocation of Cd by wheat

seedlings (Chapter 6). In carrying out this last objective, it was also possible to determine if solution chemistry was modified by contact with actively growing root tissue, and to determine if this modification was specific to each cultivar.

The information gathered in the course of this research provides insight into the relationship between different forms of dissolved Cd and root morphological characteristics, and accumulation of Cd by root tissue. The accumulated Cd in the roots of plants represents the total amount that is potentially available for translocation to harvestable plant organs. Furthermore, it provides some information into cultivar differences in root to shoot translocation of accumulated Cd.

CHAPTER 2:

THE INFLUENCE OF CITRATE AND INORGANIC IONS ON ACCUMULATION OF CADMIUM BY DURUM WHEAT: EXCEPTIONS TO THE FREE ION MODEL?

2.1 Introduction

Plants accumulate ions which are dissolved in soil solution, and soil characteristics (CEC, pH, organic content, and Cd concentration) affect accumulation by influencing the concentration and speciation of Cd in the soil solution; a higher pH, CEC, or soil organic matter content tend to reduce the proportion of dissolved Cd, since they result in a greater proportion of Cd bound to soil particles. In the soil solution (containing dissolved ligands; L^{z-}), Cd exists as the free ion (Cd^{2+}), or as one of several metal ligand complexes (CdL_n^{2-nz}), which are in equilibrium with each other ($Cd^{2+} + L^{z-} \rightleftharpoons CdL_n^{2-nz}$). The actual proportion of the total dissolved Cd present as the free ion depends on the type and concentration of ligands dissolved in solution, as well as other factors, such as the concentration of inorganic ions, solution pH and temperature.

There is considerable evidence, for both aquatic organisms and higher plants, that accumulation of, or toxicity to, dissolved metals such as Cd correlate best with the concentration of the free ion (Cd^{2+}), and not to the total concentration of the dissolved metal (Campbell, 1995). This has led to the formation of the Free Ion Model, or Free Ion Activity Model (FIM or FIAM) to explain the effects of dissolved metals on organisms which are exposed to them (Morel and Hering, 1993; Parker and Pedler, 1997). This model assumes that 1) the effect of the metal is proportional to the extent of occupancy of cell surface binding sites by the free ion and not a complexed form, 2) there are no other metals in the exposure solution which interact with either dissolved ligands or cell surface binding sites and 3) the rate limiting step in the process is the metal interacting with cell surface binding sites (i.e. diffusion to these sites is not rate limiting). Some recent studies,

however, have indicated that exceptions to the FIM exist (Campbell, 1995). Smolders and McLaughlin (1996a; b) found that increasing the concentration of Cl in the exposure solution resulted in enhanced accumulation of Cd by Swiss chard in relation to solution Cd^{2+} concentration. Increasing Cl^- concentration in solution resulted in a higher concentration of $CdCl_n^{2-n}$ species, and the authors suggested that Cd accumulation was increased due to uptake of these species, or enhanced diffusion of Cd^{2+} to the uptake sites. In a study on the effect of increasing the concentration of SO_4 in solution on accumulation of Cd , it was discovered that plant tissue Cd concentrations were unaffected by increasing solution SO_4 concentrations, even though the concentration of Cd^{2+} in solution was reduced significantly, leading the authors to conclude that $CdSO_4^0{}_{(aq)}$ was taken up as readily as Cd^{2+} (McLaughlin *et al.*, 1998). Srivastava and Appenroth (1995) found that addition of EDTA to a solution containing Cd significantly reduced the Cd^{2+} concentration, and also the accumulation of Cd by duckweeds (*Lemnaceae*). However, the reduction in accumulation was not as great as predicted by the reduction in Cd^{2+} concentration, and the authors attributed this to uptake of CdEDTA species through breaks in the root endodermis or dissociation of CdEDTA during treatment. A recent study with unicellular algae has demonstrated that the toxicity of Cd and Zn is not solely dependent on their free ion (Cd^{2+} and Zn^{2+}) concentrations, but that the co-presence of a low molecular weight metabolite (citrate) resulted in greater Cd and Zn toxicities than predicted for similar free ion activities (Errécalde *et al.*, 1998). The citrate was accumulated at a rate which was four times higher than Cd, leading the authors to conclude that the accidental transport of a CdCitrate complex by the citrate transporter

once in every four transport events would account for the enhanced toxicity in the presence of citrate.

In the present study, two cultivars of durum wheat (*Triticum turgidum*) which have previously been demonstrated to have different patterns of Cd accumulation and tissue distribution (Chan, 1996; Berkelaar and Hale, 2000) were used to establish the relationship between accumulation of Cd in plant roots and citrate (a LMW metabolite) in the rooting solution. Citrate is secreted from durum wheat roots (Cieslinski *et al.*, 1997), and may influence speciation of Cd at the root surface, in addition to speciation of Ca and Mg. The effects of altered Ca^{2+} and Mg^{2+} concentrations on Cd accumulation were tested in order to determine if the effects of citrate on the accumulation of Cd by plant roots were caused by the presence of Cd complexes ($CdCitrate^-$), or reductions in estimated Ca^{2+} or Mg^{2+} concentrations due to the formation of $CaCitrate^-$ or $MgCitrate^-$ complexes. The null hypothesis was: accumulation of Cd by roots of two cultivars of durum wheat is dependent only on the concentration of the free ion (Cd^{2+}), and is not influenced by the presence of citrate, or changes in estimated Ca^{2+} or Mg^{2+} concentrations. If accumulation is dependent only on the Cd^{2+} concentration in the exposure solution, then there should be a simple relationship between Cd^{2+} concentration and accumulation of Cd by roots, independent of other Cd species or concentrations of inorganic ions.

2.2 Materials and Methods

2.2.1 Experimental Design

This study was conducted as six separate experiments, each of which was a

complete factorial design (cultivar, time, and exposure solution composition) in a completely randomized design (Table 2.1). Overall, the influences of Cd^{2+} concentration (a proportion of nominal concentrations of $8.90 \cdot 10^{-9}$, $4.45 \cdot 10^{-8}$, $8.90 \cdot 10^{-8}$ or $4.45 \cdot 10^{-7}$ M added as a $Cd(NO_3)_2$ stock solution), citrate (nominal concentrations of 0, $1.00 \cdot 10^{-3}$ M or $3.00 \cdot 10^{-3}$ M), and the inorganic ions Ca (nominal concentrations of $3.00 \cdot 10^{-3}$, $1.50 \cdot 10^{-3}$ or $1.00 \cdot 10^{-3}$ M), Mg (nominal concentrations of $1.50 \cdot 10^{-3}$, $7.50 \cdot 10^{-4}$ or $5.00 \cdot 10^{-4}$ M) and K (nominal concentrations of $4.00 \cdot 10^{-3}$ or $1.40 \cdot 10^{-2}$ M) on root Cd content were evaluated in two durum wheat cultivars ('Kyle' and 'Arcola') over a range of durations of exposure to Cd^{2+} (0 to 210 mins) (Table 2.1). Soil solution Cd^{2+} concentrations rarely exceed $5 \cdot 10^{-8}$ to $1 \cdot 10^{-7}$ M in agricultural soils. The first experiment established Cd accumulation in the roots of two cultivars of durum wheat, as influenced by the estimated Cd^{2+} concentration in the root solution and duration of exposure. The remaining five experiments confirmed the results of the first experiment and measured Cd accumulation in seedling roots as influenced by citrate, Ca, Mg, or K in the rooting solution (Table 2.1). These three inorganic ions were not of primary interest in this study, but their concentrations in the exposure solutions were partially confounded by citrate concentrations, as citrate forms complexes with Ca^{2+} and Mg^{2+} as well as Cd^{2+}. Significant amounts of KOH were required to compensate for the effect of citrate additions on solution pH, and KNO_3 or K_2SO_4 were added to reduced Ca or Mg solutions to restore NO_3 or SO_4 concentrations.

2.2.2 Plant Material and Growth Conditions

Caryopsis of durum wheat (*Triticum turgidum*) cvs 'Kyle' and 'Arcola' were

Table 2.1: Factors and levels of each factor tested in each of the six experiments.

exp. #	target nominal [Cd] ($\cdot 10^{-8}$ M)	citrate (M)	Ca	Mg	K**
1	0.890, 4.45, 8.90, or 44.5	0	'control'	'control'	'control'
2	4.45 or 44.5	0 or $1.00 \cdot 10^{-3}$	'control'	'control'	'control'
3	4.45, 8.90, or 44.5	0 or $3.00 \cdot 10^{-3}$ *	'control' or ⅓+	'control' or ⅓+	'control', ⅓, or 3.5x
4	4.45, 8.90, or 44.5	0 or $3.00 \cdot 10^{-3}$ *	'control' or ½++	'control' or ½++	'control', 1.75x, 1.375x, or 3.5x
5	4.45, 8.90, or 44.5	0	'control' or ⅓++	'control' or ⅓++	'control', 2x, or 1.5x
6	4.45, 8.90, or 44.5	0	'control'	'control'	'control' or 3.5x ***

* only $8.90 \cdot 10^{-8}$ or $4.45 \cdot 10^{-7}$ M Cd solutions contained citrate

+ only $8.90 \cdot 10^{-8}$ or $4.45 \cdot 10^{-7}$ M Cd solutions contained reduced concentrations of inorganic ions

++ only $8.90 \cdot 10^{-8}$ M Cd solutions contained reduced nominal Ca or Mg concentrations

** an increase in the nominal K concentration was a confounding factor in solutions containing citrate or reduced nominal Ca or Mg concentrations, except in experiment 6

*** an increase in the nominal K concentration was confounded with an increase in nominal concentrations of NO_3, SO_4, or both NO_3 and SO_4

germinated in Petri dishes on filter paper (Whatmann #1) wetted with distilled water (Step 1, Figure 2.1). Two days after seeding, 12 germinated caryopsis were transferred to a nylon mesh with about nine holes per cm^2 which was floating (using Styrofoam strips) on modified ¾-strength Hoagland's nutrient solution (Fe^{3+} was supplied as $2.68 \cdot 10^{-5}$ M FeHEDTA and the $MnCl_2$ concentration was reduced by half) (Hoagland and Arnon, 1950) at a pH of 6.0 in an opaque 2.5 L pot (Classic 300, Nursery supplies Inc., Fairless Hills, PA) (Step 2, Figure 2.1). The nutrient solution contained nominal Ca, Mg and K concentrations of $3.0 \cdot 10^{-3}$, $1.5 \cdot 10^{-3}$ and $4.5 \cdot 10^{-3}$ M, respectively. The pot was attached to a recirculating hydroponic system in a greenhouse that provided fresh nutrient solution in order to maintain balanced concentrations of nutrient ions. Two days before cadmium exposure, each mesh was thinned to nine seedlings. Six-day old seedlings (from the time of germination) were used in experiments 1, 2, 3, 5 and 6 and seven-day old seedlings were used in experiment 4.

2.2.3 Cadmium Exposure and Solution Analysis

For the determination of cadmium content of roots, each nylon mesh with seedlings was removed from the growth solution and placed on top of an acid washed 250 mL HDPE beaker (Fisher Scientific, Ltd., Napean, ON) filled to the brim with one of the exposure solutions (Step 3, Figure 2.1). For experiments 1 and 2, all of the seedlings in a beaker were harvested at either 0, 30, 60, 90, 120, 150, 180, or 210 mins after exposure began and for experiments 3, 4, 5 and 6, seedlings were harvested at either 0, 50, 100, 150, or 200 mins after exposure began. Meshes were removed from the exposure

Figure 2.1: Experimental procedure for growing and exposing seedlings to Cd.

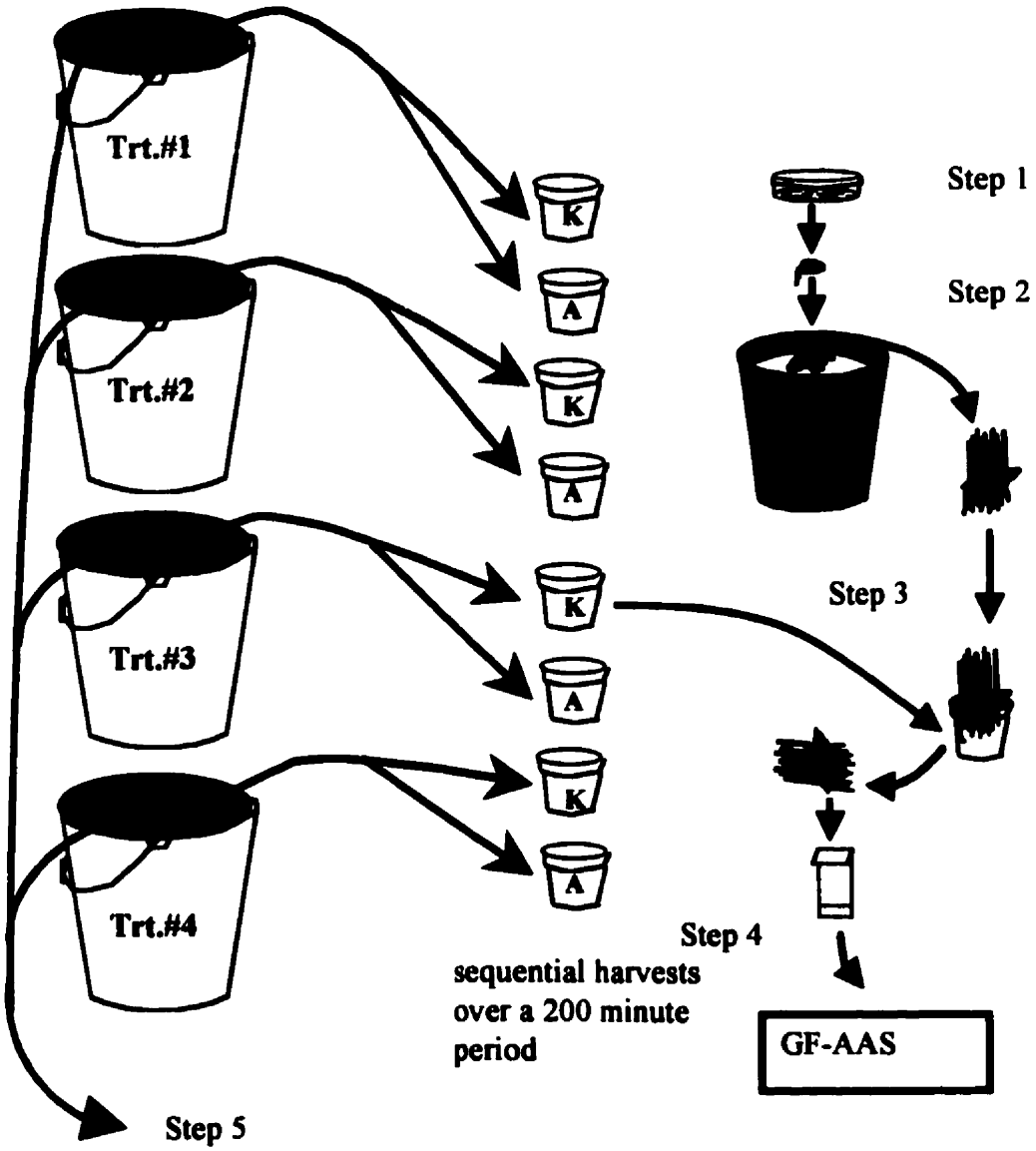
Step 1: Caryopses were germinated in Petri dishes on Whatmann #1 filter paper moistened with distilled water.

Step 2: After two days, germinated caryopses were transferred to nylon mesh squares floating on modified ¾-strength Hoagland's.

Step 3: Six days after seeding, the meshes with seedlings were transferred to 250 mL HDPE beakers containing exposure solution.

Step 4: After exposure, roots were rinsed in deionized water, harvested, dried, acid digested and analysed for Cd.

Step 5: Exposure solutions were sampled and analysed for total Cd by GF-AAS.


Trt.#1
Trt.#2
Trt.#3
Trt.#4
K
A
Step 1
Step 2
Step 3
Step 4
Step 5
sequential harvests
over a 200 minute
period
GF-AAS

solutions and roots were rinsed with deionized water, separated from shoots and placed in #1 coin envelopes (5.6 x 8.8 cm, Basics, Acton, MA) before being dried at 80°C for 48 hours (Step 4, Figure 2.1). Accumulation data represent Cd actually taken up into the symplast, as well as Cd within the apoplast. However, there was little release of ^{109}Cd from intact roots exposed to Cd concentrations which were similar to the concentrations used in this study (Hart *et al.*, 1998a). In another study, in which durum wheat seedlings were exposed to $2.0 \cdot 10^{-8}$ M ^{109}Cd, it was found that less than 5% of the ^{109}Cd present in roots was desorbed when exposure durations were 50 min or longer (Buckley *et al.*, 1997).

Exposure solutions were sampled, and the total Cd concentration was measured by GF-AAS (model SpectrAA-300 Atomic Absorption Spectrometer with a GTA-96 Graphite Tube Atomizer attachment; Varian, Australia) (Step 5, Figure 2.1). The GF-AAS was calibrated with a 1000±3 $\mu g \cdot ml^{-1}$ cadmium solution (High Purity Standards, Charleston, SC) diluted to 10 $\mu g \cdot L^{-1}$. Quality control was ensured with ICP Analytical Mixture 3 (containing Al, As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, V, and Zn; High Purity Standards, Charleston, SC) diluted to 10 $\mu g \cdot L^{-1}$ Cd and analysed along with experimental samples; the measured Cd of the internal standards was 10.0 ± 0.3 $\mu g \cdot L^{-1}$. Chemical speciation of Cd and other ions in the exposure solution was estimated using the chemical equilibrium program MINEQL$^+$ Version 3.0 (Schecher and McAvoy, 1994) using constants from NIST (Smith *et al.*, 1997). Formation constants for the complexes of interest are in Appendix B (Table B1). Modelling was done at a temperature of 25 °C and with ionic strength corrections turned on. The consistency of both the total dissolved

Cd concentration and the Cd speciation for the duration of the exposure period was verified by sampling exposure beakers both before and after exposure in experiment 1. The total Cd concentration did not change and the Cd^{2+} concentration (measured by an Ion Exchange Technique, Cantwell *et al.*, 1982) remained constant for the duration of the exposure period. For subsequent experiments, only the total Cd concentration in the exposure solutions was measured prior to exposure. Citrate can be used as a carbon source by bacteria present in the hydroponic solution, but concentrations were likely to remain stable in these experiments; the volume of solutions was high relative to the root mass, and durations of exposure were not long. Differences in measured Cd^{2+} concentrations between solutions with and without citrate (measured by an Ion Exchange Technique) were consistent with differences predicted by MINEQL$^+$.

2.2.4 Plant Digestion and Cd Analysis

Roots (about 30 mg dry weight) were completely digested using Topper and Kotuby-Amacher's method (1990) with modifications. After drying, the combined roots of the nine seedlings from each mesh were weighed and placed in acid washed Teflon digestion vessels with 1.5 mL trace metal grade HNO_3. The digestion was carried out unsealed at room temperature for 5 hours, and then the vessels were sealed and placed in an oven at 110°C overnight. Spinach leaves (NIST Standard Reference Material #1570a, US Department of Commerce, National Institute of Standards and Technology, Gaithersberg, MD) were digested with each run, and data were corrected to the NIST value for cadmium of 2.89±0.07 $\mu g \cdot g^{-1}$; results were within 10% of the NIST standard

value. Plant digests were diluted to 4.5 mL with nanopure water and the Cd concentration was measured by GF-AAS, calibrated as for exposure solution analysis, but with a similar HNO_3 concentration in the standards as in the samples to be analysed. Cadmium content of roots was expressed on a per root dry weight ($\mu g\ Cd \cdot g^{-1}$ dry weight) basis.

2.2.5 Data Analysis

Because root accumulation of Cd for each cultivar was measured as a function of multiple estimated Cd^{2+} concentrations and exposure durations, regression relationships were established for each group of data using SAS PROC GLM (SAS Institute Inc., Cary, NC) separately for each cultivar. Estimates of variation came from regression analysis; there was one replicate of each combination of treatment solution and duration of exposure, except for control solutions, of which there were eight replicates. The data were grouped for analysis as follows: the control exposure solutions from each of the six experiments (containing no citrate or changes in concentrations of inorganic ions); the citrate-containing solutions from each of three experiments; the ⅓-strength control ion concentration solutions from one experiment; reduced Ca or Mg solutions from two experiments; increased K solutions from one experiment. The estimated Cd^{2+} concentrations in the exposure solutions were transformed to their natural log (ln) as their arithmetic values were not evenly spaced. For each regression relationship, the concentration of Cd in the root tissue was related to the main effects of cultivar, time, and Cd^{2+} concentration in the exposure solution, and two and three way interactions of these parameters. As appropriate, the concentration of citrate, control solution strength, Ca,

Mg, and K and interactions involving these terms were included in the analysis. Non-significant interactions were dropped from the model, one at a time (in an iterative reduction, starting with the highest order interactions), and their sums of squares were pooled with the error term. The final regression models were then examined for main effects and interactions involving the hypothesized modifiers of Cd bioavailability. Regression relationships from the modified solutions were compared to the response surface of the control solutions by superimposition.

2.3 Results and Discussion

Throughout the discussion, the terms nominal concentration (i.e. nominal Cd concentration) and estimated ion concentration (i.e. estimated Cd^{2+} concentration) are used. Nominal concentration refers to the concentration of a compound or element added to solution (or, in the case of Cd, the total concentration measured by GF-AAS), while the estimated ion concentration is the estimated concentration of a particular chemical species after a solution has reached equilibrium, as determined by MINEQL$^+$ modelling. In all cases, accumulation of Cd by wheat roots was expressed relative to the estimated exposure solution Cd^{2+} concentration (determined by measuring the total Cd concentration in each exposure solution and estimating the proportion of the total dissolved Cd present as Cd^{2+} with MINEQL$^+$) (Table 2.2). Values presented in Table 2.2 are the proportions of various species as a percentage of their nominal concentration. For Cd, these proportions applied to exposure solutions containing different nominal Cd concentrations, since the nominal Cd concentrations in the exposure solutions were orders of magnitude below the

Table 2.2: Proportions of the various Cd species and other (Ca^{2+}, Mg^{2+} and K^{+}) significant species in the different exposure solutions. The pH of the exposure solutions was 6.0.

Species	Proportion of Species as a Percentage of Total Dissolved Ion										
	control	citrate			inorganic ions						
	control	0.001 M	0.003 M	0.003 M (balanced Ca^{2+} and Mg^{2+})	⅓-strength nutrient solution	½ Ca	½ Mg	⅓ Ca	⅓ Mg	3.5x K added as KNO_3	3.5x K added as K_2SO_4
Cd^{2+}	**87.8**	**65.2**	**29.6**	**49.6**	**93.0**	**86.9**	**87.4**	**86.6**	**87.3**	**88.3**	**68.9**
$CdSO_4^0{}_{(aq)}$	10.2	7.7	3.6	4.7	6.0	11.0	10.6	11.4	10.7	8.4	28.2
$Cd(SO_4)_2^{2-}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6
$CdNO_3^+$	1.6	1.2	0.0	1.2	0.0	1.6	1.6	1.6	1.6	2.9	1.1
$CdCitrate^-$	**0.0**	**24.9**	**64.5**	**43.3**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CdHCitrate	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ca^{2+}	91.0	71.6	35.8	56.6	95.0	90.3	90.7	90.1	90.6	91.0	77.0
$CaCitrate^-$	0.0	20.8	59.2	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mg^{2+}	93.6	73.7	37.0	58.5	96.3	93.0	93.4	92.8	93.3	94.7	80.6
$MgCitrate^-$	0.0	20.9	59.7	37.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
K^+	99.0	99.0	98.6	98.9	99.3	99.0	99.0	99.0	99.0	98.6	97.7

concentrations of other media components and therefore did not alter the speciation of these other ions. In the absence of citrate, the estimated Cd^{2+} concentration typically ranged from 87 to 93% of the nominal Cd concentration, depending on the concentration of various inorganic ions, with most (6 to 11%) of the remaining Cd present as $CdSO_4^0{}_{(aq)}$ (Table 2.2). The key question being asked in this research was whether root accumulation of Cd is dependent only on the estimated Cd^{2+} concentration, or whether $CdCitrate^-$ complexes are bioavailable as well. However, investigation of this question required manipulations of rooting solutions which resulted in changes in concentration and speciation of elements in addition to Cd and citrate. Specifically, changes in Cd speciation were partially confounded with changes in Ca and Mg speciation upon addition of citrate, the presence of citrate was partially confounded with increases in the nominal K concentration, because of the use of KOH to adjust the pH, and reduced nominal Ca or Mg concentrations in exposure solutions were partially confounded with increases in the nominal K concentration, because K was the cation used to maintain balanced nominal NO_3 and SO_4 concentrations when nominal Ca or Mg concentrations were reduced. Therefore, the effects of an increase in the nominal concentration of K, Ca, and Mg on solution speciation and plant uptake of Cd had to be characterised to validate the effects attributed to citrate. Having said this, changes in inorganic ion concentrations did not tend to affect speciation of Cd much. The exception to this was the 3.5x K solution (supplied as K_2SO_4) used in experiment 6, which also contained a nominal SO_4 concentration which was 2.4x higher than in the control solution. In this solution, the extra SO_4^{2-} shifted the equilibrium between Cd^{2+} and $CdSO_4^0{}_{(aq)}$ relatively more in favour of $CdSO_4^0{}_{(aq)}$, resulting

in an estimated Cd^{2+} concentration of 68.9% of the total dissolved Cd, with 28.2% present as $CdSO_{4\,(aq)}^{0}$ (Table 2.2).

2.3.1 Baseline Cd Accumulation

The analysis of data collected from plant roots exposed to the control exposure solutions (Table 2.3) demonstrated strong higher order interactions among $lnCd^{2+}$ concentration, exposure duration and cultivar, suggesting that the accumulation of Cd in the roots of these two durum wheat cultivars was dissimilar, and that the magnitude of the difference between them depended on both the $lnCd^{2+}$ concentration and duration of exposure (Table 2.4). The response surfaces demonstrate that accumulation of Cd in roots of 'Arcola' was greater than that for 'Kyle' (Figures 2.2 and 2.3). Accumulation of Cd by durum wheat under these conditions was less than that reported by Hart *et al.*, (1998a). In that study, durum wheat (cv Renville) exposed to $2.15 \cdot 10^{-7}$ M Cd for 60 min accumulated about 12 $nmol \cdot g^{-1}$ Cd on a fresh weight basis. Assuming that 95 g fresh weight is roughly equal to 1 g dry weight, this works out to 128 $\mu g \cdot g^{-1}$ Cd on a dry weight basis, compared with about 2.4 and 5.5 $\mu g \cdot g^{-1}$ for 'Kyle' and 'Arcola', respectively, if they were exposed to a similar Cd concentration for 60 min. The difference could be due to the fact that the exposure solution used by Hart *et al.* (1998a) contained 93% less Ca ($2.0 \cdot 10^{-4}$ M compared with $3.0 \cdot 10^{-3}$ M) and no Mg (0 M compared with $1.5 \cdot 10^{-3}$ M) than the exposure solutions used in this study. Ca^{2+} and Mg^{2+} may compete with Cd^{2+} for uptake; in a closely related study with Zn, it was demonstrated that reducing the Ca activity resulted in increased Zn uptake (Hart *et al.*, 1998b). Another possible explanation

Table 2.3: Nominal and estimated concentrations used in control exposure solutions (experiments 1 through 6).

Ion	Nominal Concentration (Estimated Concentration) (M)
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$ ($2.73 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)
K (K^{+})	$4.00 \cdot 10^{-3}$ ($3.96 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$ ($9.93 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-3}$ ($1.17 \cdot 10^{-3}$)
Cd (Cd^{2+})	$8.90 \cdot 10^{-9}$ ($7.81 \cdot 10^{-9}$)
	$4.45 \cdot 10^{-8}$ ($3.91 \cdot 10^{-8}$)
	$8.90 \cdot 10^{-8}$ ($7.81 \cdot 10^{-8}$)
	$4.45 \cdot 10^{-7}$ ($3.91 \cdot 10^{-7}$)
pH	6.0

Table 2.4: Sources of variation in content of Cd in roots exposed to control exposure solutions from each of the 6 experiments.

Source	df	F-value	p-value
Model	16	93.32	<0.0001
rep	5	4.10	0.0015
cultivar	1	0.02	0.90
time*cultivar	2	2.73	0.068
time*time*cultivar	2	7.60	0.00068
$lnCd^{2+}$*cultivar	2	10.69	<0.0001
$lnCd^{2+}$*$lnCd^{2+}$*cultivar	2	90.24	<0.0001
time*$lnCd^{2+}$*cultivar	2	198.42	<0.0001
Error	186	2.16	

Figure 2.2: Concentration of Cd in 'Kyle' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Kyle'

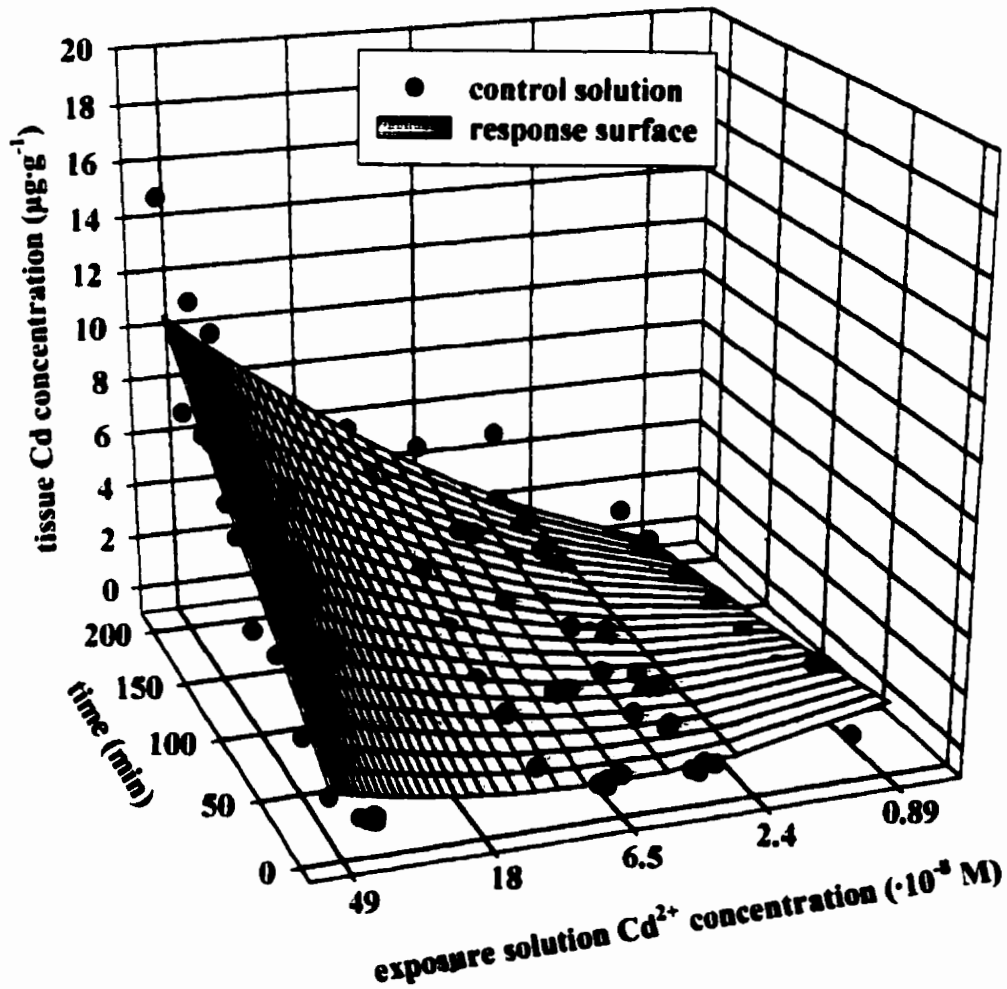
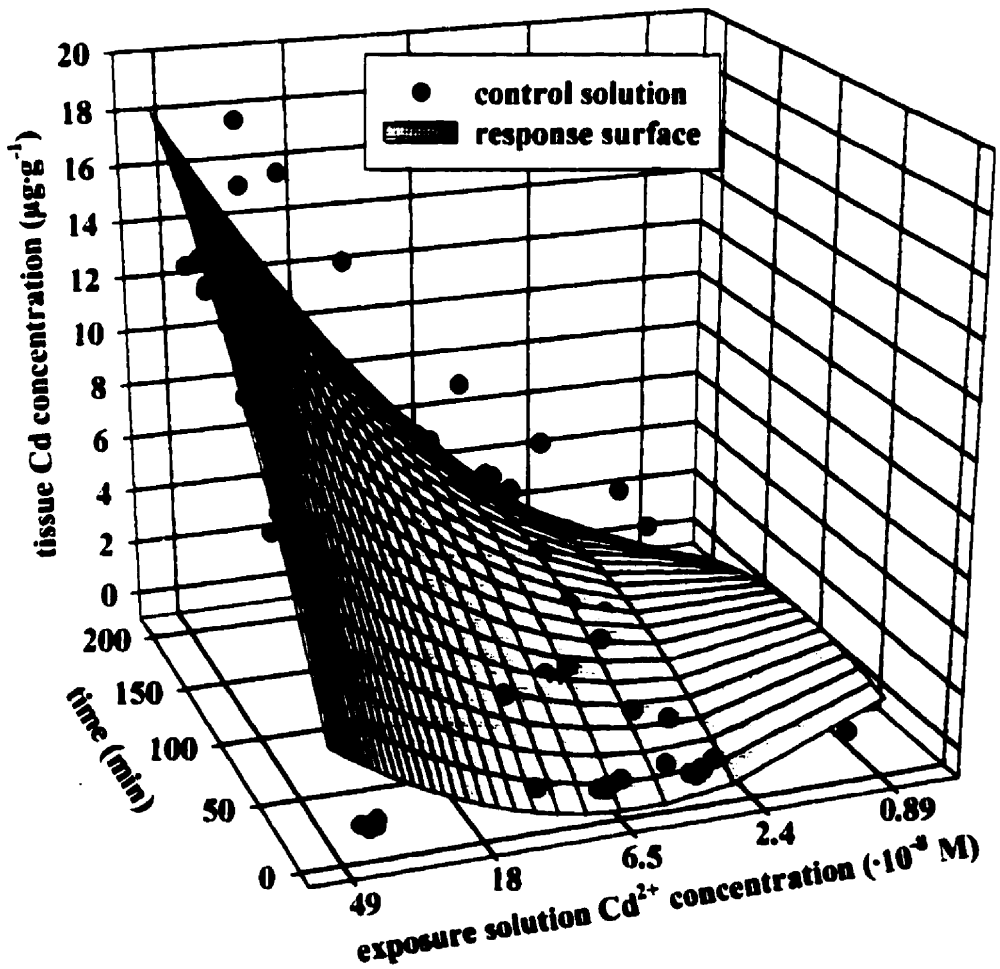

control solution
response surface
tissue Cd concentration (µg·g-1)
20
18
16
14
12
10
8
6
4
2
0
time (min)
200
150
100
50
0
49
18
6.5
2.4
0.89
exposure solution Cd2+ concentration (·10-8 M)

Figure 2.3: Concentration of Cd in 'Arcola' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Arcola'


control solution
response surface
tissue Cd concentration (µg·g⁻¹)
20
18
16
14
12
10
8
6
4
2
0
time (min)
200
150
100
50
0
49
18
6.5
2.4
0.89
exposure solution Cd2+ concentration (·10⁻⁸ M)

for the difference may have been due to the fact that the exposure solutions were strongly aerated in the study by Hart *et al.*, (1998a), which may have resulted in a narrower boundary layer surrounding the roots, and perhaps greater accumulation of Cd.

2.3.2 Modifying Effects of Citrate

When citrate was added to the exposure solution, the proportion of total Cd present as Cd^{2+} was significantly reduced; to 65.2% or 29.6% with the addition of $1.00 \cdot 10^{-3}$ M or $3.00 \cdot 10^{-3}$ M citrate, respectively (Table 2.2). Over the range of citrate concentrations, the estimated Cd^{2+} concentration ranged from $3.91 \cdot 10^{-7}$ to $1.32 \cdot 10^{-7}$ M when the nominal Cd concentration was $4.45 \cdot 10^{-7}$ M (Tables 2.3 and 2.5). Citrate also reduced the estimated Ca^{2+} and Mg^{2+} concentrations, from about 90% (control) to as low as about 35% (Table 2.2), the nominal concentrations of which were $3.00 \cdot 10^{-3}$ M and $1.50 \cdot 10^{-3}$, respectively (Tables 2.3 and 2.5). The balanced Ca^{2+} and Mg^{2+} solutions achieved similar estimated Ca^{2+} and Mg^{2+} concentrations in citrate augmented solutions as in control solutions by increasing the nominal Ca and Mg concentrations by two thirds (Table 2.5). These balanced Ca^{2+} and Mg^{2+} solutions also contained higher nominal concentrations of NO_3 (40%) and SO_4 (67%) than other solutions, as they were the counterions to Ca and Mg, respectively. Higher SO_4 (from $MgSO_4$) concentrations in these solutions resulted in slightly more $CdSO_4^0{}_{(aq)}$, than in the $3.00 \cdot 10^{-3}$ M citrate solution without balanced Ca^{2+} and Mg^{2+} concentrations, although it was still less than in the control solutions.

When tissue Cd was related to the estimated Cd^{2+} concentration, adding citrate to

Table 2.5: Nominal and estimated concentrations used in 0.001 M and 0.003 M citrate solutions, and 0.003 M citrate exposure solutions with balanced estimated Ca^{2+} and Mg^{2+} concentrations (experiments 2, 3, and 4).

Ion	Nominal Concentration (Estimated Concentration) (M)					
	$1.00 \cdot 10^{-3}$ M citrate		$3.00 \cdot 10^{-3}$ M citrate		$3.00 \cdot 10^{-3}$ M citrate, balanced Ca^{2+} & Mg^{2+}	
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$	($2.15 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$	($1.07 \cdot 10^{-3}$)	$5.00 \cdot 10^{-3}$	($2.83 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-3}$	($1.11 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$	($5.55 \cdot 10^{-4}$)	$2.50 \cdot 10^{-3}$	($1.46 \cdot 10^{-3}$)
K (K^{+})	$7.33 \cdot 10^{-3}$	($7.26 \cdot 10^{-3}$)	$1.40 \cdot 10^{-2}$	($1.38 \cdot 10^{-3}$)	$1.40 \cdot 10^{-2}$	($1.38 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$	($9.92 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$	($9.91 \cdot 10^{-3}$)	$1.40 \cdot 10^{-2}$	($1.38 \cdot 10^{-2}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-3}$	($1.21 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$	($1.30 \cdot 10^{-3}$)	$2.50 \cdot 10^{-3}$	($1.95 \cdot 10^{-3}$)
Cd (Cd^{2+})	$4.45 \cdot 10^{-8}$	($2.90 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$	($2.63 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$	($4.41 \cdot 10^{-8}$)
	$4.45 \cdot 10^{-7}$	($2.90 \cdot 10^{-7}$)	$4.45 \cdot 10^{-7}$	($1.32 \cdot 10^{-7}$)	$4.45 \cdot 10^{-7}$	($2.21 \cdot 10^{-7}$)
citrate ($citrate^{3-}$)	$1.00 \cdot 10^{-3}$	($2.10 \cdot 10^{-5}$)	$3.00 \cdot 10^{-3}$	($1.26 \cdot 10^{-4}$)	$3.00 \cdot 10^{-3}$	($6.30 \cdot 10^{-5}$)
pH	6.0		6.0		6.0	

exposure solutions containing a range of estimated exposure solution Cd^{2+} concentrations for varying durations of exposure enhanced accumulation of Cd in roots relative to control solutions (Figures 2.4 and 2.5), especially after longer durations of exposure. The statistical evidence for this is provided by interactions between time and citrate ($p=0.0015$), among time, $\ln Cd^{2+}$, and citrate ($p=0.0028$), and among $(time)^2$, $\ln Cd^{2+}$, and citrate ($p=0.0033$) (Table 2.6). Together, these interactions suggest that averaged over all estimated exposure solution Cd^{2+} concentrations, those solutions containing citrate resulted in accumulation of Cd by plant roots which was significantly different than accumulation of Cd from solutions without citrate (control), and the magnitude of the citrate effect depended on the duration of exposure and the estimated Cd^{2+} concentration in the exposure solution.

The addition of $1.00 \cdot 10^{-3}$ M citrate resulted in a minor increase in accumulation of Cd from exposure solution relative to the estimated exposure solution Cd^{2+} concentration, while the addition of $3.00 \cdot 10^{-3}$ M citrate had a much greater effect on accumulation of Cd. Accumulation of Cd from the solution containing $3.00 \cdot 10^{-3}$ M citrate with an increase in nominal Ca and Mg concentrations in order to balance the estimated Ca^{2+} and Mg^{2+} concentrations relative to the control exposure solutions, resulted in an intermediate (between $1.00 \cdot 10^{-3}$ M and $3.00 \cdot 10^{-3}$ M citrate exposure solutions) increase in Cd accumulation by plant roots. These solutions also had intermediate changes in Cd speciation relative to the control solutions (the estimated Cd^{2+} concentration was reduced from 87.8% to 49.6% and the estimated proportion of $CdCitrate^-$ increased from 0 to 43.3% of total dissolved Cd), since, compared to the other $3.00 \cdot 10^{-3}$ M citrate solution,

Figure 2.4: Concentration of Cd in 'Kyle' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with $1.00 \cdot 10^{-3}$, $3.00 \cdot 10^{-3}$ M citrate or $3.00 \cdot 10^{-3}$ M citrate with balanced Ca^{2+} and Mg^{2+} concentrations compared to the concentration of Cd in roots of 'Kyle' exposed to control exposure solutions, shown as the response surface from Figure 2.2. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Kyle': citrate effects

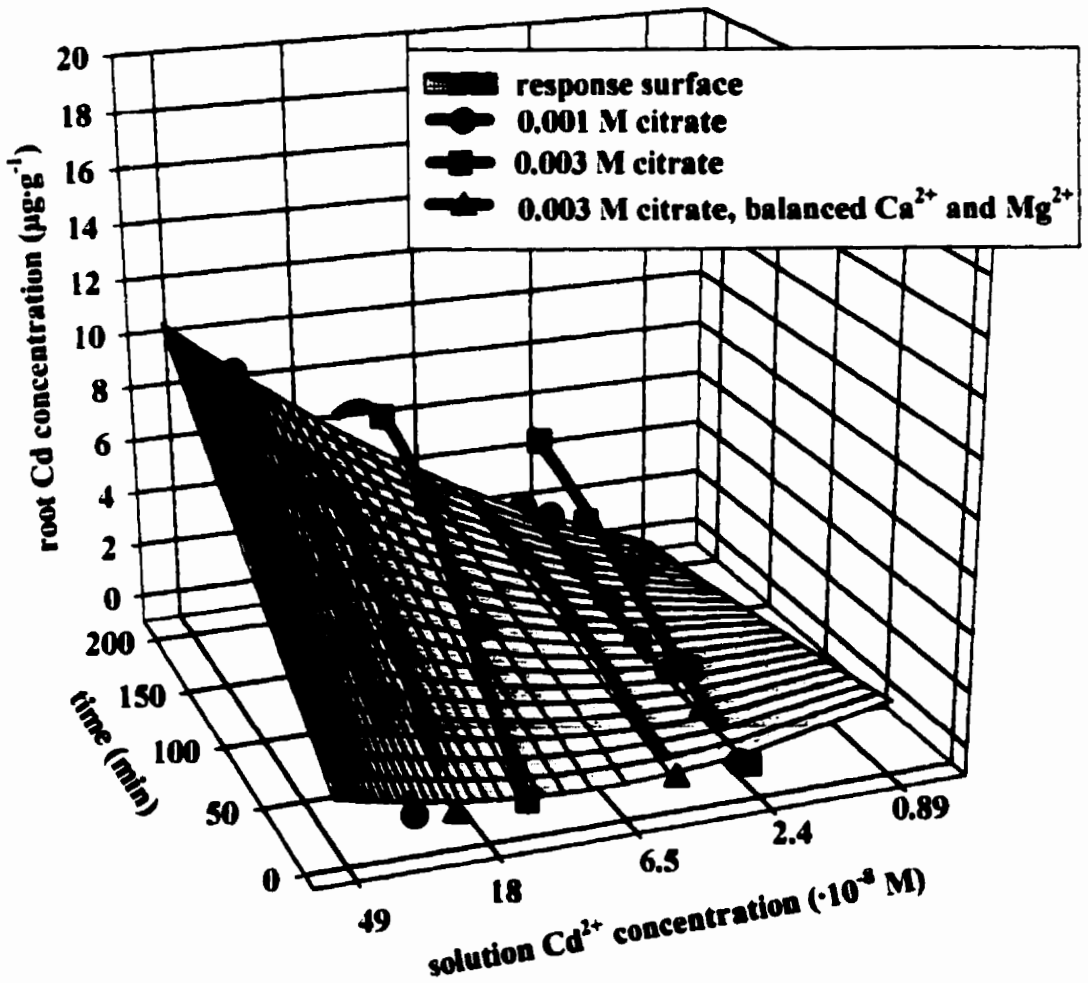


Figure 2.5: Concentration of Cd in 'Arcola' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with $1.00 \cdot 10^{-3}$, $3.00 \cdot 10^{-3}$ M citrate or $3.00 \cdot 10^{-3}$ M citrate with balanced Ca^{2+} and Mg^{2+} concentrations compared to the concentration of Cd in roots of 'Arcola' exposed to control exposure solutions, shown as the response surface from Figure 2.3. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Arcola': citrate effects

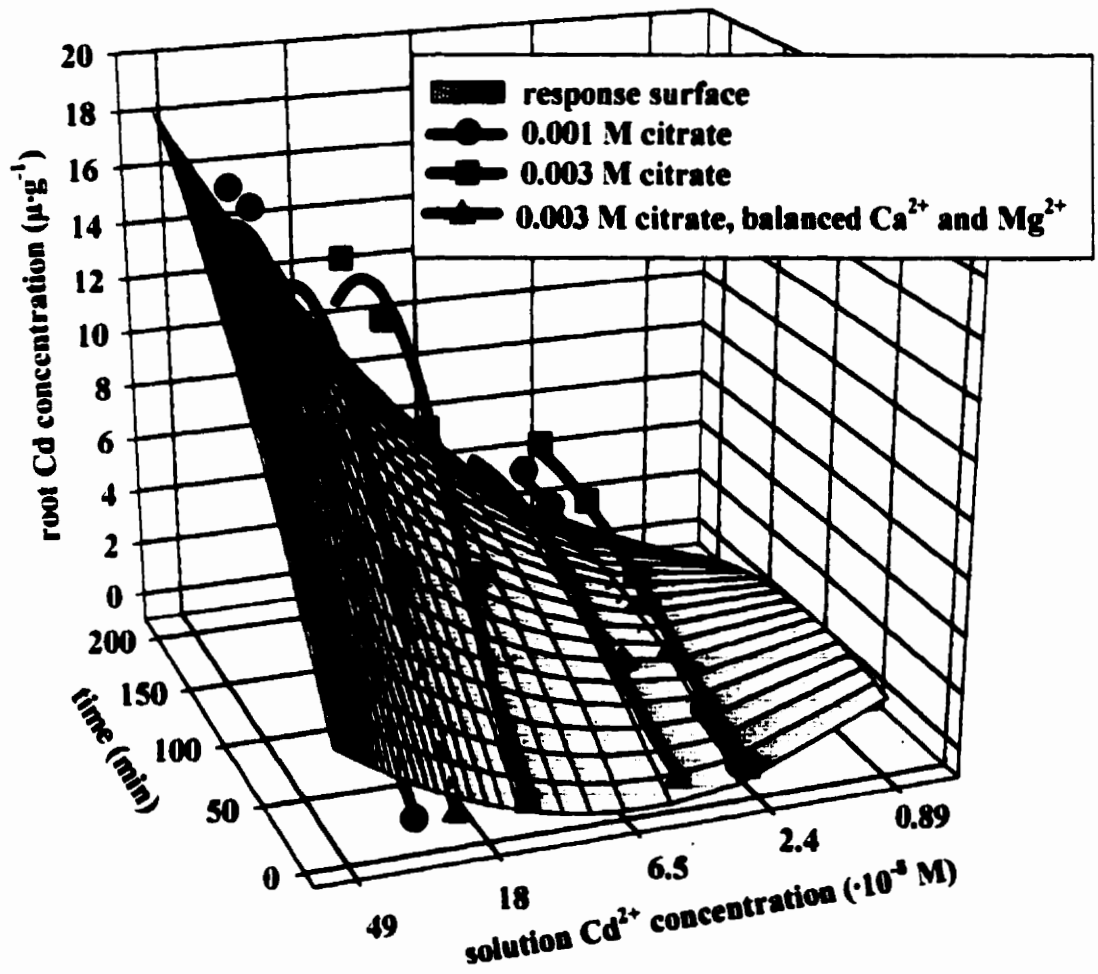


Table 2.6: Sources of variation in content of Cd in roots exposed to citrate containing exposure solutions compared with control solutions (experiments 2, 3 and 4).

