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DETERMINATION OF THE CADMIUM UPTAKE MECHANISM IN FORAGE SPECIES IN NEW ZEALAND LIVESTOCK GRAZING SYSTEMS

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Abstract

Cadmium (Cd) is a key environmental contaminant associated with long-term high application rates of superphosphate fertiliser to soils used for dairying and horticulture. Although cadmium (Cd) is considered to be a non-essential element for plants, it is effectively absorbed by the root systems of many plant species and can be subsequently transported throughout the plant. Recent studies indicate that elevated levels of Cd in New Zealand soils can lead to a cadmium concentration in forage species such as chicory (*Cichorium intybus L*) and plantain (*Plantago lanceolate L*) that is orders of magnitude higher than in ryegrass or clover. Results of such studies suggest the different abilities of pastoral species used in New Zealand to both absorb Cd from soils and to translocate it from roots to shoots. However, there have been no studies published on the Cd uptake mechanisms of common forage species used in New Zealand agriculture. Ongoing research into rhizosphere chemistry and Cd uptake mechanisms of such species will help better understand the species and cultivar variations in Cd uptake, and associated rhizosphere soil functions.

Literature reviewed for the doctoral research underpinning this manuscript has identified the research gap that must be addressed to better understand Cd uptake mechanisms in forage species. Further, this will assist in proposing advanced analytical methods to quantify the forms of plant tissue Cd in the New Zealand livestock grazing system.

Introduction

Cadmium contamination in New Zealand soils is associated with a long-term application of superphosphate fertiliser to pastoral and horticultural soils (Taylor et al., 2007). Historically, the main source of New Zealand's imported phosphate was from the Pacific Islands-Nauru. Nauru rock-phosphate is now known to be the most cadmium contaminated phosphate deposit (450 mg Cd/kg P) in the world (McLaughlin and Singh, 1999). Taylor et al. (2007) reported that the mean Cd level in all land uses of NZ soils is 0.35 mg Cd/kg soil, with horticulture and pastoral land use showing considerably higher mean concentrations at 0.50 mg Cd/kg and 0.43 mg Cd/kg soil, respectively. Cadmium contamination of New Zealand's most versatile soils threatens to limit their use for high-value vegetable, tuber and grain crops due to risk of exceedances of guideline values for Cd in food (McDowell et al., 2013; MAF, 2011). For example, the Waikato Regional Council has estimated that 16% of Waikato's pastoral soils and 22% of its horticultural soils already exceed the unofficial contamination threshold of 1 mg/kg soil cadmium (Taylor et al., 2011).

In modern livestock grazing systems, forage plant species are used due to their high drought tolerance. For example, chicory and plantain are deeper rooting plants than perennial ryegrasses, which could be useful to reduce nitrate-N leaching losses from grazed pasture systems (Li and Kemp 2005). However, a recent study has revealed that the presence of elevated levels of Cd in New Zealand soils can result in increased Cd concentrations in forage plant species relative to grasses and legumes (Stafford et al., 2016). This study reported that the tissue Cd concentration in chicory and plantain was 1.63 and 0.73 mg/kg DM, respectively. Even at extremely low total soil Cd concentrations (0.004 – 0.017 mg Cd/kg), chicory has the ability to accumulate a high Cd concentration in leaf tissues (1.6 –2.4 mg/kg DM), (Martin et al., 1996). As livestock kidney/liver Cd accumulation is related to dietary Cd intake, a practical recommendation to manage Cd accumulation in grazing livestock is to minimize grazing animals on Cd rich forages (Lee et al., 1994; Lee et al., 1996).

Such results suggest that different plants have specific mechanisms to uptake and translocate Cd to their above-ground parts (McLaughlin and Singh, 1999). In this manuscript we propose that detailed study of rhizosphere chemistry and Cd uptake mechanisms will help to understand plant species variations in Cd uptake and associated rhizosphere soil functions. Hence, this paper reviews the physiological variations of plants exerting and influence in the uptake and transportation of Cd from the rhizosphere to xylem. Various methodologies are also reviewed to identify and quantify the forms of Cd in xylem sap of forage species.