Source	df	F-value	p-value
Model	26	171.10	<0.0001
rep	2	8.25	0.00042
cultivar	1	0.47	0.49
time	1	0.35	0.56
$lnCd^{2+}$	1	4.31	0.040
citrate	3	0.73	0.54
time*cultivar	1	6.47	0.012
time*time*cultivar	2	7.81	0.00062
time*$lnCd^{2+}$	1	172.55	<0.0001
time*time*$lnCd^{2+}$	1	87.60	<0.0001
time*citrate	3	5.44	0.0015
time*$lnCd^{2+}$*citrate	3	4.93	0.0028
time*$lnCd^{2+}$*cultivar	1	36.06	<0.0001
time*time*$lnCd^{2+}$*citrate	3	4.81	0.0033
time*$lnCd^{2+}$*cultivar*citrate	3	1.82	0.15
Error	131		

relatively more citrate was associated with Ca^{2+} and Mg^{2+} (Table 2.2).

The addition of citrate to the exposure solutions (with no attempt to balance estimated Ca^{2+} or Mg^{2+} concentrations) resulted in several key changes to the exposure solution; the equilibrium between Cd^{2+} and $CdCitrate^-$ shifted in favour of $CdCitrate^-$, the estimated concentrations of Ca^{2+} and Mg^{2+} were lower (as more Ca^{2+} and Mg^{2+} associated with citrate), and the nominal K concentration in these solutions was 3.5x higher (KOH was used to adjust the pH of the exposure solutions after citrate addition; the effect of an increase in the nominal K concentration on accumulation of Cd by wheat roots will be discussed later).

The enhanced accumulation of Cd in relation to the estimated Cd^{2+} concentration by roots exposed to solutions containing citrate may be due to the presence of $CdCitrate^-$, or to decreases in estimated Ca^{2+} or Mg^{2+} concentrations since these cations may potentially compete with Cd^{2+} for uptake sites.

In this study, complexation of Cd did not result in a reduction in Cd accumulation by roots, which is an exception to the FIM. This is in contrast to several studies which demonstrated a reduction in Al toxicity as a result of complexation of Al with various peptides and organic acids, including citrate (Ojima and Ohira, 1985; Miyasaka *et al.*, 1991; Delhaize *et al.*, 1993; Basu *et al.*, 1994a; b). In another study, addition of humic acid to solution reduced the Cd^{2+} concentration in solution, and although accumulation of Cd by corn and bean was reduced, it was not reduced as much as predicted by the Cd^{2+} concentration, which is an exception to the FIM (Tyler and McBride, 1982). Similarly, the results of this study are in agreement with a recent study with unicellular algae, which

demonstrated that the toxicity of Cd and Zn was greater than predicted for similar free ion activities when citrate was included in the exposure solution (Errécalde *et al.*, 1998). In that study, citrate was accumulated at a rate which was four times higher than Cd, leading the authors to conclude that the accidental transport of a CdCitrate complex by the citrate transporter once in every four transport events would account for the enhanced toxicity in the presence of citrate. Other recent studies with Swiss chard have demonstrated that inorganically complexed forms of Cd, such as $CdCl_n^{2-n}$ and $CdSO_4^0{}_{(aq)}$, are also bioavailable to roots (Smolders and McLaughlin, 1996 a; b; McLaughlin *et al.*, 1998).

Enhanced accumulation of Cd in the presence of citrate may have been due to a reduction in the estimated Ca^{2+} and/or Mg^{2+} concentrations, since these ions may compete with Cd^{2+} for uptake. These ions carry the same charge as Cd^{2+} and Ca^{2+} has a similar ionic radius as Cd^{2+} (crystal ionic radii of Cd^{2+}, Ca^{2+} and Mg^{2+} are 0.97, 0.99, and 0.66 Å, respectively). Reduced competition from Ca^{2+} and Mg^{2+} for uptake sites could not completely explain the enhanced accumulation, however, since when the estimated Ca^{2+} and Mg^{2+} concentrations were balanced relative to the control exposure solution by increasing nominal Ca^{2+} and Mg^{2+} concentrations in the presence of $3.00 \cdot 10^{-3}$ M citrate, accumulation of Cd by plant roots in relation to the estimated solution Cd^{2+} was still enhanced.

2.3.3 Modifying Effects of Inorganic Ions (Ca^{2+} and Mg^{2+})

The ⅓-strength exposure solutions, the ½ and ⅓ Ca solutions and the ½ and ⅓ Mg solutions were designed to measure the effect of inorganic ions, in the absence of

citrate, on Cd accumulation in roots (Table 2.7). In the ⅓-strength exposure solutions, the concentrations of all inorganic ions (except Cd) in the exposure solution were reduced to ⅓ the concentrations found in the control solution. In the ½ and ⅓ Ca solutions, the nominal Ca concentration alone was reduced to ½ or ⅓ the concentration found in the control solution (the nominal NO_3 concentration was maintained by increasing the KNO_3; the nominal K concentration was increased by 1.75x in the ½ Ca exposure solution and 2.00x in the ⅓ Ca exposure solution). In the ½ and ⅓ Mg solutions, the nominal Mg alone was reduced to ½ or ⅓ the concentration found in the control solution (the nominal SO_4 concentration was maintained by adding K_2SO_4; the nominal K concentration was increased by 1.38x in the ½ Mg exposure solution and 1.50x in the ⅓ Mg exposure solution). These solutions were similar in species proportion to the control solution (Table 2.2), but were quite different in nominal and free ion concentrations (Tables 2.3 and 2.7).

Reduction of the nominal concentration of all ions in the exposure solution to ⅓ of the concentrations in the control exposure solution resulted in greater accumulation of Cd by root tissue compared to accumulation of Cd from the control solution (Figure 2.6 A and 2.7 A). There were statistical interactions between time and ion concentration ($p=0.048$), between $(time)^2$ and ion concentration ($p=0.0023$), and among time, $lnCd^{2+}$ and ion concentration ($p=0.080$) (Table 2.8). Taken together, these interactions indicate that the nominal inorganic ion concentration influenced the root Cd concentration, and that the magnitude of the influence depended on both the estimated Cd^{2+} concentration in the exposure solution and the duration of exposure. Lower concentrations of all inorganic

Table 2.7: Nominal and estimated concentrations used in 1/3 strength exposure solutions (experiment 3), ½ Ca and Mg exposure solutions (experiment 4) and ⅓ Ca and Mg exposure solutions (experiment 5).

Ion	Nominal Concentration (Estimated Concentration) (M)				
	1/3 strength nutrient solution	½ Ca	½ Mg	1/3 Ca	1/3 Mg
Ca (Ca^{2+})	$1.00 \cdot 10^{-3}$ ($9.50 \cdot 10^{-4}$)	$1.50 \cdot 10^{-3}$ ($1.35 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$ ($2.72 \cdot 10^{-3}$)	$1.00 \cdot 10^{-3}$ ($9.01 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$ ($2.72 \cdot 10^{-3}$)
Mg (Mg^{2+})	$5.00 \cdot 10^{-4}$ ($4.82 \cdot 10^{-4}$)	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)	$7.00 \cdot 10^{-4}$ ($6.54 \cdot 10^{-4}$)	$1.50 \cdot 10^{-3}$ ($1.39 \cdot 10^{-3}$)	$5.00 \cdot 10^{-4}$ ($4.67 \cdot 10^{-4}$)
K (K^{+})	$1.33 \cdot 10^{-3}$ ($1.32 \cdot 10^{-3}$)	$7.00 \cdot 10^{-3}$ ($6.93 \cdot 10^{-3}$)	$5.50 \cdot 10^{-3}$ ($5.45 \cdot 10^{-3}$)	$8.00 \cdot 10^{-3}$ ($7.92 \cdot 10^{-3}$)	$6.00 \cdot 10^{-3}$ ($5.94 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$3.33 \cdot 10^{-3}$ ($3.33 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.94 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.92 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.94 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.92 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$5.00 \cdot 10^{-4}$ ($4.37 \cdot 10^{-4}$)	$1.50 \cdot 10^{-3}$ ($1.24 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$ ($1.20 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$ ($1.27 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$ ($1.21 \cdot 10^{-3}$)
Cd (Cd^{2+})	$8.90 \cdot 10^{-8}$ ($8.28 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($7.73 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($7.78 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($7.71 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($7.77 \cdot 10^{-8}$)
	$4.45 \cdot 10^{-7}$ ($4.14 \cdot 10^{-7}$)				
pH	6.0	6.0	6.0	6.0	6.0

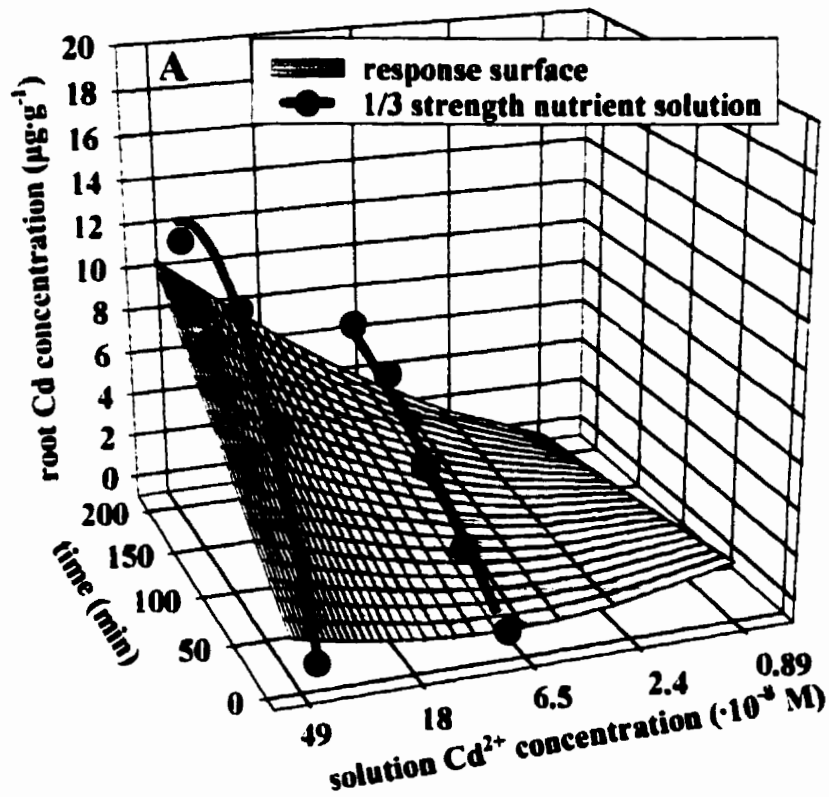
Figure 2.6 A, B and C: Concentration of Cd in 'Kyle' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with altered concentrations of inorganic ions compared to the concentration of Cd in roots of 'Kyle' exposed to control exposure solutions, shown as the response surface from Figure 2.2. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

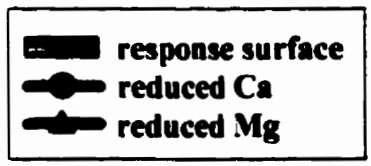
Figure 2.6 A: Concentration of all ions (except) Cd^{2+} reduced to ⅓ the concentration found in the control exposure solution.

Figure 2.6 B: Nominal concentration of Ca^{2+} or Mg^{2+} reduced to ½ the concentration found in the control exposure solution (anions balanced by adding K-salt).

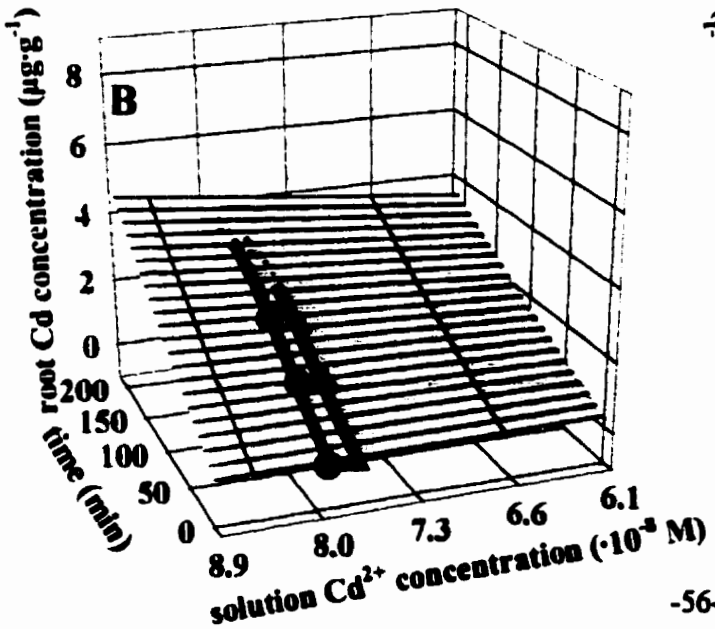
Figure 2.6 C: Nominal concentration of Ca^{2+} or Mg^{2+} reduced to ⅓ the concentration found in the control exposure solution (anions balanced by adding K-salt).

'Kyle': inorganic ion effects





effect of 50% Ca or Mg



effect of 33% Ca or Mg

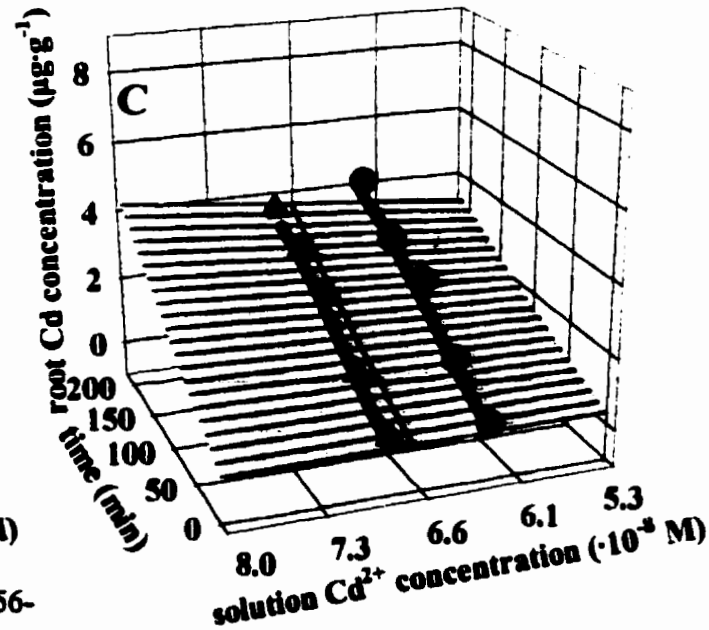


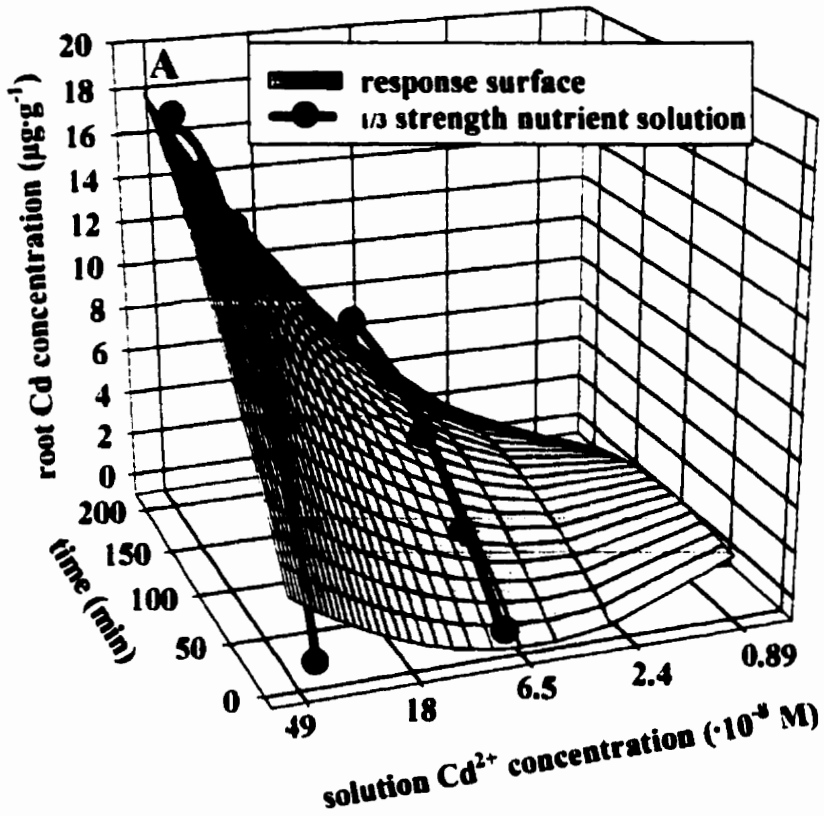
Figure 2.7 A, B, and C: Concentration of Cd in 'Arcola' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with altered concentrations of inorganic ions compared to the concentration of Cd in roots of 'Arcola' exposed to control exposure solutions, shown as the response surface from Figure 2.2. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

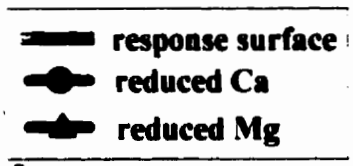
Figure 2.7 A: Concentration of all ions (except) Cd^{2+} reduced to ⅓ the concentration found in the control exposure solution.

Figure 2.7 B: Nominal concentration of Ca^{2+} or Mg^{2+} reduced to ½ the concentration found in the control exposure solution (anions balanced by adding K-salt).

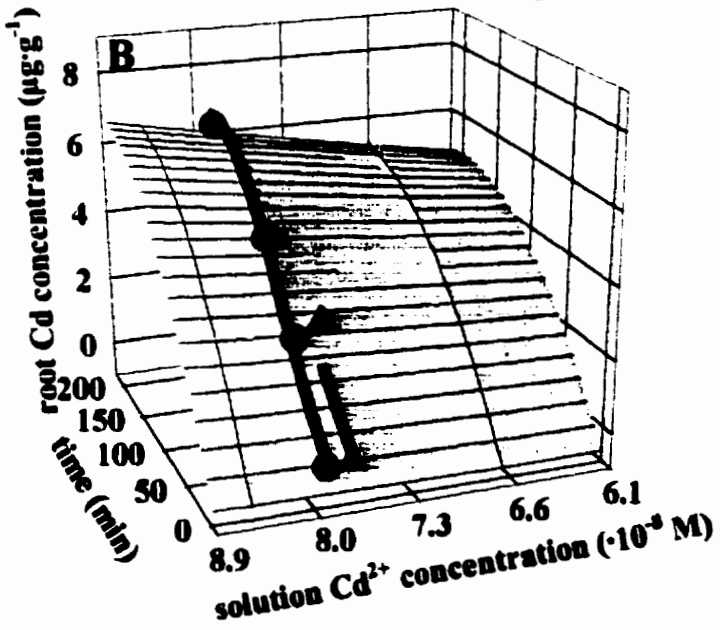
Figure 2.7 C: Nominal concentration of Ca^{2+} or Mg^{2+} reduced to ⅓ the concentration found in the control exposure solution (anions balanced by adding K-salt).

'Arcola': inorganic ion effects





effect of 50% Ca or Mg



effect of 33% Ca or Mg

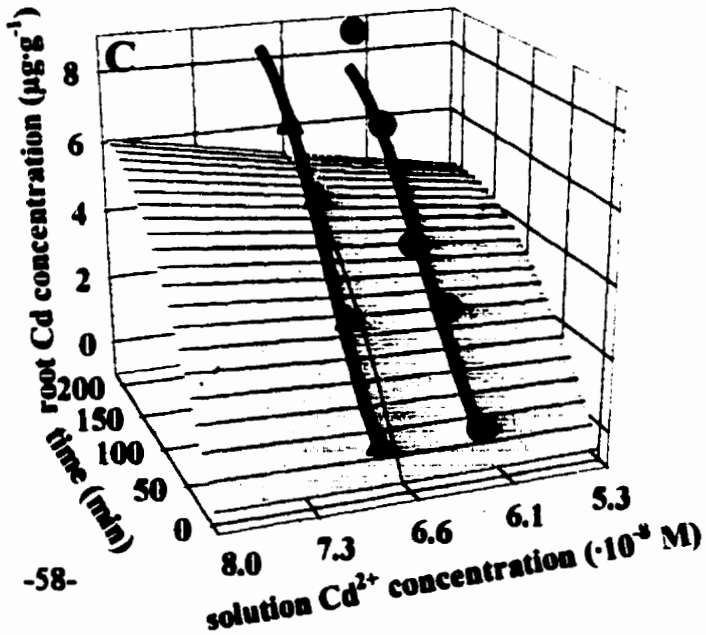


Table 2.8: Sources of variation in content of Cd in roots exposed to solutions with the concentration of all ions at ⅓ the concentration of ions found in the control exposure solutions (experiment 3).

Source	df	F-value	p-value
Model	15	204.99	<0.0001
cultivar	1	1.23	0.27
ion concentration	1	3.03	0.091
time	1	3.40	0.075
time*time	1	4.88	0.035
$lnCd^{2+}$	1	3.45	0.073
time*time*cultivar	1	14.24	0.00065
time*ion concentration	1	4.25	0.048
time*time*ion concentration	1	11.03	0.0023
$lnCd^{2+}$*ion concentration	1	3.59	0.67
time*$lnCd^{2+}$*$lnCd^{2+}$	1	9.22	0.0047
time*time*$lnCd^{2+}$	1	4.13	0.051
time*time*$lnCd^{2+}$*$lnCd^{2+}$	1	8.72	0.0059
time*$lnCd^{2+}$*cultivar	2	28.35	<0.0001
time*$lnCd^{2+}$*ion concentration	1	3.27	0.080
Error	32		

ions in the exposure solution resulted in greater accumulation of Cd by roots compared to accumulation of Cd from the control exposure solution, and the magnitude of the increase was greater after longer durations of exposure to Cd and at higher estimated concentrations of Cd^{2+} in the exposure solution. Reduction of the nominal concentrations of all ions in the exposure solution to ⅛ of the concentration found in the control solutions did not alter Cd speciation appreciably (Table 2.2), so the reason for enhanced accumulation of Cd by roots was most likely due to reduced competition for Cd^{2+} uptake. Ca^{2+} and/or Mg^{2+} are the mostly likely competitors, since these ions carry the same charge as Cd^{2+} and Ca^{2+} has a similar ionic radius as Cd^{2+}. K^{+} was not likely to compete with Cd^{2+} for uptake sites since it has a single charge and a larger ionic radius than Cd^{2+}. Nominal NO_3 and SO_4 concentrations were also reduced in these solutions, although these ions would not likely interfere with accumulation of Cd since anions are accumulated by different mechanisms than cations.

When only the nominal concentration of Ca or Mg was reduced relative to the control exposure solution, accumulation of Cd by wheat roots increased (Figures 2.6 B and C and 2.7 B and C). There were interactions between the estimated Ca^{2+} concentration and time ($p=0.0016$) and between the estimated Mg^{2+} concentration and time ($p=0.016$) (Table 2.9), indicating that Cd accumulation by wheat roots differed with reduced concentrations of Ca^{2+} or Mg^{2+} and that the magnitude of the difference depended on the duration of exposure to Cd. Lower estimated Ca^{2+} or Mg^{2+} concentrations resulted in greater accumulation of Cd, and the magnitude of the effect increased as Ca^{2+} and Mg^{2+} concentrations declined from ½ to ⅛ of control; this was possibly due to reduced

Table 2.9: Sources of variation in content of Cd in roots exposed to solutions with ½ or ⅓ the Ca or Mg (experiments 4 and 5).

Source	df	F-value	p-value
Model	19	138.87	<0.0001
rep	1	42.75	<0.0001
cultivar	1	5.01	0.028
Ca^{2+}	1	0.16	0.69
Mg^{2+}	1	7.01	0.0099
time	1	4.33	0.041
$lnCd^{2+}$	1	6.93	0.010
Mg^{2+}*cultivar	1	5.16	0.026
time*cultivar	1	4.02	0.048
time*time*cultivar	2	4.67	0.012
$lnCd^{2+}$*cultivar	1	4.97	0.029
Ca^{2+}*time	1	10.73	0.0016
Mg^{2+}*time	1	6.06	0.016
Mg^{2+}*$lnCd^{2+}$	1	6.99	0.010
time*$lnCd^{2+}$*$lnCd^{2+}$	1	31.08	<0.0001
time*time*$lnCd^{2+}$*$lnCd^{2+}$	1	85.49	<0.0001
Mg^{2+}*$lnCd^{2+}$*cultivar	1	5.08	0.027
time*$lnCd^{2+}$*cultivar	2	7.18	0.0014
Error	74		

competition with Cd^{2+} for uptake sites. It is possible that Ca^{2+} and Mg^{2+} also competed with Cd^{2+} for binding sites in the apoplast, although the low Cd^{2+} concentrations used in these experiments likely resulted in little Cd accumulation in cell walls. The possibility that Mg^{2+} might compete with Cd^{2+} for uptake has not been studied to date, although there has been some work done on the effects of Ca^{2+}. Less Cd is taken up by marine organisms, and one reason for this is thought to be enhanced competition with Ca^{2+} (Canadian Environmental Protection Act, 1994). Tyler and McBride (1982) exposed corn and bean seedlings to 0 to $1.78 \cdot 10^{-5}$ M Cd with one of two Ca concentrations; $1.0 \cdot 10^{-3}$ or $5.0 \cdot 10^{-3}$ M. They found no difference in the Cd concentration in roots, but did observe significantly higher Cd concentrations (and greater toxicity) in shoots of plants exposed to Cd with the lower Ca concentration, and hypothesized that Ca competed with Cd for translocation. It is important to note that in this study, the Ca was added as $CaSO_4$, with no apparent balancing of the nominal SO_4 concentration; the excess SO_4 would undoubtably alter Cd speciation, and possibly bioavailability (Chapter 3). More recently, McLaughlin *et al.* (1998) found that changing the nominal Ca concentration resulted in no changes in root Cd concentrations, although in that study, the nominal Ca concentration was adjusted over a narrower range ($6.6 \cdot 10^{-3}$ to $9.4 \cdot 10^{-3}$ M) than in this study ($1.0 \cdot 10^{-3}$ to $3.0 \cdot 10^{-3}$ M).

In these solutions, the nominal NO_3 and SO_4 concentrations were kept similar to those in the control exposure solutions by adding KNO_3 or K_2SO_4; the nominal K concentration was increased by 1.75x and 2.00x in the ½ and ⅓ Ca solutions, respectively, and 1.38x and 1.50x in the ½ and ⅓ Mg solutions, respectively, relative to the control

exposure solutions. The effects of an increase in the nominal K concentration on accumulation of Cd by plant roots is discussed in the following section.

2.3.4 Modifying Effects of Potassium

K was the counter cation added in solutions requiring particular anions; for example, KOH in citrate solutions in order to adjust the pH, and KNO_3 and K_2SO_4 to supply balanced nominal NO_3 and SO_4 concentrations compared with the control solutions in exposure solutions with reduced nominal Ca and Mg concentrations, respectively. K salts were chosen for these roles because it does not interact strongly with the ligands present in the exposure solutions used, so increasing the nominal K concentration did not alter speciation of other media components, including Cd (Table 2.2). Also, since K^+ is different than Cd^{2+}, Ca^{2+}, or Mg^{2+} in terms of size or charge, it was assumed to be least likely to interfere in biological processes (such as uptake) involving the other ions of interest. The effect of an increase in the nominal K concentration on accumulation of Cd by wheat roots was tested in experiment 6 by increasing the nominal K concentration by 3.5x. This increase was of a similar magnitude to the increase in the nominal K concentration (added as KOH) required to adjust the pH of solutions containing $3.00 \cdot 10^{-3}$ M citrate to 6.0. Increased nominal K concentrations had very little influence on the speciation of other exposure solution components (Table 2.2). K^+ associates weakly with the ligands present in these solutions (citrate and SO_4^{2-}); the proportion of K present as K^+ ranged from 98 to 99% in all exposure solutions (Table 2.2). The nominal K concentration was increased by adding KNO_3 or K_2SO_4 resulting in a nominal NO_3

concentration which was twice as high or a nominal SO_4 concentration which was 2.4x as high as in control solutions (Table 2.10). The addition of KNO_3 did not result in appreciable changes to the speciation of other ions, while the addition of K_2SO_4 did, since SO_4^{2-} forms complexes with Cd^{2+} as well as Ca^{2+} and Mg^{2+} (Table 2.2).

There was no main effect of KNO_3, nor were there any interactions involving KNO_3 on accumulation of Cd by wheat roots (Table 2.11). This suggests that neither an increase in the nominal K nor NO_3 concentration influenced Cd accumulation by wheat roots. There was evidence, however, that the addition of K_2SO_4 had an effect on Cd accumulation by wheat roots. There were interactions between K_2SO_4 and time ($p<0.0001$), and among K_2SO_4, time, and $\ln Cd^{2+}$ ($p=0.087$) (Table 2.11) indicating that the presence of a higher nominal K and/or SO_4 concentration had an influence on Cd in relation to the estimated concentration of Cd^{2+} in the exposure solution, and that the magnitude of the effect depended on the duration of exposure and on the concentration of Cd^{2+} in the exposure solution. In this exposure solution, the estimated proportion of total Cd present as Cd^{2+} was reduced from 87.8% to 68.9% by the presence of excess SO_4, and the estimated proportion of $CdSO_4^0{}_{(aq)}$ was increased from 10.2% to 28.2% (Table 2.2). This reduction in the estimated Cd^{2+} concentration was similar to the reduction in the estimated Cd^{2+} concentration observed upon the addition of $1.00 \cdot 10^{-3}$ M citrate, and that solution resulted in higher than predicted accumulation of Cd. The increased proportion of Cd present as a complex (similar to $CdCitrate^-$) was more likely the cause for the enhanced accumulation of Cd, and not an increase in the nominal K concentration, since the addition of KNO_3 did not influence accumulation of Cd. Additionally, if K^+ was to

Table 2.10: Nominal and estimated concentrations used in 3.5 x K exposure solutions (experiment 6).

Ion		Nominal Concentration (Estimated Concentration (M)			
		3.5 x K as KNO_3		3.5 x K as K_2SO_4	
Ca	(Ca^{2+})	$3.00 \cdot 10^{-3}$	($2.73 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$	($2.31 \cdot 10^{-3}$)
Mg	(Mg^{2+})	$1.50 \cdot 10^{-3}$	($1.42 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$	($1.21 \cdot 10^{-3}$)
K	(K^{+})	$1.40 \cdot 10^{-2}$	($1.38 \cdot 10^{-2}$)	$1.40 \cdot 10^{-2}$	($1.37 \cdot 10^{-3}$)
NO_3	(NO_3^{-})	$2.00 \cdot 10^{-2}$	($1.98 \cdot 10^{-2}$)	$1.00 \cdot 10^{-2}$	($9.90 \cdot 10^{-3}$)
SO_4	(SO_4^{2-})	$1.50 \cdot 10^{-3}$	($1.18 \cdot 10^{-3}$)	$5.15 \cdot 10^{-3}$	($4.19 \cdot 10^{-3}$)
Cd	(Cd^{2+})	$8.90 \cdot 10^{-8}$	($7.86 \cdot 10^{-3}$)	$8.90 \cdot 10^{-8}$	($6.13 \cdot 10^{-3}$)
		$4.45 \cdot 10^{-7}$	($3.93 \cdot 10^{-7}$)	$4.45 \cdot 10^{-7}$	($3.07 \cdot 10^{-7}$)
pH		6.0		6.0	

Table 2.11: Sources of variation in content of Cd in roots exposed to solutions with 3.5x K supplied as KNO_3 or K_2SO_4 (experiment 6).

Source	df	F-value	p-value
Model	11	156.56	<0.0001
cultivar	1	0.87	0.36
KNO_3	1	1.31	0.26
K_2SO_4	1	0.89	0.35
time	1	19.20	<0.0001
time*time	1	10.08	0.0028
$lnCd^{2+}$	1	0.00	0.95
K_2SO_4*time	1	25.07	<0.0001
time*time*$lnCd_{2+}$	1	15.85	0.00026
time*$lnCd^{2+}$*cultivar	2	83.74	<0.0001
K_2SO_4*time*$lnCd^{2+}$	1	3.07	0.087
Error	43		

have an effect on accumulation of Cd (by competition with Cd for uptake sites), an increase in the estimated K^+ concentration would likely reduce, and not enhance, accumulation of Cd by roots.

2.4 Summary and Conclusions

Our null hypothesis, that accumulation of Cd by roots of two cultivars of durum wheat is dependent only on the concentration of the free ion (Cd^{2+}), and is not influenced by the presence of citrate or altered Ca^{2+} or Mg^{2+} concentrations, can be rejected. The concentration of Cd^{2+} in the exposure solution did not predict the Cd accumulation by wheat roots from solution as Cd speciation was altered and/or concentrations of inorganic ions such as Ca^{2+} or Mg^{2+} were altered. The addition of citrate to exposure solutions resulted in accumulation of Cd in relation to the Cd^{2+} concentration in the exposure solution which was greater than accumulation from control solutions which did not contain citrate. This was an exception to the FIM. Although the presence of citrate was confounded by an increase in the nominal K concentration, K did not influence accumulation of Cd. The effect of adding citrate to the exposure solution resulted in two major changes; a shift in the equilibrium between Cd^{2+} and $CdCitrate^-$ toward $CdCitrate^-$, and reductions in estimated Ca^{2+} and Mg^{2+} concentrations. The data demonstrate that both of these changes resulted in enhanced accumulation of Cd. The presence of $CdCitrate^-$ may have enhanced accumulation of Cd in relation to the concentration of Cd^{2+} in the exposure solution in a number of different ways. One possible explanation is that the $CdCitrate^-$ complex is accumulated by roots. Perhaps a citrate transporter in the root

membrane can be fooled into accepting a CdCitrate$^-$ (Errécalde *et al.*, 1998); this would be an exception to the FIM since it predicts that only the free ion (Cd^{2+}) is taken up. A second possibility is that diffusion of Cd^{2+} to the root cell surface is the rate limiting step in the accumulation of Cd, resulting in a depletion of Cd^{2+} at the root surface relative to the bulk solution. With a significant proportion of the total dissolved Cd present as CdCitrate$^-$, the Cd^{2+} concentration at the root surface could be buffered by dissociation of CdCitrate$^-$ into citrate and Cd^{2+}, which could then be accumulated by the root tissue. If the process of dissociation is faster than diffusion of Cd^{2+} from the bulk solution to the root surface and Cd accumulation by root tissue, then the presence of a complexed form of Cd which can easily dissociate could result in a relatively higher concentration of Cd^{2+} at the root surface than if the dissolved Cd was present mostly as Cd^{2+}. This scenario would be a case where the assumptions of the FIM were not met, since the FIM assumes that the rate limiting step in the interaction a between dissolved metal and the biological organism is binding to cell surface binding sites, and not diffusion to the site. This later possibility will be discussed more in depth in Chapter 4. Reductions in estimated Ca^{2+} and Mg^{2+} concentrations may have resulted in decreased competition with Cd^{2+} for uptake sites; this would also be a situation where the assumptions of the FIM were not being met, since the FIM assumes that cell surface binding sites are specific for the metal causing the effect (Cd), and do not bind with other metals (i.e. Ca or Mg).

CHAPTER 3:

THE INFLUENCE OF EDTA AND SO_4 ON ACCUMULATION OF CADMIUM BY DURUM WHEAT: EXCEPTIONS TO THE FREE ION MODEL?

3.1 Introduction

The background literature regarding accumulation of Cd by plants and the FIM has been discussed previously in Chapter 2 (***2.1 Introduction***), and will not be discussed again here. The data presented in this chapter were collected from experiments very similar in nature to the ones presented in the previous chapter. In the experiments discussed in this chapter, however, EDTA and SO_4^{2-} were the ligands added to the exposure solutions instead of citrate. While citrate is a natural organic compound known to be secreted by roots of durum wheat (Cieslinski *et al.*, 1997), EDTA (ethylenediamine tetraacetic acid) is a synthetic organic compound, and SO_4^{2-} is an inorganic anion found in soil solution, and is required for plant growth. These compounds share a common ability to form complexes with Cd^{2+} (as well as other metals), specifically $CdCitrate^-$, $CdEDTA^{2-}$, or $CdSO_4^0{}_{(aq)}$.

In the present study, two cultivars of durum wheat *(Triticum turgidum)* which have been demonstrated previously to have different patterns of Cd accumulation and tissue distribution (Chan, 1996; Berkelaar and Hale, 1999) were used to establish the relationship between accumulation of Cd in plant roots and EDTA or an increase in the nominal SO_4 concentration in the exposure solution. The two null hypotheses were: 1) accumulation of Cd by roots of two cultivars of durum wheat is dependent only on the concentration of the free ion (Cd^{2+}) and is not influenced by the presence of EDTA, and 2) accumulation of Cd by roots of two cultivars of durum wheat is dependent only on the concentration of the free ion (Cd^{2+}) and is not influenced by an increase in the nominal SO_4 concentration.

3.2 Materials and Methods

3.2.1 Experimental Design

This study was conducted as three separate experiments, each of which was a complete factorial design (cultivar, time, and exposure solution composition) in a completely randomized design (Table 3.1). Overall, the influences of Cd^{2+} concentration (a proportion of nominal concentrations of $8.90 \cdot 10^{-9}$, $4.45 \cdot 10^{-8}$, $8.90 \cdot 10^{-8}$, or $4.45 \cdot 10^{-7}$ M), EDTA (nominal concentrations of 0, $8.9 \cdot 10^{-8}$ or $3.0 \cdot 10^{-7}$ M), and SO_4 (nominal concentrations of $1.50 \cdot 10^{-3}$ M or $1.50 \cdot 10^{-2}$ M added as $MgSO_4$, K_2SO_4, or half $MgSO_4$ and half K_2SO_4) on root Cd content were evaluated in two durum wheat cultivars ('Kyle' and 'Arcola') over a range of durations of exposure to Cd^{2+} (0 to 210 min) (Table 3.1). The same baseline Cd accumulation from control solutions established in Chapter 2 was used in this chapter, while the remaining two experiments measured Cd accumulation in seedling roots as influenced by EDTA and SO_4 (Table 3.1).

3.2.2 Plant Material and Growth Conditions

Plant material used and growth conditions were as described in section *2.2.2 Plant Material and Growth Conditions*. Six-day old seedlings were used in all experiments.

3.2.3 Cadmium Exposure and Solution Analysis

Cadmium exposure and analysis of exposure solutions were as described in section *2.2.3 Cadmium Exposure and Solution Analysis*. For experiment 1, seedlings were harvested at either 0, 30, 60, 90, 120, 150, 180, or 210 min after exposure began, and for

Table 3.1: Factors and levels of each factor tested in each of the three experiments.

exp. #	target nominal [Cd] ($\cdot 10^{-8}$ M)	EDTA ($\cdot 10^{-8}$ M)	SO_4	Mg	K**
1	0.890, 4.45, 8.90, or 44.5	0	'control'	'control'	'control'
2	4.45, 8.90, or 44.5	0, 8.90, or 30.0*	'control'	'control'	'control'
3	4.45 or 44.5	0	'control' or 10 x	'control', 5x, or 10x	'control', 4.75x, or 16x

* the $8.90 \cdot 10^{-8}$ M Cd solution contained $8.90 \cdot 10^{-8}$ M EDTA; the $4.45 \cdot 10^{-7}$ M total Cd solution contained $3.00 \cdot 10^{-7}$ M EDTA

experiments 2 and 3, seedlings were harvested at either 0, 50, 100, 150, or 200 min after exposure began.

3.2.4 Plant Digestion and Cd Analysis

Digestion of tissue samples and analysis of samples for Cd were as described in section *2.2.4 Plant Digestion and Cd Analysis*.

3.2.5 Data Analysis

Data were analysed in a manner similar to that described in section *2.2.5 Data Analysis*. Data were grouped for analysis as follows: the control exposure solutions from each experiment (analysed previously and presented in section *2.3.1 Baseline Cd Accumulation*); the EDTA containing solutions from one experiment; the solutions containing a tenfold higher nominal SO_4 concentration from the other experiment.

3.3 Results and Discussion

Throughout the discussion, the terms nominal concentration (i.e. nominal Cd concentration) and estimated ion concentration (i.e. estimated Cd^{2+} concentration) are used. Nominal concentration refers to the concentration of a compound or element added to solution, while the estimated ion concentration is the estimated concentration of a particular chemical species after a solution has reached equilibrium, determined by MINEQL$^+$ modelling. Accumulation of Cd by wheat roots was related to the estimated exposure solution Cd^{2+} concentration (determined by measuring the total Cd concentration

in each exposure solution and estimating the proportion of the total dissolved Cd present as Cd^{2+} with MINEQL^{+}) in all cases (Table 3.2). Values presented in Table 3.2 are the proportions of various species as a percentage of their total concentration. For the control and SO_4 solutions, the proportions in the table applied to solutions containing different nominal Cd concentrations. Since the nominal Cd concentrations used in these exposure solutions were orders of magnitude below the concentrations of other media components, changing the nominal concentration of Cd did not alter the speciation of these other ions. EDTA has a very high affinity for Cd^{2+}, and was present at concentrations similar to those of Cd^{2+}, so proportional speciation which is specific to the nominal Cd and EDTA concentrations used in each solution are included in the table. In the control solutions, the estimated Cd^{2+} concentration was 87.8% of the nominal dissolved Cd concentration, with most (10.2%) of the remaining Cd present as $CdSO_4^0{}_{(aq)}$ (Table 3.2).

3.3.1 Baseline Cd Accumulation

The response surfaces for root Cd concentrations in 'Kyle' and 'Arcola' have been previously presented in section *2.3.1 Baseline Cd Accumulation*, in Tables 2.3 and 2.4 and Figures 2.1 and 2.2. Since the chemical composition of the control solutions will be discussed in later sections, Table 2.3 is repeated in this chapter as Table 3.3.

3.3.2 Modifying Effects of EDTA

When EDTA was added to the exposure solution, the proportion of total Cd present as Cd^{2+} was significantly reduced (Table 3.2). The target pairs of nominal Cd

Table 3.2: Proportions of the various Cd species and other significant species in the different exposure solutions. The pH of the exposure solutions was 6.0.