Influence of rhizosphere properties on Cd bioavailability

Bioavailability may be defined as the fraction of an element (e.g. Cd) in soil that can interact with a biological target such as soil microbes and plants (Geebelen et al., 2003). Rhizosphere is the volume of soil around living plant roots that is influenced by root activity (Hinsinger, 1998). Generally, Cd bioavailability depends on the physical, chemical and biological processes operating in the rhizosphere (McLaughlin and Singh, 1999). These processes are related to plant root associated microorganisms, rhizosphere pH, and ligands secreted from plant roots to the rhizosphere such as siderophores, organic acids and protons (Jeyakumar et al., 2010)

Root exudation of chelating compounds and Cd bioavailability

Root secreted Cd complexing ligands can be categorised into two divisions; organic ligands (carbohydrates, organic acids, humic acids, polypeptides, proteins, amino acids, nucleic acid) and inorganic ligands (Cl^-, SO_4^{-2}, NH_4^+, CO_3^{-2}, PO_4^{-3}) (Hinsinger et al., 2006). These ligands influence Cd uptake by plants (Collins et al., 2003). Han et al. (2006) showed that root exuded acetic acid (250µM) from maize plants increased Cd uptake by 110%. Duarte et al. (2007) reported that the application of 25 µM citric acid increased Cd uptake from 27% (in control) to 61% in sea purslane plants. Kim et al. (2010) found that oxalic acid (37.6 mg/kg) and citric acid (40.3 mg/kg) in the root exudate of barnyard grass enhanced Cd uptake by 52%. Tao et al. (2016) observed that increasing the soil Cd concentration from 0 to 2.81 mg Cd/kg led to elevated levels of root secreted oxalic acid concentration in *Sedum alfredii* from 2.61 to 12.13 mg/kg FW. They also observed that the increased concentration of the Cd-oxalic complex, increased the plant Cd concentration from 19 to 487 mg Cd/kg. McLaughlin and Singh (1999) reported that Cl^- levels in soil increase plant Cd uptake by forming inorganic complexes of $CdCl^+$ and $CdCl_2$. Hattori et al. (2006) observed that high soil Cl^- concentration (1370 mg Cl^- /kg) approximately doubled the leave tissue Cd concentration compared with control leave tissue Cd concentration (>10 mg Cd/kg). However, in our knowledge, there is no reference available in the literature on the effect of root exudates on Cd bioavailability of chicory and plantain.

Rhizosphere soil characteristics and Cd bioavailability

The rhizosphere soil solution pH differs by up to 2.5 pH-units from that of the bulk soil solution, depending on the plant species and buffering capacity of the bulk soil (Marschner, 1996). This difference can be attributed to a combination of mechanisms, including: (i) cation exchange capacity (ii) root exudation and respiration (iii) redox-coupled processes involving changes in the oxidation state of Fe and Mn (Hinsinger et al., 2006). Xian and In shokohifard (1989) reported that a decrease in soil pH from 7.0 to 4.6 increased the Cd bioavailability for plant uptake. Loganathan et al. (2012) indicated that Cd sorption on metal oxides increased sharply and regularly when the pH increased from 6 to 9. Wang et al. (2006) reported that the Cd hyperaccumulator alpine penny grass growing in loamy soil showed a 40% increase in shoot tissue Cd concentration at pH of 4.74 when compared to pH 7.27. Spinach grown in loam soil showed a decrease in shoot Cd concentration from 2.607 to 1.565 mg Cd/kg when the soil pH increases from pH 5.89 to 6.80 (He and Singh, 1995). Gray et al. (1999) found that shoot Cd concentration in clover and wheat decreased by 76% and 41%, respectively when pH increased from 5.5 to 7.0 in a recent soil. In a further example, shoot Cd accumulation in ryegrass decreased from 10% to 20% when soil pH changed from pH 5 to pH 7 in a clay soil (Eriksson, 1989). In contrast, Hatch et al. (1998) found that the shoot Cd concentration of ryegrass increased from 49 to 412 mg Cd/kg when the pH of the flowing solution culture increased from 5.5–7.0. This apparently anomalous result may be due to the possible availability of other metals which compete with Cd for plant uptake at lower pH (Lux et al., 2010).

The type and amount of other trace elements in soil is another important soil characteristic which affects plant Cd uptake. For example, the chemical similarity of Zn makes it a competing ion for Cd in its uptake by plants. Zaho et al. (2006) in the analysis of Cd and Zn interactions in rockcress found that increasing the amount of Zn in hydroponic culture from 0 to 65.38 mg Zn/L resulted in decreased Cd accumulation by 10-fold in shoot. Similarly, Han et al. (2006) reported that Cd accumulation in maize roots increased 3-fold under Zn deficiency in a hydroponic culture, whereas there was 87% reduction in total root Cd, when Zn concentration increased to 0.16 mg Zn/L.