Species	Proportion of Species as a percentage of the Total Concentration of Dissolved Ion					
	control	**EDTA**			**10x SO_4**	
	control	$8.90 \cdot 10^{-8}$ M EDTA ($7.67 \cdot 10^{-8}$ M Cd)	$3.00 \cdot 10^{-7}$ M EDTA ($4.32 \cdot 10^{-7}$ M Cd)	added as $MgSO_4$	added as K_2SO_4	added as $MgSO_4$ and K_2SO_4
Cd^{2+}	**87.8**	**13.6**	**28.7**	**60.8**	**55.0**	**57.6**
$CdSO_4^0{}_{(aq)}$	**10.2**	1.6	3.3	**34.4**	**38.8**	**36.9**
$Cd(SO_4)_2^{2-}$	0.0	0.0	0.0	3.9	5.3	4.6
$CdNO_3^+$	1.6	0.0	0.0	0.0	0.0	0.0
$CdEDTA^{2-}$	**0.0**	**84.4**	**67.2**	0.0	0.0	0.0
Ca^{2+}	91.0	91.0	91.0	71.2	66.6	68.6
$CaEDTA^{2-}$	0.0	0.0	0.0	0.0	0.0	0.0
$CaSO_4^0{}_{(aq)}$	7.3	7.3	7.3	27.8	32.5	30.4
Mg^{2+}	93.6	93.6	93.6	75.0	70.6	72.6
$MgEDTA^{2-}$	0.0	0.0	0.0	0.0	0.0	0.0
$MgSO_4^0{}_{(aq)}$	6.4	6.4	6.4	25.0	29.4	27.4
K^+	99.0	99.0	99.0	96.5	95.9	96.2

Table 3.3: Nominal and estimated concentrations used in control exposure solutions (experiment 1).

Ion	Nominal Concentration (Estimated Concentration) (M)
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$ ($2.73 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)
K (K^{+})	$4.00 \cdot 10^{-3}$ ($3.96 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$ ($9.93 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-3}$ ($1.17 \cdot 10^{-3}$)
Cd (Cd^{2+})	$8.90 \cdot 10^{-9}$ ($7.81 \cdot 10^{-9}$)
	$4.45 \cdot 10^{-8}$ ($3.91 \cdot 10^{-8}$)
	$8.90 \cdot 10^{-8}$ ($7.81 \cdot 10^{-8}$)
	$4.45 \cdot 10^{-7}$ ($3.91 \cdot 10^{-7}$)
pH	6.0

and EDTA concentrations were $8.90 \cdot 10^{-8}$ M Cd and $8.90 \cdot 10^{-8}$ M EDTA, and $4.45 \cdot 10^{-7}$ M Cd and $3.00 \cdot 10^{-7}$ M EDTA. If these concentrations had been precisely met, then the estimated Cd^{2+} concentration would have been approximately 30% of the nominal Cd concentration in each solution. Since the measured total Cd concentrations in each exposure solution containing EDTA were slightly less ($7.67 \cdot 10^{-8}$ M and $4.32 \cdot 10^{-7}$ M), the proportion of dissolved Cd present as Cd^{2+} was estimated to be 13.6 and 28.7%, respectively, though this assumes that the nominal EDTA concentrations actually present in each solution were exactly the target concentrations of $8.90 \cdot 10^{-8}$ M and $3.00 \cdot 10^{-7}$ M. The actual EDTA concentrations were not measured.

Unlike when citrate was added to the exposure solution, EDTA did not alter speciation of Ca or Mg in the exposure solution, so changes in Cd speciation were not confounded with changes in estimated Ca^{2+} or Mg^{2+} concentrations. While EDTA will form complexes with Ca^{2+} and Mg^{2+}, the concentration of EDTA required to appreciably alter Cd speciation was not high enough to reduce the estimated Ca^{2+} or Mg^{2+} concentrations. Also, solutions containing EDTA did not require much KOH to adjust the pH to 6.0, so speciation of Cd was not confounded by an increase in the nominal K concentration, either. With the addition of EDTA the equilibrium between Cd^{2+} and $CdEDTA^{2-}$ shifted in favour of $CdEDTA^{2-}$ (Tables 3.3 and 3.4).

When tissue Cd was related to the estimated Cd^{2+} concentration, adding EDTA to exposure solutions containing Cd^{2+} resulted in enhanced accumulation of Cd in roots relative to the control solution (Figures 3.1 and 3.2), particularly as durations of exposure increased. The evidence for this is the interactions between time and EDTA ($p<0.0001$)

Table 3.4: Nominal and estimated concentrations used in exposure solutions containing EDTA (experiment 2).

Ion	Nominal Concentration (Free-Ion Concentration) (M)	
	$8.90 \cdot 10^{-8}$ M EDTA with $7.67 \cdot 10^{-8}$ M Cd	$3.00 \cdot 10^{-7}$ M EDTA with $4.32 \cdot 10^{-7}$ M Cd
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$ ($2.73 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$ ($2.73 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)
K (K^{+})	$4.00 \cdot 10^{-3}$ ($3.96 \cdot 10^{-3}$)	$4.00 \cdot 10^{-3}$ ($3.96 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$ ($9.93 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.93 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-3}$ ($1.17 \cdot 10^{-3}$)	$1.50 \cdot 10^{-3}$ ($1.17 \cdot 10^{-3}$)
Cd (Cd^{2+})	$7.67 \cdot 10^{-8}$ ($1.04 \cdot 10^{-8}$)	$4.32 \cdot 10^{-7}$ ($1.24 \cdot 10^{-7}$)
EDTA ($EDTA^{4-}$)	$8.90 \cdot 10^{-8}$ ($2.86 \cdot 10^{-17}$)	$3.00 \cdot 10^{-7}$ ($1.08 \cdot 10^{-17}$)
pH	6.0	6.0

Figure 3.1: Concentration of Cd in 'Kyle' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with EDTA compared to the concentration of Cd in roots of 'Kyle' exposed to control exposure solutions, shown as the response surface from Figure 2.2. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Kyle': EDTA effects

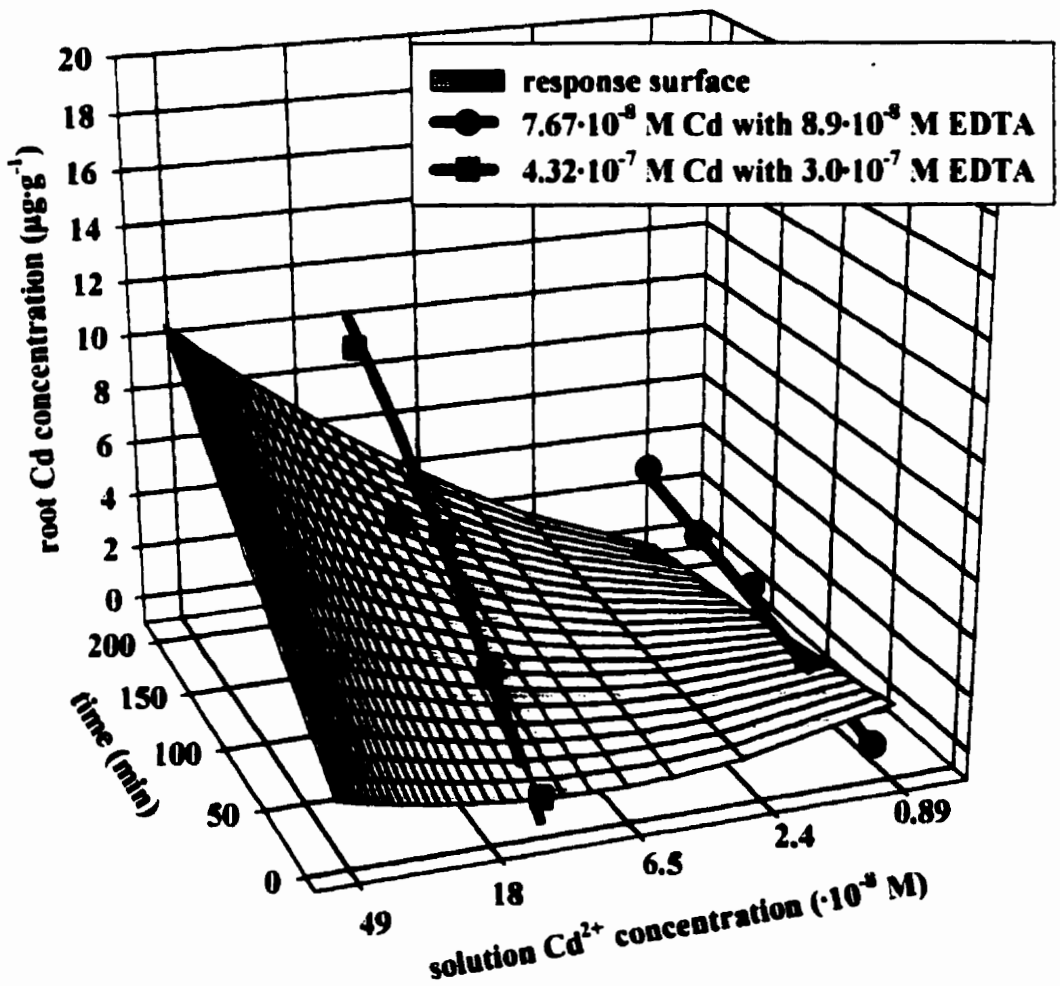
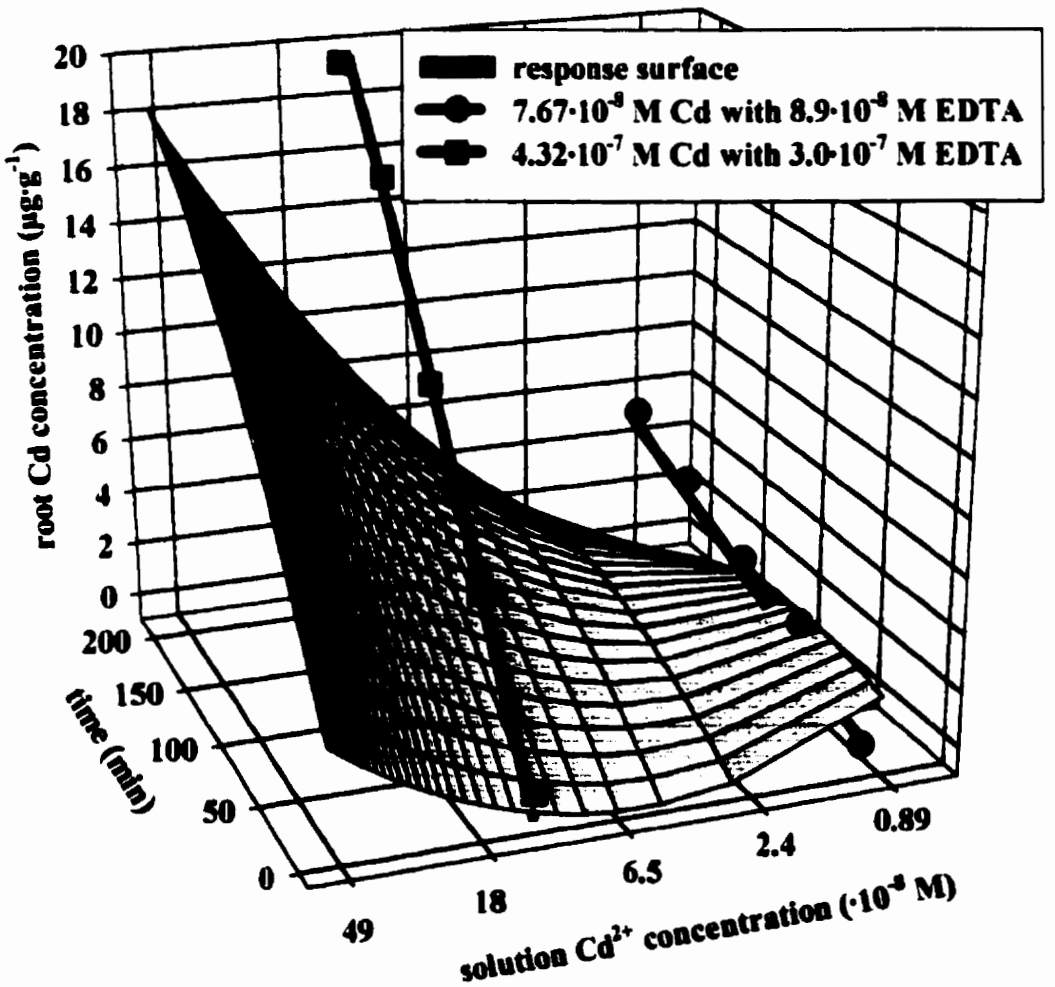


Figure 3.2: Concentration of Cd in 'Arcola' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes along with EDTA compared to the concentration of Cd in roots of 'Arcola' exposed to control exposure solutions, shown as the response surface from Figure 2.3. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Arcola': EDTA effects



and between $lnCd^{2+}$ and EDTA ($p<0.0001$) (Table 3.5). The interaction between time and EDTA indicates that, averaged over both cultivars and all estimated exposure solution Cd^{2+} concentrations, those solutions which contained EDTA resulted in accumulation of Cd by plant roots which was significantly different than accumulation of Cd from solutions without EDTA (control), and the magnitude of the difference depended on the duration of exposure. The interaction between $lnCd^{2+}$ and EDTA indicated that averaged over both cultivars and all durations of exposure, those solutions which contained EDTA resulted in accumulation of Cd by plant roots which was significantly different than accumulation of Cd from solutions without EDTA (control), and the magnitude of the difference depended on the estimated concentration of Cd^{2+} in the exposure solution.

Exposure to solutions which contained EDTA resulted in greater accumulation of Cd by wheat roots when the accumulation was related to the concentration of Cd^{2+} in the exposure solution, and the magnitude of the increase was greater with longer durations of exposure or greater concentrations of Cd^{2+} in the exposure solution. Similarly to the effect of adding citrate to the exposure solution, adding EDTA resulted in a significant reduction in the proportion of dissolved Cd present as Cd^{2+}, but did not result in the decrease in accumulation of Cd by wheat roots which would have been predicted by the FIM.

The addition of EDTA to the exposure solution altered speciation of Cd without altering speciation of other ions present in the exposure solution, or requiring the addition of significant amounts of KOH to adjust the pH of the exposure solution to 6.0. The increased accumulation of Cd by wheat roots in relation to the exposure solution Cd^{2+}

Table 3.5: Sources of variation in content of Cd in roots exposed to solutions with or without EDTA from experiment 2.

Source	df	F-value	p-value
Model	11	183.54	<0.0001
cultivar	1	0.00	0.99
time	1	3.00	0.092
$lnCd^{2+}$	1	0.06	0.81
EDTA	1	8.79	0.0054
time*cultivar	1	2.89	0.097
time*$lnCd^{2+}$	1	120.91	<0.0001
time*time*$lnCd^{2+}$	1	6.89	0.013
time*EDTA	1	102.05	<0.0001
$lnCd^{2+}$*EDTA	1	19.17	<0.0001
time*$lnCd^{2+}$*cultivar	1	133.81	<0.0001
time*cultivar*EDTA	1	45.98	<0.0001
Error	36		

concentration was not due to decreased concentrations of ions which may have competed with Cd^{2+} for uptake, such as Ca^{2+} or Mg^{2+}, but was due to the presence of $CdEDTA^{2-}$. EDTA is a large, synthetic chelating agent of the formula $(COOHCH_2)_2N(CH_2)_2N(CH_2COOH)_2$. It is a diamine with four acetic acid groups, and is very efficient at binding metals. EDTA is thought to be unable to cross phytoplankton cell membranes (Jackson and Morgan, 1978) or other biological membranes (Simkiss and Taylor, 1995), although in a study on Fe accumulation by castor oil (*Ricinus communis*) and dwarf bean (*Phaseolus vulgaris*) FeEDTA was found in the phloem sap of plants grown in nutrient solution containing FeEDTA (Maas *et al.*, 1988). This indicates that some EDTA must have crossed biological membranes, although FeEDTA was only a small portion of the Fe measured in the phloem. One possible explanation for enhanced accumulation of Cd in the presence of EDTA is the uptake of the $CdEDTA^{2-}$ complex. A second possibility is that $CdEDTA^{2-}$ increases uptake of Cd by roots by altering the chemistry in the rhizosphere in a way that results in an increase in accumulation of Cd^{2+}, such as enhancing diffusion of Cd to the root surface.

The results are in agreement with a study on duckweeds (*Lemnaceae*) exposed to Cd and EDTA (Srivastava and Appenroth, 1995). In that study, addition of EDTA to a solution containing Cd significantly reduced the Cd^{2+} concentration, and also the accumulation of Cd by duckweeds, but the reduction in accumulation was not as great as predicted by the reduction in Cd^{2+} concentration. The authors attributed this to uptake of CdEDTA species through breaks in the root endodermis or dissociation of CdEDTA during treatment. In another study, absorption of Cd by duckweeds (*Lemna paucicostata*)

was not reduced much by addition of EDTA, while absorption of Cu was reduced (Nasu *et al.*, 1983).

3.3.3 Modifying Effects of an Increase in the Nominal SO_4 Concentration

Increasing the nominal concentration of SO_4 tenfold relative to the control solution resulted in a decrease in the proportion of total Cd present as Cd^{2+}, since sulphur in general, including SO_4^{2-}, is a ligand for Cd^{2+} (Table 3.2). SO_4 was added to solution with a counter ion. For this experiment the nominal SO_4 concentration was increased by three methods; by adding $MgSO_4$, which resulted in a tenfold increase in the nominal Mg concentration compared with the nominal Mg concentration in the control exposure solution; by adding K_2SO_4, which resulted in a nominal K concentration which was 7.75x higher than in the control exposure solution, and by adding half of the excess SO_4 as $MgSO_4$ and half as K_2SO_4, resulting in a fivefold increase in the nominal Mg concentration while the nominal K concentration was increased by 4.75 times compared with the nominal concentrations of Mg and K found in the control exposure solutions (Table 3.6). The proportion of Cd present as Cd^{2+} decreased from 87.8% to about 57% (or by about 35%); the precise amount of the reduction depended on the counterion for SO_4 (Table 3.2). The greatest reduction in the estimated Cd^{2+} concentration occurred when SO_4 was added as K_2SO_4, and the smallest reduction in the estimated Cd^{2+} concentration occurred when SO_4 was added as $MgSO_4$, since the extra Mg^{2+} present competed with Cd^{2+} to form complexes with SO_4^{2-} (Table 3.2). K^+ does not form very strong complexes with SO_4^{2-}.

Increasing the nominal SO_4 concentration also affected Ca and Mg speciation,

Table 3.6: Nominal and estimated concentrations used in exposure solutions containing a tenfold increase in the nominal SO_4 concentration (experiment 3).

Ion	Nominal Concentration (Estimated Concentration) (M)		
	SO_4 concentration increased by adding $MgSO_4$	SO_4 concentration increased by adding K_2SO_4	SO_4 concentration increased by adding $MgSO_4$ and K_2SO_4
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$ ($2.14 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$ ($2.00 \cdot 10^{-3}$)	$3.00 \cdot 10^{-3}$ ($2.06 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-2}$ ($1.13 \cdot 10^{-2}$)	$1.50 \cdot 10^{-3}$ ($1.06 \cdot 10^{-3}$)	$7.50 \cdot 10^{-3}$ ($5.45 \cdot 10^{-3}$)
K (K^{+})	$4.00 \cdot 10^{-3}$ ($3.86 \cdot 10^{-3}$)	$3.10 \cdot 10^{-2}$ ($2.97 \cdot 10^{-2}$)	$1.90 \cdot 10^{-2}$ ($1.83 \cdot 10^{-2}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$ ($9.95 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.85 \cdot 10^{-3}$)	$1.00 \cdot 10^{-2}$ ($9.89 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-2}$ ($1.03 \cdot 10^{-2}$)	$1.50 \cdot 10^{-2}$ ($1.24 \cdot 10^{-2}$)	$1.50 \cdot 10^{-2}$ ($1.14 \cdot 10^{-2}$)
Cd (Cd^{2+})	$8.90 \cdot 10^{-8}$ ($5.41 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($4.90 \cdot 10^{-8}$)	$8.90 \cdot 10^{-8}$ ($5.13 \cdot 10^{-8}$)
	$4.45 \cdot 10^{-7}$ ($2.71 \cdot 10^{-7}$)	$4.45 \cdot 10^{-7}$ ($2.48 \cdot 10^{-7}$)	$4.45 \cdot 10^{-7}$ ($2.56 \cdot 10^{-7}$)
pH	6.0	6.0	6.0

since SO_4^{2-}, like citrate, forms complexes with Ca^{2+} and Mg^{2+} as well as Cd^{2+}. So in all exposure solutions which had increased nominal SO_4 concentration, the estimated Ca^{2+} concentration was 25% less than in the control exposure solution, and when the nominal SO_4 concentration was increased by adding K_2SO_4, the estimated Mg^{2+} concentration was also about 25% less than in the control exposure solution (Table 3.5). When the nominal SO_4 concentration was increased by adding $MgSO_4$, or half $MgSO_4$ and half K_2SO_4, the proportion of dissolved Mg present as Mg^{2+}, but not the estimated Mg^{2+} concentration was reduced relative to the control exposure solution, since there was a five- or tenfold increase in the nominal Mg concentration (Table 3.5). In these solutions, the estimated Mg^{2+} concentration was increased sevenfold when the nominal SO_4 concentration was increased by adding $MgSO_4$ and almost threefold when the nominal SO_4 concentration was increased by adding both K_2SO_4 and $MgSO_4$.

The nominal K concentration in the exposure solutions where the nominal SO_4 concentration was increased by adding K_2SO_4 or both K_2SO_4 and $MgSO_4$ increased by 6.5x and 3.6x, respectively, and results from the previous chapter demonstrate that when the nominal K concentration was increased 3.5x by adding KNO_3, accumulation of Cd by wheat roots was not altered.

When the tissue Cd concentration was related to the estimated Cd^{2+} concentration, increasing the nominal SO_4 concentration in the exposure solution to tenfold that found in the control exposure over a range of estimated exposure solution Cd^{2+} concentrations and durations of exposure, increased or did not change the Cd accumulation by roots (Figures 3.3 and 3.4). The superimposed data points and regression lines in the figures

Figure 3.3: Concentration of Cd in 'Kyle' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes with a tenfold increase in the SO_4 concentration compared to the concentration of Cd in roots of 'Kyle' exposed to control exposure solutions, shown as the response surface from Figure 2.2. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Kyle': SO_4 effects

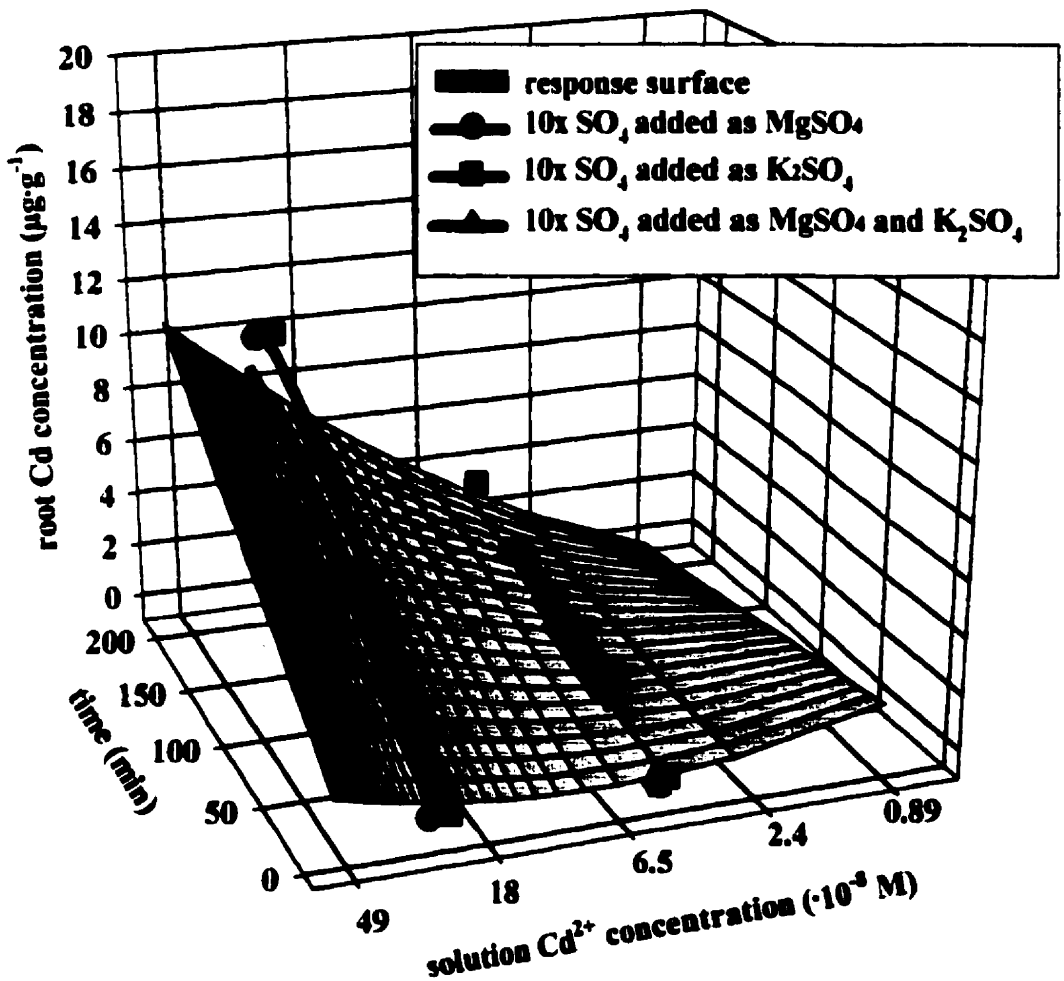
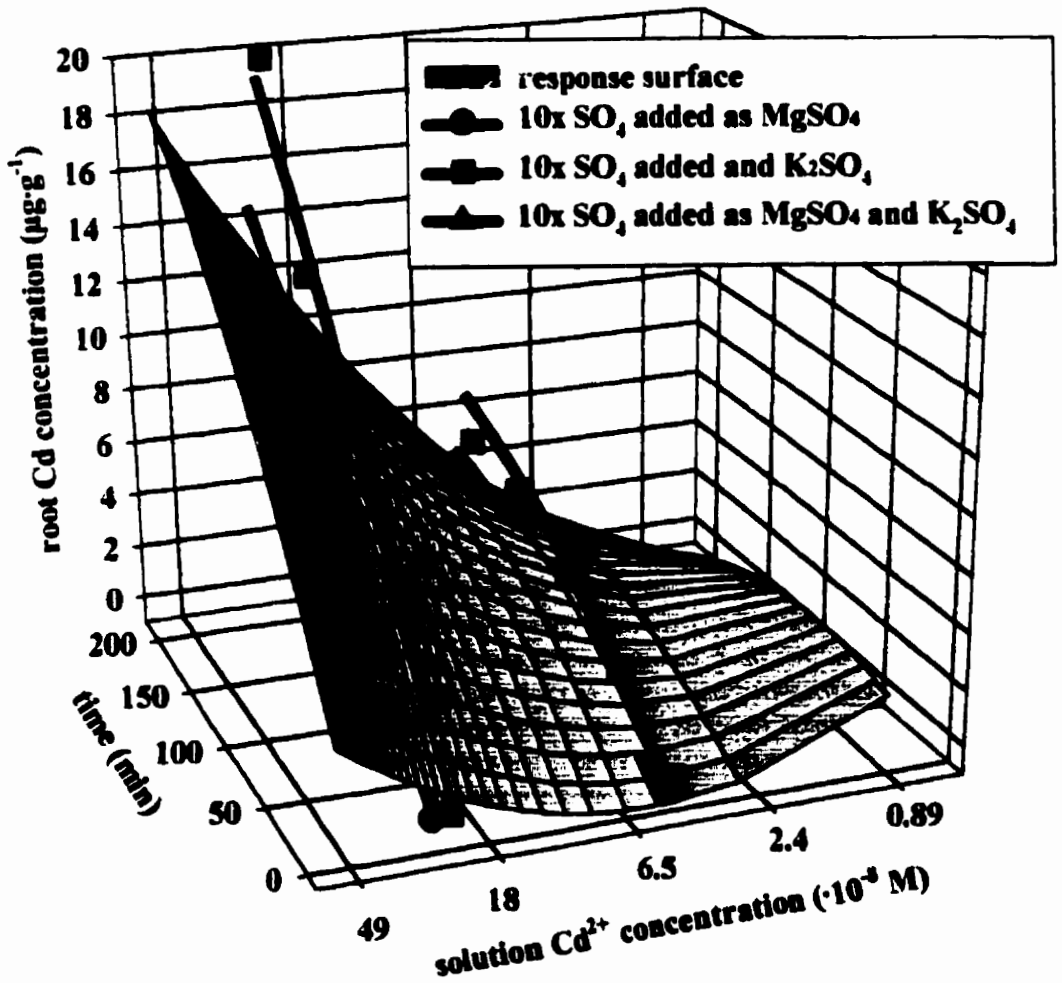


Figure 3.4: Concentration of Cd in 'Arcola' roots exposed to a range of Cd^{2+} concentrations for 0 to 200 minutes with a tenfold increase in the SO_4 concentration compared to the concentration of Cd in roots of 'Arcola' exposed to control exposure solutions, shown as the response surface from Figure 2.3. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

'Arcola': SO_4 effects



demonstrate that the effect of SO_4 on accumulation of Cd by root tissue was dependent on how the nominal SO_4 concentration was increased. When the nominal SO_4 concentration was increased by adding $MgSO_4$ or by adding both $MgSO_4$ and K_2SO_4, accumulation of Cd did not seem to be influenced, while when the nominal SO_4 concentration was increased by adding K_2SO_4, Cd accumulation by roots was increased in relation to accumulation from the control exposure solution. Similarly to when EDTA or citrate are added to the exposure solution, accumulation was especially enhanced after longer durations of exposure. Those exposure solutions in which the increase in the nominal SO_4 concentration was achieved by adding $MgSO_4$, or a combination of $MgSO_4$ and K_2SO_4 likely did not result in an enhanced accumulation of Cd by wheat roots because the excess Mg^{2+} competed with Cd^{2+} for uptake (Chapter 2). Results from the experiments reported on in Chapter 2 also demonstrate that the nominal K concentration in the exposure solution did not influence Cd accumulation by roots. K^+ is a larger ion with a single charge, and is not likely to compete with Cd^{2+} for uptake.

The evidence for the effect of SO_4 on accumulation of Cd by wheat roots is the interactions between time and SO_4 ($p=0.059$), among time, cultivar and SO_4 ($p<0.0001$) and among time, $lnCd^{2+}$ and SO_4 ($p=0.0063$) (Table 3.7). The interaction between time and SO_4 indicates that solutions with an increased nominal SO_4 concentration resulted in accumulation of Cd by plant roots which was significantly different than accumulation of Cd from solutions with a lower SO_4 concentration (control), and the magnitude of the difference depended on the duration of exposure. The interaction among time, cultivar and SO_4 indicates that, averaged over all Cd^{2+} concentrations in the exposure solution,

Table 3.7: Sources of variation in content of Cd in roots exposed to solutions containing Cd with nominal SO_4 concentrations of 0.00150 M or 0.0150 M added as $MgSO_4$, K_2SO_4, or as a combination of $MgSO_4$ and K_2SO_4.

Source	df	F-value	p-value
Model	19	99.18	<0.0001
cultivar	1	0.17	0.68
time	1	12.41	0.00084
$lnCd^{2+}$	1	1.16	0.29
SO_4	3	0.03	0.99
time*time	1	3.15	0.081
time*$lnCd^{2+}$	1	259.55	<0.0001
time*SO_4	3	2.63	0.059
time*$lnCd^{2+}$*cultivar	1	85.78	<0.0001
time*cultivar*SO_4	4	9.50	<0.0001
time*$lnCd^{2+}$*SO_4	3	4.54	0.0063
Error	58		

'Kyle' and 'Arcola' had different Cd concentrations in their roots, that the magnitude of the cultivar difference in root Cd concentration depended on the duration of exposure to Cd, and that different nominal SO_4 concentrations resulted in different levels of accumulation of Cd. Similarly, the interaction among time, $lnCd^{2+}$, and SO_4 indicated that the concentration of Cd in roots depended on the estimated Cd^{2+} concentration the roots were exposed to, and that the magnitude of this difference depended on the duration of exposure and the nominal SO_4 concentration in the exposure solution.

Solutions which contained an increase in the nominal SO_4 concentration with similar nominal Mg concentrations (i.e. SO_4 added as K_2SO_4) resulted in greater accumulation of Cd by wheat roots. Similar to the effect of adding citrate or EDTA to the exposure solution, increasing the nominal SO_4 concentration reduced the proportion of dissolved Cd present as Cd^{2+}, but did not result in a decrease in accumulation of Cd by wheat roots. These results are in agreement with a recent study on the effects of SO_4 on accumulation of Cd by Swiss chard which demonstrated no reduction in accumulation even though the estimated Cd^{2+} concentration was reduced as a result of enhanced formation of $CdSO_4^0{}_{(aq)}$ complexes in the presence of additional SO_4 (McLaughlin *et al.*, 1998). In that study, the authors suggested that $CdSO_4^0{}_{(aq)}$ was taken up as easily as Cd^{2+}.

In all of the exposure solutions with increased nominal SO_4 concentration, the estimated Ca^{2+} concentration was reduced by about 25%, and in the exposure solution where the nominal SO_4 was increased by adding K_2SO_4, both the estimated Ca^{2+} and Mg^{2+} concentrations were reduced by about 25%. From Chapter 2, reductions in estimated Ca^{2+} or Mg^{2+} concentrations of 50 or almost 70% resulted in increased Cd accumulation in

wheat roots, so in this case, a reduction in the estimated Ca^{2+} and Mg^{2+} concentrations as a result of the formation of $CaSO_4^0{}_{(aq)}$ and $MgSO_4^0{}_{(aq)}$ complexes may be partially responsible for the observed increase in Cd accumulation.

Conversely, an increase in the estimated Mg^{2+} concentration might be expected to result in a decrease in Cd accumulation (Chapter 2). In solutions where the nominal SO_4 concentration was increased by adding $MgSO_4$ or a combination of $MgSO_4$ and K_2SO_4, the estimated Mg^{2+} concentration was increased by 7.1 and 2.9x, respectively. These large increases in the estimated Mg^{2+} concentration could result in increased competition with Cd^{2+} for accumulation. This may explain why there was no increase in Cd accumulation by roots exposed to solutions containing both an increase in the nominal SO_4 concentration along with a substantial increase in the estimated Mg^{2+} concentration.

The nominal K concentration in the exposure solution was also increased when the nominal SO_4 concentration was increased by adding K_2SO_4 or half K_2SO_4 and half $MgSO_4$. Data presented in the previous chapter indicate that when the nominal K concentration was increased by 3.5x, accumulation was unaffected. In these solutions, the nominal K^+ concentration was increased by 3.6 to 6.5x, .

3.4 Summary and Conclusions

Our null hypotheses, that accumulation of Cd by roots of two cultivars of durum wheat is dependent only on the concentration of the free ion (Cd^{2+}), and is not influenced by the presence of EDTA or an increase in the nominal SO_4 concentration, can be rejected. The concentration of Cd^{2+} in the exposure solution did not predict the Cd concentration in

wheat roots exposed to solutions with altered Cd speciation. The addition of EDTA to exposure solutions resulted in accumulation of Cd in relation to the Cd^{2+} concentration in the exposure solution which was greater than accumulation from control solutions which did not contain EDTA, and in this case, changes in Cd speciation were not confounded by changes in Ca, Mg or K concentrations. Adding EDTA to the exposure solution resulted in a shift in the equilibrium between Cd^{2+} and $CdEDTA^{2-}$ toward $CdEDTA^{2-}$.

Increasing the nominal SO_4 concentration in the exposure solutions also resulted in accumulation of Cd in relation to the Cd^{2+} concentration in the exposure solution which was greater than accumulation from control solutions. This was the case only if the nominal Mg concentrations were not increased as well, since Mg^{2+} competes with Cd^{2+} for uptake. In the solution where the nominal SO_4 concentration was increased without an increase in the nominal Mg concentration, Cd accumulation was enhanced. In this solution, the estimated Ca^{2+} and Mg^{2+} concentrations were reduced by about 25%, which may have resulted in enhanced Cd accumulation as a result of reduced competition between Cd^{2+} and Ca^{2+} or Mg^{2+}.

The presence of $CdEDTA^{2-}$ or an increase in the concentration of $CdSO_4^0{}_{(aq)}$ may have resulted in enhanced accumulation of Cd in relation to the concentration of Cd^{2+} in the exposure solution in a number of different ways. One possible explanation is that the $CdEDTA^{2-}$ and $CdSO_4^0{}_{(aq)}$ complexes were accumulated by roots. This would be an exception to the FIM since it predicts that only the free ion (Cd^{2+}) is taken up. In the case of $CdEDTA^{2-}$ this is unlikely, since EDTA is a large, synthetic molecule and it is not likely that biological membranes are very permeable to EDTA. A second possibility is that

diffusion of Cd^{2+} to the root cell surface was the rate limiting step in the accumulation of Cd, resulting in a depletion of Cd^{2+} at the root surface relative to the bulk solution. With a significant proportion of the total dissolved Cd present as a complex, the Cd^{2+} concentration at the root surface may have been buffered by dissociation of $CdEDTA^{2-}$ or $CdSO_4^0{}_{(aq)}$ into EDTA or SO_4^{2-} and Cd^{2+} which in its free ion form could then be accumulated by the root tissue. If the process of dissociation was faster than diffusion of Cd^{2+} from the bulk solution to the root surface and accumulation of Cd by root tissue, then the presence of a complexed form of Cd which can easily dissociate could result in a relatively higher concentration of Cd^{2+} at the root surface than if the dissolved Cd was present mostly as Cd^{2+}. This scenario would be a case where the assumptions of the FIM were not met, since the FIM assumes that the rate limiting step in the interaction between dissolved metal and the biological organism is binding to cell surface binding sites, and not diffusion to the site. This scenario is considered in greater depth in the following chapter.

In the case of SO_4, reductions in estimated Ca^{2+} and Mg^{2+} concentrations may have resulted in decreased competition with Cd^{2+} for uptake sites; this would also be a situation where the assumptions of the FIM were not being met, since the FIM assumes that cell surface binding sites are specific for the metal causing the effect (Cd), and do not bind with other metals (Ca or Mg).

CHAPTER 4:

THE EFFECT OF STIRRING THE SOLUTION IN WHICH WHEAT ROOTS ARE EXPOSED: IS DIFFUSION ACROSS THE BOUNDARY LAYER IN HYDROPONIC SOLUTION THE RATE LIMITING STEP IN ACCUMULATION OF CADMIUM?

4.1 Introduction

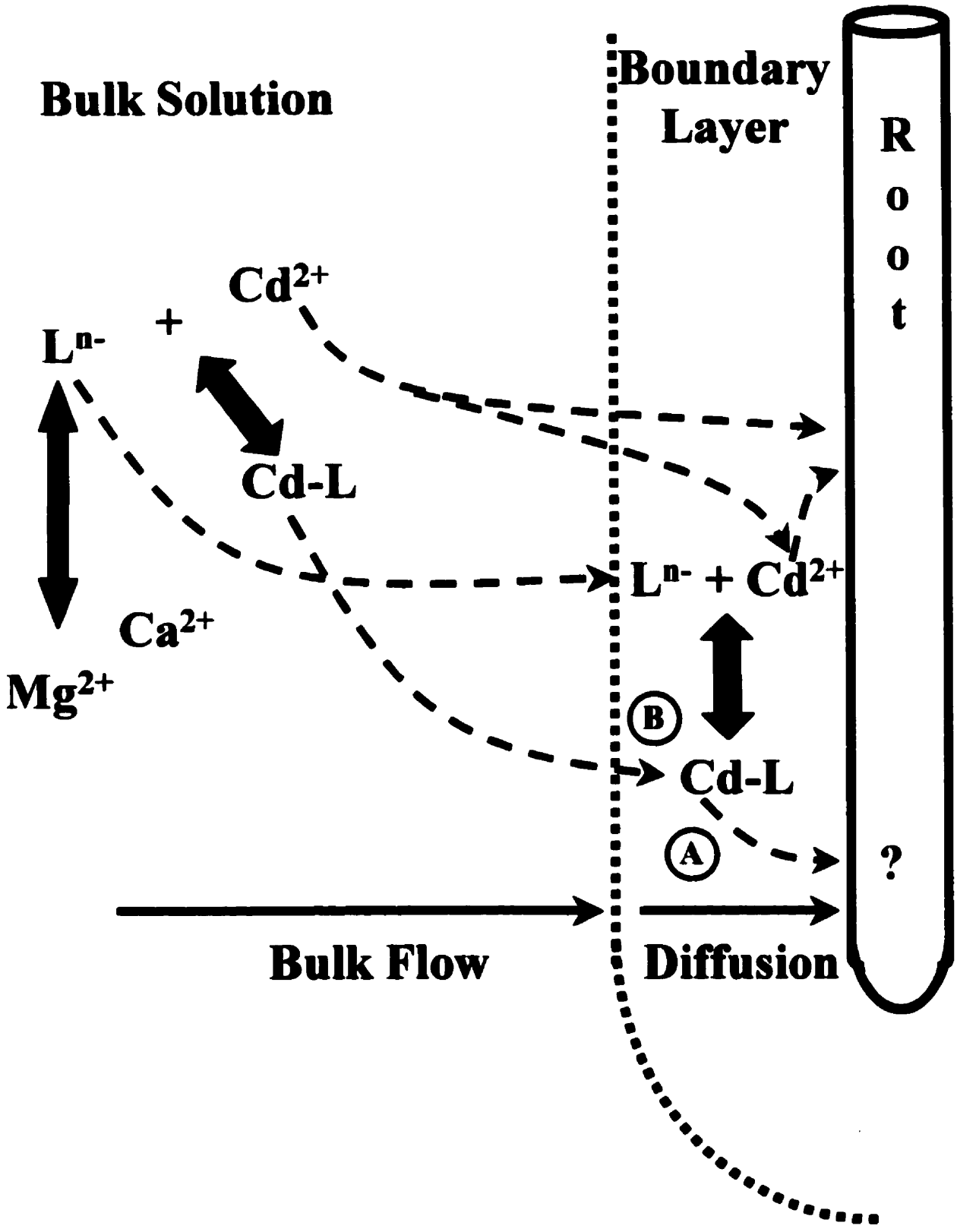
Results from the previous two chapters suggest that relating accumulation of Cd by roots of 'Kyle' and 'Arcola' wheat seedlings to the concentration of Cd^{2+} in the exposure solution works well as long as estimated Ca^{2+} and Mg^{2+} concentrations were kept constant among various exposure solutions and as long as there was not a significant proportion of dissolved Cd present complexed with dissolved ligands such as citrate, EDTA or SO_4^{2-}, resulting in $CdCitrate^-$, $CdEDTA^{2-}$ or $CdSO_4^0{}_{(aq)}$.

If the estimated Ca^{2+} or Mg^{2+} concentrations were reduced, then Cd accumulation by wheat roots was enhanced, likely as a result of reduced competition with Ca^{2+} and/or Mg^{2+} for uptake sites on the root surface. This is consistent with the observation that marine organisms tend to accumulate less Cd, which is thought to be due to competition between Cd^{2+} and Ca^{2+} for uptake (Canadian Environmental Protection Act, 1994).

If a large portion of the dissolved Cd was present in complexed forms, such as $CdCitrate^-$, $CdEDTA^{2-}$, or $CdSO_4^0{}_{(aq)}$, then accumulation of Cd by wheat roots was greater than would be predicted by the estimated Cd^{2+} concentration in the exposure solution. In this case, enhanced accumulation of Cd by the roots may have been due to either; 1) accumulation of Cd complexes (Figure 4.1 labelled '*A*'), and/or 2) enhanced diffusion of dissolved Cd in a complexed form across the boundary layer surrounding the roots (Figure 4.1, labelled '*B*'), and subsequent dissociation to Cd^{2+} and uptake.

Several studies have concluded that in the presence of complexed forms of Cd, bioaccumulation was greater than predicted due to the accumulation of Cd complexes. In a study on the influence of citrate on the toxicity of Cd and Zn to the alga *Selenastrum*

Figure 4.1: Model of how solution chemistry and a boundary layer might influence accumulation of Cd by a root.


Bulk Solution
Boundary Layer
Root
Cd^{2+}
+
L^{n-}
Cd-L
$L^{n-} + Cd^{2+}$
Ca^{2+}
Mg^{2+}
B
Cd-L
A
?
Bulk Flow
Diffusion

capricornutum, the authors maintained equal estimated Cd^{2+} concentrations in solutions without citrate, with $1 \cdot 10^{-4}$ M citrate, or with $1 \cdot 10^{-4}$ M NTA (a membrane impermeable metal buffer) by increasing the nominal Cd concentration in solutions containing citrate or NTA (Errécalde *et al.*, 1998). According to the FIM, toxicity of Cd would be expected to be similar among the three solutions, but it was discovered that the toxicity of Cd in the solution containing citrate was greater than in the solutions containing no citrate or NTA. Furthermore, by using [^{14}C]-citrate, the authors were able to demonstrate that citrate was accumulated by the alga, and that if one in four transport events were a $CdCitrate^-$ complex rather than citrate, the extra Cd accumulated could be accounted for. In a study on the effects of EDTA and iron on accumulation of Cd^{2+} in duckweeds (*Lemnaceae*), it was discovered that the presence of the CdEDTA complex resulted in accumulation of Cd which was greater than predicted by the estimated Cd^{2+} concentration, and the authors attributed this to dissociation of the CdEDTA complex during the exposure (Srivastava and Appenroth, 1995). The effect of Cl (Smolders and McLaughlin, 1996a; b) and SO_4 (McLaughlin *et al.*, 1998) on accumulation of Cd by Swiss chard has also been investigated. In both studies, the presence of complexed Cd ($CdCl_n^{2-n}$ or $CdSO_4^0{}_{(aq)}$) resulted in accumulation of Cd by Swiss chard which was greater than predicted by the estimated Cd^{2+} concentration. The authors attributed this to accumulation of $CdCl_n^{2-n}$ or $CdSO_4^0{}_{(aq)}$, or, in the case of Cl, possibly to enhanced diffusion of Cd^{2+} to uptake sites. Enhanced diffusion could be achieved by the dissociation of $CdCl_n^{2-n}$ into Cl^- and Cd^{2+} near the root surface, resulting in Cd^{2+} available for uptake.