Moreover, Fe and Cl are other antagonistic micronutrients compete with Cd for uptake in plants when they co-occur in soil solutions (McLaughlin and Singh, 1999). Ueno et al. (2008) reported that introduction of 2.8 mg Fe/L to hydroponic culture reduced the Cd concentration in rockcress by 3.6 fold. In contrast, Hattori et al. (2006) reported that high Cl concentration in soil approximately doubled the Cd accumulation in sunflower leaves, as we discussed in the previous section.

Rhizosphere microorganisms and Cd bioavailability

Rhizosphere microorganisms are symbiotically associated with the roots of most plant species where they obtain food and in return they regulate the uptake of plant nutrients and trace elements (Jones et al., 2004). The uptake of Cd by plants is associated with the presence of siderophores and chelators, these being synthesized by rhizosphere microorganisms which enhance Cd bioavailability. Rhizosphere microbial inoculation can lead to enhanced production of organic acids and phytochelatins which reduce the pH of soil, thereby enhancing Cd bioavailability (Kamnev et al., 2005).

A study conducted by Li et al. (2007) reported that soil inoculation with *Burkholderia cepacia* significantly enhanced Cd uptake in a perennial herb by a range of 36.5–243%. This was attributed to the production of siderophores which increased Cd uptake by forming Cd-siderophore complexes. Chauhan and Rai (2009) found a 2.5 and 1.8-fold increase in Cd uptake by Indian mustard plants in the presence of *Pseudomonas fluorescens* and *Trichoderma harzianum*, respectively. They reported that these two microorganisms secrete siderophores and

organic acids to the soil solution which increased the Cd bioavailability. Furthermore, Guo et al. (1996) reported that inoculation of soil with micohorrizal fungus (*Glomus mosseae*) increased uptake of Cd by up to 37% and 41 % in beans and maize, respectively.

However, the influence of microorganisms is not always to increase uptake. Dong et al. (2007) reported a decrease in Cd bioavailability in soil after inoculation and this was attributed to the formation of insoluble metal sulphides as a consequence of H_2S released into soil in a changed rhizosphere pH environment. Tripathi et al. (2005) reported that *Pseudomonas putida* in mung bean plant decreased the Cd concentration in shoots from 24 to 12 mg Cd/kg when the soil Cd concentration was 12.36 mg Cd/kg. They observed that bacteria secrete the compound pyoverdine, which binds with Cd in soil to decrease Cd bioavailability.

Cd uptake by root

Cadmium uptake across a plant's root plasma membrane is influenced by the electrochemical potential difference between the activity of Cd^{2+} in the cytosol of a plant cell and the root apoplast (Clemens et al., 2002). This large negative potential might exceed -200 mV, providing the driving force to uptake Cd^{2+} ions from soil solution (McLaughlin and Singh, 1999).

Non-essential metals such as Cd compete with essential micronutrients (example: Fe and Zn) and gain access into the plant cell via the transport systems (transmembrane carriers) operating for micronutrient uptake (McLaughlin and Singh, 1999). Lombi et al. (2002) reported that Cd uptake in alpine pennycress was enhanced 3-fold in a Fe deficient soil (0.28 mg Fe/kg) relative to the Fe sufficient soil (3.36 mg Fe/kg). A study conducted by Cohen et al. (1998) reported that Fe deficiency conditions in pea induce the expression of Fe transmembrane carrier (IRT1). They also found that the Cd uptake 7-fold higher when soil had no Fe where the sufficient level Fe in soil was of 0.56 mg Fe/kg. Han et al. (2006) reported that Zn membrane transporter-Zip enhance the Cd uptake by 3-fold in maize the hydroponic culture Zn concentration increase from 0 to 0.163 mg Zn/L. Further, Lindberg et al. (2004) reported that cadmium uptake into the cytosol of wheat partly takes place by channels permeable to calcium and potassium.