The ability of complexed forms of Cd to result in enhanced diffusion of Cd to

uptake sites assumes the existence of a boundary layer around the root surface and that diffusion of Cd^{2+} from the bulk solution to the root surface is the rate limiting step in the process of Cd^{2+} uptake by the roots. The presence of complexed forms of Cd in the exposure solution could result in enhanced diffusion of Cd to the root surface by diffusing through the layer as a complex (such as $CdCitrate^{-}$) and then dissociating into the free ion (Cd^{2+}) and citrate as Cd^{2+} is taken up and its concentration declines.

By this method, the Cd^{2+} concentration at the root surface could be buffered by the presence of soluble, easily dissociable, complexed forms of Cd. In the absence of these complexed forms of Cd, the Cd^{2+} taken up by the root would have to be replaced by Cd^{2+} from the bulk solution via diffusion through the unstirred layer to the root surface. If this process of diffusion is slower than membrane transport, then the Cd^{2+} concentration near the root surface would decline, creating a zone of Cd^{2+} depletion around areas of Cd uptake on the root. This could in turn result in a slower rate of uptake. If a boundary layer is the rate limiting step in accumulation of Cd, then reducing the thickness of the boundary layer by swirling the exposure solutions during exposure to Cd may result in enhanced accumulation of Cd by wheat roots.

The presence of a boundary layer and its effect on accumulation of metals has been considered previously. Jackson and Morgan (1978) carried out theoretical calculations with the goal of determining whether complexed forms of Fe could result in greater rates of diffusion across the boundary layer surrounding marine phytoplankton. Robinson (1986) concluded that, due to the lack of a large boundary layer surrounding the root, ion absorption from solution was limited by the capacity of the root for uptake, though he

allowed that in dilute solutions, diffusive flux may contribute to limited uptake. In aquatic plants, the supply of CO_2 (as HCO_3^-) can be limited by diffusion; supply of CO_2 can be increased by increasing the water flow over the surface of the plants, which reduces the thickness of the unstirred layer surrounding the plants (Raven *et al.*, 1985). More recently, Hudson (1998) concluded that if uptake rates approach diffusion limitation (resulting in a reduction in the concentration of the ion being accumulated at the root surface), dissociation of labile complexes could enhance the diffusion process. In such a scenario, the rate of uptake would not only depend on the concentration of the free ion (Cd^{2+}), but also the concentration of the complexed species which could easily dissociate to the free ion. In the exposure solutions used in the experiments on durum wheat (Chapters 2 and 3), $CdCitrate^-$, $CdEDTA^{2-}$ and $CdSO_4^0{}_{(aq)}$ may meet this criterion.

The interaction between trace metals and aquatic organisms has been studied more extensively than the interaction between trace metals and plants. According to Tessier *et al.* (1994), when physical transport (i.e. diffusion) becomes the rate limiting process in the movement of dissolved metals from the bulk solution through a diffusion layer, and finally into a cell, the flux (J; $mol \cdot cm^{-2} \cdot s^{-1}$) can be predicted by the following equation: $J = D_{avg} [M_t] / \delta$; where D_{avg} ($cm^2 \cdot s^{-1}$) is the average diffusion coefficient for the different species of the dissolved metal; $[M_t]$ (mol) is the total dissolved metal concentration; and δ (cm) is the thickness of the boundary layer. In contrast to the FIM, accumulation under these circumstances is proportional to the total metal concentration, and not the concentration of the free ion. This is reasonable, since under these circumstances, one of the assumptions of the FIM, that diffusion is not rate limiting, is not true. The results

presented in Chapters 2 and 3 demonstrate that accumulation of Cd from solutions containing low Cd concentrations with or without citrate, EDTA, or SO_4 was not proportional to the concentration of the free ion, but proportional to the total Cd concentration. When citrate, EDTA, or SO_4 were added to exposure solutions, the Cd^{2+} concentration was reduced, but accumulation of Cd by wheat roots remained similar. This observation is consistent with the relationship between dissolved metals and aquatic organisms when diffusion is rate limiting.

The goal of this study was to determine the effect of solution turbulence on accumulation of Cd. It was assumed that swirling the exposure solution would cause enough movement in the bulk solution so that the thickness of the boundary layer would be less in the swirled than in the non-swirled exposure solutions. The null hypothesis was that the Cd concentration in roots exposed to a range of estimated Cd^{2+} concentrations for 0 to 200 min and swirled does not differ from the Cd concentration in roots similarly exposed and not swirled. With no way to actually measure the thickness of the boundary layer, however, a lack of difference in root Cd concentration in roots exposed to Cd in swirled or non-swirled solutions would mean one of two things. Either the boundary layer was reduced in thickness, but was not the rate limiting step in movement of Cd from the bulk solution into the root, or the swirling of the solutions was not enough to influence the thickness of the boundary layer. The presence of root hairs may impede the effect of swirling on reducing boundary layer thickness.

Accumulation data presented in Chapter 2 along with morphology data presented in Chapter 4 allow determinations of metal flux into 'Kyle' and 'Arcola' roots.

Furthermore, making certain assumptions about the thickness of a hypothetical boundary layer, the flux of Cd from the bulk solution to the root surface can be roughly estimated. In theory, if diffusion of Cd through a boundary layer is rate limiting, then the flux of Cd into wheat roots should be the same as flux of Cd from the bulk solution to the root surface. Theoretical calculations are presented at the end of this chapter.

4.2 Materials and Methods

4.2.1 Plant Material and Growth Conditions

Plant material used and growth conditions were as described in section *2.2.2 Plant Material and Growth Conditions*. Six-day old seedlings were used in the experiment.

4.2.2 Cadmium Exposure and Solution Analysis

Cadmium exposure and analysis of exposure solutions were as described in section *2.2.3 Cadmium Exposure and Solution Analysis*. All of the seedlings in a beaker were harvested after either 0, 50, 100, 150, or 200 mins of exposure to Cd. Swirling of solutions was achieved by placing beakers on a shaker (Orbit Shaker, Lab-Line Instruments, Inc., Melrose Park, ILL) rotating at 125 rpm on a bench in the greenhouse.

4.2.3 Plant Digestion and Cd Analysis

Digestion of tissue samples and analysis of samples for Cd were as described in section *2.2.4 Plant Digestion and Cd Analysis*.

4.2.4 Data Analysis

The root cadmium accumulation experiment was conducted as a completely randomized factorial design. There were three target nominal Cd concentrations ($4.45 \cdot 10^{-8}$, $8.90 \cdot 10^{-8}$, and $4.45 \cdot 10^{-7}$ M), two cultivars ('Kyle' and 'Arcola'), five harvest times (0, 50, 100, 150, and 200 min), and two levels of turbulence (swirled or non-swirled) for a total of 60 experimental units (meshes of nine seedlings). Estimates of variation came from regression analysis. Cadmium content of roots was expressed as the concentration of Cd on a dry weight basis ($\mu g \cdot g^{-1}$). The tissue Cd accumulation data were analysed relative to the actual exposure Cd^{2+} concentrations (determined by measuring the total Cd concentration in each treatment solution and using MINEQL$^+$ to estimate the proportion available as the free ion, Cd^{2+}) in the treatment solutions, rather than the target concentrations. The Cd^{2+} concentrations in the exposure solutions were transformed for data analysis using a natural log (ln) transformation because the exposure solution Cd^{2+} concentrations were not evenly spaced. The data were analysed using SAS PROC GLM (SAS Institute Inc., Cary, NC). The initial model tested the contribution to variation in root tissue Cd concentrations of the main effects of cultivar, swirling, exposure duration (time) and the natural log of exposure Cd^{2+} concentration ($lnCd^{2+}$) and all two and three way interactions involving cultivar, swirling, time and $lnCd^{2+}$. Non-significant interactions were dropped from the model, one at a time (in an iterative reduction, starting with the highest order interactions), and their sums of squares were pooled with the error term. The final model for the analysis of Cd concentration in root tissue included cultivar, swirling, time, $lnCd^{2+}$, cultivar*time, time*$lnCd^{2+}$*swirling and cultivar*time*$lnCd^{2+}$.

4.3 Results and Discussion

4.3.1 Swirling Experiment

The three nominal Cd concentrations in the exposure solutions were $4.56 \cdot 10^{-8}$, $9.01 \cdot 10^{-8}$ and $4.80 \cdot 10^{-7}$ M. From MINEQL^{+}, the estimated Cd^{2+} concentrations were 87.8% of the nominal dissolved Cd concentration, or $4.00 \cdot 10^{-8}$, $7.91 \cdot 10^{-8}$ and $4.22 \cdot 10^{-7}$ M Cd^{2+}. The components of the exposure solutions are presented in Table 4.1.

Accumulation of Cd by 'Kyle' and 'Arcola' roots exposed to this range of estimated Cd^{2+} concentrations in the exposure solution for 0 to 200 mins and swirled or not is shown in Figure 4.2 A to E. As in previous experiments, there was a significant interaction among cultivar, time, and $\ln Cd^{2+}$ ($p<0.0001$; Table 4.2). The basis of this interaction was that 'Arcola' roots accumulated more Cd than 'Kyle' roots, and that the magnitude of the difference was greater when seedlings were exposed to higher concentrations of Cd^{2+} in the exposure solution for longer durations of time. There was a significant interaction among cultivar, swirling and $\ln Cd^{2+}$ ($p=0.016$; Table 4.2), and the basis for this interaction was that 'Arcola' seedlings exposed to Cd^{2+} and swirled had less Cd compared with seedlings exposed to Cd^{2+} and not swirled, although this only appeared to be the case for seedlings exposed to the highest concentration of Cd^{2+} ($4.22 \cdot 10^{-7}$ M Cd^{2+}). Swirling did not influence accumulation of Cd by roots of 'Kyle' seedlings; data points and regression equations for 'Kyle' seedlings exposed to Cd^{2+} and swirled or not swirled are superimposed (Figure 4.2 A to E).

Our hull hypothesis, that swirling the exposure solutions does not result in increased accumulation of Cd by wheat seedlings, cannot be rejected. Swirling did not

Table 4.1: Nominal and estimated concentrations used in the exposure solutions.

Ion	Nominal Concentration (Estimated Concentration) (M)
Ca (Ca^{2+})	$3.00 \cdot 10^{-3}$ ($2.73 \cdot 10^{-3}$)
Mg (Mg^{2+})	$1.50 \cdot 10^{-3}$ ($1.40 \cdot 10^{-3}$)
K (K^{+})	$4.00 \cdot 10^{-3}$ ($3.96 \cdot 10^{-3}$)
NO_3 (NO_3^{-})	$1.00 \cdot 10^{-2}$ ($9.93 \cdot 10^{-3}$)
SO_4 (SO_4^{2-})	$1.50 \cdot 10^{-3}$ ($1.17 \cdot 10^{-3}$)
Cd (Cd^{2+})	$4.56 \cdot 10^{-8}$ ($4.00 \cdot 10^{-8}$)
	$9.01 \cdot 10^{-8}$ ($7.91 \cdot 10^{-8}$)
	$4.80 \cdot 10^{-7}$ ($4.22 \cdot 10^{-7}$)
pH	6.0

Figure 4.2 A to E: Accumulation of Cd by 'Kyle' and 'Arcola' roots as affected by swirling. 'Kyle' and 'Arcola' seedlings were exposed to $3.91 \cdot 10^{-8}$ to $3.91 \cdot 10^{-7}$ M Cd^{2+} for 0 to 200 mins and swirled (dashed line; closed symbols) or not (solid line; open symbols). The solution Cd^{2+} concentrations are on a natural log (ln) scale.

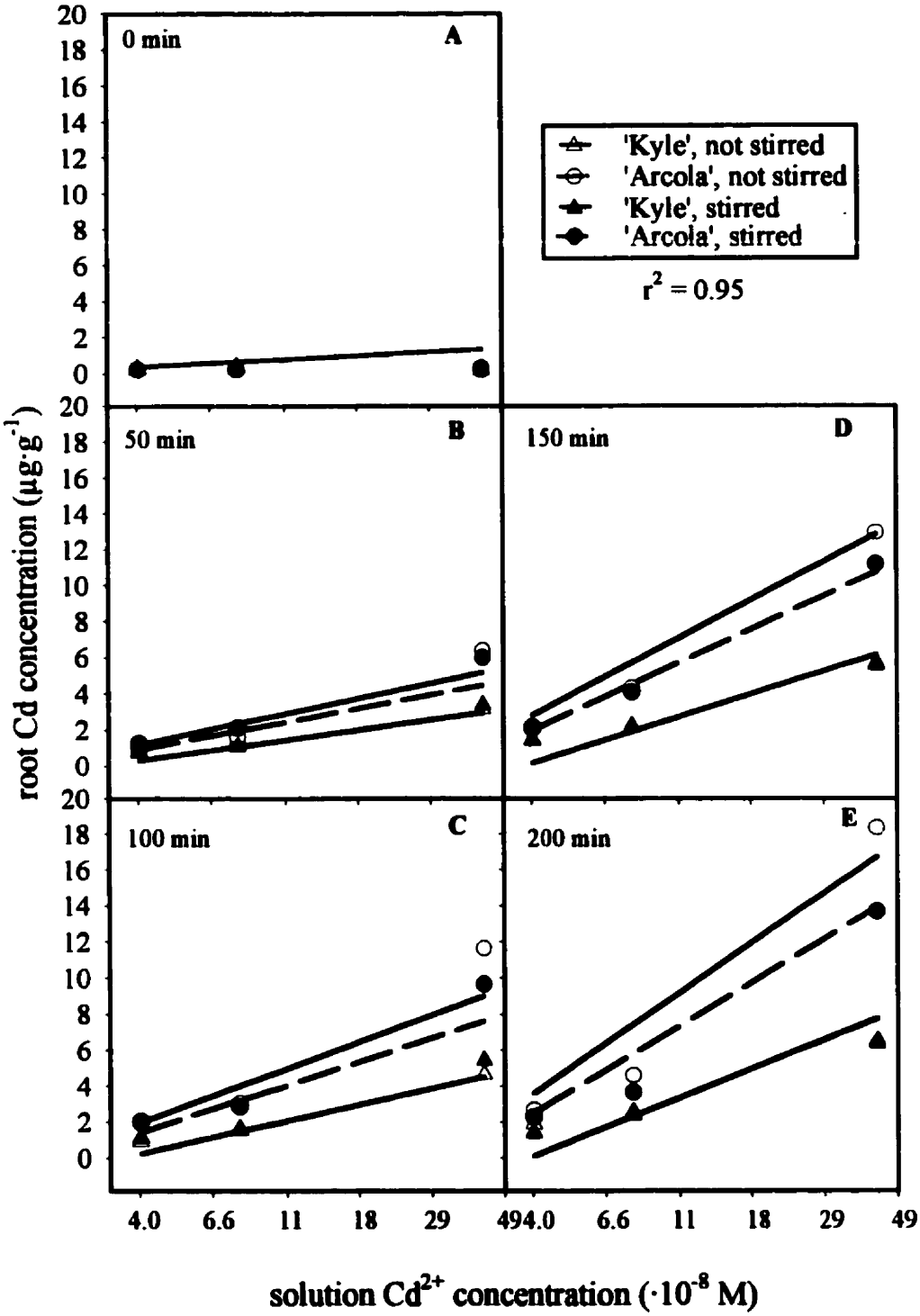

0 min
A
50 min
B
150 min
D
100 min
C
200 min
E
'Kyle', not stirred
'Arcola', not stirred
'Kyle', stirred
'Arcola', stirred
r² = 0.95
root Cd concentration (µg·g⁻¹)
solution Cd²⁺ concentration (·10⁻⁸ M)
4.0
6.6
11
18
29
49

Table 4.2: Sources of variation in content of Cd in roots of 'Kyle' and 'Arcola' exposed to $4.00 \cdot 10^{-8}$ M to $4.22 \cdot 10^{-7}$ M Cd^{2+} for 0 to 200 mins and swirled or not.

Source	df	F value	p value
Model	8	129.00	<0.0001
swirling	(1)	0.98	0.33
cultivar	(1)	0.25	0.62
time	(1)	26.29	<0.0001
$lnCd^{2+}$	(1)	4.36	0.042
time*cultivar	(1)	23.55	<0.0001
cultivar*time*$lnCd^{2+}$	(1)	101.16	<0.0001
cultivar*$lnCd^{2+}$*swirling	(1)	6.21	0.016
Error	51		
Corrected Total	59		

result in an increase in Cd accumulation by wheat roots, but instead appeared to result in a decrease in accumulation of Cd by 'Arcola' roots, but only at one Cd^{2+} dose. The underlying hypothesis of this experiment was that diffusion through an unstirred layer surrounding the root surface was the rate limiting step in accumulation of Cd from the hydroponic solution, and that swirling the exposure solutions would result in more movement in the bulk solution, and therefore a narrower unstirred layer surrounding the root surface through which diffusion would have to occur. The fact that swirling the exposure solution did not result in an increase in Cd accumulation may have been due to one of two reasons. It may have been that diffusion was not the rate limiting step in the accumulation of Cd by wheat roots from the bulk solution, in which case narrowing the unstirred layer would not be expected to increase accumulation since some other step in the process of accumulation of Cd (i.e. membrane transport) was already rate limiting.

A second possibility still assumes that diffusion through an unstirred layer was the rate limiting step in the accumulation of Cd by wheat roots, but that the unstirred layer around the roots was not influenced by swirling the exposure solutions. It is difficult to know what the thickness of the unstirred layer might have been, and how that thickness might have changed by swirling the exposure solutions. Perhaps in this experiment the thickness was influenced very little, or not at all. The influence of swirling the exposure solutions on how well the exposure solutions were mixed was tested by adding a crystal of K_2MnO_4 to swirled and non-swirled beakers filled with exposure solution, and observing the mixing of the purple color through the solution. The purple color of the K_2MnO_4 spread through the solution of swirled beakers much more quickly than non-swirled

solutions. However, this only indicates how much movement occurred in the bulk solution; the influence of swirling on the actual boundary layer is not known.

From Figure 4.2 A to E, it appears that when 'Arcola' seedlings were swirled, accumulation of Cd was less compared with non-swirled exposure solutions. There were 30 pairs of data exposed to similar Cd^{2+} concentrations for similar durations of time and swirled or not swirled. In three of these pairs ('Arcola' exposed to $4.22 \cdot 10^{-7}$ M Cd^{2+} for 100, 150, or 200 mins), accumulation of Cd by seedlings exposed to Cd in swirled exposure solutions was 15 to 20% lower than accumulation from non-swirled solutions; many of the other pairs of data were nearly superimposed. The effect did not appear consistently in all swirled experimental units, or even in all swirled experimental units of 'Arcola', so we conclude that swirling did not enhance accumulation of Cd in this experiment.

4.3.2 Theoretical Calculations

If accumulation of Cd by wheat roots is limited by the rate of diffusion to the root surface, then these two estimates of flux should be similar. If diffusion is not rate limiting, then it would be expected that the flux of Cd from the bulk solution to the root surface would be greater than actual accumulation.

4.3.2.1 Flux of Cd^{2+} into the Root

The flux of Cd^{2+} from the bulk solution into the root can be described by:

$$(1) \qquad J = w \cdot A^{-1} \cdot t^{-1}$$

where **J** is flux ($\mu g \cdot cm^{-2} \cdot s^{-1}$); **w** is the mass of Cd accumulated; **A** is the surface area of the root (cm^2); and **t** is the duration of exposure (s). From the response surfaces presented in Chapter 2 (Figures 2.2 and 2.3) 'Kyle' and 'Arcola' roots exposed to $3.91 \cdot 10^{-8}$ M Cd^{2+} for 100 min accumulated 1.27 and 2.66 $\mu g \cdot g^{-1}$ Cd, respectively, while 'Kyle' and 'Arcola' roots exposed to $3.91 \cdot 10^{-7}$ M Cd^{2+} accumulated 4.94 and 10.17 $\mu g \cdot g^{-1}$ Cd, respectively. The dry weights of 'Kyle' and 'Arcola' roots were 0.0371 and 0.0414 g, respectively, and the surface areas (**A**) of 'Kyle' and 'Arcola' roots were 35.4 and 49.8 cm^2, respectively (Table 5.2). The total mass of Cd accumulated by each cultivar can be determined by multiplying the tissue Cd concentration ($\mu g \cdot g^{-1}$) by the mass of tissue. Therefore, 'Kyle' and 'Arcola' seedlings accumulated 0.0471 and 0.110 µg of Cd (**w**) when exposed to $3.91 \cdot 10^{-8}$ M Cd^{2+} for 100 min (**t**), and 0.183 and 0.421 µg of Cd (**w**) when exposed to $3.91 \cdot 10^{-7}$ M Cd^{2+} for 100 min (**t**). Entering these numbers into the equation above results in fluxes of Cd (**J**) of $2.22 \cdot 10^{-7}$ and $3.68 \cdot 10^{-7}$ $\mu g \cdot cm^{-2} \cdot s^{-1}$ for 'Kyle' and 'Arcola' exposed to $3.91 \cdot 10^{-8}$ M Cd^{2+}, and $8.62 \cdot 10^{-7}$ and $1.41 \cdot 10^{-6}$ $\mu g \cdot cm^{-2} \cdot s^{-1}$ for 'Kyle' and 'Arcola' exposed to $3.91 \cdot 10^{-7}$ M Cd^{2+}.

4.3.2.2 Flux of Cd^{2+} Through a Boundary Layer

The flux of Cd^{2+} from the bulk solution, through the boundary layer and to the root surface can be determined by:

$$(2) \qquad J = (C_1 - C_2) \cdot D \cdot x^{-1}$$

where **J** is flux ($\mu g \cdot cm^{-2} \cdot s^{-1}$); C_1 is the Cd concentration in the bulk solution (at the edge of the boundary layer) ($\mu g \cdot cm^{-3}$); C_2 is the Cd concentration at the root surface ($\mu g \cdot cm^{-3}$); **D**

is the diffusion coefficient ($cm^2 \cdot s^{-1}$); and **x** is the thickness of the boundary layer (cm). In order to do these calculations, several assumptions need to be made. These assumptions, and how the estimate of flux would change as the various parameters change, are discussed below. The Cd^{2+} concentrations in the bulk solution ranged from $3.91 \cdot 10^{-8}$ M to $3.91 \cdot 10^{-7}$ M, or 4.39 to 43.9 $\mu g \cdot L^{-1}$ (ppb) (C_1); the concentration at the root surface is assumed to be 0 $\mu g \cdot L^{-1}$ (C_2). The diffusion coefficient for Cd in water is $7.17 \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$ (**D**) at 25°C (Li and Gregory, 1974), and the thickness of the boundary layer is assumed to be 0.05 cm (**x**). Entering these values into equation (2) results in a flux (**J**) of $6.30 \cdot 10^{-7}$ $\mu g \cdot cm^{-2} \cdot s^{-1}$ when the Cd^{2+} concentration in solution was $3.91 \cdot 10^{-8}$ M and $6.30 \cdot 10^{-6}$ M when the Cd^{2+} concentration in solution was $3.91 \cdot 10^{-7}$ M. As a comparison, when seedlings were exposed to $3.91 \cdot 10^{-8}$ M Cd^{2+}, the flux of Cd to 'Kyle' and 'Arcola' roots (based on accumulation of Cd) was 35 and 58%, respectively, of the estimated flux across the boundary layer, and when seedlings were exposed to $3.91 \cdot 10^{-7}$ M Cd^{2+}, the flux of Cd to 'Kyle' and 'Arcola' roots (based on accumulation of Cd) was 14 and 22%, respectively, of the estimated flux across the boundary layer. The fact that these estimates are within an order of magnitude indicates that it is very possible that the rate limiting step in accumulation of Cd under these conditions is the rate of diffusion of Cd from the bulk solution to the root surface, across the boundary layer.

4.3.2.3 Assumptions

In estimating flux to the roots from Cd accumulation, it is assumed that flux is similar over the entire root surface. This assumption is likely not true; Piñeros *et al.*

(1998) demonstrated that uptake was greater near the root tip. This would result in a greater flux in one location of the root, and less flux elsewhere, resulting in a region of the root where diffusion was more likely to be rate limiting and other areas where diffusion would be less likely to be rate limiting.

In estimating flux through the boundary layer, the first assumption made was that the Cd^{2+} concentration at the root surface was 0 $\mu g \cdot L^{-1}$. It is possible that it was greater than 0 $\mu g \cdot L^{-1}$, since uptake may not have been instantaneous, and this would result in slower diffusion through the boundary layer, since the concentration gradient, which is the driving force of diffusion, would be less. If, instead of 0 $\mu g \cdot L^{-1}$, the concentration at the root surface was half that of the bulk solution, flux across the boundary layer would be half of what was presented above.

The diffusion coefficient for Cd is a value for 25 °C, and a lower temperature would result in a slightly lower flux through the boundary layer. Another assumption was the choice of 0.05 cm for the thickness of the boundary layer. Thicknesses of 2 to $5 \cdot 10^{-3}$ cm have been proposed for the boundary layer surrounding phytoplankton cells, which are assumed to be spheres with radii ranging from 10^{-3} to 10^{-2} cm (Whitfield and Turner, 1979). Plant roots have a much larger radius, and often have root hairs up to 0.15 cm long (Salisbury and Ross, 1992), which would result in significantly thicker boundary layers. If the boundary layer was thicker than 0.05 cm, then flux through the boundary layer would be less, while a thinner boundary layer would result in a greater flux of Cd. A boundary layer of 0.01 cm, for example, would result in a flux five times greater than the value presented.

4.3.3 Future Experiments

To further test this hypothesis, it may be possible to actually measure the Cd^{2+} concentration at the root surface of wheat roots exposed to solutions containing low Cd^{2+} concentrations. If diffusion is rate limiting, then the Cd^{2+} concentration at the root surface would begin to decline, as the root accumulated Cd^{2+}. Piñeros *et al.* (1998) were able to measure flux of Cd^{2+} into wheat roots using a microelectrode and a similar electrode could be applied to this question. The electrode measures Cd^{2+} concentration, specifically. The influence of ligands could be tested as well, by doing similar measurements in solution containing similar Cd^{2+} concentrations with and without ligands. If, after time, the Cd^{2+} concentration at the root surface decreased more rapidly in solutions not containing ligands compared with solutions containing complexed forms of Cd, such as $CdCitrate^{-}$, $CdEDTA^{2-}$, or $CdSO_4^0{}_{(aq)}$, then this would provide evidence in favour of diffusion through a boundary layer as the rate limiting step in movement of Cd from the bulk solution into wheat roots.

CHAPTER 5:

THE RELATIONSHIP BETWEEN ROOT MORPHOLOGY AND CADMIUM ACCUMULATION IN SEEDLINGS OF TWO DURUM WHEAT CULTIVARS

5.1 Introduction

There is considerable inter and intraspecific variation in both the amount of Cd taken up by plants and its distribution among various tissues within the plant. Plants typically have higher concentrations of Cd in roots than in stems and leaves, with even lower concentrations of Cd found in fruits, grains or seeds (Coughtrey and Martin, 1978; Jastrow and Koeppe, 1980; Kubota *et al.*, 1992). There is considerable variation in plant tissue Cd concentrations, both within and among species (Baker and Walker, 1990). Differences in the shoot Cd content among species (or cultivars) may be expected to relate to differences in net uptake of Cd, since accumulation of Cd by root tissue is the source of Cd available for translocation to other tissues. A higher rate of accumulation by roots may be due to physiological factors, such as a higher density or efficiency (K_m) of uptake sites, or differences in rates of transpiration and water use. Lower accumulation by roots has been attributed to secretion of polypeptides or organic acids such as malate or citrate by some plants. This mechanism has been shown to confer tolerance to Al, presumably as a result of reduced bioavailability (and therefore uptake) of Al due to complexation with exudates (Miyasaka *et al.*, 1991; Delhaize *et al.*, 1993; Basu *et al.*, 1994a; b). Secretion of low molecular weight organic acids into nutrient solutions varies significantly among durum wheat cultivars (Cieslinski *et al.*, 1997).

Physical factors may also influence uptake of metals by plants. Root morphology has been shown to influence uptake of mineral elements: increased phosphorus supply has been related to the density and length of root hairs or differences in root length/shoot weight ratios (Itoh and Barber, 1983; Föhse *et al.*, 1988). Bowen and Rovira (1971)

demonstrated that the majority of phosphate and sulphate was accumulated by lateral roots of the seminal root system of 14 day old wheat seedlings, and suggested that varieties which produce more lateral roots may be better at utilizing phosphorus. Horst *et al.* (1993) studied two cultivars of wheat and demonstrated that phosphorus efficiency (the ability to grow and yield better in P-deficient soil) was related to several characteristics, including root diameter and length of root hairs. In a study on root morphology of wheat genotypes differing in zinc efficiency, it was observed that the Zn-efficient genotype tended to have longer and thinner roots than the Zn-inefficient genotype (Dong *et al.*, 1995). Using a cadmium-selective microelectrode to measure Cd^{2+} flux along roots of *Thlaspi caerulescens* (a Zn/Cd hyperaccumulator), *Thlaspi arvense* (a related nonaccumulator) and *Triticum aestivum*, Piñeros *et al.* (1998) demonstrated that the flux of Cd^{2+} to the roots was greatest near the root tip, but occurred along the whole length of the root. This suggests that both the number of root tips in a root system, and the total surface area may influence the amount of Cd accumulated by a plant. Nutrient uptake from soil is also dependent on root architecture, or the spatial configuration of the root system (Lynch, 1995), though uptake from hydroponic solutions may not be influenced by root architecture since the roots are free to move around.

In this experiment, seedlings of two cultivars of durum wheat (*Triticum turgidum*) which are known to accumulate high and lower concentrations of Cd in the grain were used to establish the co-incidence of root tissue accumulation of Cd and various root morphological parameters. Cadmium content data were expressed as the mass of Cd accumulated per experimental unit and on a per dry weight basis, and then converted to

per surface area and root tip bases in order to determine if the method of expressing the Cd content of root tissue could alter the conclusions related to cultivar differences in Cd accumulation. The null hypothesis being tested was that while the cultivars differed in accumulation of Cd in root tissues, their root morphology was not different.

5.2 Materials and Methods

5.2.1 Experimental Design

This study was conducted in two parts: the Cd accumulation by 'Kyle' and 'Arcola' root tissue was determined in three independent replicates of a completely randomized design, which were carried out at different times; and the root morphology of 'Kyle' and 'Arcola' was characterized in five independent replicates of a completely randomized design, which were carried out at different times. The same populations of 'Kyle' and 'Arcola' seed were used in both studies. The same plants could not be used for both determinations, since root morphology determinations would result in Cd efflux from roots and cross contamination of tissue samples, and determinations of tissue Cd concentrations are destructive. The root Cd accumulation and morphology data were analysed separately to establish cultivar specific differences in Cd accumulation over time, and cultivar specific root morphology characteristics.

5.2.2 Plant Material and Growth Conditions

Plant material used and growth conditions were as described in section *2.2.2 Plant Material and Growth Conditions*. Six-day old seedlings (from the time of germination)

were used in all experiments.

5.2.3 Cadmium Exposure and Solution Analysis

Cadmium exposure and analysis of exposure solutions were as described in section *2.2.3 Cadmium Exposure and Solution Analysis*. All of the seedlings in a beaker were harvested after either 0, 50, 100, 150, or 200 mins of exposure to Cd.

5.2.4 Plant Digestion and Cd Analysis

Digestion of tissue samples and analysis of samples for Cd were as described in section *2.2.4 Plant Digestion and Cd Analysis*. The total mass of Cd accumulated by the roots in each mesh equalled the mass of Cd in its respective plant digest; the concentration of Cd present in the plant tissue equalled the mass of Cd present in the plant digest divided by the mass of tissue digested.

5.2.5 Morphological Analysis

For each mesh containing nine seedlings, the following parameters were measured: total root length, root surface area, root volume, number of root tips, root dry weight, shoot area and shoot dry weight. Root length, surface area, volume and number of root tips were determined using the Winrhizo software package (Version 3.9, Régent Instruments Inc., Québec, Canada) with an attached scanner (Hewlett Packard Scanjet 4C/T) fitted with an overhead lamp. Shoots were cut just above the caryopsis and shoot area determined with a leaf area meter (LI-3100 area Meter, LI-COR Inc., Lincoln, NE).

Caryopses with attached roots were floated in deionized water in a plexiglass tray and placed in the scanner, which was set at 300 dpi. Root morphology data were separated into 12 classes based on root diameter ranging from 0-0.1 mm to 1.0-1.1 mm and finally >1.1 mm. In the analysis of data, the lowest class (0-0.1 mm) and the four higher classes (0.8-0.9 mm, 0.9-1.0 mm, 1.0-1.1 mm and >1.1 mm) were not included: the lowest size class was dominated by dust and/or small scratches in the tray, while the higher size classes were the caryopses. Dirt and air bubbles were digitally excluded from the analysis of scanned roots using the software. After morphological analysis, the roots were separated from the seeds, placed in #1 coin envelopes and dried at 80°C in a drying oven for 48 hrs before being weighed. Ratios of shoot area:root area, root volume:root dry weight and the number of root tips: root dry weight were calculated from the raw data.

5.2.6 Data Analysis

The root cadmium accumulation experiment was conducted as a completely randomized factorial design. In each of the three replicates, there were three target total Cd concentrations ($4.45 \cdot 10^{-8}$, $8.90 \cdot 10^{-8}$, and $4.45 \cdot 10^{-7}$ M), two cultivars ('Kyle' and 'Arcola') and five harvest times (0, 50, 100, 150, and 200 min) for a total of 90 experimental units (meshes of nine seedlings). Cadmium content of roots was expressed as both the concentration of Cd ($\mu g\ Cd \cdot g^{-1}$ root dry weight) and the total mass of Cd accumulated by each experimental unit (μg Cd; not normalized for tissue dry weight). In each case, the tissue Cd accumulation data were analysed relative to the actual exposure Cd^{2+} concentrations (determined by measuring the total Cd concentration in each

treatment solution and using MINEQL$^+$ to estimate the proportion available as the free ion) in the treatment solutions, rather than the target concentrations. The Cd^{2+} concentrations were 87.8% of the total, measured Cd concentration in all cases. The Cd^{2+} concentrations in the exposure solutions were transformed for data analysis using a natural log (ln) transformation because the exposure solution Cd^{2+} concentrations were not evenly spaced. The data were analysed using SAS PROC GLM (SAS Institute Inc., Cary, NC). The initial model tested the contribution to variation in root tissue Cd concentrations of the main effects of replicate, cultivar, exposure duration (time) and the natural log of exposure Cd^{2+} concentration ($lnCd^{2+}$) and all two and three way interactions involving cultivar, time and $lnCd^{2+}$. Non-significant interactions were dropped from the model, one at a time (in an iterative reduction, starting with the highest order interactions), and their sums of squares were pooled with the error term. The final model for the analysis of Cd concentration in root tissue included replicate, cultivar, time, $lnCd^{2+}$, cultivar*time, time*$lnCd^{2+}$ and cultivar*time*$lnCd^{2+}$, and the final model for the analysis of the mass of Cd in root tissue was similar, except the cultivar*time term in the model was pooled with the error term (Table 5.1). Because the significant interactions ruled out cultivar comparison using main effects, cultivars were declared different in Cd accumulation if the interaction among cultivar, time and $lnCd^{2+}$ was significant ($p \leq 0.05$), indicating that a cultivar difference existed which was dependent on both the duration of exposure and exposure solution Cd^{2+} concentration.

For the morphology experiment, the data were analysed using SAS PROC GLM. The model tested the significance of cultivar ('Kyle' and 'Arcola'), replicate (1 through 5)

and the interaction between cultivar and replicate for each parameter. There were 56 experimental units (meshes with nine seedlings); four of each cultivar in rep 1 and six of each cultivar in reps 2 through 5. The interaction between replicate and cultivar was significant for most parameters, and this was due to among-replicate variation in the magnitude (but not the direction) of the differences between the two cultivars; differences presented (Table 5.2) are averaged over replicates. Ratios of root surface area:root dry weight ($cm^2 \cdot g^{-1}$) and the number of root tips:root dry weight ($tips \cdot g^{-1}$) were determined for each cultivar. The numerator and denominator of these ratios were analysed for their degree of correlation using SAS PROC CORR to determine the Pearson's correlation coefficient, which was found to be high for each ratio. These ratios were then used to convert the root Cd content per unit of root dry weight ($\mu g\ Cd \cdot g^{-1}$) from the Cd accumulation study, to estimates of root Cd content per unit of root surface area ($\mu g\ Cd \cdot cm^{-2}$) and 1000 root tips ($\mu g \cdot 1000\ tips^{-1}$) by converting the data to per root tip ($\mu g \cdot tip^{-1}$) and then multiplying by 1000. The two derived response parameters were then analysed using SAS PROC GLM as previously described for Cd per unit of root dry weight, and the final model in each case was the same as that for dry weight expressions of Cd content, except that the cultivar*time interaction was non-significant and therefore pooled with the error term (Table 5.3).

5.3 Results and Discussion

In this study, seedlings were exposed to cadmium in their rooting medium in order to determine if there was a cultivar difference in root Cd accumulation. This was followed

by an in-depth analysis of seedling root morphology of the same two cultivars in order to determine if any morphological characteristics predominated in one cultivar relative to the other. The results of the experiment characterising the relationships between root Cd concentration and cultivar over a range of solution Cd^{2+} concentrations and exposure durations are presented in Figures 5.1A-E. The analysis of variance for these data demonstrated that there was an interaction among duration of exposure (time), exposure solution Cd^{2+} concentration ($lnCd^{2+}$) and cultivar ($p<0.0001$) (Table 5.1). This indicates that the Cd concentrations in the roots of the two cultivars were different, but that the magnitude of this difference was dependent on both the concentration of Cd^{2+} in the exposure solution and the duration of exposure. Additionally, the interaction also indicates that the main effects of cultivar, time and $lnCd^{2+}$ are not reliable estimates of statistical significance. The bases of this three way interaction were: 'Arcola' had a greater concentration of Cd in its roots than 'Kyle'; and the difference between the cultivars was greater when exposed to higher concentrations and after longer durations of exposure. The difference in Cd concentration between the cultivars ranged from 0% (with no exposure to Cd^{2+}) to about 30% (after 150 to 200 minutes of exposure to the highest concentration of Cd^{2+}). There were differences in Cd concentration of root tissue from replicate to replicate ($p=0.0034$) (Table 5.1) which could be due to differences in environmental conditions such as humidity or light levels, among replicates, either during exposure or in the days leading up to exposure. The relationships between cultivars, and among concentrations of Cd^{2+} and durations of exposure were the same, however, and results presented are averaged over the replicates. Statistical analysis of root Cd content

Figure 5.1 A to E: Cadmium content of roots of 'Kyle' and 'Arcola' exposed to $3.91 \cdot 10^{-8}$ to $3.91 \cdot 10^{-7}$ M Cd^{2+} ($4.45 \cdot 10^{-8}$ to $4.45 \cdot 10^{-7}$ M total cadmium) for 0 to 200 minutes. The solution Cd^{2+} concentrations are on a natural log (ln) scale.

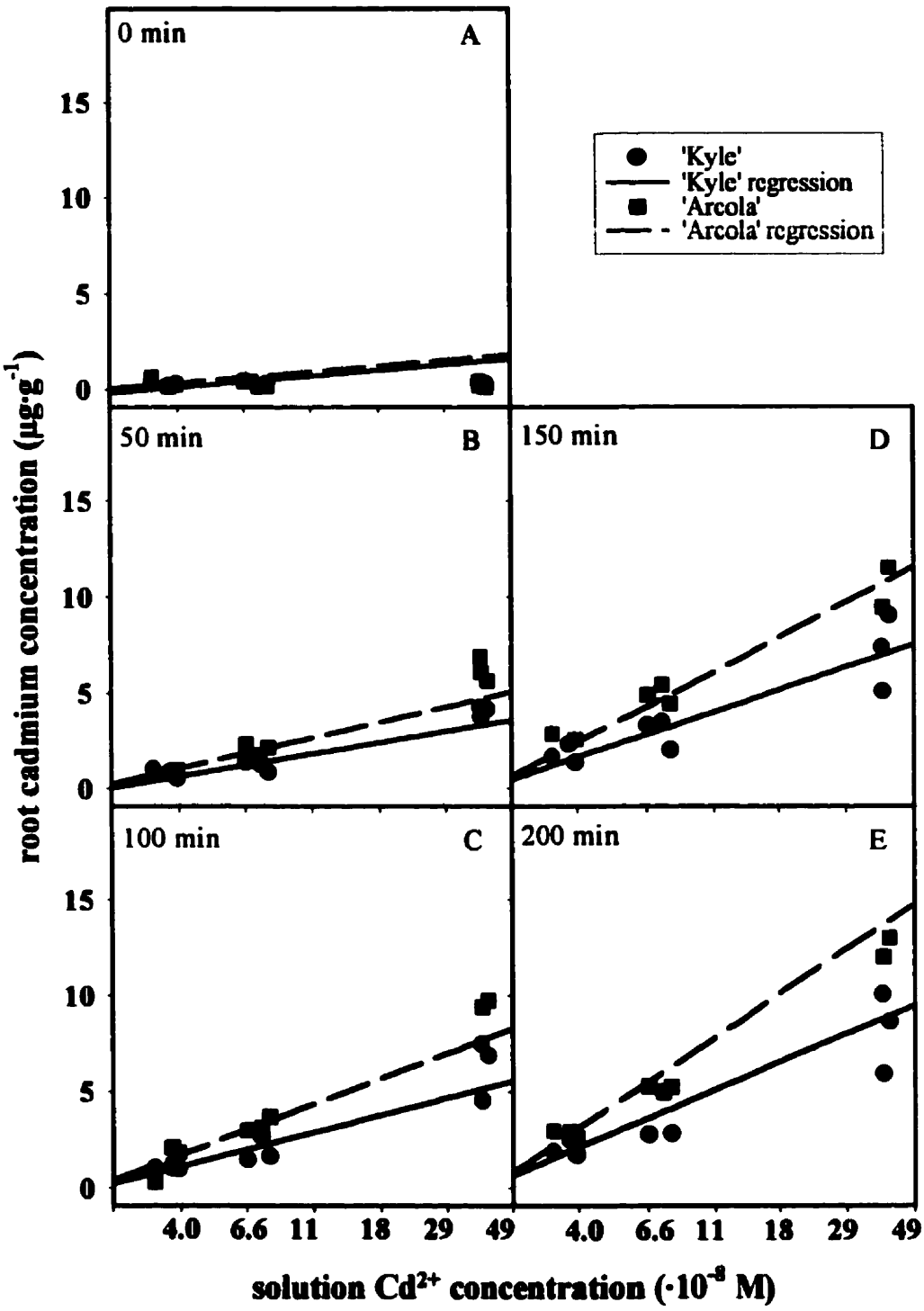

0 min
A
50 min
B
150 min
D
100 min
C
200 min
E
'Kyle'
'Kyle' regression
'Arcola'
'Arcola' regression
root cadmium concentration (µg·g-1)
solution Cd2+ concentration (·10-8 M)
0
5
10
15
4.0
6.6
11
18
29
49

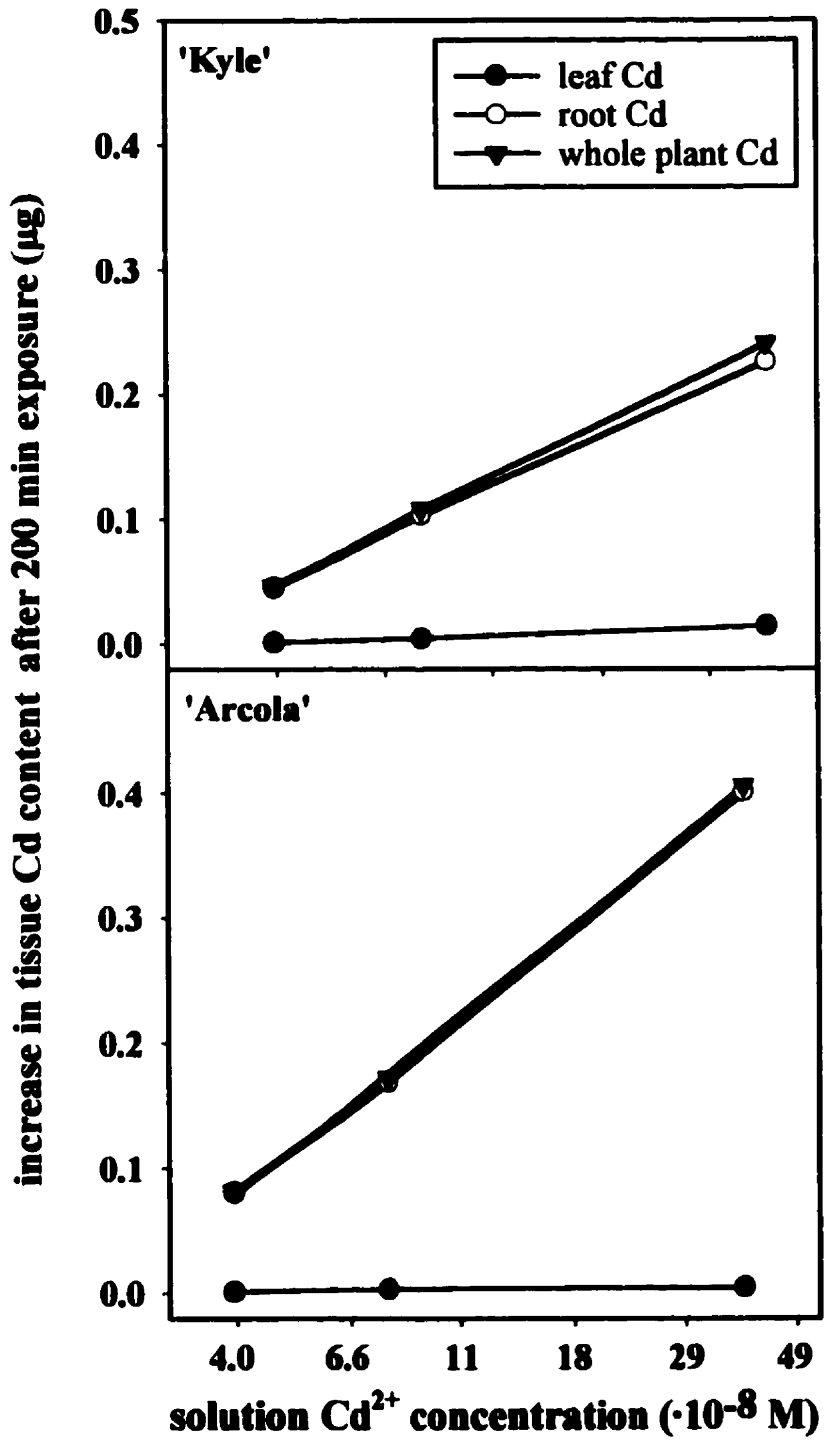
Table 5.1: Sources of variation in content of Cd in roots of 'Kyle' and 'Arcola' expressed on concentration (μg $Cd \cdot g^{-1}$ dw) or mass of Cd per experimental unit bases (μg Cd).

	Cd concentration in tissue (μg $Cd \cdot g^{-1}$ dw)			Amount of Cd (μg Cd)		
Source	df	F value	p value	df	F value	p value
Model	8	114.7	<0.0001	7	74.03	<0.0001
replicate	-2	6.13	0.0034	-2	4.35	0.016
cultivar	-1	0.45	0.5	-1	0.86	0.36
time	-1	10.49	0.0018	-1	0.16	0.69
$lnCd^{2+}$	-1	12.34	0.0007	-1	8.19	0.0033
cultivar*time	-1	3.43	0.0677			
time*$lnCd^{2+}$	-1	125.99	<0.0001	-1	43.06	<0.0001
cultivar*time*$lnCd^{2+}$	-1	24.99	<0.0001	-1	7.3	0.0085
Error	77			77		
Corrected Total	85			84		

expressed as the mass of Cd in the root tissue (not normalized for the mass of tissue) was similar to that for the Cd concentration in roots (Table 5.1). Once again, a significant interaction among cultivar, $lnCd^{2+}$ and time ($p=0.0085$) (Table 5.1), is the basis for concluding that there was a significant cultivar difference in accumulation of Cd. The difference in the mass of Cd in the roots of the two cultivars ranged from 0% (with no exposure to Cd^{2+}), to about 35% (after 150 to 200 minutes of exposure to the highest concentration of Cd^{2+}) (data not shown). Whether the Cd content of roots was expressed as the mass of Cd accumulated by the root system, or as the mass of Cd accumulated normalized for tissue dry weight, roots of 'Kyle' seedlings contained less Cd than roots of 'Arcola' seedlings. This is an example of variation in accumulation of Cd by cultivars of the same species. Differences in accumulation of Cd in the root tissue of these seedlings did not reflect known patterns of accumulation by the grain of these cultivars, indicating that the different patterns of Cd accumulation by the grain of these cultivars is not as a result of differences at the root:soil solution interface, but may possibly be due to differences in root:shoot mobility of Cd (Chan, 1996).

Variation in the accumulation of an element in root tissue is the net result of variation in uptake, efflux and translocation to shoots. Accumulation data represents both apoplastic and symplastic Cd, although it is possible that most Cd was located in the symplast (Buckley *et al.*, 1997; Hart *et al.*, 1998a). Results from a preliminary study indicate that under the conditions, and the durations of time the seedlings were exposed to Cd, very little Cd was translocated to shoots; almost all of the Cd accumulated by the plants during this study remained in the roots (Figure 5.2). Piñeros *et al.* (1998)

Figure 5.2: Increase in mass of Cd in root, shoot and whole plant exposed to a range of Cd^{2+} concentrations for 200 minutes. The solution Cd^{2+} concentrations are on a natural log (ln) scale.


'Kyle'
leaf Cd
root Cd
whole plant Cd
'Arcola'
0.5
0.4
0.3
0.2
0.1
0.0
4.0
6.6
11
18
29
49
increase in tissue Cd content after 200 min exposure (µg)
solution Cd2+ concentration (·10-8 M)

demonstrated with a microelectrode that Cd^{2+} efflux from the roots of wheat seedlings was minor. Therefore, root morphology, from the perspective of element uptake, was investigated as a basis for differential Cd content (not normalized for root dry weight) of 'Kyle' and 'Arcola' roots. Ion uptake by roots is a function of the maximum rate of uptake (V_{max}) and affinity of the metal for the uptake site (K_m) as well as opportunity for exchange with soil solution (surface area, root tips) (Marschner, 1995). 'Arcola' root systems generally had more mass than 'Kyle'. 'Kyle' roots had 10.4% less dry weight; 29.7% less total root length; 27.6 % less root surface area; 28.3 % less root volume; and 21.2 % fewer root tips than 'Arcola' (all with $p<0.001$, Figure 5.3, Table 5.2). The differences in root surface area and volume between the two cultivars can be explained simply by the extra root length of 'Arcola'; 'Arcola' roots likely had more branching as indicated by the greater number of root tips. The proportions of roots in each root diameter class were similar for the two cultivars. These results suggest that greater root-Cd content of 'Arcola' than 'Kyle' could be explained by differences in root morphology. This is consistent with the study by Piñeros *et al.* (1998), which determined that the region of a root within 1500 μm of the tip was the most active in Cd^{2+} uptake. So, it is reasonable that 'Arcola' roots would accumulate more Cd, per unit time, than 'Kyle'.

It is more usual in element uptake studies with plants to express root accumulation of metal as a concentration, on the basis of root dry weight. Because of cultivar-specific variation in tissue arrangement or density, the interpretation of cultivar differences in root accumulation, based on concentration, may be influenced by disproportionate cultivar

Figure 5.3 A and B: Digital scans of 'Kyle' (A) and 'Arcola' (B) roots generated with the Winrhizo scanner. Images were used to determine total root length, root surface area, root volume, and the number of root tips.

'Kyle' roots

A

'Arcola' roots

B

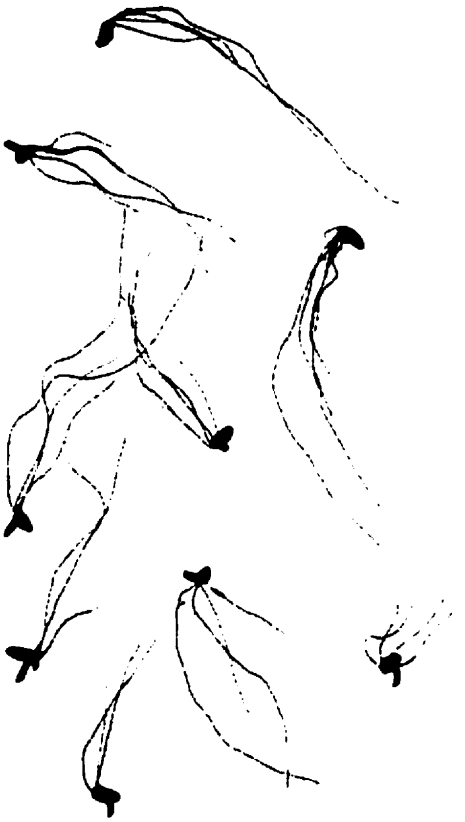


Table 5.2: Morphological characteristics of six-day old 'Kyle' and 'Arcola' seedlings grown in the absence of Cd (percentage differences are: (('Arcola'-'Kyle')/'Arcola')*100%).

Parameter	'Kyle'	'Arcola'	% difference	p value
root dry weight (g)	0.0371	0.0414	10.4	0.0014
total root length (cm)	189.1	268.9	29.7	<0.0001
root surface area (cm^2)	35.4	48.9	27.6	<0.0001
root volume (cm^3)	0.381	0.531	28.3	<0.0001
number of root tips	83	105.3	21.2	0.0002
shoot dry weight (g)	0.115	0.123	6.06	0.03
shoot area (cm^2)	18.6	24.3	23.4	<0.0001
shoot area:root surface area ($cm^2 \cdot cm^{-2}$)	0.516	0.480	7.54	0.041
root surface area:root dry weight ($cm^2 \cdot g^{-1}$)	956.5	1191	19.7	<0.0001
number of root tips:root dry weight ($tips \cdot g^{-1}$)	2305	2559	9.93	0.0002

differences in various morphological parameters. 'Kyle' had 19.7 % less total surface area per unit of tissue dry weight than 'Arcola' (Table 5.2), indicating that, if accumulation was dependant on root surface area, 'Arcola' could have a higher Cd concentration than 'Kyle' due to the fact that for a given mass of root tissue, 'Arcola' roots had more surface area than 'Kyle' roots. Likewise, 'Kyle roots had 9.93% fewer root tips per dry weight of tissue than 'Arcola' roots, so if Cd accumulation is dependant on the number of root tips, 'Arcola' roots would have a higher concentration of Cd than 'Kyle' for similar reasons (Table 5.2). These results are consistent with the relationship between P uptake and root morphology. Rubio *et al.* (1997) examined the root characteristics in relationship to P uptake in *Paspalum dilatum*. Compared to control plants, waterlogged plants had similar root biomass, but greater specific root length ($cm \cdot g^{-1}$ dw) and P uptake per unit mass of root. These data show that the waterlogged plants had finer roots, and this change in morphology was associated with enhanced P uptake. Mature Norway spruce (*Picea abies* (L.) Karst) were treated for five years, *in situ*, with various nutrient regimes, following which the uptake of ^{32}P and ^{35}S was evaluated (Clemensson-Lindell and Asp, 1995). Compared to control plants, the fine roots (<1 mm) in two of the nutrient regimes resulted in plants with lower specific root length and higher P uptake (ammonium sulphate regime) or greater specific root length ($cm \cdot g^{-1}$) and lower P uptake (complete nutrient solution regime). A study of Zn-efficient genotypes of wheat (*Triticum aestivum* L. cvs Excalibur and Gatcher) demonstrated that the Zn-efficient cv. (Excalibur) had: a greater proportion of fine roots with a diameter less than 0.2 mm than the two less Zn-efficient cultivars, early in the growth period; and, longer and thinner roots (Dong *et al.*, 1995). Zn and Cd

compete with each other for uptake by plant roots, suggesting similar pathways; so this study also corroborates our suggestion that 'Arcola' roots accumulate more Cd because of greater specific root area ($cm^2 \cdot g^{-1}$).

Shoot characteristics may also be important sources of variation in the amount of Cd accumulated by root tissue, since water and ions move from soil into roots, and from roots to shoots because of transpirational pull and root pressure; the latter is thought by some to be the dominant force driving water and ion movement within young seedlings (Marschner, 1995). If rates of transpiration controlled accumulation of Cd in roots of these seedlings, then differences in shoot area or the ratio of shoot area:root area would be expected to be consistent with differences in root Cd accumulation, assuming that transpiration rates per unit area were similar between the two cultivars. 'Kyle' had 23.4% less shoot area than 'Arcola' ($p<0.0001$). 'Kyle' had a ratio of shoot area:root surface area which was 7.54% greater than that of 'Arcola' ($p=0.0041$)., which means that 'Kyle' shoots were 'supported' by less root surface area than 'Arcola' shoots. This suggests that transpirational pull would play no role in differential Cd accumulation, unless the transpiration rates of the shoots were different on a per unit area basis. Additionally, Marschner (1995) suggests that in young plants, such as these, root pressure may account for all of the movement of water from root to shoot.

Since surface area or the number of root tips may be more closely related to mechanisms of Cd accumulation by roots than dry weight, cultivar differences in Cd content were additionally normalized using the ratios of root area:root dry weight and number of root tips:root dry weight to convert root Cd content data normalized for dry

weight ($\mu g\ Cd \cdot g^{-1}$). This allowed the comparison of the root Cd content data relative to surface area ($\mu g\ Cd \cdot cm^{-2}$) or the number of root tips ($\mu g\ Cd \cdot 1000$ root $tips^{-1}$) typically achieved by the two cultivars. The Pearson's correlation coefficients for these ratios were 0.70 and 0.89 for root surface area:root dry weight for 'Kyle', and 'Arcola', respectively, and 0.54 and 0.62 for numbers of root tips:root dry weight for 'Kyle' and 'Arcola', respectively. These derived data for root Cd accumulation were analysed as were the original data, to determine the relationship among Cd accumulation by roots, cultivar, and exposure solution Cd^{2+} concentration and duration of exposure. In both of these analyses, there was a significant three way interaction among cultivar, time and $lnCd^{2+}$. Significant sources of variation were the same as those determined for root tissue Cd concentration per unit of dry weight except there was no interaction between cultivar and time (Table 5.3). There was a similarity in the significant sources of variation for the different expressions of root Cd content, however, the magnitudes of the differences between cultivars varied. When Cd content was normalized for dry weight, the Cd content of roots of 'Kyle' ranged from 0 to 30% less than 'Arcola'. When Cd content was normalized for surface area, the Cd content of roots of 'Kyle' ranged from about 0 to 15% less Cd than 'Arcola', and when the amount of Cd was normalized for 1000 root tips, the Cd content of roots of 'Kyle' ranged from 0 to 25% less than 'Arcola'. The roots of 'Kyle' contained less Cd than roots of 'Arcola', regardless of the morphological basis upon which Cd content was expressed, suggesting that there was a physiological basis (i.e. K_m, V_{max}) for the cultivar difference, in addition to morphological differences that influenced Cd accumulation.

Table 5.3: Sources of variation in content of Cd in roots of 'Kyle' and 'Arcola' expressed on surface area and 1000 root tips bases.

	surface area			1000 root tips		
Source	df	F value	p value	df	F value	p value
Model	7	119.98	<0.0001	7	124.81	<0.0001
replicate	-2	6.76	0.002	-2	6.08	0.0035
cultivar	-1	0.08	0.7736	-1	0.05	0.8323
time	-1	9.34	0.0028	-1	9.68	0.0026
$lnCd^{2+}$	-1	11.81	0.0009	-1	12	0.001
time*$lnCd^{2+}$	-1	117.34	<0.0001	-1	120.11	<0.0001
cultivar*time*$lnCd^{2+}$	-1	7.94	0.0061	-1	26.16	<0.0001
Error	78			78		
Corrected Total	85			85		

The null hypothesis, that while the cultivars differ in accumulation of Cd in root tissues, their root morphology is not different, can be rejected. Clearly, roots of 'Kyle' seedlings contained less Cd than roots of 'Arcola', and this was true whether the Cd content was expressed as the mass of Cd per experimental unit (μg Cd), on a per dry weight (μg Cd $\cdot g^{-1}$), per surface area (μg$\cdot cm^{-2}$), or per 1000 root tips (μg Cd$\cdot$1000 root $tips^{-1}$) basis. Patterns of root Cd accumulation observed in these young plants were not consistent with previously identified patterns of grain accumulation of these cultivars (Chan, 1996). Lower accumulation of Cd in root tissues co-occurred with fewer root tips, and smaller surface area; lower concentrations of Cd in root tissue co-occurred with smaller ratios of surface area:dry weight but not number of root tips:dry weight. While root accumulation of Cd is not necessarily a good predictor of Cd that is available for translocation to shoots, it does represent the total amount of metal that is potentially available for translocation. Reducing/enhancing the uptake of metals by plants are strategies for breeding food crops, or phytoremediation cultivars, respectively; this study identifies some root characteristics that might be capable of manipulation, or might explain varied Cd uptake of the same cultivar under different soil or environmental conditions.

CHAPTER 6:

ACCUMULATION OF CADMIUM BY ROOTS AND SUBSEQUENT TRANSLOCATION TO SHOOTS OF TWO DURUM WHEAT CULTIVARS

6.1 Introduction

The previous chapters have examined how Cd speciation in the exposure solution influenced accumulation of Cd by the roots of six-day old wheat seedlings and how the difference in the root Cd content of 'Kyle' and 'Arcola' seedlings was consistent with cultivar differences in root morphological characteristics such as surface area and the numbers of root tips. These two cultivars, 'Kyle' and 'Arcola', were studied because when grown under similar conditions, the grain of 'Kyle' contains more Cd than that of 'Arcola'. This difference is not consistent with differences in accumulation of Cd by the roots of these cultivars. Data from Chapter 5 demonstrated that roots of six-day old 'Arcola' seedlings contained more Cd than 'Kyle' seedlings, and a previous study in our laboratory demonstrated no difference between these cultivars in the concentration of Cd of mature root tissue, and suggested that the difference in grain accumulation of these cultivars was due to an internal mechanism which reduced translocation of Cd to the aerial portions of the plant (Chan, 1996).

Cd gets into the symplast by crossing membranes of individual root cells. Once inside the symplast, Cd can bind with functional groups (-SH or -COOH) on proteins or carbohydrates. Cd^{2+} has the ability to induce the synthesis of phytochelatin synthase, an enzyme responsible for producing phytochelatin, a polypeptide which can complex Cd^{2+} and therefore prevent it from interacting with other cell macromolecules (Grill *et al.*, 1989; Rauser, 1995). There are several types of phytochelatins, and these compounds are analogous to metallothioneins found in animals.

Complexed Cd exists as one of two classes of Cd-binding complexes, called low

molecular weight (LMW) or high molecular weight (HMW) complexes based roughly on their migration in gel filtration chromatography (Rauser, 1995). LMW complexes appear to be made up of the γ-Glu-Cys peptide plus chelated Cd, while HMW complexes appear to be groups of γ-Glu-Cys peptides, chelated Cd and S^{2-} (Rauser, 1995). There is evidence to suggest that Cd^{2+} is pumped into the vacuole by a Cd^{2+}/H^{+} antiport (Salt and Wagner, 1993), and phytochelatins (with or without chelated Cd) are pumped into the vacuole by a MgATP driven pump (Salt and Rauser, 1995). Together, these observations provide evidence to suggest that Cd is sequestered in the vacuole. In a study on Cd exposed tobacco plants, virtually all of the Cd and Cd-binding peptides in leaves were found in the vacuoles of leaf cells (Vögeli-Lange and Wagner, 1990).

The degree to which different species, or cultivars within a species, can form complexes with Cd^{2+} and transport these complexes into the vacuole of root cells may be responsible for how mobile Cd is within the plant once it is accumulated. For example, if one species or cultivar has a higher rate of phytochelatin synthesis or a greater ability to transport Cd into the vacuole of root cells, then relatively less Cd will be available for transport to shoots or be available to cause toxic effects in plant tissue. In populations of Cd-tolerant and Cd-sensitive *Silene vulgaris*, for example, differential sensitivity to Cd did not appear to result from different phytochelatin levels (de Knecht *et al.*, 1992; 1994). Tolerant plants had a lower rate of phytochelatin synthesis, and the authors suggest that a possible reason for differential sensitivity may instead be related to the rate or efficiency of sequestering Cd in the vacuole.

Cd may also form complexes with organic compounds which instead of being

sequestered in the vacuole, may then be transported from the root to the shoot in the vascular tissue. Xylem vessels are non-living at maturity, and cations such as Cd^{2+} are pumped out of the symplast by a proton driven antiport (Marschner, 1995). Cations in the xylem sap interact with negatively charged sites in the cell walls of xylem vessels, resulting in a translocation rate slower than that of water or neutral or negatively charged ions. Xylem sap contains organic compounds in addition to nutrient ions, and White *et al.* (1981) suggest that polyvalent cations exist mainly as complexes. Intact plants preincubated for 24 hours in a solution containing citrate, and then exposed to a solution containing Cd, had a twofold increase in total Cd accumulation compared with plants exposed to Cd but not preincubated with citrate (Senden *et al.*, 1995). All of this extra Cd was transported to shoots; root accumulation was not significantly increased, while root to shoot transport of Cd was increased five to sixfold. Citrate was detected in xylem exudates and speciation calculations suggested that Cd in the xylem may have existed as a $CdCitrate^-$ complex. In the same study, exposure to Cd concomitant with citrate resulted in no increase in Cd accumulation. Perhaps differences observed between species or cultivars in root to shoot transport of Cd exist because there are cultivar differences in the level of production of mobile complexes of Cd.

It has long been recognised that plant roots secrete a wide variety of organic compounds, including organic acids (Vancura, 1964; Uren and Reisenauer, 1988). Exudation of both citric and malic acid from the roots of various species has been observed in response to Al stress , and for both snapbean (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.), tolerant cultivars tended to secrete more exudates than

sensitive cultivars (Delhaize *et al.*, 1993; Miyasaka *et al.*, 1991; Basu *et al.*, 1994b). Recently, exudates from cultivars of durum wheat (*Triticum turgidum*) were identified in sterile nutrient solutions (Cieslinski *et al.*, 1997), though the influence of these exudates on speciation of Cd in solution, or on bioavailability of Cd is not known. Exudation of organic acids varied among cultivars, but exudation of the sum of all organic acids measured was higher in 'Kyle', a higher grain-Cd accumulator than in 'DT627' and 'DT637', which are lower grain-Cd accumulators. It is possible Cd speciation in exposure solutions may be influenced differentially by cultivars of durum wheat.

The goals of this experiment were threefold. The first was to characterise the accumulation of Cd by root and shoot tissue of three week old 'Kyle' and 'Arcola' seedlings exposed to nominal Cd concentrations of $4.45 \cdot 10^{-8}$ M or $4.45 \cdot 10^{-7}$ M for three days in order to determine how the two cultivars, which are known to differ in their grain accumulation of Cd, differed in root and/or shoot accumulation of Cd. The second was to determine how adding a small amount of citrate ($1.00 \cdot 10^{-4}$ M) to the exposure solution might influence translocation of Cd from roots to shoots of 'Kyle' and 'Arcola' seedlings exposed to $4.45 \cdot 10^{-7}$ M Cd. The third was to characterise changes to Cd speciation in the exposure solution (percent of dissolved Cd present as Cd^{2+}) resulting from prolonged contact between the exposure solution and root tissue. The three null hypotheses were: 1) there is no difference in root or shoot tissue Cd concentrations of 'Kyle' or 'Arcola' seedlings exposed to solutions containing similar Cd concentrations; 2) adding citrate to the exposure solution does not influence root to shoot translocation of Cd accumulation by shoot tissue of 'Kyle' and 'Arcola' seedlings; and 3) the proportion of dissolved Cd

present as Cd^{2+} (initially 87.8%) is not changed by prolonged contact with roots of 'Kyle' or 'Arcola' seedlings.

6.2 Materials and Methods

6.2.1 Experimental Design

To determine the nominal Cd concentration and Cd^{2+} concentration in the exposure solutions, the experimental design was a complete three-way factorial experiment, with two target nominal Cd concentrations in the exposure solution ($4.45 \cdot 10^{-8}$ and $4.45 \cdot 10^{-7}$ M), two cultivars ('Kyle' and 'Arcola'), and ten durations of exposure (0, 8, 16, 24, 32, 40, 48, 56, 64, and 72 hours). Blanks with no plants and containing Cd at each exposure solution concentration were set up and sampled every other harvest time (8, 24, 40, 56, and 72 hours). Also, 'Kyle' and 'Arcola' were exposed to an exposure solution containing $4.45 \cdot 10^{-7}$ M Cd and $1.00 \cdot 10^{-4}$ citrate and sampled at 8, 24, 40, 56, and 72 hours to see how citrate might influence shoot accumulation of Cd. Each experimental unit consisted of a pot filled with one of the exposure solutions and with either no seedlings (Blanks), or two seedlings of 'Kyle' or 'Arcola'. A 250 mL sample of the exposure solution from each experimental unit was collected during each harvest and analysed for Cd^{2+} (estimated by an Ion Exchange Technique) and total Cd by GF-AAS.

To determine plant tissue Cd accumulation, the experimental design was a four-way factorial experiment, with two target nominal Cd concentrations in the exposure solutions ($4.45 \cdot 10^{-8}$ and $4.45 \cdot 10^{-7}$ M), two cultivars ('Kyle' and 'Arcola'), two tissues (root and shoot) and ten durations of exposure (0, 8, 16, 24, 32, 40, 48, 56, 64, and 72

hours). Additionally, a pot each of 'Kyle' and Arcola' seedlings exposed to $4.45 \cdot 10^{-7}$ M Cd and $1.00 \cdot 10^{-4}$ citrate were harvested at 8, 24, 40, 56, and 72 hours to see how citrate might influence translocation of Cd to shoots.

Root and shoot tissues were collected from each experimental unit; there were 100 tissue samples in total. Each experimental unit consisted of a Styrofoam tray containing two seedlings (one per rockwool cube (2.5 x 2.5 x 3.8 cm, Grodan, Denmark)) which were 21 days old at the beginning of the exposure period.

6.2.2 Plant Material and Growth Conditions

Caryopses of durum wheat (*Triticum turgidum*) cvs 'Kyle' and 'Arcola' were germinated in Petri dishes on filter paper (Watmann #1) wetted with distilled water (Step 1, Figure 6.1). The following day, germinated seeds were transferred to rockwool cubes wetted with deionized water in the greenhouse (Step 2, Figure 6.1). Three days after seeding, rockwool cubes with growing seedlings were placed in holes drilled in a 2.5 cm thick Styrofoam tray which was cut to fit in the top of an opaque 2.5 L pot (Classic 300, Nursery supplies Inc., Fairless Hills, PA) filled with a modified ¾-strength Hoagland's nutrient solution (Fe^{3+} was supplied as $2.68 \cdot 10^{-5}$ M Fe-HEDTA and the $MnCl_2$ concentration was reduced by half) (Hoagland and Arnon, 1950) (Step 3, Figure 6.1). The pH of the solution was maintained at 5.8 to 6.2 by an Argus control system which monitored the pH and automatically added dilute HNO_3 or KOH as required to maintain the pH within the desired range.

The pot was part of a recirculating hydroponic system in a greenhouse and was

Figure 6.1: Experimental procedure for growing and exposing seedlings to Cd.

Step 1: Caryopses were germinated in Petri dishes on Whatmann #1 filter paper moistened with distilled water.

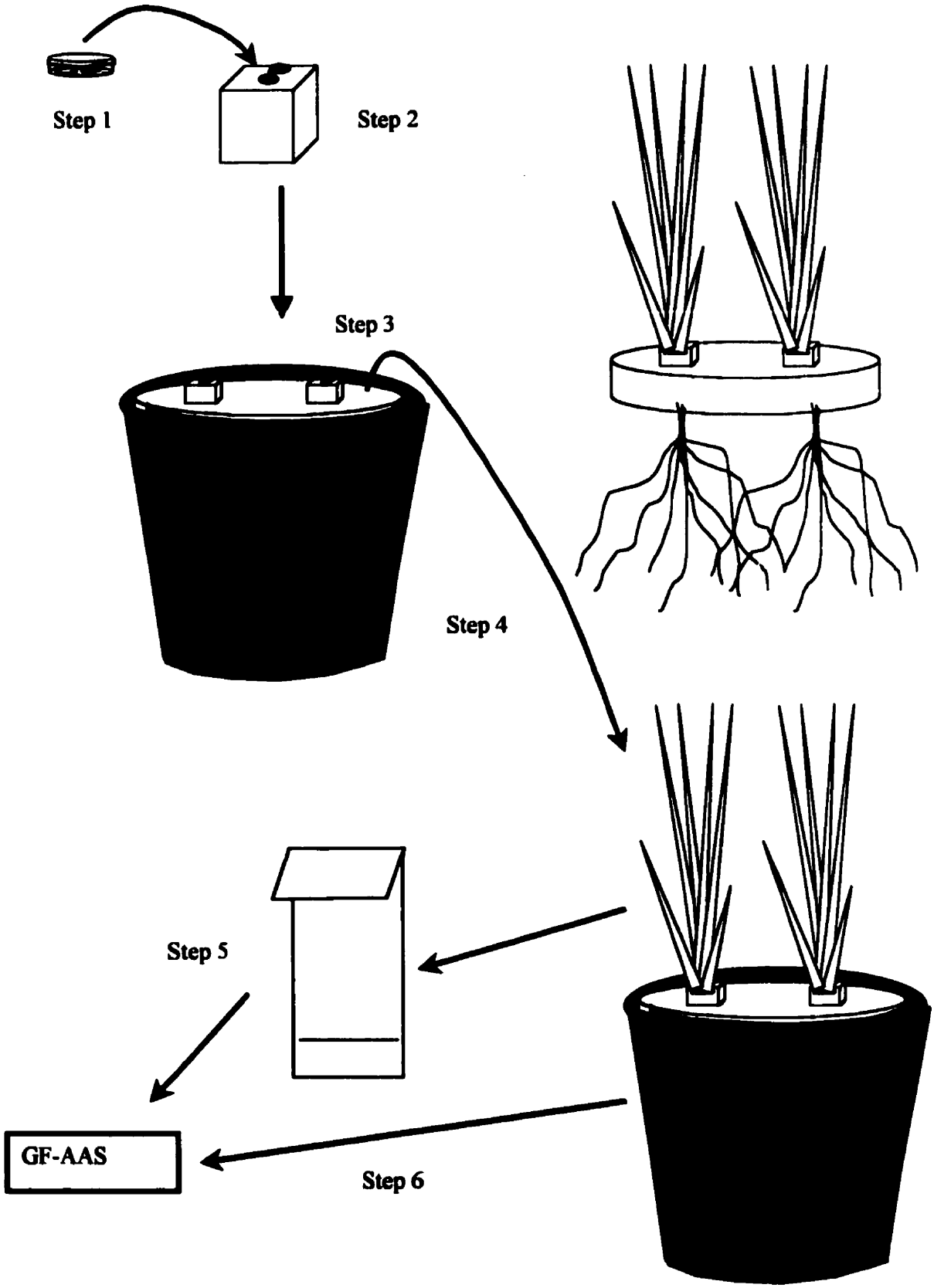
Step 2: After two days, germinated caryopses were transferred to rockwool cubes in a greenhouse.

Step 3: The following day, rockwool cubes were transferred to Styrofoam trays floating on pots part of a recirculating hydroponic system.

Step 4: Three-week old seedlings were transferred to pots containing exposure solution.

Step 5: Roots and shoots were harvested, rinsed, dried, acid digested with HNO_3, and analysed for Cd by GF-AAS.

Step 6: Exposure solutions were sampled, and Cd^{2+} and total Cd determined by and IET and GF-AAS.


Step 1
Step 2
Step 3
Step 4
Step 5
Step 6
GF-AAS

attached to one of four reservoirs, each containing 80 L of nutrient solution. Each reservoir was attached to 14 pots (two rows of seven pots each, for a total of 56 pots in 8 rows) each containing two seedlings per pot. Nutrient solution was added to each pot through a line which emitted about 4 $L \cdot hr^{-1}$ of nutrient solution, which drained through an overflow tube back into the main reservoir. Beginning a week after the seedlings were established in the hydroponic system, 20 L of the nutrient solution in each reservoir were replaced every other day in order to maintain relatively constant nutrient concentrations. Water lost through transpiration was replaced daily. On the 11^{th} and 16^{th} days after seeding, a commercial FeEDTA (Plant Products Co. Ltd., Brampton, ON) solution was applied to the foliage to prevent Fe deficiency symptoms. For each foliar spray, 0.2 g of FeEDTA and two drops of Tween 80 were added to 650 mL of distilled water and all of the solution was evenly sprayed onto the plant canopy.

6.2.3 Cadmium Exposure and Solution Analysis

6.2.3.1 Exposure Set-up and Exposure Solution Sampling

At the beginning of the exposure period, the seedlings (25 'Kyle' and 25 'Arcola') were transferred to opaque 2.5 L pots (Classic 300, Nursery supplies Inc., Fairless Hills, PA) containing the exposure solution (Step 4, Figure 6.1). Additionally, there were five pots with no seedlings containing $4.45 \cdot 10^{-8}$ M Cd and five pots with no seedlings containing $4.45 \cdot 10^{-7}$ M Cd. These pots were not part of the recirculating hydroponic system, but were sitting on a bench in the greenhouse. The exposure solutions used to expose 20 pots each of 'Kyle' and 'Arcola' contained $3.00 \cdot 10^{-3}$ M $CaNO_3$, $1.50 \cdot 10^{-3}$ M

$MgSO_4$, $4.00 \cdot 10^{-3}$ M KNO_3 and $4.45 \cdot 10^{-8}$ or $4.45 \cdot 10^{-7}$ M $Cd(NO_3)_2$ at a pH of 6.0. Five pots each of 'Kyle' and 'Arcola' contained the above, with $4.45 \cdot 10^{-7}$ M Cd and $1.0 \cdot 10^{-4}$ M citrate.

The experimental set-up and initial harvest was done at 3:00 PM, 21 days after seeding. Subsequent harvests were made every 8 hours (11:00 PM, 7:00 AM and 3:00 PM, daily) until the 24th day after seeding at 3:00 PM. For each harvest, two experimental units containing 'Kyle' seedlings and two experimental units containing 'Arcola' seedlings were harvested; one of each cultivar exposed to $4.45 \cdot 10^{-8}$ M Cd and the other exposed to $4.45 \cdot 10^{-7}$ M Cd. Every second harvest, beginning 8 hours after exposure (8, 24, 49, 56, and 72 hours), plants exposed to Cd with citrate were harvested. The Styrofoam tray was removed from the solution, the roots and shoots were separated, rinsed with deionized water, placed in paper bags and dried in a drying oven at 80 °C for 48 hours (Step 5, Figure 6.1). Additionally, 250 mL of the exposure solution from each pot was sampled after the plant material was removed, and during every second harvest (8, 24, 49, 56, and 72 hours), one of the blank pots containing each Cd concentration was sampled (Step 6, Figure 6.1). Solution samples were kept refrigerated at 4 °C in acid washed 250 mL HDPE bottles until analysed. The pH of the exposure solutions from the remaining, unharvested experimental units was re-adjusted to 6.0 during each harvest. At the end of the first and second day of the three day exposure, plants and day two of the exposure a commercial complete micronutrient (Fe, Mn, Zn, Cu, B and Mo) (Plant Products Co. Ltd., Brampton, ON) solution was applied to the foliage to prevent nutrient deficiency, since the exposure solutions contained none of these micronutrients . For each foliar spray, 0.2

g of the formulation and two drops of Tween 80 were added to 650 mL of distilled water and all of the solution was evenly sprayed onto the plant canopy.

6.2.3.2 Measurement of Cd^{2+} Concentration

Each solution sample was split into two volumes; a 50 mL volume for analysis of total Cd, and a 200 mL volume to be passed through a cation exchange column in order to estimate the Cd^{2+} concentration. The Cd^{2+} concentration was measured by the method of Cantwell *et al.* (1982), with modifications (Fortin and Campbell, 1997). Analytical grade cation exchange resin (0.1000±0.0002 g AG 50W-X8, Bio-Rad Laboratories, Hercules, CA) was packed in each of eight poly-prep columns (0.8 cm x 4.0 cm, Bio-Rad Laboratories, Hercules, CA) to which 250 mL reservoirs and two way stopcocks (Bio-Rad Laboratories, Hercules, CA) were attached. Resin was converted to the Na form by passing 1 M NaOH through the resin, and then the resin was conditioned by passing 0.2 M $NaNO_3$ at a pH of 6.0 through the column at a rate of 6 $mL \cdot min^{-1}$ until the pH of the eluent was also 6.0. The 0.2 M $NaNO_3$ was made in a 20 L reservoir with nanopure water and supplied to the reservoirs through Teflon lines (0.2 cm, Bio-Rad Laboratories, Hercules, CA) to ensure a continuous supply.

The $NaNO_3$ concentration in each sample was brought to 0.2 M by adding 3.40 g $NaNO_3$, and the pH of the samples was adjusted to 6.0 with HNO_3 or NaOH and then run through the resin at a rate of 6 $mL \cdot min^{-1}$. Free Cd^{2+} in the sample exchanged with Na bound to the resin and remained trapped in the resin until equilibrium was reached between the Cd^{2+} dissolved in the sample passing though the resin and Cd bound to the

resin. Once all of the sample passed through the resin, N_2 gas was forced through the resin to remove interstitial solution. Finally, HNO_3 (50 mL of 1.5 M trace metal grade) was passed through the resin in order to exchange the Cd bound to the resin with H^+, and this eluent was collected and analysed for Cd.

The concentration of Cd^{2+} present in the sample was related to the amount of Cd eluted from the resin by the equation $[Cd^{2+}] = [Cd_{meas}] \cdot 0.05\ L \div (k \cdot m)$, where $[Cd^{2+}]$ was the Cd^{2+} concentration in the original sample, $[Cd_{meas}]$ was the Cd concentration measured (by GF-AAS) in the final eluent, 0.05 L was the volume of the eluent, m was the mass of resin used and k was the distribution coefficient ($L \cdot g^{-1}$) which was determined by 'calibrating' the columns. Columns were 'calibrated' by passing samples of known Cd^{2+} (determined by MINEQL$^+$ Version 3.0 (Schecher and McAvoy, 1994) using constants from NIST (Smith *et al.*, 1997)) through the resin, measuring $[Cd_{meas}]$ (by GF-AAS), and solving for k in the equation above. The constant k could then be used to determine the Cd^{2+} concentrations of samples with similar Ca^{2+} and Mg^{2+} concentrations, since k is altered by changes in concentrations of these ions. For each run (of eight samples) the $NaNO_3$ solution used to condition the resin and the HNO_3 solution used to elute the Cd trapped in the resin were also sampled and measured for Cd to ensure that there was no contamination; these samples never had detectable Cd concentrations.

6.2.3.3 Analysis of Solutions Samples by GF-AAS

Analysis of solution samples for the total Cd concentration was as described in section *2.2.3 Cadmium Exposure and Solution Analysis*. To determine the Cd^{2+}

concentration, it was necessary to analyse the acidified samples eluted from cation exchange columns with HNO_3. These samples were analysed similarly except all blanks, calibration, and quality control samples had similar amounts of acid added to them as were in the samples to be analysed. The Cd^{2+} concentration was determined using the equation above.

6.2.4 Plant Digestion and Cd Analysis

Digestion of tissue and analysis of samples for Cd were as described in section *2.2.4 Plant Digestion and Cd Analysis*, with a few minor changes. After drying, the plant tissues were ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) and samples of approximately 0.2 g were weighed to four decimal places and placed in acid washed Teflon digestion vessels with 3 mL trace metal grade HNO_3. After digestion, samples were diluted to 10 mL with nanopure water. Citrus leaves (NIST Standard Reference Material #1572, US Department of Commerce, National Institute of Standards and Technology, Gaithersberg, MD) were digested with each run, and data were corrected to the NIST value for cadmium of 0.03 ± 0.01 $\mu g \cdot g^{-1}$.

6.2.5 Data Analysis

To test for the effect of cultivar on loss of Cd and Cd^{2+} from the exposure solutions over time, the data were plotted for each exposure solution separately by cultivar (full model) and pooled for cultivars (reduced model) using SAS PROC NLIN (SAS Institute Inc., Cary, NC). The equation used to fit the data was: solution Cd (or Cd^{2+}) =

$b_0 + (b_1 \cdot e^{(-b2 \cdot time)})$. Reduction in error sum of squares was tested by: $F = (((ESS_{red} - ESS_{full}) / (df_{red} - df_{full})) / EMS_{full})$. The calculated F-value was compared with the tabulated F. An example calculation is presented in Appendix C.

Data collected from Blanks were analysed using SAS PROC REG. Additionally, the proportion of Cd present as Cd^{2+} (expressed as a percentage) was analysed using SAS PROC GLM. The model tested the significance of exposure duration (time) for each target Cd concentration in the exposure solution.

To test for cultivar differences in root or shoot Cd, the data for the root and shoot concentration of Cd over time were plotted separately by cultivar (full model) and pooled for cultivars (reduced model) using SAS PROC NLIN. The equation used to fit the data was: tissue $Cd = b_0 \cdot (1 - e^{(-b1 \cdot time)}) + b_2$. Bounds were placed on the estimate of b_2; it was not permitted to fall below 0, and consequently in all equations generated, the estimate for b_2 was 0 and it could be removed from the equation. This was done so that the equation would not result in predictions of tissue Cd concentration which were negative at time 0. Cd accumulation by roots or shoots of 'Kyle' or 'Arcola' seedlings from the same exposure solutions were compared by examining the reduction in error sum of squares, as previously described for Cd and Cd^{2+} concentrations in solutions.

The effects of citrate on the total Cd and Cd^{2+} concentration in solution was analysed using SAS PROC GLM; the final model tested the effects of cultivar, duration of exposure (time) and citrate. To test whether citrate influenced accumulation of Cd by root or shoots of 'Kyle' or 'Arcola' seedlings, the data for the root and shoot concentration of Cd over time were plotted separately by citrate (full model), and pooled

for citrate concentrations (reduced model) using SAS PROC NLIN. The analysis was similar to that used for testing whether cultivar influenced Cd accumulation.

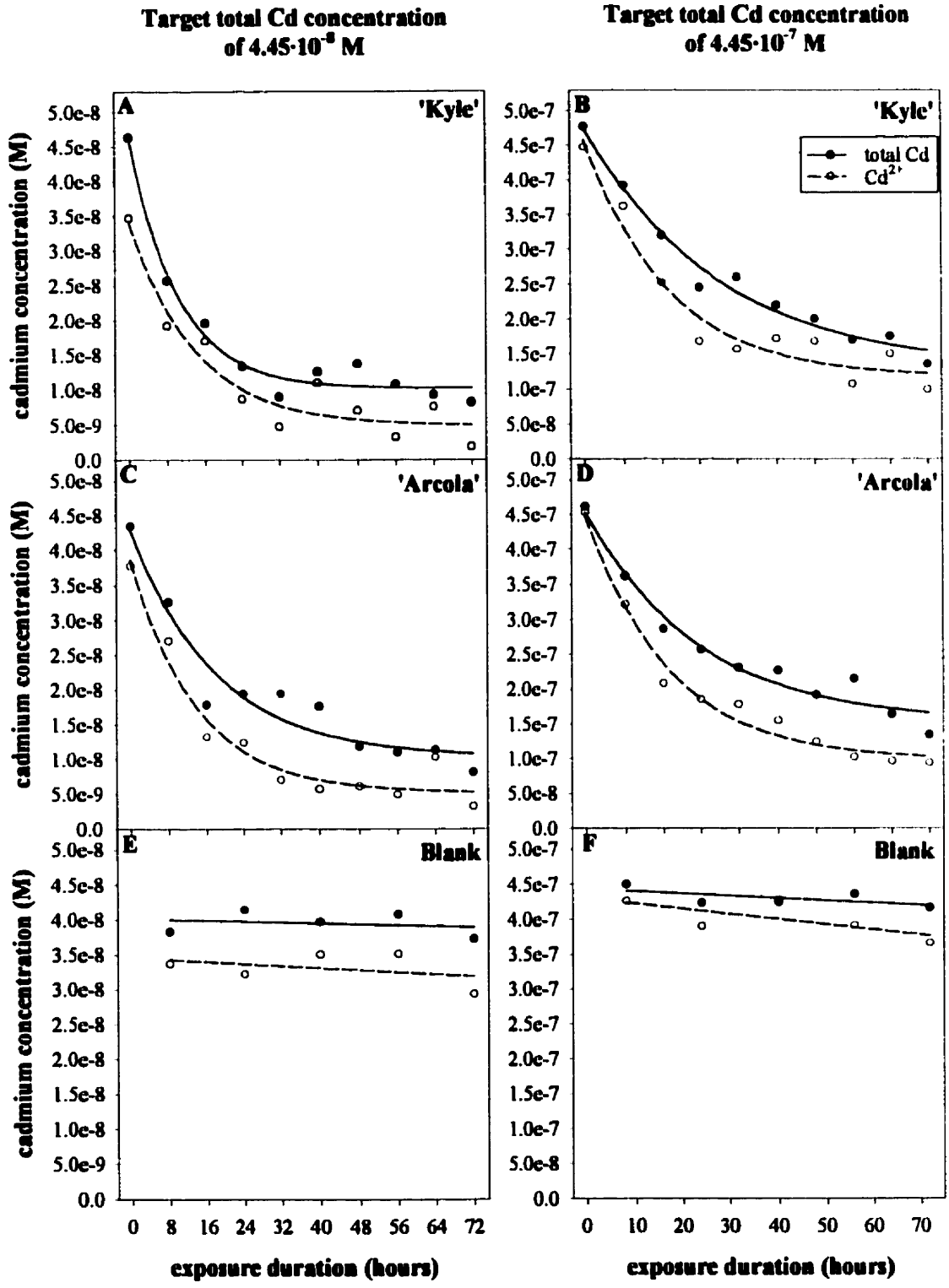
The effects of duration of exposure, nominal solution Cd concentration and cultivar on the proportion of Cd^{2+} in solution was analysed using SAS PROC GLM; the final model tested the effects of cultivar, duration of exposure (time), nominal solution Cd concentration, duration of exposure*cultivar, and duration of exposure*nominal solution Cd concentration.

6.3 Results and Discussion

6.3.1 Exposure Solution

The presence of plant roots rapidly depleted the exposure solution of Cd. When 'Kyle' and 'Arcola' seedlings were placed in exposure solutions initially containing $4.45 \cdot 10^{-8}$ M Cd (±10%), total Cd concentrations dropped to about $1.3 \cdot 10^{-8}$ to $1.8 \cdot 10^{-8}$ M (or 30 to 40% of the initial Cd concentration) after only 24 hours of exposure, and to about $1.1 \cdot 10^{-8}$ M Cd (25% of the initial Cd concentration) by 48 or 72 hours (Figure 6.2 A and C). Similarly, when 'Kyle' and 'Arcola' seedlings were placed in exposure solutions initially containing $4.45 \cdot 10^{-7}$ M Cd (±10%), total Cd concentrations dropped to about $2.7 \cdot 10^{-7}$ (60% of the initial Cd concentration) after only 24 hours of exposure, to about $1.9 \cdot 10^{-7}$ M Cd (40% of the initial Cd concentration) by 48 hrs, and to $1.6 \cdot 10^{-7}$ M Cd (or 35% of the initial Cd concentration) by 72 hours of exposure (Figure 6.2 B and D). The reduction in concentration of Cd^{2+} over time was greater than the reduction in total Cd concentration, and will be discussed later. Obviously, the Cd dose was not constant over

Figure 6.2A to F: Total Cd and Cd^{2+} concentrations in exposure solutions containing target total concentrations of $4.45 \cdot 10^{-8}$ M (A, C, and E) or $4.45 \cdot 10^{-7}$ M Cd (B, D, and F) used to expose 'Kyle' seedlings (A and B), 'Arcola' seedlings (C and D), or Blanks (E and F) for 0 to 72 hours.


Target total Cd concentration of 4.45·10⁻⁸ M
Target total Cd concentration of 4.45·10⁻⁷ M
A
'Kyle'
B
'Kyle'
total Cd
Cd2+
C
'Arcola'
D
'Arcola'
E
Blank
F
Blank
cadmium concentration (M)
5.0e-8
4.5e-8
4.0e-8
3.5e-8
3.0e-8
2.5e-8
2.0e-8
1.5e-8
1.0e-8
5.0e-9
0.0
5.0e-7
4.5e-7
4.0e-7
3.5e-7
3.0e-7
2.5e-7
2.0e-7
1.5e-7
1.0e-7
5.0e-8
0.0
0 8 16 24 32 40 48 56 64 72
0 10 20 30 40 50 60 70
exposure duration (hours)
exposure duration (hours)

the duration of exposure. Regression relationships for separate cultivars were not significantly different from that for the pooled cultivars, indicating that there was no significant difference in the doses of total Cd or Cd^{2+} between cultivars (Table 6.1).

It appears that in this case, if constant doses are desired over the course of a long term exposure (greater than a few hours), exposure solutions in stagnant exposure systems should be replaced every 8 hours at least, and even at this frequency, the dose would vary by as much as 40% in the case of an initial dose of $4.45 \cdot 10^{-8}$ M Cd, and by 20% in the case of an initial dose of $4.45 \cdot 10^{-7}$ M Cd. The Cd was rapidly removed from the solution by the plant roots, and not by precipitation or binding to the pot surface. This was demonstrated by the fact that pots with $4.45 \cdot 10^{-8}$ or $4.45 \cdot 10^{-7}$ M Cd and no plants in them (Blanks) appeared to have remarkably stable Cd concentrations (Figure 6.2 E and F) and the slopes of the regression equations were not significantly different from zero (Table 6.2). This suggests that Cd or Cd^{2+} in the exposure solutions did not bind to the walls of the HDPE pots, the Styrofoam tray, or the rockwool cubes. Another way to supply constant free ion concentrations over time is through the use of chelator-buffered solutions, where the free ion concentration of Cd^{2+}, for example, would be buffered by the dissociation of complexed Cd species (Bell, *et al.*, 1991). In studies such as this, however, the introduction of complexed Cd species (which may influence Cd accumulation) may complicate the interpretation of data, and it may be better to buffer the Cd^{2+} dose through the use of recirculating hydroponic systems with large volumes of exposure solution..

Table 6.1: Calculated F-value for the reduction in error sum of squares resulting from including cultivar in the regression models for the depletion of Cd and Cd^{2+} from solution, and Cd accumulation by roots and shoots.

$F_{0.05,3,14} = 3.34$ and $F_{0.01,3,14} = 5.56$.

Solution Cd and Cd^{2+} concentrations	
Solution	F-value
$4.45 \cdot 10^{-8}$ M Cd, total Cd concentration	2.62
$4.45 \cdot 10^{-8}$ M Cd, Cd^{2+} concentration	0.664
$4.45 \cdot 10^{-7}$ M Cd, total Cd concentration	0.844
$4.45 \cdot 10^{-7}$ M Cd, Cd^{2+} concentration	0.877
Tissue Cd concentrations	
Tissue	F-value
roots of plants exposed to $4.45 \cdot 10^{-8}$ M Cd	1.04
shoots of plants exposed to $4.45 \cdot 10^{-8}$ M Cd	13.2*
roots of plants exposed to $4.45 \cdot 10^{-7}$ M Cd	0.863
shoots of plants exposed to $4.45 \cdot 10^{-7}$ M Cd	2.77

Table 6.2: ANOVA table of regressions of Cd^{2+} and total Cd concentrations measured from Blank pots.

$4.45 \cdot 10^{-8}$ M Cd Blank						
	total Cd concentration			Cd^{2+} concentration		
Variable	estimate	df	p-value	estimate	df	p-value
intercept	4.52	1	0.00018	3.89	1	0.00065
slope	-0.00191	1	0.68	-0.00415	1	0.51
$4.45 \cdot 10^{-7}$ M Cd Blank						
	total Cd concentration			Cd^{2+} concentration		
Variable	estimate	df	p-value	estimate	df	p-value
intercept	49.84	1	<0.0001	48.3	1	0.00019
slope	-0.0383	1	0.23	-0.0842	1	0.17

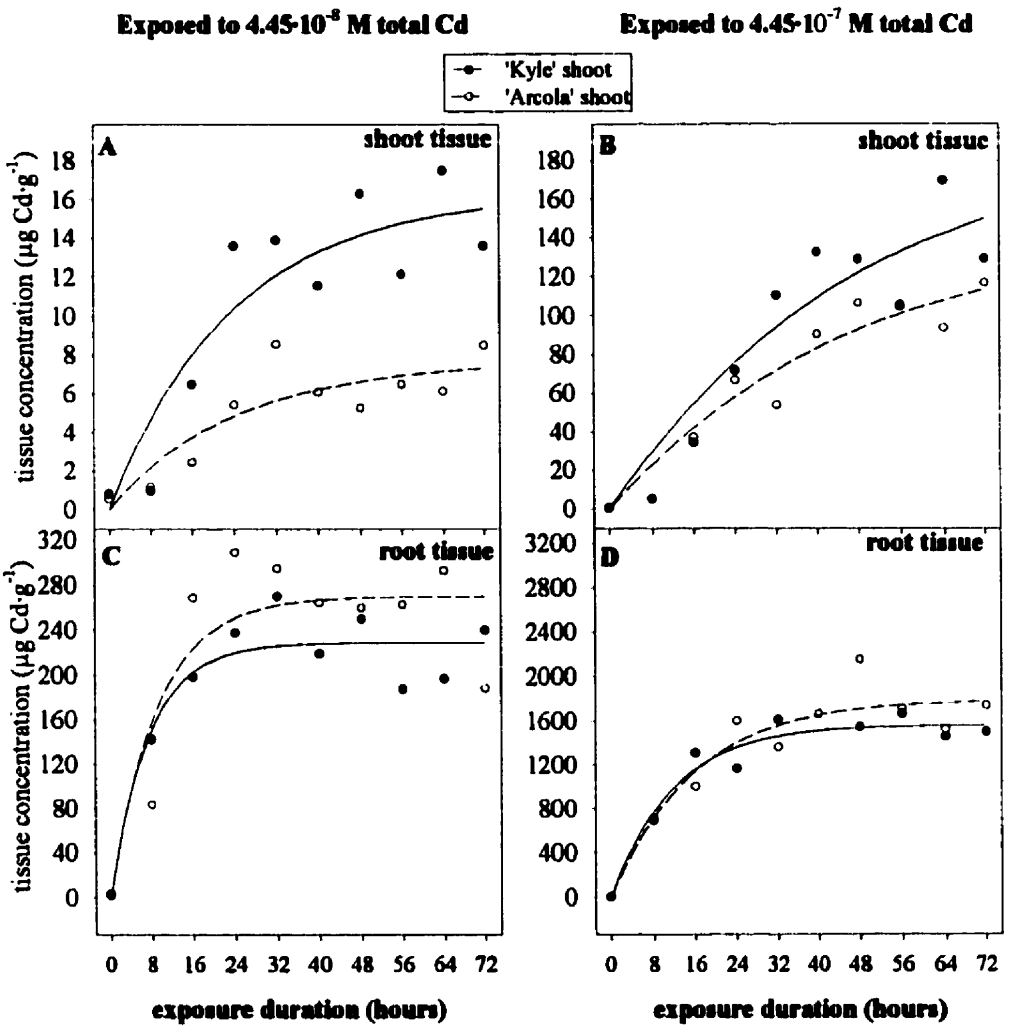
6.3.2 Plant Accumulation

In both cultivars, and at both exposure solution Cd concentrations, the concentration of Cd in root tissue initially increased rapidly before beginning to plateau after 24 hours of exposure (Figure 6.3 C and D). Regression lines were drawn through the data using an asymptotic function (tissue Cd = $b_0 \cdot (1 - e^{(-b1 \cdot time)}) + b_2$); the plateau in root Cd concentration occurred when Cd concentrations in the exposure solutions had dropped to around half of the initial concentrations. Further uptake by roots after 24 hours may have been matched by translocation to shoots or efflux from roots, resulting in no further increase in root concentration of Cd .

For each dose used, the tissue Cd concentration in 'Kyle' and 'Arcola' roots were not significantly different ($p>0.05$, Table 6.1). Not surprisingly, roots of 'Kyle' and 'Arcola' seedlings exposed to $4.45 \cdot 10^{-7}$ M Cd had a Cd concentration which was approximately ten times greater than roots of 'Kyle' or 'Arcola' seedlings exposed to $4.45 \cdot 10^{-8}$ M Cd.

Accumulation of Cd by shoots of 'Kyle' and 'Arcola' seedlings was described by regression equations of the same format used to describe root Cd concentrations (Figure 6.3 A and B). Shoot Cd concentrations also increased rapidly at first and then began to plateau, although this plateau was not as distinctive as that for Cd concentration in root tissue. Shoot Cd concentrations were about 5% that of root Cd concentrations. While Cd concentrations in 'Kyle' and 'Arcola' roots were not different, Cd concentrations in shoots were different when seedlings were exposed to $4.45 \cdot 10^{-8}$ M Cd ($p<0.01$, Table 6.1), but not when seedlings were exposed to $4.45 \cdot 10^{-7}$ M Cd ($p>0.05$, Table 6.1).

Figure 6.3 A to D: Concentration of Cd in shoots (A and B) and roots (C and D) of 'Kyle' and 'Arcola' seedlings exposed to target total Cd concentrations of $4.45 \cdot 10^{-8}$ M (A and C) and $4.45 \cdot 10^{-7}$ M (B and D) for 0 to 72 hours.


Exposed to 4.45·10⁻⁸ M total Cd
Exposed to 4.45·10⁻⁷ M total Cd
'Kyle' shoot
'Arcola' shoot
A
shoot tissue
B
shoot tissue
C
root tissue
D
root tissue
tissue concentration (µg Cd·g⁻¹)
tissue concentration (µg Cd·g⁻¹)
exposure duration (hours)
exposure duration (hours)

'Arcola' shoots contained a Cd concentration which was 100% greater than 'Kyle' when exposed to $4.45 \cdot 10^{-8}$ M Cd, and 34% greater when exposed to $4.45 \cdot 10^{-7}$ M Cd, although this latter difference is not statistically significant.

A physiological mechanism is likely the basis of greater root to shoot translocation of Cd in 'Kyle' seedlings than 'Arcola' seedlings, as it appears that the mechanism responsible for this difference is saturable, since the cultivar difference was only significant when the seedlings were exposed to $4.45 \cdot 10^{-8}$ M Cd. Perhaps 'Arcola' seedlings immobilize relatively more Cd in the roots by complexation with ligands or sequestration in vacuoles of root cells. This mechanism may have had a maximum capacity and may explain the smaller difference between the cultivars exposed to a higher Cd concentration. The membranes of vacuoles contain a pump for Cd^{2+} (Salt and Wagner, 1993) and phytochelatin (Salt and Rauser, 1995), and it may be that one or both of these pumps are more active in 'Arcola' than in 'Kyle', resulting in less Cd in the cytoplasm available for translocation in 'Arcola'. Alternatively, 'Kyle' seedlings may have mobilized more Cd by complexation with ligands which were then transported to the shoots. Plants preincubated with citrate demonstrated greater root to shoot translocation of Cd, possibly in the form of a $CdCitrate^-$ complex (Senden *et al.*, 1995), so it is possible that a cultivar difference in tissue citrate levels may result in a cultivar difference in Cd mobility.

The difference in translocation of Cd between 'Kyle' and 'Arcola' may have been due to differences in transpiration. Neither total leaf surface area nor transpiration rates were measured in this experiment, and the rate of transpiration may influence translocation of ions (Marschner, 1995).

Similarly to root tissue Cd concentrations, shoot tissues contained a Cd concentration which was ten times higher when seedlings were exposed to $4.45 \cdot 10^{-7}$ M Cd compared with $4.45 \cdot 10^{-6}$ M Cd.

6.3.3 Effect of Citrate in the Exposure Solution on Cd Concentration in the Exposure Solution and Plant Tissue

Adding $1.00 \cdot 10^{-4}$ M citrate to the exposure solution influenced solution chemistry very little: using MINEQL$^+$, the proportion of Cd present as Cd^{2+} was predicted to be 87.8% in the absence of citrate, and 85.3% when the citrate concentration was $1.00 \cdot 10^{-4}$ M. The nominal Cd concentration and Cd^{2+} concentration over time in exposure solutions with or without citrate used to expose 'Kyle' and 'Arcola' seedlings were similar ($p=0.36$ Table 6.3, Figure 6.4 A and B). Interestingly, however, solutions which contained $1.00 \cdot 10^{-4}$ M citrate actually had higher Cd^{2+} concentrations as measured by the ion exchange technique than solutions without citrate ($p=0.0041$, Table 6.3), and the reason for this is unclear. One possible explanation might be the use of the ion exchange technique; the ion exchange columns must be calibrated for given Ca^{2+} and Mg^{2+} concentrations, since in the resin, Ca^{2+} and Mg^{2+} are competing with Cd^{2+} for binding sites. If the citrate present complexed some of the Ca^{2+} and Mg^{2+} present, then the use of the technique would result in artificially high Cd^{2+} measurements. Another possibility is that the presence of citrate influenced the secretion of root exudates, resulting in a smaller proportion of complexed Cd in citrate solutions than solutions without citrate.

Accumulation of Cd by root or shoot tissue of 'Arcola' seedlings exposed to

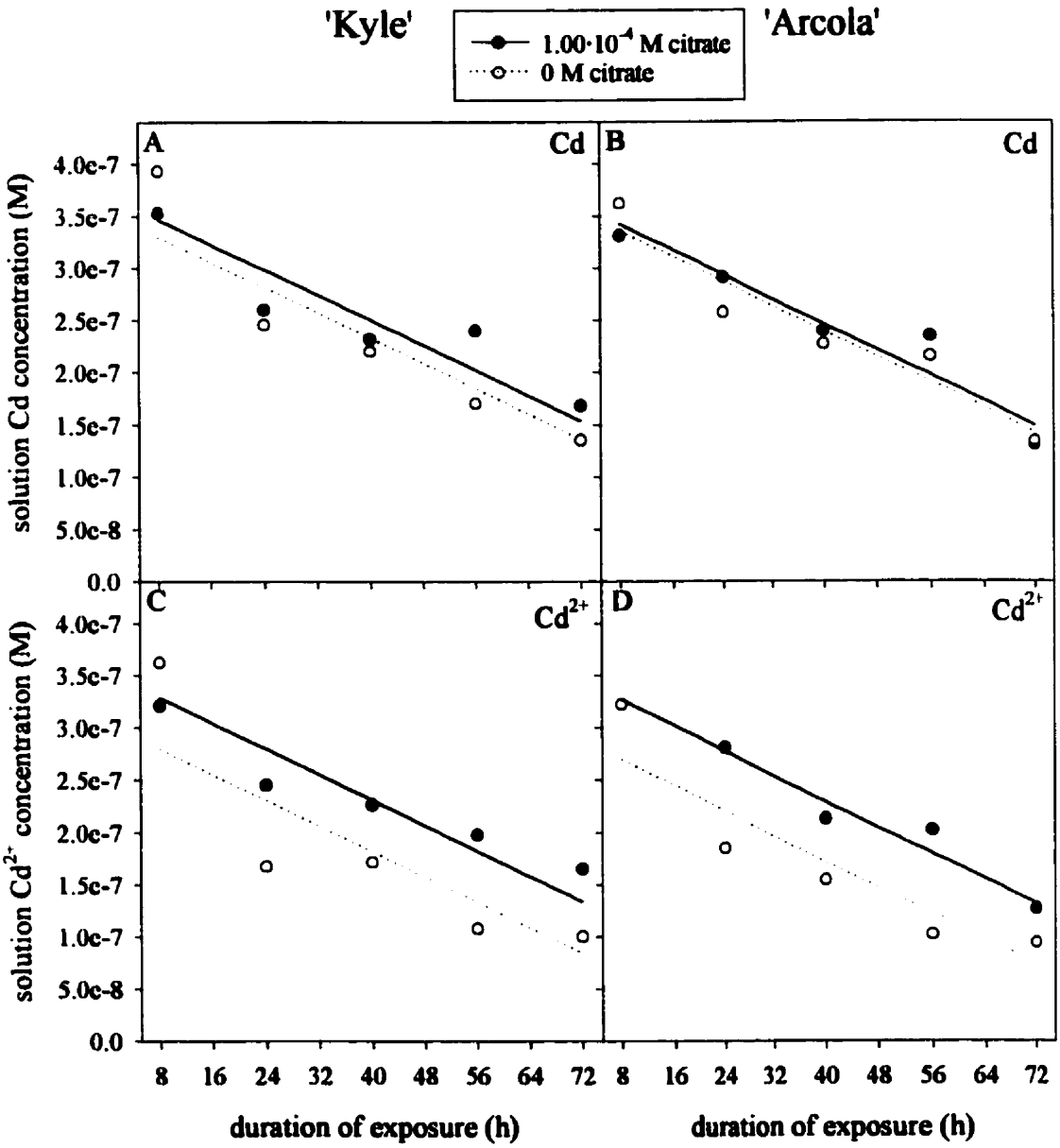
Table 6.3: Sources of variation in Cd and Cd^{2+} concentration in exposure solutions with or with citrate and in 'Kyle' or 'Arcola' seedlings exposed to these solutions.

Total Cd Concentration in Exposure Solution			
Source	df	F-value	p-value
Model	3	397.72	<0.0001
cultivar	(1)	0.00	0.96
time	(1)	110.12	<0.0001
citrate	(1)	0.87	0.36
Error	16		

Cd^{2+} Concentration in Exposure Solution			
Source	df	F-value	p-value
Model	3	28.75	<0.0001
cultivar	(1)	0.15	0.70
time	(1)	74.87	<0.0001
citrate	(1)	11.23	0.0041
Error	16		

Cd Concentration in 'Kyle' and 'Arcola' Tissue	
Cultivar and Tissue	F-value (df) [$F_{3, 10, 0.05} = 3.71$]
'Kyle' roots	5.38*
'Kyle' shoots	3.96*
'Arcola' roots	0.70
'Arcola' shoots	2.10

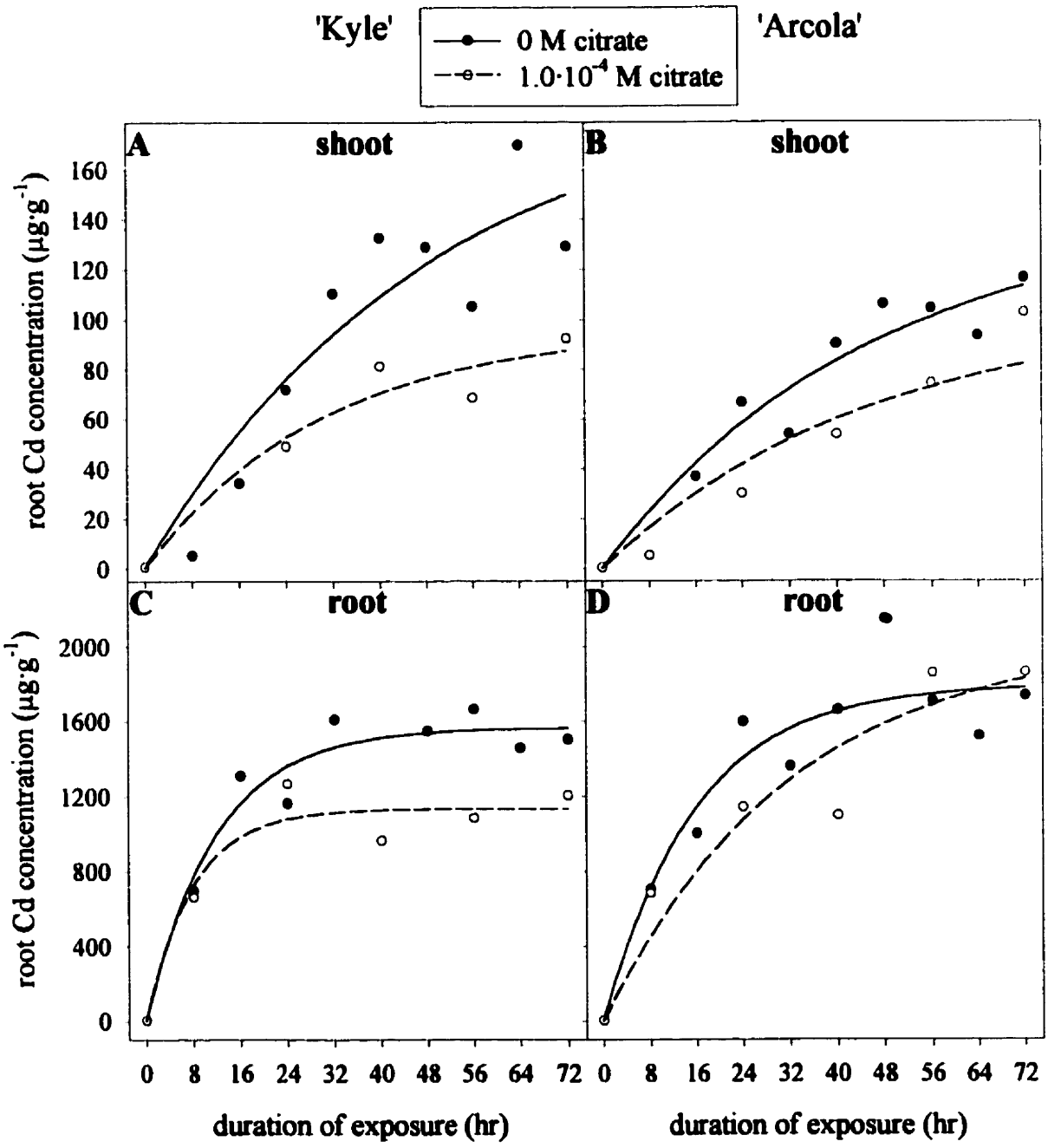
Figure 6.4 A to D: Total Cd (A and B) and Cd^{2+} concentrations (C and D) in solutions containing 0 M or $1.00 \cdot 10^{-4}$ M citrate used to exposed 'Kyle' (A and C) or 'Arcola' seedlings for 8 to 72 hours.


'Kyle'
'Arcola'
1.00·10⁻⁴ M citrate
0 M citrate
A
Cd
B
Cd
C
Cd2+
D
Cd2+
solution Cd concentration (M)
solution Cd2+ concentration (M)
4.0e-7
3.5e-7
3.0e-7
2.5e-7
2.0e-7
1.5e-7
1.0e-7
5.0e-8
0.0
8 16 24 32 40 48 56 64 72
duration of exposure (h)

$4.45 \cdot 10^{-7}$ M Cd were not influenced by citrate in the exposure solution, but roots and shoots of 'Kyle' seedlings contained less Cd when the exposure solution contained citrate (Figure 6.5 A to D; Table 6.3). It appeared that the addition of citrate to the exposure solution resulted in less accumulation of Cd by 'Kyle'; the effect on 'Arcola' was not significant. The lower accumulation by shoots was associated with lower Cd concentrations in roots. Accumulation by roots did not appear to differ during the first 24 hours of exposure, but was less for the final 48 hours of the exposure. Senden *et al.* (1995) found that preincubation with citrate resulted in increase shoot accumulation of Cd, while tomato plants exposed to Cd along with citrate showed no difference in Cd accumulation.

The amount of citrate added to the exposure solution was not enough to alter Cd speciation very much, so it seems unlikely that the difference in accumulation was due to changes in Cd speciation; Figure 6.3 demonstrates that speciation did not appear to differ between the two cultivars. Since the exposures were not done under sterile conditions, perhaps the citrate was a carbon source for bacteria which were competing with the wheat seedlings for Cd. The apparent cultivar difference in response to citrate is intriguing, and deserves further study. Only five experimental units of each cultivar were exposed to Cd and citrate in this experiment, and the small sample size makes it difficult to reach solid conclusions. Further experiments with a greater number of samples and at least two nominal Cd concentrations should be carried out in order to determine if there is an effect of citrate on accumulation of Cd, and if this effect is cultivar specific.

Figure 6.5 A to D: Concentration of Cd in shoots (A and B) and roots (C and D) of 'Kyle' (A and C) and 'Arcola' (B and D) seedlings exposed to a target total Cd concentration of $4.45 \cdot 10^{-7}$ M with 0 M or $1.00 \cdot 10^{-4}$ M citrate for 8 to 72 hours.


'Kyle'
'Arcola'
0 M citrate
1.0·10⁻⁴ M citrate
A
shoot
B
shoot
C
root
D
root
root Cd concentration (µg·g⁻¹)
root Cd concentration (µg·g⁻¹)
0
20
40
60
80
100
120
140
160
0
400
800
1200
1600
2000
0 8 16 24 32 40 48 56 64 72
0 8 16 24 32 40 48 56 64 72
duration of exposure (hr)
duration of exposure (hr)

6.3.4 Proportion of Dissolved Cd as Cd^{2+} Over 72 Hours of Exposure

Prior to contact between the exposure solution and plant roots, it is possible to model solution chemistry if the solution pH and concentrations of the various ions present in the exposure solution are known, and entered into MINEQL$^+$. When this is done, the estimated Cd^{2+} concentration was predicted to be 87.8% of the total dissolved Cd concentration. The value for Cd^{2+} can then be used to calibrate cation exchange columns which can then be used to measure the Cd^{2+} concentration in solutions in contact with plant roots for a period of time. The effect of prolonged contact between the exposure solution and roots of 'Kyle' or 'Arcola' seedlings on speciation of Cd is shown in Figure 6.6 A and B. There were significant interactions between duration of exposure and cultivar (Blank, 'Kyle' or 'Arcola') ($p=0.042$, Table 6.4) and duration of exposure and target total exposure solution Cd concentration ($4.45 \cdot 10^{-8}$ M or $4.45 \cdot 10^{-7}$ M) ($p=0.041$, Table 6.3) in the proportion of measured Cd^{2+} . The significant interaction between duration of exposure and cultivar can be explained by the fact that the proportion of Cd^{2+} in Blank pots did not change with longer durations of exposure, while the proportion of Cd^{2+} in exposure solution in contact with 'Kyle' or 'Arcola' seedlings was reduced as the duration of exposure went on. If the Blanks are left out of the statistical analysis, there was no significant difference between the cultivars, which indicates that 'Kyle' and 'Arcola' did not have a cultivar specific influence on Cd^{2+} concentration. The interaction between duration of exposure and target total exposure solution Cd concentration can be explained by the fact that the reduction in the proportion of Cd^{2+} with longer durations of exposure was greater in pots initially containing $4.45 \cdot 10^{-8}$ M Cd than in pots containing

Figure 6.6 A and B: Proportion of dissolved Cd present as Cd^{2+} (estimated by an ion exchange technique) in solutions containing total target Cd concentrations of $4.45 \cdot 10^{-8}$ M or $4.45 \cdot 10^{-7}$ M and in contact with 'Kyle' or 'Arcola' seedlings or no seedlings (Blanks) for 0 to 72 hours.

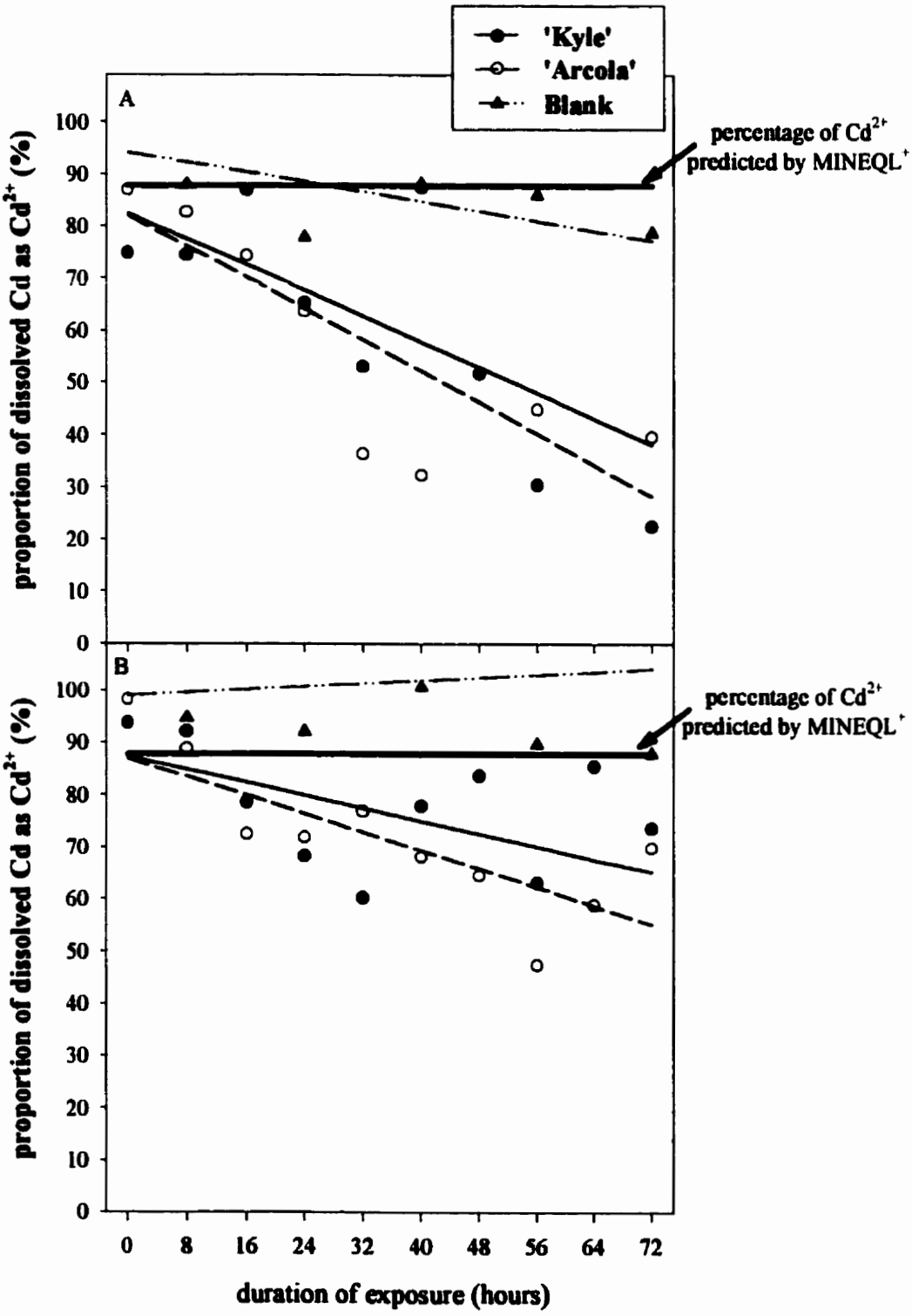

'Kyle'
'Arcola'
Blank
A
B
percentage of Cd^{2+} predicted by MINEQL+
proportion of dissolved Cd as Cd^{2+} (%)
0
10
20
30
40
50
60
70
80
90
100
0
8
16
24
32
40
48
56
64
72
duration of exposure (hours)

Table 6.4: ANOVA table of factors that influence the proportion of total dissolved Cd present as Cd^{2+}.

Source	df	F-value	p-value
Model	7	13.33	<0.0001
cultivar	(2)	0.36	0.70
duration of exposure	(1)	22.27	<0.0001
target total Cd concentration	(1)	0.63	0.43
duration of exposure*cultivar	(2)	3.43	0.042
duration of exposure*target total Cd concentration	(1)	4.44	0.041
Error	40		
Corrected Total	47		

$4.45 \cdot 10^{-7}$ M Cd.

It can be concluded that the action of plant roots altered solution chemistry in a way that resulted in a lower proportion of dissolved Cd present in the free ion form (Cd^{2+}) compared with pots not containing roots (Blanks). 'Kyle' and 'Arcola' roots may have altered solution pH, removed nutrient ions from solution, or secreted organic compounds into the solution. The pH of the exposure solution was slightly reduced after exposure to 'Kyle' and 'Arcola' roots, but lowering solution pH would be expected to increase, and not decrease, the proportion of Cd^{2+} in solution. The pH of all exposure solutions was adjusted to 6.0 during each harvest and, more importantly, prior to adding samples to the cation exchange column. Therefore, the observed changes in the proportion of Cd^{2+} were not due to changes in solution pH.

Removing ions from the exposure solution could have altered the proportion of Cd^{2+}. If concentrations of all ions were depleted at a similar rate, then speciation would not change much, although the proportion of Cd^{2+} would have increased slightly. If the SO_4^{2-} concentration was reduced relative to that of other ions, the proportion of Cd^{2+} would have increased because of dissociation of $CdSO_4^0{}_{(aq)}$. If, however, concentrations of Ca^{2+} or Mg^{2+} were reduced relative to other ions, especially SO_4^{2-}, the proportion of Cd present as Cd^{2+} would have been reduced. This would happen because in the exposure solution, there were $CaSO_4^0{}_{(aq)}$ and $MgSO_4^0{}_{(aq)}$ complexes, and if Ca^{2+} or Mg^{2+} were removed from solution, these complexes would have dissociated to maintain equilibrium, resulting in a higher SO_4^{2-} concentration which would then form complexes with free Cd^{2+}. However, it is not likely that this mechanism could explain the observed reduction in the

proportion of Cd^{2+}. According to MINEQL$^+$, if total Ca and Mg concentrations were reduced to one third their initial concentrations, the proportion of Cd^{2+} would only have dropped from 87.8% to 84.7%, and the observed reduction in the proportion of Cd^{2+} was far greater than this.

Plants secrete various organic compounds from their roots into the surrounding media, which can mobilize nutrient ions or complex non-essential metal ions (Al^{3+}). Cieslinski *et al.* (1997) identified various organic ions in sterile nutrient solutions used to culture seedlings. In that experiment, plants were grown for fourteen days before solutions were analysed, but seedlings were only three days old at the beginning of the experiment, and weighed only 30 mg (dry weight) at harvest. In this experiment, plants were 21 days old and weighed 1500 to 2000 mg (dry weight) at harvest. 'Kyle' and 'Arcola' seedlings were likely secreting compounds into the exposure solutions during this three day exposure, and some of these compounds could have resulted in a reduction in the proportion of Cd^{2+} by forming complexes (ie. $CdCitrate^-$). If the total concentration of these ligands increased with time, relatively more of the dissolved Cd remaining in solution would be in the complexed form, and not Cd^{2+}. Also, since it is likely that root exudation was independent of target Cd concentrations in the exposure solution, it is probable that the speciation of Cd would be more greatly influenced at $4.45 \cdot 10^{-8}$ M Cd than at $4.45 \cdot 10^{-7}$ M Cd.

The first null hypothesis, that there is no difference in the Cd concentration in roots or shoots of 'Kyle' or 'Arcola' seedlings exposed to similar Cd concentrations, can be rejected. While root concentrations of the two cultivars were not different, 'Arcola'

shoots contained less Cd than 'Kyle' shoots when the Cd concentration in the exposure solution was $4.45 \cdot 10^{-8}$ M. There was an internal mechanism which permitted relatively less root to shoot movement of Cd in 'Arcola' seedlings compared with 'Kyle' seedlings. The Cd doses, while not consistent over time, were similar for the two cultivars.

The second null hypothesis, that adding $1.00 \cdot 10^{-4}$ M citrate to the exposure solution does not influence accumulation of Cd, can not be rejected. This concentration of citrate did not influence speciation, and accumulation of Cd by roots or shoots of 'Kyle' or 'Arcola' from solutions containing $4.45 \cdot 10^{-7}$ M Cd with or without citrate, did not significantly differ.

The final null hypothesis, that the proportion of dissolved Cd present as Cd^{2+} in the exposure solution is not influenced by roots of 'Kyle' and 'Arcola' seedlings, can be rejected. The total Cd concentration in the exposure solution was reduced by its accumulation in root tissue, and the proportion of Cd present as Cd^{2+} was also decreased. Prior to contact with roots of 'Kyle' and 'Arcola' seedlings, 87.8% of the dissolved Cd was estimated to be Cd^{2+}, and this proportion decreased to 30% in the $4.45 \cdot 10^{-8}$ M Cd solution and 60% in the $4.45 \cdot 10^{-7}$ M Cd solution after 3 days. Exudation of organic compounds which formed complexes with Cd^{2+} in solution seems to be the most likely mechanism for the change, as neither pH nor inorganic ion concentration were likely to change enough to result in the observed changes in Cd speciation.

This study provides further evidence that the difference in grain accumulation of Cd by 'Kyle' and 'Arcola' seedlings is due to an internal mechanism which results in reduced mobility of Cd in 'Arcola' seedlings compared with 'Kyle' seedlings. Speciation

of Cd in the exposure solutions, and likely soil solution surrounding roots in the environment, appears to be influenced by exudation of organic compounds which result in a lower proportion of Cd^{2+} in solution. How this influences bioavailability of Cd is unclear, since results from previous chapters indicate that, in the short term, accumulation was not reduced when Cd speciation was altered in this manner. It is possible that these Cd-complexes are in equilibrium with dissolved Cd^{2+} and have a similar bioavailability.

CHAPTER 7:

SUMMARY

7.1 Free Ion Model

The first objective of the research presented in this thesis was to determine how the bioavailability of dissolved Cd was influenced by altering exposure solution chemistry. This was done by adding compounds (both natural and synthetic, organic and inorganic) which formed soluble complexes with Cd (CdL_n^{2-nz}) or by altering concentrations of Ca^{2+} and Mg^{2+}, since these ions might compete with Cd^{2+} for uptake. The study was a test of the Free Ion Model (FIM), which relates toxicity or accumulation of a dissolved metal to the free ion (M^{z+}) concentration of the metal in solution. This model assumes that 1) the effect of the metal is proportional to the extent of occupancy of cell surface binding sites (by the free ion and not a complexed form), 2) there are no other metals which interact with either dissolved ligands or cell surface binding sites and 3) the rate limiting step in the process is the metal interacting with cell surface binding sites; diffusion to these sites is not rate limiting.

The results presented in chapters 2 and 3 demonstrate exceptions to the FIM. The free ion concentration of Cd, Cd^{2+}, did not accurately predict accumulation of Cd by root tissue. Addition of citrate, EDTA, or excess SO_4^{2-} resulted in the formation of Cd complexes in solution, and when complexed forms of Cd were present, the solution Cd^{2+} concentration underestimated the accumulation of Cd by wheat roots.

Altering the Ca^{2+} and Mg^{2+} concentrations in solution influenced Cd accumulation by wheat roots, indicating that these ions may share a similar uptake mechanism. Citrate and SO_4^{2-} formed complexes with Ca^{2+} and Mg^{2+} as well as Cd^{2+}. It appears that the second assumption of the FIM was not met, since Cd^{2+} was competing with Ca^{2+} and Mg^{2+}

for both the dissolved ligands (citrate and SO_4^{2-}) and the cell surface binding sites. However, Cd^{2+} was not competing with Ca^{2+} or Mg^{2+} to form complexes with EDTA.

The results demonstrate that Cd^{2+} was not the only form of dissolved Cd which was bioavailable to the wheat roots. When the proportion of Cd^{2+} was reduced by adding ligands which complexed Cd, accumulation did not decrease. This was true when the complexed form of Cd was $CdCitrate^-$, $CdEDTA^{2-}$, or $CdSO_4^0{}_{(aq)}$. There are two possible explanations for the apparent bioavailability of complexed forms of Cd. The first possible explanation is that some complexed forms of Cd are able to cross cell membranes. Citrate is known to be secreted by durum wheat roots (Cieslinski *et al.*, 1997), and it has been shown to be accumulated by the alga *Selenastrum capricornutum* (Errécalde *et al.*, 1998), so it is possible that $CdCitrate^-$ was accumulated by wheat roots as well. Membranes of plant roots are quite permeable to small, charged ions; SO_4^{2-} is an essential plant nutrient and is accumulated by tissue. It is possible that $CdSO_4^0{}_{(aq)}$ was also accumulated, although this species is uncharged. The chelating agent EDTA is a large, synthetic compound, and it is a little more difficult to believe that it is very membrane permeable, so it is less probable that $CdEDTA^{2-}$ complexes were accumulated by roots, yet similar enhancement of Cd accumulation was noted with EDTA as with citrate.

The second possible explanation is that the third assumption of the FIM (that diffusion to uptake sites is not rate limiting) was not being met. This assumes the presence of an unstirred, or boundary layer, surrounding roots in solution. In this scenario, accumulation of Cd resulted in a depletion of Cd immediately surrounding the root surface, since replenishment of Cd to this region of the solution from the bulk solution was

slower than actual uptake of Cd by the wheat roots. Assuming that the free ion, Cd^{2+}, was the form of Cd accumulated, complexed forms of Cd would have dissociated once the Cd^{2+} began to decline, since the free ionic and complexed forms of Cd were in equilibrium with each other. In such a situation, the Cd^{2+} concentration surrounding the root would be buffered by the dissociation of Cd complexes, resulting in enhanced diffusion of Cd to the root surface. The results of the swirling experiment presented in Chapter 4 did not demonstrate enhanced accumulation of Cd; however, it was not possible to directly measure the thickness of the boundary layer, or what influence swirling had on this layer. Theoretical calculations of the flux through the boundary layer indicate that it is very possible that diffusion was rate limiting, and the observation that accumulation of Cd was closely related to the total Cd concentration in solution is consistent with the model by Tessier *et al.* (1994) of metal accumulation when diffusion is rate limiting.

Accumulation data presented represent both the Cd taken up by root cells plus Cd present in the apoplast. Free Cd^{2+} in the apoplast of the root would be in equilibrium with Cd bound to the cell wall, and as the Cd^{2+} concentration in the apolastic solution declined (due to uptake), equilibrium would shift to maintain the free ion (Cd^{2+}) concentration in the apoplastic solution. Under conditions where diffusion through the boundary layer was rate limiting, it is possible that very little Cd was bound to cell walls, as the Cd^{2+} concentration in the apoplastic solution would be very low. Hart *et al.* (1998) demonstrated that when roots were exposed to low ^{109}Cd concentrations, very little ^{109}Cd was removed by exchange with cold Cd.

The results presented in chapters 2, 3 and 4 of the thesis provide valuable

information about which forms of dissolved Cd are available to wheat roots. The results demonstrate that attempting to relate accumulation of dissolved Cd to the Cd^{2+} concentration can underestimate accumulation of Cd by plant tissue. This is relevant to regulating soil water for the protection of crops. It is also relevant for those wishing to clean up metal contaminated soils through the use of phytoremediation. In soils, boundary layers around roots may be larger than around roots in hydroponic solution, so diffusion may be what determines accumulation of ions which are present in low concentrations. It may be necessary to use total dissolved metal, and not free ion concentrations, to protect crops. For phytoremediation, the use of compounds to solubilize Cd (soluble chelators) may result in enhanced accumulation of dissolved metals despite having low free ion concentrations in the soil solution.

While the results presented in this section of the thesis demonstrate exceptions to the FIM, they do not clearly explain why this exception occurred. Future research should focus on answering the question of whether or not diffusion of metals to uptake sites is rate limiting. It may be possible to do this with the use of metal microelectrodes, which can determine free ion metal concentrations on a very localized scale. Under conditions where plants are exposed to very low concentrations of dissolved metals, it seems reasonable to develop models to relate accumulation to the total dissolved metal concentration, the rate of diffusion, and the thickness of the boundary layer. It is also important to determine the metal concentration above which the rate of diffusion is no longer the rate limiting step in accumulation of the metal by the plant, since above this concentration, it is the plant characteristics which would determine the rate of

accumulation.

7.2 Effect of Morphology on Accumulation

The second objective was to determine if differences in Cd accumulation by root tissue of two cultivars of durum wheat could be related to observed differences in root morphology of the two cultivars. The results presented in Chapter 5 demonstrate that differences in the root Cd concentration of 'Kyle' and 'Arcola' were consistent with observed cultivar differences in root characteristics thought to be important in ion uptake.

Root systems of 'Arcola' had more surface area and root tips, and greater ratios of surface area:root dry weight and number of rot tips:root dry weight, and this was associated with the observation that 'Arcola' contained more Cd (μg Cd), and had higher concentrations of Cd (μg $Cd \cdot g^{-1}$ root) in its roots than 'Kyle'.

There is often a large amount of variation in the amount of Cd taken up by different species (or cultrivars) growing in similar soils, and part of the reason may be due to differences in root morphology. In addition, the same plant growing in different soils containing similar amounts of Cd may accumulate different amounts of Cd, and while some of this variation is undoubtably due to differences in bioavailability of Cd, it is entirely possible that some of this variation may be due to differences in root morphology resulting from altered soil conditions the plants were growing in. Closely related species, or cultivars of the same species, may differ in root morphology, and this may partially explain differences in Cd accumulation from soils of similar Cd concentration and chemistry. Environmental conditions, such as soil moisture or nutrient content, can

influence the root morphology of a species, and these differences may be responsible for variation in Cd accumulation.

Further research could be done to test and expand this hypothesis. The study examined two cultivars of durum wheat which differed in their Cd accumulation by root tissue. There are many more cultivars of durum wheat, with a range of Cd accumulation, and the study should be expanded to include some more cultivars. It may also be possible to grow a single durum wheat cultivar under conditions with different nutrient levels, in order to result in differences in root morphology. If differences in root morphology exist, and these differences are consistent with observed differences in Cd accumulation upon exposure to Cd under similar conditions, then this theory would be strengthened. It would also be interesting to attempt these experiments in soil grown plants, although analysis of root morphology would be complicated in that case.

7.3 Cultivar Differences in Root to Shoot Translocation of Cd

The objective of the experiment reported in chapter 6 was to see if reported differences in grain accumulation were reflected by differences in root accumulation or root to shoot translocation of Cd by wheat seedlings. The results of this experiment demonstrate that root Cd concentrations in 'Kyle' and 'Arcola' seedlings were not significantly different from each other, but that shoot Cd concentrations were significantly lower in 'Arcola' than 'Kyle', but only when seedlings were exposed to low Cd concentrations. The cultivar difference in root Cd concentration was not significant in this case due to the smaller sample size of the study. These differences are consistent with

previously reported cultivar differences in grain accumulation of Cd.

The results suggest that Cd is more mobile in 'Arcola' than 'Kyle'. 'Kyle' may have an increased capacity to form mobile Cd complexes which are transported to the shoot, or 'Arcola' may have an increased capacity to complex Cd and immobilize it in the roots. Whatever the mechanism, it appears to be saturable, since the cultivar difference was lost when seedlings were exposed to higher Cd concentrations. Experiments with the goal of evaluating Cd speciation within the various tissues may help determine the fate of Cd once accumulated, and cultivar differences in mobility of Cd. Perhaps an analysis of xylem contents may answer this question. Research in this direction would be very valuable to those people involved in phytoremediation of metal contaminated soils, since in that field, it is important to have species which not only accumulate high concentrations of metals from soil, but also translocate a large portion of the metals to shoots, which could then be easily harvested and disposed.

LIST OF REFERENCES

Ahner, B. A. and Morel, F. F. M.. 1995. Phytochelatin production in marine algae. 2. Induction by various metals. Limnol. & Oceanogr. **40**: 658-665.

Baker, A. J. M. 1981. Accumulators and excluders - Strategies in the response of plants to heavy metals. J. Plant Nutr. **3**: 643-654.

Baker, A. J. and Walker, P. L. 1990. Ecophysiology of metal uptake by tolerant plants. *In* Heavy metal tolerance in plants: Evolutionary aspects. *Edited by* A. Jonathon Shaw. CRC Press Inc., Boca Raton, Fla. pp. 156-177.

Basu, U., Basu, A., and Taylor, G. J. 1994a. Differential exudation of polypeptides by roots of aluminum-resistant and aluminum-sensitive cultivars of *Triticum aestivum* L. in response to aluminum stress. Plant Physiol. **106**: 151-158.

Basu, U., Godbold, D., and Taylor, G. J. 1994b. Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. J. Plant Physiol. **144**: 747-753.

Bell, , P. F., Chaney, R. L., and Angle, J. S. 1991. Determination of the copper^{2+} activity required by maize using chelator-buffered nutrient solutions. Soil Sci. Am. J. **55**: 1366-1374.

Berkelaar, E. and Hale, B. 2000. The relationship between root morphology and cadmium accumulation in seedlings of two durum wheat cultivars. Can. J. Bot. (In Press).

Bowen, G. D. and Rovira, A. D. 1971. Relationship between root morphology and nutrient uptake. *In* Colloquium on plant analysis and fertilizer problems, 6th, Tel-Aviv, 1970. Recent advances in plant nutrition. *Edited by* R. M. Samish. Gordon and Breach Science Publishers, New York, NY. pp. 293-305.

Buckley, W. T., Buckley, K. E., and Grant, C. A. 1997. Adsorption, adsorption and translocation of cadmium in high-cadmium and low-cadmium accumulating lines of durum wheat. *In* Forth international conference on the biogeochemistry of trace elements, extended abstracts. *Edited by* I. S. Iskandar, S. E. Hardey, A. C. Chang, and G. M. Pierzynski. United States Cold Regions Research and Engineering Laboratory, Hanover New Hampshire. pp 129-130.

Campbell, P. G. C. 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D. R. Turner, John Wiley & Sons, Chichester, England. pp 45-102.

Canadian Environmental Protection Act. 1994. Priority substances list assessment report: Cadmium and its compounds. Canadian Department of the Environment.

Cantwell, F. F., Nielsen, J. S. and Hrudey, S. E. 1982. Free nickel ion concentration in sewage by an ion exchange column-equilibration method. Anal. Chem. **54**: 1498-1503.

Chan, D. Y. 1996. The influence of lifestage and cultivar on the distribution of cadmium in durum wheat (*Triticum turgidum* L. var durum, cvs Kyle and Arcola). M.Sc. thesis, Department of Horticultural Science, University of Guelph, Guelph, ON.

Chanmugathas, P. and Bollag, J.-M. 1988. A column study of the biological mobilization and speciation of cadmium in soil. Arch. Environ. Contam. Toxicol. **17**: 229-237.

Cherian, M. G., O'Heany, J. and Kusiak, R. A. 1985. Health effects of cadmium and its inorganic compounds. Prepared for the Ontario Ministry of Labour. Occupational Health and Safety Division.

Cieslinski, G., Van Rees, K. C., Szmigielska, A. M., and Huang, P. M. 1997. Low molecular weight organic acids released from roots of durum wheat and flax into sterile nutrient solutions. J. Plant Nutr. **20**: 753-764.

Clemensson-Lindell, A. and Asp, H. 1995. Fine-root morphology and uptake of ^{32}P and ^{35}S in a Norway spruce (*Picea abies* (L.) Karst.) stand subjected to various nutrient and water supplies. Plant Soil. **173**:147-155.

Coughtrey, P.J. and Martin, M.H. 1978. Cadmium uptake and distribution in tolerant and non-tolerant populations of *Holcus lanatus* grown in solution culture. OIKO. **30**: 555-560.

Delhaize, E., Ryan, P. R. and Randall, P. J. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.): II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol. **103**: 695-702.

de Knecht, J. A., Koevoets, P. L. M., Verkleij, J. A. C. and Ernst, W. H. O. 1992. Evidence against a role for phytochelatins in naturally selected increased cadmium tolerance in *Silene vulgaris* (Moench) Garcke. New Phytol. **122**: 681-688.

de Knecht, J. A., van Dillen, M., Koevoets, P. L. M., Schat, H., Verkleij, J. A. C., and Ernst, W. H. O. 1994. Phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*: Chain length distribution and sulfide incorporation. Plant Physiol. **104**: 255-261.

Department of the Environment. 1980. Cadmium in the environment and its significance to man. Pollution Paper No. 17, Central Directorate on Environmental Pollution. Her Majesty's Stationary Office, London.

Dong, B., Rengel, Z., and Graham, R. D. 1995. Root morphology of wheat genotypes differing in zinc efficiency. J. Plant Nutr. **18**: 2761-2773.

Errécalde, O., Seidl, M., and Campbell, P. G. C. 1998. Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga *Selenastrum capricornutum*: An exception to the free-ion model. Wat. Res. **132(2)**: 419-429.

Fortin, C. and Campbell, P. G. C. 1997. Peran ion-exchange technique for free-metal ion measurements (Cd^{2+}, Zn^{2+}): Applications to complex aqueous media. Int. J. Env. Anal. Chem. 1-38

Föhse, D., Classen, N., and Jungk, A. 1988. Phosphorus efficiency of plants. I. External and internal P requirement and P uptake efficiency of different plant species. Plant Soil. **110**: 101-109.

Garrett, R. G. 1994. The distribution of cadmium in A horizon soils in the prairies of Canada and adjoining United States. Current Research **1994-B**; Geological Survey of Canada, 73-82.

Grill, E., Löffler, S., Winnacker, E.-L., and Zenk, M. H. 1989. Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc. Natl. Acad. Sci. USA. **86**: 6838-6842.

Hart, J. J., Welch, R. M., Norvell, W. A., Sullivan, L. A., and Kochian, L. V. 1998a. Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol. **116**: 1413-1420.

Hart, J. J., Norvell, W. A., Welch, R. M., Sullivan, L. A., and Kochian, L. V. 1998b. Characterization of zinc uptake, binding, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol. **118**: 219-226.

Hoagland, D. R. and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Expt. Sta. **Cir. 347.** Agricultural Productions, Univ. of Calif., Berkeley, Calif., 94720.

Horst, W. J., Abdou, M., and Wiesler, F. 1993. Genotypic differences in phosphorus efficiency of wheat. Plant Soil. **155/156**: 293-296.

Hudson, R. J. M. 1998. Which aqueous species control the rates of trace metal uptake by aquatic biota? Observations and predictions of non-equilibrium effects. Sci. Total Environ. **219**: 95-115.

Itoh, S. and Barber, S. A. 1983. Phosphorus uptake by six plant species as related to root hairs. Agron. J. **75**: 457-461.

Jackson, G. A. and Morgan, J. J. 1978. Trace metal-chelator interactions and phytoplankton growth in seawater media: Theoretical analysis and comparison with reported observations. Limnol. & Oceanogr. **23**: 268-282.

Jalil, A., Selles, F. and Clark, J. M. 1994. Effect of cadmium on growth and the uptake of cadmium and other elements by durum wheat. J. Plant Nutr. **17(11)**: 1839-1858.

Jastrow, J. and Koeppe, D. E. 1980. Uptake and effects of cadmium in higher plants. *In* Cadmium in the environment, Part I: Ecological cycling. *Edited by* J. O. Nriagu. John Wiley & Sons, Inc., New York, NY. pp. 608-638.

Kubota, J., Welch, R. M., and Van Campen, D. R. 1992. Partitioning of cadmium, copper, lead and zinc amongst above-ground parts of seed and grain crops in selected locations in the USA. Environ. Geochem. Health. **14**: 91-100.

Lester, J. N. 1987. Heavy metals in wastewater and sludge treatment processes. Vol. I: Sources, analysis, and legislation. CRC Press Inc., Boca Raton, FL.

Li, Y.-H. And Gregory, S. 1974. Diffusion of ions in sea water and deep-sea sediments. Geochim. Cosmochim. Acta. **38**: 703-714.

Lynch, J. 1995. Root architecture and plant productivity. Plant Physiol. **109**: 7-13.

Marschner, H. 1995. Mineral nutrition of higher plants. Second edition. Academic Press. Harcourt Brace & Company, Publishers, London.

Maas, F. M., van de Wetering, D. A. M., van Beusichem, M. L., Bienfait, H. F. 1988. Characterization of phloem iron and its possible role in regulation of Fe-efficiency reactions. Plant Physiol. **87:** 167-171.

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

McLaughlin, M. J., Andrew, S. J., Smart, M. K., and Smolders, E. 1998. Effects of sulfate on cadmium uptake by Swiss chard: I. Effects of complexation and calcium competition in nutrient solutions. Plant Soil. **202**: 211-216.

Miyasaka, S. C., Buta, J. G., Howell, R. K., and Foy, C. D. 1991. Mechanism of aluminum tolerance in snapbeans: Root exudation of citric acid. Plant Physiol. **96**: 737-743.

Morel, F. M. M. and Hering, J. G. 1993. Principles and applications of aquatic chemistry. John Wiley & Sons, Inc., New York, NY.

Nasu, Y., Kugimoto, M., Tanaka, O., and Takimoto, A. 1983. Comparative studies on the absorption of cadmium and copper in *Lemna paucicostata*. Environ. Pollut. Ser. A **32:** 201-209.

Ojima, K. and Ohira, K. 1985. Reduction of aluminum toxicity by addition of a conditioned medium from aluminum-tolerant cells of carrot. Plant Cell Physiol. **26**: 281-286.

Parker, D. R. and Pedler, J. F. 1997. Reevaluating the free-ion activity model of trace metal availability to higher plants. Plant Soil. **196**: 223-228.

Piñeros, M. A., Shaff, J. E., and Kochian, L. V. 1998. Development, characterization, and application of a cadmium-selective microelectrode for the measurement of cadmium fluxes in roots of *Thlaspi* species and wheat. Plant Physiol. **116**: 1393-1401.

Rauser, W. E. 1995. Phytochelatins and related peptides: structure, biosynthesis, and function. Plant Physiol. **109**: 1141-1149.

Rauser, W. E. and Meuwly, P. 1995. Retention of cadmium in roots of maize seedlings: Role of complexation by phytochelatins and related thiol peptides. Plant Physiol. **109**: 195-202.

Raven, J. A., Osborne, B. A., and Johnston, A. M. 1985. Uptake of CO_2 by aquatic vegetation. Plant Cell Environ. **8**: 417-425.

Robinson, D. 1986. Limits to nutrient inflow rates in roots and root systems. Physiol. Plantarum. **68**: 551-559.

Rubio, G., Oesterheld, M., Alvarex, C.R. and Lavado, R.S. 1997. Mechanisms for the increase in phosphorus uptake of waterlogged plants: soil phosphorus availability, root morphology and uptake kinetics. Oecologia **112**: 150-155.

Salisbury, F. B. and Ross, F. B. 1992. Plant physiology, Forth Edition. Wadsworth Publishing Company, Belmont, California.

Salt, D. E. and Rauser, W. E. 1995. MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol. **107**: 1293-1301.

Salt, D. E. and Wagner, G. J. 1993. Cadmium transport across tonoplast of vesicles from oat roots: Evidence for a Cd^{2+}/H^{+} antiport activity. J. Biol. Chem. **268**: 12297-12302.

Schecher, W. D. and McAvoy, D. C. 1994. MINEQL^{+}: A chemical equilibrium program for personal computers. Users Manual, Version 3.0. Environmental Research Software, Hallowell, Maine.

Senden, M. H. M. N., van der Meer, A. J. G. M., Verburg, T. G., and Wolterbeek, H. Th. 1995. Citric acid in tomato plant roots and its effect on cadmium uptake and distribution. Plant Soil. **171**: 333-339.

Sharpe, A. G. 1992. Inorganic chemistry. (Third Edition) Longman Group Limited, Essex, England.

Simkiss, K., and Taylor, M. G. 1995. Transport of metals across membranes. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D. R. Turner, John Wiley & Sons, Chichester, England. pp1-44.

Smith, R. M., Martell, A. E., and Motekaitis, R. J. 1997. NIST critical stability constants of metal complexes database [NIST Standard Reference Database 46]. Version 4.0. Gaithersburg (MD): U.S. Department of Commerce **41**.

Smolders, E. and McLaughlin, M. J. 1996a. Chloride increases cadmium uptake in Swiss chard in a resin-buffered nutrient solution. Soil Sci. Soc. Am. J. **60** :1443-1447.

Smolders, E. and McLaughlin, M. J. 1996b. Effect of Cl on Cd uptake by Swiss chard in nutrient solutions. Plant Soil. **179**: 57-64.

Srivastava, A.and Appenroth, K.-J. 1995. Interaction of EDTA and iron on the accumulation of Cd^{2+} in duckweeds (*Lemnaceae*). J. Plant Physiol. **146**: 173-176.

Taylor, G. J. 1987. Exclusion of metals from the symplasm: A possible mechanism of metal tolerance in higher plants. J. Plant Nutr. **10**: 1213-1222.

Taylor, G. J. and Foy, C. D. 1985. Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. **63**: 2181-2186.

Tessier, A., Buffle, J., and Campbell, P. G. C. 1994. Uptake of trace metals by aquatic organisms. *In* Chemical and biological regulation of aquatic systems. *Edited by* J. Buffle and R. R. De Vitre. CRC Press, Inc., Boca Raton, FL. pp 197-230.

Topper, K. and Kotuby-Amacher, J. 1990. Evaluation of a closed vessel acid digestion method for plant analysis using inductively coupled plasma spectroscopy. Commun. in Soil Sci. Plant Anal. **21**: 1437-1455.

Turner, R. G. and Marshall, C. 1972. The accumulation of zinc by subcellular fractions of roots of *Agrostis tenuis* Sibth. in relation to zinc tolerance. New Phytol. **71(4)**: 671-676.

Tyler, L. D. and McBride, M. B. 1982. Influence of Ca, pH and humic acid on Cd uptake. Plant Soil. **64**: 259-262.

Uren, N. C. and Reisenauer, H. M. 1988. *In* The role of root exudation in nutrient acquisition. *Edited by* B. Tinker and A. Lauchli. Advances in Plant Nutrition, Praeger, New York. pp 79-114.

Vancura, V. 1964. Root exudates from plants: Analysis of root exudates of barley and wheat in their initial phases of growth. Plant Soil. **21(2)**: 231-248.

Vögeli-Lange, R. and Wagner, G. J. 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Plant Physiol. **92**: 1086-1093.

Wagner, G. J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. **51**: 173-212.

Weis, M. and Barclay, G. F. 1985. Distribution of heavy metal and organic contaminants in plants and soils of Windsor and Essex County, Ontario. J. Great Lakes Res. **11(3)**: 339-346.

White, M. C., Decker, A. M., and Chaney, R. L. 1981. Metal complexation in xylem fluid. I. Chemical composition of tomato and soybean stem exudate. Plant Physiol. **67**: 292-300.

Whitfield, M. and Turner, D. R. 1979. Critical assessment of the relationship between biological thermodynamic and electrochemical availability. *In* Chemical modeling in aqueous systems - Speciation, sorption, solubility, and kinetics. *Edited by* E. A. Jenne. American Chemical Society Symposium Series 93. Washinfton, D. C. pp 657-680.

WHO. 1989. 33rd report of the joint FAO/WHO Codex committee on food additives. Evaluation of certain food additives and contaminants. WHO Technical Report Series 776.

Zhang, F., Romheld, V., and Marschner, H. 1991. Release of zinc mobilizing root exudates in different plant species as affected by zinc nutritional status. J. Plant Nutr. **14**: 675-686.

APPENDIX A:

RAW DATA

Table A.1: Raw data gathered from experiment 1 (Chapter 2). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure [Cd^{2+}] from MINEQL^{+}. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure [Cd^{2+}] (ppb)	ln[Cd^{2+}]	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	1.509500	1.325341	0.281670	0.69475	0.0161
	2	Kyle	30	1.509500	1.325341	0.281670	1.96593	0.0173
	3	Kyle	60	1.509500	1.325341	0.281670	0.73157	0.0197
1.0 ppb	4	Kyle	90	1.509500	1.325341	0.281670	0.80260	0.0205
Cd	5	Kyle	120	1.509500	1.325341	0.281670	0.70643	0.0212
	6	Kyle	150	1.509500	1.325341	0.281670	0.74386	0.0176
	7	Kyle	180	1.509500	1.325341	0.281670	0.95384	0.0267
	8	Kyle	210	1.509500	1.325341	0.281670	1.06437	0.0228
	9	Kyle	0	6.079500	5.337801	1.674814	1.89212	0.0084
	10	Kyle	30	6.079500	5.337801	1.674814	1.12371	0.0200
	11	Kyle	60	6.079500	5.337801	1.674814	1.37483	0.0228
5.0 ppb	12	Kyle	90	6.079500	5.337801	1.674814	1.83029	0.0172
Cd	13	Kyle	120	6.079500	5.337801	1.674814	1.60470	0.0230
	14	Kyle	150	6.079500	5.337801	1.674814	2.07602	0.0179
	15	Kyle	180	6.079500	5.337801	1.674814	1.99470	0.0160
	16	Kyle	210	6.079500	5.337801	1.674814	2.51552	0.0182
	17	Kyle	0	15.765000	13.841670	2.627684	1.21105	0.0150
	18	Kyle	30	15.765000	13.841670	2.627684	1.80033	0.0180
	19	Kyle	60	15.765000	13.841670	2.627684	1.74890	0.0206
10.0 ppb	20	Kyle	90	15.765000	13.841670	2.627684	2.43995	0.0181
Cd	21	Kyle	120	15.765000	13.841670	2.627684	3.30453	0.0207
	22	Kyle	150	15.765000	13.841670	2.627684	3.01715	0.0222
	23	Kyle	180	15.765000	13.841670	2.627684	4.65332	0.0147
	24	Kyle	210	15.765000	13.841670	2.627684	5.61891	0.0193
	25	Kyle	0	68.275000	59.945450	4.093435	1.27931	0.0155
	26	Kyle	30	68.275000	59.945450	4.093435	1.99735	0.0206
	27	Kyle	60	68.275000	59.945450	4.093435	3.58073	0.0212
50.0 ppb	28	Kyle	90	68.275000	59.945450	4.093435	3.26926	0.0157
Cd	29	Kyle	120	68.275000	59.945450	4.093435	6.67743	0.0227
	30	Kyle	150	68.275000	59.945450	4.093435	8.11042	0.0195
	31	Kyle	180	68.275000	59.945450	4.093435	7.94412	0.0206
	32	Kyle	210	68.275000	59.945450	4.093435	14.90531	0.0245

	33	Arcola	0	1.509500	1.325341	0.281670	0.84903	0.0220
	34	Arcola	30	1.509500	1.325341	0.281670	0.90680	0.0148
	35	Arcola	60	1.509500	1.325341	0.281670	1.67647	0.0261
1.0 ppb	36	Arcola	90	1.509500	1.325341	0.281670	1.85796	0.0139
Cd	37	Arcola	120	1.509500	1.325341	0.281670	1.21344	0.0269
	38	Arcola	150	1.509500	1.325341	0.281670	1.27888	0.0257
	39	Arcola	180	1.509500	1.325341	0.281670	1.58085	0.0185
	40	Arcola	210	1.509500	1.325341	0.281670	1.99049	0.0234
	41	Arcola	0	6.079500	5.337801	1.674814	0.72901	0.0216
	42	Arcola	30	6.079500	5.337801	1.674814	1.34160	0.0279
	43	Arcola	60	6.079500	5.337801	1.674814	2.65701	0.0197
5.0 ppb	44	Arcola	90	6.079500	5.337801	1.674814	2.77909	0.0211
Cd	45	Arcola	120	6.079500	5.337801	1.674814	3.65684	0.0199
	46	Arcola	150	6.079500	5.337801	1.674814	4.82690	0.0147
	47	Arcola	180	6.079500	5.337801	1.674814	4.47084	0.0117
	48	Arcola	210	6.079500	5.337801	1.674814	6.97857	0.0185
	49	Arcola	0	15.765000	13.841670	2.627684	0.61213	0.0196
	50	Arcola	30	15.765000	13.841670	2.627684	2.58039	0.0169
	51	Arcola	60	15.765000	13.841670	2.627684	2.81848	0.0281
10.0 ppb	52	Arcola	90	15.765000	13.841670	2.627684	4.39649	0.0198
Cd	53	Arcola	120	15.765000	13.841670	2.627684	5.32652	0.0255
	54	Arcola	150	15.765000	13.841670	2.627684	6.35729	0.0281
	55	Arcola	180	15.765000	13.841670	2.627684	.	
	56	Arcola	210	15.765000	13.841670	2.627684	12.11884	0.0165
	57	Arcola	0	68.275000	59.945450	4.093435	5.43358	0.0078
	58	Arcola	30	68.275000	59.945450	4.093435	8.02297	0.0140
	59	Arcola	60	68.275000	59.945450	4.093435	7.93261	0.0205
50.0 ppb	60	Arcola	90	68.275000	59.945450	4.093435	13.20033	0.0212
Cd	61	Arcola	120	68.275000	59.945450	4.093435	14.53785	0.0225
	62	Arcola	150	68.275000	59.945450	4.093435	16.96146	0.0251
	63	Arcola	180	68.275000	59.945450	4.093435	20.10247	0.0241
	64	Arcola	210	68.275000	59.945450	4.093435	.	0.0183
	65	Kyle	0	14.340000	12.461460	2.522641	0.89881	0.0202
	66	Kyle	30	14.340000	12.461460	2.522641	2.54325	0.0257
10.0 ppb	67	Kyle	60	14.340000	12.461460	2.522641	3.10297	0.0170
Cd; 1/2	68	Kyle	90	14.340000	12.461460	2.522641	2.64739	0.0205
[Ca] &	69	Kyle	120	14.340000	12.461460	2.522641	3.18680	0.0178
1.75 x	70	Kyle	150	14.340000	12.461460	2.522641	4.12189	0.0185
[K]	71	Kyle	180	14.340000	12.461460	2.522641	3.38064	0.0217
	72	Kyle	210	14.340000	12.461460	2.522641	5.68614	0.0131

Table A.2: Raw data gathered from experiment 2 (Chapter 2). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure $[Cd^{2+}]$ from MINEQL^{+}. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure $[Cd^{2+}]$ (ppb)	$\ln[Cd^{2+}]$	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	4.590000	4.030020	1.393771	0.51154	0.0126
	2	Kyle	30	4.590000	4.030020	1.393771	0.57225	0.0210
	3	Kyle	60	4.590000	4.030020	1.393771	1.11323	0.0201
5.0 ppb Cd	4	Kyle	90	4.590000	4.030020	1.393771	1.23595	0.0176
	5	Kyle	120	4.590000	4.030020	1.393771	1.05655	0.0239
	6	Kyle	150	4.590000	4.030020	1.393771	1.56207	0.0240
	7	Kyle	180	4.590000	4.030020	1.393771	2.11910	0.0135
	8	Kyle	210	4.590000	4.030020	1.393771	4.77733	0.0140
	9	Kyle	0	4.849500	3.161874	1.151165	0.36335	0.0168
	10	Kyle	30	4.849500	3.161874	1.151165	0.66959	0.0158
5.0 ppb	11	Kyle	60	4.849500	3.161874	1.151165	1.12514	0.0212
Cd; 0.001	12	Kyle	90	4.849500	3.161874	1.151165	1.05561	0.0162
M Citrate	13	Kyle	120	4.849500	3.161874	1.151165	1.36532	0.0176
	14	Kyle	150	4.849500	3.161874	1.151165	1.73753	0.0220
	15	Kyle	180	4.849500	3.161874	1.151165	2.54390	0.0114
	16	Kyle	210	4.849500	3.161874	1.151165	1.86271	0.0201
	17	Kyle	0	54.595000	47.934410	3.869834	0.41475	0.0188
	18	Kyle	30	54.595000	47.934410	3.869834	2.27482	0.0106
	19	Kyle	60	54.595000	47.934410	3.869834	5.19286	0.0103
50.0 ppb	20	Kyle	90	54.595000	47.934410	3.869834	5.77207	0.0194
Cd	21	Kyle	120	54.595000	47.934410	3.869834	6.69360	0.0181
	22	Kyle	150	54.595000	47.934410	3.869834	4.27230	0.0138
	23	Kyle	180	54.595000	47.934410	3.869834	7.80322	0.0184
	24	Kyle	210	54.595000	47.934410	3.869834	11.03775	0.0081
	25	Kyle	0	52.380000	34.151760	3.530814	0.42449	0.0083
	26	Kyle	30	52.380000	34.151760	3.530814	3.20221	0.0097
50.0 ppb	27	Kyle	60	52.380000	34.151760	3.530814	4.14624	0.0129
Cd; 0.001	28	Kyle	90	52.380000	34.151760	3.530814	4.52408	0.0214
M Citrate	29	Kyle	120	52.380000	34.151760	3.530814	8.12953	0.0134
	30	Kyle	150	52.380000	34.151760	3.530814	6.12806	0.0215
	31	Kyle	180	52.380000	34.151760	3.530814	8.38722	0.0195
	32	Kyle	210	52.380000	34.151760	3.530814	8.48543	0.0212

	33	Arcola	0	4.590000	4.030020	1.393771	0.38102	0.0187
	34	Arcola	30	4.590000	4.030020	1.393771	0.86183	0.0293
	35	Arcola	60	4.590000	4.030020	1.393771	1.22268	0.0217
5.0 ppb Cd	36	Arcola	90	4.590000	4.030020	1.393771	2.27719	0.0236
	37	Arcola	120	4.590000	4.030020	1.393771	2.36172	0.0224
	38	Arcola	150	4.590000	4.030020	1.393771	2.66381	0.0266
	39	Arcola	180	4.590000	4.030020	1.393771	2.66096	0.0246
	40	Arcola	210	4.590000	4.030020	1.393771	3.17665	0.0228
	41	Arcola	0	4.849500	3.161874	1.151165	0.29938	0.0188
	42	Arcola	30	4.849500	3.161874	1.151165	1.03545	0.0227
5.0 ppb	43	Arcola	60	4.849500	3.161874	1.151165	1.60813	0.0250
Cd; 0.001	44	Arcola	90	4.849500	3.161874	1.151165	2.07683	0.0173
M Citrate	45	Arcola	120	4.849500	3.161874	1.151165	2.08063	0.0187
	46	Arcola	150	4.849500	3.161874	1.151165	2.54364	0.0245
	47	Arcola	180	4.849500	3.161874	1.151165	2.91447	0.0297
	48	Arcola	210	4.849500	3.161874	1.151165	3.29603	0.0265
	49	Arcola	0	54.595000	47.934410	3.869834	0.30664	0.0233
	50	Arcola	30	54.595000	47.934410	3.869834	3.85930	0.0259
	51	Arcola	60	54.595000	47.934410	3.869834	7.02715	0.0131
50.0 ppb	52	Arcola	90	54.595000	47.934410	3.869834	7.43493	0.0277
Cd	53	Arcola	120	54.595000	47.934410	3.869834	10.40544	0.0229
	54	Arcola	150	54.595000	47.934410	3.869834	12.06056	0.0211
	55	Arcola	180	54.595000	47.934410	3.869834	14.77300	0.0255
	56	Arcola	210	54.595000	47.934410	3.869834	12.51856	0.0303
	57	Arcola	0	52.380000	34.151760	3.530814	0.25825	0.0137
	58	Arcola	30	52.380000	34.151760	3.530814	.	0.0246
50.0 ppb	59	Arcola	60	52.380000	34.151760	3.530814	7.25853	0.0255
Cd; 0.001	60	Arcola	90	52.380000	34.151760	3.530814	9.40392	0.0274
M citrate	61	Arcola	120	52.380000	34.151760	3.530814	11.63371	0.0264
	62	Arcola	150	52.380000	34.151760	3.530814	11.63479	0.0190
	63	Arcola	180	52.380000	34.151760	3.530814	15.25351	0.0235
	64	Arcola	210	52.380000	34.151760	3.530814	15.22689	0.0162

Table A.3: Raw data gathered from experiment 3 (Chapter 2). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure [Cd^{2+}] from MINEQL^{+}. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure [Cd^{2+}] (ppb)	ln[Cd^{2+}]	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	4.793167	4.208400	1.437083	0.19974	0.0350
	2	Kyle	50	4.793167	4.208400	1.437083	0.86941	0.0291
5.0 ppb Cd	3	Kyle	100	4.793167	4.208400	1.437083	1.08934	0.0305
	4	Kyle	150	4.793167	4.208400	1.437083	2.33619	0.0298
	5	Kyle	200	4.793167	4.208400	1.437083	2.52862	0.0281
	6	Kyle	0	9.414667	8.266077	2.112160	0.18301	0.0239
10.0 ppb	7	Kyle	50	9.414667	8.266077	2.112160	1.27925	0.0237
Cd	8	Kyle	100	9.414667	8.266077	2.112160	2.68718	0.0191
	9	Kyle	150	9.414667	8.266077	2.112160	3.49859	0.0327
	10	Kyle	200	9.414667	8.266077	2.112160	4.96260	0.0343
	11	Kyle	0	51.550000	45.260900	3.812444	0.28626	0.0288
50.0 ppb	12	Kyle	50	51.550000	45.260900	3.812444	4.17093	0.0336
Cd	13	Kyle	100	51.550000	45.260900	3.812444	6.89532	0.0342
	14	Kyle	150	51.550000	45.260900	3.812444	9.03666	0.0276
	15	Kyle	200	51.550000	45.260900	3.812444	8.66336	0.0337
10.0 ppb	16	Kyle	0	9.289333	8.639080	2.156296	0.24816	0.0298
Cd; 1/3	17	Kyle	50	9.289333	8.639080	2.156296	1.80345	0.0226
nutrient	18	Kyle	100	9.289333	8.639080	2.156296	3.51104	0.0328
solution	19	Kyle	150	9.289333	8.639080	2.156296	6.05229	0.0299
	20	Kyle	200	9.289333	8.639080	2.156296	6.77514	0.0328
50.0 ppb	21	Kyle	0	50.833333	47.275000	3.855982	0.18007	0.0321
Cd; 1/3	22	Kyle	50	50.833333	47.275000	3.855982	7.98630	0.0210
nutrient	23	Kyle	100	50.833333	47.275000	3.855982	11.44006	0.0266
solution	24	Kyle	150	50.833333	47.275000	3.855982	8.55689	0.0310
	25	Kyle	200	50.833333	47.275000	3.855982	11.59580	0.0303
	26	Kyle	0	10.020667	2.966117	1.087254	0.24725	0.0271
10.0 ppb	27	Kyle	50	10.020667	2.966117	1.087254	1.60934	0.0383
Cd; 0.003	28	Kyle	100	10.020667	2.966117	1.087254	3.23971	0.0287
M Citrate	29	Kyle	150	10.020667	2.966117	1.087254	3.32509	0.0278
	30	Kyle	200	10.020667	2.966117	1.087254	4.78829	0.0273
	31	Kyle	0	51.530000	15.252880	2.724768	0.27309	0.0301
50.0 ppb	32	Kyle	50	51.530000	15.252880	2.724768	4.22631	0.0326
Cd; 0.003	33	Kyle	100	51.530000	15.252880	2.724768	6.76315	0.0307
M Citrate	34	Kyle	150	51.530000	15.252880	2.724768	8.21503	0.0314
	35	Kyle	200	51.530000	15.252880	2.724768	5.91811	0.0290

	36	Arcola	0	4.793167	4.208400	1.437083	0.15510	0.0281
	37	Arcola	50	4.793167	4.208400	1.437083	0.79774	0.0380
5.0 ppb Cd	38	Arcola	100	4.793167	4.208400	1.437083	2.12091	0.0280
	39	Arcola	150	4.793167	4.208400	1.437083	6.49973	0.0350
	40	Arcola	200	4.793167	4.208400	1.437083	2.93802	0.0318
	41	Arcola	0	9.414667	8.266077	2.112160	0.16258	0.0220
10.0 ppb	42	Arcola	50	9.414667	8.266077	2.112160	1.74190	0.0279
Cd	43	Arcola	100	9.414667	8.266077	2.112160	3.13622	0.0315
	44	Arcola	150	9.414667	8.266077	2.112160	5.44716	0.0220
	45	Arcola	200	9.414667	8.266077	2.112160	4.96081	0.0246
	46	Arcola	0	51.550000	45.260900	3.812444	0.14076	0.0319
50.0 ppb	47	Arcola	50	51.550000	45.260900	3.812444	5.64934	0.0293
Cd	48	Arcola	100	51.550000	45.260900	3.812444	9.77729	0.0215
	49	Arcola	150	51.550000	45.260900	3.812444	11.52395	0.0350
	50	Arcola	200	51.550000	45.260900	3.812444	13.03558	0.0330
10.0 ppb	51	Arcola	0	9.289333	8.639080	2.156296	0.18755	0.0254
Cd; 1/3	52	Arcola	50	9.289333	8.639080	2.156296	2.38741	0.0282
nutrient	53	Arcola	100	9.289333	8.639080	2.156296	4.94073	0.0283
solution	54	Arcola	150	9.289333	8.639080	2.156296	5.77445	0.0337
	55	Arcola	200	9.289333	8.639080	2.156296	6.90817	0.0261
50.0 ppb	56	Arcola	0	50.833333	47.275000	3.855982	0.16889	0.0299
Cd; 1/3	57	Arcola	50	50.833333	47.275000	3.855982	8.24198	0.0237
nutrient	58	Arcola	100	50.833333	47.275000	3.855982	14.90236	0.0209
solution	59	Arcola	150	50.833333	47.275000	3.855982	15.79036	0.0244
	60	Arcola	200	50.833333	47.275000	3.855982	17.20422	0.0287
	61	Arcola	0	10.020667	2.966117	1.087254	0.18644	0.0371
10.0 ppb	62	Arcola	50	10.020667	2.966117	1.087254	1.32573	0.0322
Cd; 0.003	63	Arcola	100	10.020667	2.966117	1.087254	3.06183	0.0310
M Citrate	64	Arcola	150	10.020667	2.966117	1.087254	4.10189	0.0296
	65	Arcola	200	10.020667	2.966117	1.087254	4.62515	0.0260
	66	Arcola	0	51.530000	15.252880	2.724768	0.21063	0.0340
50.0 ppb	67	Arcola	50	51.530000	15.252880	2.724768	5.95558	0.0278
Cd; 0.003	68	Arcola	100	51.530000	15.252880	2.724768	9.33773	0.0397
M Citrate	69	Arcola	150	51.530000	15.252880	2.724768	11.66521	0.0273
	70	Arcola	200	51.530000	15.252880	2.724768	12.52919	0.0282

Table A.4: Raw data gathered from experiment 4 (Chapter2). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure [Cd^{2+}] from MINEQL^{+}. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure [Cd^{2+}] (ppb)	ln[Cd^{2+}]	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	5.032639	4.418657	1.485836	0.30404	0.0298
	2	Kyle	50	5.032639	4.418657	1.485836	0.53348	0.0357
5.0 ppb Cd	3	Kyle	100	5.032639	4.418657	1.485836	1.02803	0.0322
	4	Kyle	150	5.032639	4.418657	1.485836	1.35816	0.0304
	5	Kyle	200	5.032639	4.418657	1.485836	1.75082	0.0302
	6	Kyle	0	10.008379	8.787357	2.173314	0.28741	0.0308
10.0 ppb	7	Kyle	50	10.008379	8.787357	2.173314	0.84911	0.0347
Cd	8	Kyle	100	10.008379	8.787357	2.173314	1.67395	0.0353
	9	Kyle	150	10.008379	8.787357	2.173314	2.01733	0.0320
	10	Kyle	200	10.008379	8.787357	2.173314	2.84646	0.0391
	11	Kyle	0	49.198915	43.196647	3.765763	0.19835	0.0240
50.0 ppb	12	Kyle	50	49.198915	43.196647	3.765763	3.75977	0.0358
Cd	13	Kyle	100	49.198915	43.196647	3.765763	4.55873	0.0361
	14	Kyle	150	49.198915	43.196647	3.765763	5.10229	0.0391
	15	Kyle	200	49.198915	43.196647	3.765763	5.93172	0.0394
10.0 ppb	16	Kyle	0	10.158836	5.038783	1.617165	0.16134	0.0287
Cd; 0.003	17	Kyle	50	10.158836	5.038783	1.617165	0.67772	0.0294
M Citrate;	18	Kyle	100	10.158836	5.038783	1.617165	1.64874	0.0364
bal Ca^{2+} &	19	Kyle	150	10.158836	5.038783	1.617165	2.24139	0.0393
Mg^{2+}	20	Kyle	200	10.158836	5.038783	1.617165	2.29190	0.0415
50.0 ppb	21	Kyle	0	51.459486	25.523905	3.239615	0.20219	0.0408
Cd; 0.003	22	Kyle	50	51.459486	25.523905	3.239615	3.61822	0.0423
M Citrate;	23	Kyle	100	51.459486	25.523905	3.239615	4.80847	0.0364
bal Ca^{2+} &	24	Kyle	150	51.459486	25.523905	3.239615	5.72437	0.0267
Mg^{2+}	25	Kyle	200	51.459486	25.523905	3.239615	5.47724	0.0384
10.0 ppb	26	Kyle	0	10.268293	8.923147	2.188649	0.19682	0.0332
Cd; 1/2 Ca	27	Kyle	50	10.268293	8.923147	2.188649	1.48966	0.0389
& 1.75 x	28	Kyle	100	10.268293	8.923147	2.188649	2.41491	0.0328
K	29	Kyle	150	10.268293	8.923147	2.188649	2.44041	0.0345
	30	Kyle	200	10.268293	8.923147	2.188649	3.17324	0.0364
10.0 ppb	31	Kyle	0	9.939379	8.687017	2.161830	0.16965	0.0283
Cd; 1/2	32	Kyle	50	9.939379	8.687017	2.161830	1.40638	0.0293
Mg &	33	Kyle	100	9.939379	8.687017	2.161830	2.08470	0.0450
1.375 x K	34	Kyle	150	9.939379	8.687017	2.161830	2.16598	0.0389
	35	Kyle	200	9.939379	8.687017	2.161830	2.62874	0.0399

	36	Arcola	0	5.032639	4.418657	1.485836	0.25660	0.0309
	37	Arcola	50	5.032639	4.418657	1.485836	0.96356	0.0314
5.0 ppb Cd	38	Arcola	100	5.032639	4.418657	1.485836	1.87173	0.0326
	39	Arcola	150	5.032639	4.418657	1.485836	2.57336	0.0361
	40	Arcola	200	5.032639	4.418657	1.485836	2.65645	0.0331
	41	Arcola	0	10.008379	8.787357	2.173314	0.20114	0.0310
10.0 ppb	42	Arcola	50	10.008379	8.787357	2.173314	2.14564	0.0396
Cd	43	Arcola	100	10.008379	8.787357	2.173314	3.72438	0.0347
	44	Arcola	150	10.008379	8.787357	2.173314	4.45687	0.0340
	45	Arcola	200	10.008379	8.787357	2.173314	5.25091	0.0335
	46	Arcola	0	49.198915	43.196647	3.765763	0.18909	0.0377
50.0 ppb	47	Arcola	50	49.198915	43.196647	3.765763	6.09868	0.0466
cd	48	Arcola	100	49.198915	43.196647	3.765763	9.44559	0.0376
	49	Arcola	150	49.198915	43.196647	3.765763	9.46172	0.0417
	50	Arcola	200	49.198915	43.196647	3.765763	12.03053	0.0339
10.0 ppb	51	Arcola	0	10.158836	5.038783	1.617165	0.13634	0.0449
Cd; 0.003	52	Arcola	50	10.158836	5.038783	1.617165	2.18947	0.0487
M Citrate;	53	Arcola	100	10.158836	5.038783	1.617165	3.10923	0.0403
bal Ca^{2+} &	54	Arcola	150	10.158836	5.038783	1.617165	3.77237	0.0386
Mg^{2+}	55	Arcola	200	10.158836	5.038783	1.617165	3.34440	0.0501
50.0 ppb	56	Arcola	0	51.459486	25.523905	3.239615	0.15861	0.0351
Cd; 0.003	57	Arcola	50	51.459486	25.523905	3.239615	6.39362	0.0294
M Citrate;	58	Arcola	100	51.459486	25.523905	3.239615	7.32744	0.0371
bal Ca^{2+} &	59	Arcola	150	51.459486	25.523905	3.239615	10.32347	0.0346
Mg^{2+}	60	Arcola	200	51.459486	25.523905	3.239615	12.01875	0.0360
10.0 ppb	61	Arcola	0	10.268293	8.923147	2.188649	0.12049	0.0310
Cd; 1/2 Ca	62	Arcola	50	10.268293	8.923147	2.188649	2.65202	0.0338
& 1.75 x	63	Arcola	100	10.268293	8.923147	2.188649	4.61052	0.0361
K	64	Arcola	150	10.268293	8.923147	2.188649	4.51952	0.0432
	65	Arcola	200	10.268293	8.923147	2.188649	6.48299	0.0213
10.0 ppb	66	Arcola	0	9.939379	8.687017	2.161830	0.14890	0.0334
Cd; 1/2	67	Arcola	50	9.939379	8.687017	2.161830	3.02141	0.0350
Mg &	68	Arcola	100	9.939379	8.687017	2.161830	3.75447	0.0385
1.375 x K	69	Arcola	150	9.939379	8.687017	2.161830	3.64445	0.0460
	70	Arcola	200	9.939379	8.687017	2.161830	5.18112	0.0384

Table A.5: Raw data gathered from experiment 5 (Chapters 2 and 3). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure $[Cd^{2+}]$ from MINEQL^{+}. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure $[Cd^{2+}]$ (ppb)	ln$[Cd^{2+}]$	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	4.220670	3.705748	1.309885	0.39250	0.0366
	2	Kyle	50	4.220670	3.705748	1.309885	1.03780	0.0215
5.0 ppb Cd	3	Kyle	100	4.220670	3.705748	1.309885	1.06770	0.0342
	4	Kyle	150	4.220670	3.705748	1.309885	1.66190	0.0332
	5	Kyle	200	4.220670	3.705748	1.309885	1.92480	0.0331
	6	Kyle	0	8.463330	7.430804	2.005634	0.46800	0.0308
10.0 ppb	7	Kyle	50	8.463330	7.430804	2.005634	1.37580	0.0296
Cd	8	Kyle	100	8.463330	7.430804	2.005634	1.49370	0.0370
	9	Kyle	150	8.463330	7.430804	2.005634	3.30840	0.0307
	10	Kyle	200	8.463330	7.430804	2.005634	2.79510	0.0404
	11	Kyle	0	48.838300	42.880027	3.758406	0.37760	0.0282
50.0 ppb	12	Kyle	50	48.838300	42.880027	3.758406	4.24980	0.0279
Cd	13	Kyle	100	48.838300	42.880027	3.758406	7.48780	0.0355
	14	Kyle	150	48.838300	42.880027	3.758406	7.38260	0.0375
	15	Kyle	200	48.838300	42.880027	3.758406	10.09200	0.0357
	16	Kyle	0	8.62267	1.17268	0.159294	0.3436	0.0258
10.0 ppb	17	Kyle	50	8.62267	1.17268	0.159294	1.0832	0.0358
Cd &	18	Kyle	100	8.62267	1.17268	0.159294	2.1852	0.0351
EDTA	19	Kyle	150	8.62267	1.17268	0.159294	2.3002	0.0304
	20	Kyle	200	8.62267	1.17268	0.159294	3.2622	0.0328
	21	Kyle	0	48.53330	13.92906	2.633977	0.399	0.0328
50.0 ppb	22	Kyle	50	48.53330	13.92906	2.633977	2.6636	0.031
Cd &	23	Kyle	100	48.53330	13.92906	2.633977	5.7078	0.0318
EDTA	24	Kyle	150	48.53330	13.92906	2.633977	4.4778	0.0338
	25	Kyle	200	48.53330	13.92906	2.633977	9.2682	0.0286
	26	Kyle	0	7.946000	6.881236	1.928798	0.54740	0.0386
10.0 ppb	27	Kyle	50	7.946000	6.881236	1.928798	1.44330	0.0273
Cd; 1/3 Ca	28	Kyle	100	7.946000	6.881236	1.928798	2.89310	0.0273
& 2 x K	29	Kyle	150	7.946000	6.881236	1.928798	3.33180	0.0374
	30	Kyle	200	7.946000	6.881236	1.928798	4.37250	0.0297
10.0 ppb	31	Kyle	0	8.742670	7.632351	2.032396	0.60960	0.0319
Cd; 1/3	32	Kyle	50	8.742670	7.632351	2.032396	1.46070	0.0378
Mg & 1.5	33	Kyle	100	8.742670	7.632351	2.032396	2.76870	0.0404
x K	34	Kyle	150	8.742670	7.632351	2.032396	3.35830	0.0420
	35	Kyle	200	8.742670	7.632351	2.032396	3.84100	0.0328

	36	Arcola	0	4.220670	3.705748	1.309885	0.64830	0.0335
	37	Arcola	50	4.220670	3.705748	1.309885	0.85760	0.0444
5.0 ppb Cd	38	Arcola	100	4.220670	3.705748	1.309885	0.34330	0.0425
	39	Arcola	150	4.220670	3.705748	1.309885	2.86280	0.0410
	40	Arcola	200	4.220670	3.705748	1.309885	2.98010	0.0469
	41	Arcola	0	8.463330	7.430804	2.005634	0.42720	0.0338
10.0 ppb	42	Arcola	50	8.463330	7.430804	2.005634	2.32690	0.0289
Cd	43	Arcola	100	8.463330	7.430804	2.005634	3.01360	0.0452
	44	Arcola	150	8.463330	7.430804	2.005634	4.90750	0.0468
	45	Arcola	200	8.463330	7.430804	2.005634	5.28320	0.0308
	46	Arcola	0	48.838300	42.880027	3.758406	0.41570	0.0400
50.0 ppb	47	Arcola	50	48.838300	42.880027	3.758406	6.89870	0.0422
Cd	48	Arcola	100	48.838300	42.880027	3.758406	18.42300	0.0355
	49	Arcola	150	48.838300	42.880027	3.758406	16.81800	0.0445
	50	Arcola	200	48.838300	42.880027	3.758406	20.53300	0.0401
	51	Arcola	0	8.62267	1.17268	0.159294	0.2169	0.0296
10.0 ppb	52	Arcola	50	8.62267	1.17268	0.159294	2.5952	0.0427
Cd &	53	Arcola	100	8.62267	1.17268	0.159294	3.1003	0.0321
EDTA	54	Arcola	150	8.62267	1.17268	0.159294	4.3599	0.0443
	55	Arcola	200	8.62267	1.17268	0.159294	5.4097	0.0407
	56	Arcola	0	48.53330	13.92906	2.633977	0.4043	0.0261
50.0 ppb	57	Arcola	50	48.53330	13.92906	2.633977	5.2691	0.0341
Cd &	58	Arcola	100	48.53330	13.92906	2.633977	10.817	0.0414
EDTA	59	Arcola	150	48.53330	13.92906	2.633977	16.635	0.0403
	60	Arcola	200	48.53330	13.92906	2.633977	19.539	0.0415
	61	Arcola	0	7.946000	6.881236	1.928798	0.47890	0.0403
10.0 ppb	62	Arcola	50	7.946000	6.881236	1.928798	2.89290	0.0468
Cd; 1/3 Ca	63	Arcola	100	7.946000	6.881236	1.928798	3.92690	0.0388
& 2 x K	64	Arcola	150	7.946000	6.881236	1.928798	6.65600	0.0418
	65	Arcola	200	7.946000	6.881236	1.928798	8.79930	0.0354
10.0 ppb	66	Arcola	0	8.742670	7.632351	2.032396	0.39710	0.0319
Cd; 1/3	67	Arcola	50	8.742670	7.632351	2.032396	2.89080	0.0410
Mg & 1.5	68	Arcola	100	8.742670	7.632351	2.032396	5.51020	0.0444
x K	69	Arcola	150	8.742670	7.632351	2.032396	6.88950	0.0373
	70	Arcola	200	8.742670	7.632351	2.032396	9.26130	0.0382

Table A.6: Raw data gathered from experiment 6 (Chapter 3). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure $[Cd^{2+}]$ from MINEQL$^+$. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure $[Cd^{2+}]$ (ppb)	$\ln[Cd^{2+}]$	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	9.390670	8.245008	2.109608	0.37340	0.0350
10.0 ppb	2	Kyle	50	9.390670	8.245008	2.109608	1.40840	0.0466
Cd	3	Kyle	100	9.390670	8.238270	2.108790	1.51660	0.0618
	4	Kyle	150	9.390670	8.238270	2.108790	2.18320	0.0449
	5	Kyle	200	9.390670	8.238270	2.108790	3.32740	0.0515
	6	Kyle	0	50.355000	43.634410	3.775846	0.40640	0.0402
50.0 ppb	7	Kyle	50	50.355000	43.634410	3.775846	3.62350	0.0440
Cd	8	Kyle	100	50.355000	43.634410	3.775846	3.77900	0.0506
	9	Kyle	150	50.355000	43.634410	3.775846	7.12700	0.0460
	10	Kyle	200	50.355000	43.634410	3.775846	8.07230	0.0422
	11	Kyle	0	10.018220	8.846088	2.179975	0.35140	0.0374
10.0 ppb	12	Kyle	50	10.018220	8.846088	2.179975	1.21535	0.0355
Cd; 3.5x K	13	Kyle	100	10.018220	8.846088	2.179975	1.79380	0.0519
as KNO_3	14	Kyle	150	10.018220	8.846088	2.179975	1.90610	0.0466
	15	Kyle	200	10.018220	8.846088	2.179975	2.73980	0.0518
	16	Kyle	0	50.293330	44.409010	3.793442	0.37180	0.0474
50.0 ppb	17	Kyle	50	50.293330	44.409010	3.793442	3.54460	0.0402
Cd; 3.5x K	18	Kyle	100	50.293330	44.409010	3.793442	4.30020	0.0456
as KNO_3	19	Kyle	150	50.293330	44.409010	3.793442	6.71390	0.0403
	20	Kyle	200	50.293330	44.409010	3.793442	5.81840	0.0394
	21	Kyle	0	9.786890	6.743167	1.908530	0.40460	0.0470
10.0 ppb	22	Kyle	50	9.786890	6.743167	1.908530	1.42840	0.0429
Cd; 3.5x K	23	Kyle	100	9.786890	6.743167	1.908530	2.24730	0.0435
as K_2SO_4	24	Kyle	150	9.786890	6.743167	1.908530	2.24290	0.0346
	25	Kyle	200	9.786890	6.743167	1.908530	7.17770	0.0401
	26	Kyle	0	74.676770	51.452295	3.940655	0.32720	0.0336
50.0 ppb	27	Kyle	50	74.676770	51.452295	3.940655	5.12500	0.0467
Cd; 3.5x K	28	Kyle	100	74.676770	51.452295	3.940655	6.87500	0.0454
as K_2SO_4	29	Kyle	150	74.676770	51.452295	3.940655	9.79020	0.0354
	30	Kyle	200	74.676770	51.452295	3.940655	10.33760	0.0320
	36	Arcola	0	9.390670	8.238270	2.108790	0.27610	0.0431
10.0 ppb	37	Arcola	50	9.390670	8.238270	2.108790	1.94440	0.0466
Cd	38	Arcola	100	9.390670	8.238270	2.108790	3.58760	0.0413
	39	Arcola	150	9.390670	8.238270	2.108790	2.95270	0.0598
	40	Arcola	200	9.390670	8.238270	2.108790	5.57240	0.0453

	41	Arcola	0	50.355000	43.634410	3.775846	0.35352	0.0455
50.0 ppb	42	Arcola	50	50.355000	43.634410	3.775846	5.81260	0.0465
Cd	43	Arcola	100	50.355000	43.634410	3.775846	13.62600	0.0476
	44	Arcola	150	50.355000	43.634410	3.775846	19.03670	0.0409
	45	Arcola	200	50.355000	43.634410	3.775846	11.82000	0.0422
	46	Arcola	0	10.018220	8.846088	2.179975	0.44991	0.0436
10.0 ppb	47	Arcola	50	10.018220	8.846088	2.179975	2.18760	0.0455
Cd; 3.5x K	48	Arcola	100	10.018220	8.846088	2.179975	3.28720	0.0464
as KNO_3	49	Arcola	150	10.018220	8.846088	2.179975	3.13480	0.0472
	50	Arcola	200	10.018220	8.846088	2.179975	5.59770	0.0498
	51	Arcola	0	50.293330	44.409010	3.793442	0.25070	0.0396
50.0 ppb	52	Arcola	50	50.293330	44.409010	3.793442	4.91140	0.0390
Cd; 3.5x K	53	Arcola	100	50.293330	44.409010	3.793442	9.07360	0.0420
as KNO_3	54	Arcola	150	50.293330	44.409010	3.793442	10.68990	0.0577
	55	Arcola	200	50.293330	44.409010	3.793442	14.85960	0.0543
	56	Arcola	0	9.786890	6.743167	1.908530	0.24750	0.0457
10.0 ppb	57	Arcola	50	9.786890	6.743167	1.908530	1.99610	0.0468
Cd; 3.5x K	58	Arcola	100	9.786890	6.743167	1.908530	5.35480	0.0432
as K_2SO_4	59	Arcola	150	9.786890	6.743167	1.908530	5.53200	0.0453
	60	Arcola	200	9.786890	6.743167	1.908530	10.06800	0.0429
	61	Arcola	0	74.676770	51.452295	3.940655	0.22710	0.0432
50.0 ppb	62	Arcola	50	74.676770	51.452295	3.940655	9.83540	0.0449
Cd; 3.5x K	63	Arcola	100	74.676770	51.452295	3.940655	11.39765	0.0512
as K_2SO_4	64	Arcola	150	74.676770	51.452295	3.940655	14.48840	0.0528
	65	Arcola	200	74.676770	51.452295	3.940655	18.49860	0.0385

Table A.7: Raw data gathered from experiment 7 (Chapter 3). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure [Cd^{2+}] from MINEQL$^+$. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure [Cd^{2+}] (ppb)	ln[Cd^{2+}]	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	9.435000	8.283930	2.114317	0.21974	0.0429
10.0 ppb	2	Kyle	50	9.435000	8.283930	2.114317	1.28171	0.0422
Cd	3	Kyle	100	9.435000	8.283930	2.114317	2.25398	0.0433
	4	Kyle	150	9.435000	8.283930	2.114317	2.29705	0.0423
	5	Kyle	200	9.435000	8.283930	2.114317	3.43473	0.0391
	6	Kyle	0	51.891667	45.560883	3.819050	0.44469	0.0353
50.0 ppb	7	Kyle	50	51.891667	45.560883	3.819050	5.19131	0.0425
Cd	8	Kyle	100	51.891667	45.560883	3.819050	4.82968	0.0336
	9	Kyle	150	51.891667	45.560883	3.819050	6.73475	0.0387
	10	Kyle	200	51.891667	45.560883	3.819050	11.15155	0.0396
10.0 ppb	11	Kyle	0	8.871333	5.393771	1.685245	0.30159	0.0372
Cd & 10x	12	Kyle	50	8.871333	5.393771	1.685245	0.98881	0.0451
SO_4^{2-} as	13	Kyle	100	8.871333	5.393771	1.685245	1.34656	0.0443
$MgSO_4$	14	Kyle	150	8.871333	5.393771	1.685245	1.57534	0.0447
	15	Kyle	200	8.871333	5.393771	1.685245	2.04199	0.0482
50.0 ppb	16	Kyle	0	50.178333	30.508427	3.418003	0.28167	0.0315
Cd & 10x	17	Kyle	50	50.178333	30.508427	3.418003	2.79463	0.0456
SO_4^{2-} as	18	Kyle	100	50.178333	30.508427	3.418003	4.35027	0.0372
$MgSO_4$	19	Kyle	150	50.178333	30.508427	3.418003	5.86802	0.0334
	20	Kyle	200	50.178333	30.508427	3.418003	10.05851	0.0368
10.0 ppb	21	Kyle	0	9.383600	5.160980	1.641126	0.29842	0.0359
Cd & 10x	22	Kyle	50	9.383600	5.160980	1.641126	1.76685	0.0477
SO_4^{2-} as	23	Kyle	100	9.383600	5.160980	1.641126	1.63977	0.0494
K_2SO_4	24	Kyle	150	9.383600	5.160980	1.641126	2.55922	0.0506
	25	Kyle	200	9.383600	5.160980	1.641126	3.50668	0.0375
50.0 ppb	26	Kyle	0	47.988333	26.393583	3.273121	0.26778	0.0404
Cd & 10x	27	Kyle	50	47.988333	26.393583	3.273121	2.57039	0.0416
SO_4^{2-} as	28	Kyle	100	47.988333	26.393583	3.273121	4.64987	0.0428
K_2SO_4	29	Kyle	150	47.988333	26.393583	3.273121	7.04631	0.0431
	30	Kyle	200	47.988333	26.393583	3.273121	10.11581	0.0376
10.0 ppb	31	Kyle	0	9.814000	5.652864	1.732162	0.33505	0.0384
Cd & 10x	32	Kyle	50	9.814000	5.652864	1.732162	0.88931	0.0358
SO_4^{2-} as	33	Kyle	100	9.814000	5.652864	1.732162	1.64842	0.0412
$MgSO_4$	34	Kyle	150	9.814000	5.652864	1.732162	2.57267	0.0256
and K_2SO_4	35	Kyle	200	9.814000	5.652864	1.732162	2.40974	0.0344

50.0 ppb	36	Kyle	0	50.771667	29.244480	3.375691	0.32634	0.0363
Cd & 10x	37	Kyle	50	50.771667	29.244480	3.375691	3.15652	0.0435
SO_4^{2-} as	38	Kyle	100	50.771667	29.244480	3.375691	5.09954	0.0472
$MgSO_4$	39	Kyle	150	50.771667	29.244480	3.375691	6.70550	0.0325
and K_2SO_4	40	Kyle	200	50.771667	29.244480	3.375691	6.56766	0.0488
	41	Arcola	0	9.435000	8.283930	2.114317	0.20542	0.0392
10.0 ppb	42	Arcola	50	9.435000	8.283930	2.114317	2.05089	0.0452
Cd	43	Arcola	100	9.435000	8.283930	2.114317	3.15842	0.0539
	44	Arcola	150	9.435000	8.283930	2.114317	4.08095	0.0349
	45	Arcola	200	9.435000	8.283930	2.114317	5.39031	0.0522
	46	Arcola	0	51.891667	45.560883	3.819050	0.18152	0.0452
50.0 ppb	47	Arcola	50	51.891667	45.560883	3.819050	6.97268	0.0413
Cd	48	Arcola	100	51.891667	45.560883	3.819050	9.56518	0.0473
	49	Arcola	150	51.891667	45.560883	3.819050	21.86947	0.0449
	50	Arcola	200	51.891667	45.560883	3.819050	20.50977	0.0529
10.0 ppb	51	Arcola	0	8.871333	5.393771	1.685245	0.18717	0.0542
Cd & 10x	52	Arcola	50	8.871333	5.393771	1.685245	1.16769	0.0445
SO_4^{2-} as	53	Arcola	100	8.871333	5.393771	1.685245	1.52685	0.0388
$MgSO_4$	54	Arcola	150	8.871333	5.393771	1.685245	2.15103	0.053
	55	Arcola	200	8.871333	5.393771	1.685245	3.32349	0.0409
50.0 ppb	56	Arcola	0	50.178333	30.508427	3.418003	0.21436	0.0399
Cd & 10x	57	Arcola	50	50.178333	30.508427	3.418003	4.91311	0.0373
SO_4^{2-} as	58	Arcola	100	50.178333	30.508427	3.418003	9.43817	0.0443
$MgSO_4$	59	Arcola	150	50.178333	30.508427	3.418003	10.19280	0.0439
	60	Arcola	200	50.178333	30.508427	3.418003	11.09189	0.0468
10.0 ppb	61	Arcola	0	9.383600	5.160980	1.641126	0.19279	0.0421
Cd & 10x	62	Arcola	50	9.383600	5.160980	1.641126	1.98370	0.0435
SO_4^{2-} as	63	Arcola	100	9.383600	5.160980	1.641126	3.43239	0.0431
K_2SO_4	64	Arcola	150	9.383600	5.160980	1.641126	4.88398	0.0466
	65	Arcola	200	9.383600	5.160980	1.641126	5.09204	0.0481
50.0 ppb	66	Arcola	0	47.988333	26.393583	3.273121	0.21329	0.0424
Cd & 10x	67	Arcola	50	47.988333	26.393583	3.273121	6.27922	0.0472
SO_4^{2-} as	68	Arcola	100	47.988333	26.393583	3.273121	10.40091	0.0413
K_2SO_4	69	Arcola	150	47.988333	26.393583	3.273121	13.53960	0.0499
	70	Arcola	200	47.988333	26.393583	3.273121	19.96858	0.0354
10.0 ppb	71	Arcola	0	9.814000	5.652864	1.732162	0.15353	0.053
Cd & 10x	72	Arcola	50	9.814000	5.652864	1.732162	1.10492	0.0416
SO_4^{2-} as	73	Arcola	100	9.814000	5.652864	1.732162	2.56686	0.0499
$MgSO_4$	74	Arcola	150	9.814000	5.652864	1.732162	4.49748	0.0386
and K_2SO_4	75	Arcola	200	9.814000	5.652864	1.732162	3.61516	0.0448
50.0 ppb	76	Arcola	0	50.771667	29.244480	3.375691	0.19239	0.0447
Cd & 10x	77	Arcola	50	50.771667	29.244480	3.375691	4.22572	0.0446
SO_4^{2-} as	78	Arcola	100	50.771667	29.244480	3.375691	8.50025	0.044
$MgSO_4$	79	Arcola	150	50.771667	29.244480	3.375691	11.61755	0.0468
and K_2SO_4	80	Arcola	200	50.771667	29.244480	3.375691	21.18786	0.0374

Table A.8: Raw data gathered from experiment 8 (Chapter 4). Exposure [Cd] and root [Cd] were determined by GF-AAS and exposure $[Cd^{2+}]$ from MINEQL$^+$. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	Sample	cultivar	time (min.)	exposure [Cd] (ppb)	exposure $[Cd^{2+}]$ (ppb)	ln$[Cd^{2+}]$	root [Cd] (ppm)	root mass (g)
	1	Kyle	0	5.124000	4.498872	1.503827	0.25514	0.0337
	2	Kyle	50	5.124000	4.498872	1.503827	0.79391	0.0297
5.0 ppb Cd	3	Kyle	100	5.124000	4.498872	1.503827	0.92626	0.0253
	4	Kyle	150	5.124000	4.498872	1.503827	1.42235	0.0284
	5	Kyle	200	5.124000	4.498872	1.503827	1.89128	0.0303
	6	Kyle	0	10.130667	8.894725	2.185458	0.29748	0.0237
10.0 ppb	7	Kyle	50	10.130667	8.894725	2.185458	1.47714	0.0185
Cd	8	Kyle	100	10.130667	8.894725	2.185458	1.57880	0.0317
	9	Kyle	150	10.130667	8.894725	2.185458	2.18736	0.0309
	10	Kyle	200	10.130667	8.894725	2.185458	2.55777	0.0266
	11	Kyle	0	53.993333	47.406147	3.858752	0.21736	0.0327
50.0 ppb	12	Kyle	50	53.993333	47.406147	3.858752	3.15338	0.0259
Cd	13	Kyle	100	53.993333	47.406147	3.858752	4.69351	0.0281
	14	Kyle	150	53.993333	47.406147	3.858752	5.67707	0.032
	15	Kyle	200	53.993333	47.406147	3.858752	6.53056	0.0354
	16	Kyle	0	5.124000	4.498872	1.503827	0.29335	0.0245
5.0 ppb Cd	17	Kyle	50	5.124000	4.498872	1.503827	0.74895	0.0306
& swirled	18	Kyle	100	5.124000	4.498872	1.503827	1.12477	0.0245
	19	Kyle	150	5.124000	4.498872	1.503827	1.47478	0.0276
	20	Kyle	200	5.124000	4.498872	1.503827	1.39168	0.0247
	21	Kyle	0	10.130667	8.894725	2.185458	0.38896	0.0291
10.0 ppb	22	Kyle	50	10.130667	8.894725	2.185458	1.10170	0.0289
Cd &	23	Kyle	100	10.130667	8.894725	2.185458	1.64261	0.0265
swirled	24	Kyle	150	10.130667	8.894725	2.185458	2.20115	0.0284
	25	Kyle	200	10.130667	8.894725	2.185458	2.39685	0.0329
	26	Kyle	0	53.993333	47.406147	3.858752	0.28457	0.0305
50.0 ppb	27	Kyle	50	53.993333	47.406147	3.858752	3.40381	0.0225
Cd &	28	Kyle	100	53.993333	47.406147	3.858752	5.45600	0.029
swirled	29	Kyle	150	53.993333	47.406147	3.858752	5.58402	0.0318
	30	Kyle	200	53.993333	47.406147	3.858752	6.36969	0.0298
	31	Arcola	0	5.124000	4.498872	1.503827	0.22060	0.0318
	32	Arcola	50	5.124000	4.498872	1.503827	0.92832	0.0357
5.0 ppb Cd	33	Arcola	100	5.124000	4.498872	1.503827	2.04382	0.0344
	34	Arcola	150	5.124000	4.498872	1.503827	2.16416	0.0327
	35	Arcola	200	5.124000	4.498872	1.503827	2.65291	0.0286

	36	Arcola	0	10.130667	8.894725	2.185458	0.22568	0.0393
10.0 ppb	37	Arcola	50	10.130667	8.894725	2.185458	1.59564	0.0353
Cd	38	Arcola	100	10.130667	8.894725	2.185458	3.06031	0.0309
	39	Arcola	150	10.130667	8.894725	2.185458	4.29791	0.0359
	40	Arcola	200	10.130667	8.894725	2.185458	4.59840	0.0333
	41	Arcola	0	53.993333	47.406147	3.858752	0.19472	0.0343
50.0 ppb	42	Arcola	50	53.993333	47.406147	3.858752	6.39419	0.0277
Cd	43	Arcola	100	53.993333	47.406147	3.858752	11.63507	0.0352
	44	Arcola	150	53.993333	47.406147	3.858752	12.94768	0.0335
	45	Arcola	200	53.993333	47.406147	3.858752	18.32126	0.0395
	46	Arcola	0	5.124000	4.498872	1.503827	0.22975	0.0463
5.0 ppb Cd	47	Arcola	50	5.124000	4.498872	1.503827	1.26240	0.0236
& swirled	48	Arcola	100	5.124000	4.498872	1.503827	1.97008	0.0351
	49	Arcola	150	5.124000	4.498872	1.503827	2.05323	0.0373
	50	Arcola	200	5.124000	4.498872	1.503827	2.27326	0.037
	51	Arcola	0	10.130667	8.894725	2.185458	0.21901	0.0358
10.0 ppb	52	Arcola	50	10.130667	8.894725	2.185458	2.11698	0.0254
Cd &	53	Arcola	100	10.130667	8.894725	2.185458	2.86713	0.0378
swirled	54	Arcola	150	10.130667	8.894725	2.185458	4.08259	0.0319
	55	Arcola	200	10.130667	8.894725	2.185458	3.65619	0.0291
	56	Arcola	0	53.993333	47.406147	3.858752	0.29291	0.023
50.0 ppb	57	Arcola	50	53.993333	47.406147	3.858752	6.00963	0.0284
Cd &	58	Arcola	100	53.993333	47.406147	3.858752	9.65299	0.0346
swirled	59	Arcola	150	53.993333	47.406147	3.858752	11.20967	0.0401
	60	Arcola	200	53.993333	47.406147	3.858752	13.68714	0.0303

Table A.9: Morphology data used in Chapter 5; root and shoot morphological characteristics collected from meshes of 'Kyle' and 'Arcola' seedlings.

cultivar	rep	plant #	diameter class (mm)	root d.w. grams	root len. cm	root area cm^2	root vol. cm^3	# root tips	leaf d.w. g	leaf area cm^2
Kyle	1	1	0.1-0.8	.	229.27	34.35	0.31	62	.	.
Kyle	1	2	0.1-0.8	0.0314	186.83	27.29	0.21	68	.	.
Kyle	1	3	0.1-0.8	0.0347	203.26	28.09	0.28	91	.	.
Kyle	1	4	0.1-0.8	0.0345	213.49	31.72	0.28	69	.	.
Arcola	1	1	0.1-0.8	.	230.4	34.03	0.26	73	.	.
Arcola	1	2	0.1-0.8	0.0391	262.1	38.54	0.33	77	.	.
Arcola	1	3	0.1-0.8	0.036	243.71	34.66	0.31	94	.	.
Arcola	1	4	0.1-0.8	0.0318	220.05	33.73	0.3	103	.	.
Kyle	2	5	0.1-0.8	0.0324	136.87	27.57	0.3	94	0.1181	17.3
Kyle	2	6	0.1-0.8	0.0337	137.97	27.29	0.29	79	0.1219	19.345
Kyle	2	7	0.1-0.8	0.0353	170.35	33.79	0.36	88	0.1027	18.12
Kyle	2	8	0.1-0.8	0.0359	146.66	30.06	0.35	96	0.1265	18.93
Kyle	2	9	0.1-0.8	0.0369	158.41	30.33	0.31	81	0.1049	16.375
Kyle	2	10	0.1-0.8	0.0359	158.87	32.05	0.36	79	0.1098	18.56
Arcola	2	5	0.1-0.8	0.0489	305.59	58.2	0.64	132	0.1444	27.385
Arcola	2	6	0.1-0.8	0.0459	294.79	56.88	0.68	122	0.1372	28.47
Arcola	2	7	0.1-0.8	0.0555	345.91	67.13	0.78	158	0.1535	31.375
Arcola	2	8	0.1-0.8	0.0515	332.91	64.76	0.74	165	0.1152	27.165
Arcola	2	9	0.1-0.8	0.0375	269.85	50.45	0.57	133	0.1143	23.525
Arcola	2	10	0.1-0.8	0.0412	256.54	51.26	0.49	109	0.1196	25.4
Kyle	3	11	0.1-0.8	0.0444	274.78	53.02	0.59	89	0.1344	22.375
Kyle	3	12	0.1-0.8	0.0497	254.35	51.24	0.61	239	0.1432	21.095
Kyle	3	13	0.1-0.8	0.0352	207.37	40.01	0.44	72	0.1239	23.25
Kyle	3	14	0.1-0.8	0.0421	111.14	24.22	0.3	119	0.1367	22.31
Kyle	3	15	0.1-0.8	0.0399	186.55	39.25	0.45	113	0.1193	18.83
Kyle	3	16	0.1-0.8	0.0387	218.68	41.98	0.49	99	0.1256	20.12
Arcola	3	11	0.1-0.8	0.0477	330.05	60.66	0.67	155	0.145	28.675
Arcola	3	12	0.1-0.8	0.0472	318.5	60.46	0.7	119	0.1455	27.87
Arcola	3	13	0.1-0.8	0.042	275	54.63	0.68	135	0.1161	23.32
Arcola	3	14	0.1-0.8	0.0425	290.17	54.33	0.66	131	0.1244	21.655
Arcola	3	15	0.1-0.8	0.0389	306.379	55.7	0.71	161	0.114	21.015
Arcola	3	16	0.1-0.8	0.0456	292.24	55.09	0.58	118	0.1256	20.12

Kyle	4	17	0.1-0.8	0.0325	167.42	34.79	0.4	100	0.1078	17.825
Kyle	4	18	0.1-0.8	0.0346	182.74	35.52	0.36	112	0.1098	16.335
Kyle	4	19	0.1-0.8	0.0369	157.76	31.2	0.34	92	0.1133	17.385
Kyle	4	20	0.1-0.8	0.0403	172.66	35.97	0.42	85	0.1129	19.185
Kyle	4	21	0.1-0.8	0.0356	148.59	29.89	0.33	75	0.1011	14.595
Kyle	4	22	0.1-0.8	0.0393	182.12	35.18	0.38	87	0.1457	20.14
Arcola	4	17	0.1-0.8	0.0344	216.51	39.55	0.41	74	0.1217	21.25
Arcola	4	18	0.1-0.8	0.0433	267.96	50.73	0.56	97	0.1311	25.205
Arcola	4	19	0.1-0.8	0.041	235.2	45.59	0.5	75	0.1256	26.455
Arcola	4	20	0.1-0.8	0.0393	242.38	44.05	0.46	78	0.1181	21.91
Arcola	4	21	0.1-0.8	0.0392	251.26	47.57	0.56	96	0.1059	21.62
Arcola	4	22	0.1-0.8	0.0475	277.87	50.13	0.51	66	0.1628	28.685
Kyle	5	23	0.1-0.8	0.041	224.96	41.84	0.47	63	0.1102	18.43
Kyle	5	24	0.1-0.8	0.0317	183.34	33.41	0.35	65	0.0958	14.13
Kyle	5	25	0.1-0.8	0.0401	211.97	38.88	0.39	48	0.1027	15.795
Kyle	5	26	0.1-0.8	0.0424	230.72	43.66	0.48	62	0.1168	19.51
Kyle	5	27	0.1-0.8	0.0349	195.35	37.07	0.41	74	0.1136	20.11
Kyle	5	28	0.1-0.8	0.0316	164.43	30.5	0.33	78	0.1016	16.785
Arcola	5	23	0.1-0.8	0.0395	239.66	43.36	0.47	88	0.12	23.135
Arcola	5	24	0.1-0.8	0.0414	293.34	52.35	0.58	108	0.1299	25.25
Arcola	5	25	0.1-0.8	0.0325	226.83	40.02	0.44	69	0.0937	17.865
Arcola	5	26	0.1-0.8	0.0383	259.09	43.75	0.41	94	0.1169	24.26
Arcola	5	27	0.1-0.8	0.0325	214.55	39.57	0.45	58	0.0972	19.12
Arcola	5	28	0.1-0.8	0.0371	229.33	41.29	0.43	61	0.1088	22.755

Table A.10: Solution and plant tissue data used in Chapter 6. To convert Cd concentrations from ppb ($\mu g \cdot L^{-1}$) to M, multiply by $8.90 \cdot 10^{-9}$.

treatment	cv	tissue	time (h)	target [Cd]	solution [Cd]	solution [Cd^{2+}]	solution [citrate] (M)	tissue [Cd]	dry wt.	total tissue Cd
'Kyle'; root tissue; 5.0 ppb Cd	Kyle	root	0	5	5.209	3.8994	0	2.160	0.28	0.605
	Kyle	root	8	5	2.894	2.1579	0	141.992	0.36	51.117
	Kyle	root	16	5	2.202	1.9146	0	197.930	0.29	57.400
	Kyle	root	24	5	1.502	0.9803	0	237.231	0.33	78.286
	Kyle	root	32	5	1.013	0.5377	0	269.647	0.3	80.894
	Kyle	root	40	5	1.415	1.2375	0	218.433	0.34	74.267
	Kyle	root	48	5	1.545	0.7987	0	249.648	0.28	69.901
	Kyle	root	56	5	1.212	0.3697	0	186.900	0.31	57.939
	Kyle	root	64	5	1.049	0.8551	0	196.237	0.32	62.796
	Kyle	root	72	5	0.926	0.2086	0	239.052	0.35	83.668
'Kyle'; shoot tissue; 5.0 ppb Cd	Kyle	shoot	0	5	5.209	3.8994	0	0.817	1.11	0.907
	Kyle	shoot	8	5	2.894	2.1579	0	0.937	1.35	1.265
	Kyle	shoot	16	5	2.202	1.9146	0	6.490	0.97	6.295
	Kyle	shoot	24	5	1.502	0.9803	0	13.570	1.22	16.555
	Kyle	shoot	32	5	1.013	0.5377	0	13.875	1.24	17.205
	Kyle	shoot	40	5	1.415	1.2375	0	11.551	1.28	14.785
	Kyle	shoot	48	5	1.545	0.7987	0	16.288	1.12	18.243
	Kyle	shoot	56	5	1.212	0.3697	0	12.125	1.51	18.308
	Kyle	shoot	64	5	1.049	0.8551	0	17.486	1.46	25.530
	Kyle	shoot	72	5	0.926	0.2086	0	13.593	1.56	21.205
'Arcola'; root tissue; 5.0 ppb Cd	Arcola	root	0	5	4.883	4.2464	0	3.440	.	.
	Arcola	root	8	5	3.671	3.0383	0	82.987	0.23	19.087
	Arcola	root	16	5	2.004	1.4895	0	268.649	0.3	80.595
	Arcola	root	24	5	2.188	1.3932	0	308.672	0.2	61.734
	Arcola	root	32	5	2.187	0.7956	0	294.550	0.21	61.855
	Arcola	root	40	5	1.985	0.6436	0	263.709	0.23	60.653
	Arcola	root	48	5	1.334	0.6915	0	259.138	0.33	85.516
	Arcola	root	56	5	1.242	0.5574	0	262.074	0.32	83.864
	Arcola	root	64	5	1.282	1.1610	0	292.406	0.35	102.342
	Arcola	root	72	5	0.912	0.3615	0	187.484	0.3	56.245
'Arcola'; shoot tissue; 5.0 ppb Cd	Arcola	shoot	0	5	4.883	4.2464	0	0.521	.	.
	Arcola	shoot	8	5	3.671	3.0383	0	1.146	0.9	1.032
	Arcola	shoot	16	5	2.004	1.4895	0	2.427	1.04	2.525
	Arcola	shoot	24	5	2.188	1.3932	0	5.424	0.8	4.339
	Arcola	shoot	32	5	2.187	0.7956	0	8.511	0.85	7.234
	Arcola	shoot	40	5	1.985	0.6436	0	6.088	0.92	5.601
	Arcola	shoot	48	5	1.334	0.6915	0	5.263	1.41	7.420
	Arcola	shoot	56	5	1.242	0.5574	0	6.462	1.43	9.240
	Arcola	shoot	64	5	1.282	1.1610	0	6.109	1.47	8.980
	Arcola	shoot	72	5	0.912	0.3615	0	8.470	1.36	11.520

	Kyle	root	0	50	53.66	50.3117	0	2.207	0.25	0.552
	Kyle	root	8	50	44.2	40.7230	0	694.983	0.24	166.796
'Kyle';	Kyle	root	16	50	36.06	28.3773	0	1308.116	0.23	300.867
root tissue;	Kyle	root	24	50	27.62	18.8525	0	1161.991	0.32	371.837
50.0 ppb	Kyle	root	32	50	29.26	17.6243	0	1608.984	0.22	353.976
Cd	Kyle	root	40	50	24.74	19.2929	0	520.832	0.31	161.458
	Kyle	root	48	50	22.53	18.8568	0	1548.102	0.32	495.393
	Kyle	root	56	50	19.16	12.0971	0	1664.223	0.37	615.763
	Kyle	root	64	50	19.77	16.9005	0	1456.082	0.26	378.581
	Kyle	root	72	50	15.25	11.2397	0	1500.668	0.3	450.200
	Kyle	shoot	0	50	53.66	50.3117	0	0.572	0.96	0.549
	Kyle	shoot	8	50	44.2	40.7230	0	5.256	0.98	5.151
'Kyle';	Kyle	shoot	16	50	36.06	28.3773	0	34.298	0.9	30.868
shoot	Kyle	shoot	24	50	27.62	18.8525	0	71.668	1.17	83.852
tissue;	Kyle	shoot	32	50	29.26	17.6243	0	110.514	0.9	99.463
50.0 ppb	Kyle	shoot	40	50	24.74	19.2929	0	132.510	1.1	145.761
Cd	Kyle	shoot	48	50	22.53	18.8568	0	128.838	1.33	171.354
	Kyle	shoot	56	50	19.16	12.0971	0	105.361	1.63	171.738
	Kyle	shoot	64	50	19.77	16.9005	0	169.927	0.98	166.528
	Kyle	shoot	72	50	15.25	11.2397	0	129.114	1.62	209.164
	Arcola	root	0	50	51.85	50.9493	0	2.447	.	.
	Arcola	root	8	50	40.71	36.1727	0	702.869	0.31	217.889
'Arcola';	Arcola	root	16	50	32.22	23.3831	0	1002.675	0.31	310.829
root tissue;	Arcola	root	24	50	28.95	20.8178	0	1598.242	0.24	383.578
50.0 ppb	Arcola	root	32	50	26	19.9997	0	1358.974	0.28	380.513
Cd	Arcola	root	40	50	25.49	17.3809	0	1660.493	0.23	381.913
	Arcola	root	48	50	21.58	13.9279	0	2149.088	0.26	558.763
	Arcola	root	56	50	24.18	11.4833	0	1708.605	0.27	461.323
	Arcola	root	64	50	18.44	10.8587	0	1521.454	0.35	532.509
	Arcola	root	72	50	15.1	10.5444	0	1737.925	0.33	573.515
	Arcola	shoot	0	50	51.85	50.9493	0	0.383	1.08	0.413
	Arcola	shoot	8	50	40.71	36.1727	0	.	1.27	.
'Arcola';	Arcola	shoot	16	50	32.22	23.3831	0	36.833	1.13	41.621
shoot	Arcola	shoot	24	50	28.95	20.8178	0	66.597	0.98	65.265
tissue;	Arcola	shoot	32	50	26	19.9997	0	53.846	1.21	65.154
50.0 ppb	Arcola	shoot	40	50	25.49	17.3809	0	90.118	0.88	79.303
Cd	Arcola	shoot	48	50	21.58	13.9279	0	106.226	1.15	122.159
	Arcola	shoot	56	50	24.18	11.4833	0	104.429	1.3	135.758
	Arcola	shoot	64	50	18.44	10.8587	0	93.447	1.49	139.236
	Arcola	shoot	72	50	15.1	10.5444	0	116.546	1.72	200.460
'Kyle';	Kyle	root	8	50	39.75	36.0944	0.0001	663.176	0.23	152.530
root tissue;	Kyle	root	24	50	29.21	27.5761	0.0001	1265.811	0.28	354.427
50.0 ppb	Kyle	root	40	50	26.06	25.4457	0.0001	963.062	0.29	279.288
Cd &	Kyle	root	56	50	26.95	22.2379	0.0001	1084.265	0.28	303.594
citrate	Kyle	root	72	50	18.95	18.5952	0.0001	1204.127	0.34	409.403

'Kyle'; shoot tissue; 50.0 ppb Cd & citrate	Kyle	shoot	8	50	39.75	36.0944	0.0001	.	0.83	.
	Kyle	shoot	24	50	29.21	27.5761	0.0001	48.877	1.09	53.276
	Kyle	shoot	40	50	26.06	25.4457	0.0001	81.220	1.12	90.966
	Kyle	shoot	56	50	26.95	22.2379	0.0001	68.426	1.27	86.900
	Kyle	shoot	72	50	18.95	18.5952	0.0001	92.333	1.89	174.509
'Arcola'; root tissue; 50.0 ppb Cd & citrate	Arcola	root	8	50	37.31	36.1987	0.0001	684.679	0.29	198.557
	Arcola	root	24	50	32.77	31.5754	0.0001	1142.035	0.24	274.088
	Arcola	root	40	50	26.92	23.9609	0.0001	1096.559	0.3	328.968
	Arcola	root	56	50	26.38	22.7587	0.0001	1858.865	0.19	353.184
	Arcola	root	72	50	14.81	14.2381	0.0001	1860.930	0.28	521.060
'Arcola'; shoot tissue; 50.0 ppb Cd & citrate	Arcola	shoot	8	50	37.31	36.1987	0.0001	5.125	1.15	5.894
	Arcola	shoot	24	50	32.77	31.5754	0.0001	30.031	0.93	27.929
	Arcola	shoot	40	50	26.92	23.9609	0.0001	53.534	1.23	65.847
	Arcola	shoot	56	50	26.38	22.7587	0.0001	74.311	0.95	70.596
	Arcola	shoot	72	50	14.81	14.2381	0.0001	102.626	1.52	155.992
Blank; 5.0 ppb Cd	Blank	.	8	5	4.306	3.7907	0	.	.	.
	Blank	.	24	5	4.658	3.6252	0	.	.	.
	Blank	.	40	5	4.465	3.9371	0	.	.	.
	Blank	.	56	5	4.584	3.9443	0	.	.	.
	Blank	.	72	5	4.19	3.2991	0	.	.	.
Blank; 50.0 ppb Cd	Blank	.	8	50	50.56	47.8972	0	.	.	.
	Blank	.	24	50	47.56	43.7960	0	.	.	.
	Blank	.	40	50	47.63	47.9492	0	.	.	.
	Blank	.	56	50	49	43.9669	0	.	.	.
	Blank	.	72	50	46.78	41.0769	0	.	.	.

APPENDIX B:

FORMATION CONSTANTS (LOG K)

Table B1: Formation constants (log K) used by MINEQL$^+$ to calculate proportions of various complexes. The log K values are corrected to 0 ionic strength (i = 0).

complex	formation constant (log K)
CdCitrate$^-$	4.97
CdHCitrate	9.47
CdEDTA^{2-}	18.26
$CdSO_4^0{}_{(aq)}$	2.46
$Cd(SO_4)_2^{2-}$	3.50
$CdNO_3^+$	0.50
CaCitrate$^-$	4.85
MgCitrate$^-$	4.84
CaEDTA^{2-}	12.41
MgEDTA^{2-}	10.61
$CaSO_4^0{}_{(aq)}$	2.30
$MgSO_4^0{}_{(aq)}$	2.23

APPENDIX C:

STATISTICAL COMPARISON OF REGRESSION EQUATIONS: AN EXAMPLE CALCULATION

In chapter 6, regression lines were determined for accumulation of Cd by roots and shoots of 'Kyle' and 'Arcola' seedlings over time. Regression equations were also determined for the concentration of Cd and Cd^{2+} in solutions which 'Kyle' and 'Arcola' seedlings were exposed to. It was important to be able to compare pairs of regression equations, of the same form, in order to determine, for example, whether or not accumulation of Cd by shoot tissue of 'Kyle' or 'Arcola' seedlings over time was significantly different, or not.

For this example, we would like to determine if accumulation of Cd by 'Kyle' and 'Arcola' shoots exposed to a target, total Cd concentration of $4.45 \cdot 10^{-8}$ M over time is significantly different or not. The data used in this analysis are in Table C.1. In order to compare regression lines, two analysis are first required. First, regression equations for each cultivar must be determined using SAS PROC NLIN (SAS Institute Inc., Cary, NC). This is called the full model, since it considers cultivar a significant term in the model. The SAS program is as follows:

```
proc nlin;
 model shootCd = b0 * (1 - exp (-b1 * time)) + b2;
 by cultivar;
 parms b0 = 15   b1 = 0.1   b2 = 0 to 5;
 bounds 0 < b2 < 5;
run;
```

The second analysis is similar, except that the "by cultivar;" line is removed. The result is called the reduced model, since the effect of cultivar is not included in the model, and a single regression equation is determined for the data from both cultivars. The SAS output for the full and reduced models are in tables C.2 and C.3, respectively.

Table C.1: Raw data used in example statistical analysis: comparison of Cd accumulation by 'Kyle' and 'Arcola' seedlings exposed to $4.45 \cdot 10^{-8}$ M Cd.

Cultivar	time (h)	shoot [Cd] ($\mu g \cdot g^{-1}$)
'Kyle'	0	0.817
'Kyle'	8	0.937
'Kyle'	16	6.490
'Kyle'	24	13.570
'Kyle'	36	13.875
'Kyle'	40	11.551
'Kyle'	48	16.288
'Kyle'	56	12.125
'Kyle'	64	17.486
'Kyle'	72	13.593
'Arcola'	0	0.521
'Arcola'	8	1.146
'Arcola'	16	2.427
'Arcola'	24	5.424
'Arcola'	36	8.519
'Arcola'	40	6.088
'Arcola'	48	5.263
'Arcola'	56	6.462
'Arcola'	64	6.109
'Arcola'	72	8.470

Table C.2: ANOVA tables and parameter estimates from the full model.

'Kyle'			
source	df	sum of squares	mean square
regression	3	1402.82	467.61
residual	7	53.77	7.68
uncorrected total	10	1456.59	
corrected total	9	317.43	

'Arcola'			
source	df	sum of squares	mean square
regression	3	308.79	102.93
residual	7	16.12	2.30
uncorrected total	10	134.91	
corrected total	9	70.68	

'Kyle': shoot [Cd] = $16.29 \cdot (1 - e^{(-0.04211 \cdot time)}) + 0$

'Arcola': shoot [Cd] = $7.68 \cdot (1 - e^{(-0.04146 \cdot time)}) + 0$

Table C.3: ANOVA tables and parameter estimates from the reduced model.

Both 'Kyle' and 'Arcola' together			
source	df	sum of squares	mean square
regression	3	1513.97	504.66
residual	17	267.53	15.74
uncorrected total	20	1781.50	
corrected total	19	546.64	

both 'Kyle' and 'Arcola': shoot [Cd] = $11.99 \cdot (1 - e^{(-0.04191 \cdot time)}) + 0$

Once these analysis are completed, whether or not the two cultivars accumulate significantly different amounts of Cd in their shoots is determined by calculating an F-value to test whether or not including cultivar in the model (full model) resulted in a significant reduction in the error sum of squares over the reduced model. This is done by the formula:

$$\text{F-value} = \frac{(\text{Error SS}_{(\text{reduced model})} - \text{Error SS}_{(\text{full model})}) / (\text{df Error}_{(\text{reduced model})} - \text{df Error}_{(\text{full model})})}{(\text{Error SS}_{(\text{full model})} / \text{df Error}_{(\text{full model})})}$$

The error sum of squares (Error SS) for the full model is determined by summing the error sum of squares for 'Kyle' and 'Arcola' (Table C.2) and the df error for the full model is determined by summing the df for 'Kyle' and 'Arcola' (Table C.2). Putting the values from Tables B2 and B3 in the equation, we get:

$$\text{F-value} = ((267.53 - (53.77 + 16.12)) / (17 - (7 + 7))/((53.77 + 16.12) / (7+7))$$

$$= (197.64 / 3) / (69.89 / 14)$$

$$= (68.88 / 4.99)$$

$$= 13.20$$

The calculated F-value is then compared against the tabulated F-values to determine significance. In this case, $F_{0.05, 3, 14} = 3.34$ and $F_{0.01,3,14} = 5.56$, so we can say that the cultivars significantly differ ($p<0.01$) in their shoot Cd concentration when they are exposed to $4.45 \cdot 10^{-8}$ M Cd for 72 hours.