Translocation of Cd through xylem

Table 1 shows the various methodologies used to measure Cd translocating forms in different plant saps. After root uptake Cd^{2+} is translocated to shoots via the apoplast of the xylem vessels (Senden and Wolterbeek, 1990) and is adsorbed into negatively charged sites present in the cell wall (Conn and Gilliham, 2010). However, the formation of Cd complexes in xylem sap will protect Cd ions from adsorption, and lead to the transport of Cd through the xylem vessel (Sheoran et al., 2010). Salt et al. (1995) reported that citric acid was identified as the major ligand for Cd transport in tomato plants, where >50% of Cd is transported as Cd-citric acid complex. Wei et al. (2007) identified malic acid as the major Cd translocating ligand in the xylem sap of oil seed rape. They observed an increase in malate concentration by 83% that led to an increase of xylem sap Cd concentration by a factor of 172. Nakamura et al. (2008) also found maleic acid as the major ligand for Cd transport in oil seed rape. These authors observed that an increase in xylem malate concentration of 11% led to an increase in xylem sap Cd concentration by factor of 6.5. However, Ueno et al. (2008) reported that in rockcress the free Cd^{2+} concentration in xylem sap increased by 85.7% when the soil Cd concentration increased from 0.05–1.12 mg Cd/kg (Table 1). They concluded that Cd translocation in rockcress is an energy dependent process and that Cd^{2+} does not need to be complexed to organic acids for translocation.

Table 1:Summary of various methodologies used to measure forms and amount of Cd in different plant species.

Plant species	Summary of findings	Methodology	References
rock cress	• Cd occurred mainly in the free ionic form (85 %) in the xylem sap and the concentration of Cd in the xylem sap increased linearly with increasing Cd concentration in the external solution from 0.05–1.12 mg Cd/kg.	• Xylem sap was collected from plants exposed to 73.99 mg Cd/kg for 9.5 hours. • Cd concentration in xylem sap was analysed using Graphite Furnace Atomic Absorption Spectroscopy • The form of Cd in the xylem sap was identified by using the ^{113}Cd-Nuclear Magnetic Resonance (NMR) technique.	(Ueno et al., 2008)
oil seed rape	• Cd translocated as Cd maleic-complex in xylem sap. • Cd concentration in xylem sap linearly increased with xylem sap maleic concentration.	• Xylem sap was collected after plants were exposed to Cd treatment solutions of 2.11 mg Cd/L and 10.57 mg Cd/L for 10 hours. • Size exclusion and high-performance liquid chromatography was used to investigate the Cd associated chelates in the xylem sap.	(Nakamura et al., 2008)
alpine penygrass	• Cd was coordinated mainly with malate-83% in the leaves of alpine penygrass • Cd-NMR with leaf sap showed a signal at the chemical shift of around 16.9 ppm.	• Plants were grown hydroponically in a highly enriched ^{113}Cd stable isotope (10.57 mg Cd/L) • ^{113}Cd-NMR spectroscopy combined with a stable isotope (^{113}Cd) labelling technique was used to identify the form of Cd in the leaves of alpine penygrass.	(Ueno et al., 2005)

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rock cress	• Cd (80 %) was coordinated with O atom containing ligand and 20% of Cd coordinated with S atom containing ligand in leaf sap.	• Leaf sap was collected after the plants were exposed to Cd treatment solutions of 5.28 mg Cd/L and 21.14 mg Cd/L for 9 weeks. • X-ray Absorption Spectroscopy was used to investigate the Cd associated chelates in the xylem sap.	(Huguet et al., 2012)
rice.	• Cd-bound compounds indicated that phloem Cd in the rice plant exists mainly as an approximately 13 kDa complex, which is 2'-deoxymugineic acid (0.65 mM).	• Phloem sap was collected after the plants were exposed to 1.124 mg Cd/L. • Elution times for Cd-bound compounds were analysed using the size-exclusion chromatography. .	(Kato et al., 2010)

Quantifying form of Cd in plant saps

High Performance Liquid Chromatography (HPLC) can be used to identify organic compounds in plant saps. This methodology separates the organic compounds using a reverse phase column according to their polarity (Arnetoli et al., 2008). In the reverse phase column, organic compounds are partitioned leading to differential migration through the column. As a result, organic compounds elute from the column at different times enabling them to be identified depending on the retention time (Collins et al., 2004). However, in terms of Cd analysis, this method has a major limitation as it is unable to differentiate between Cd-organic complexes or free Cd^{2+} (Strobel et al., 2001). Therefore, advanced analytical techniques have been introduced for the direct identification of Cd complexes or free Cd^{2+}.

The form of Cd in plant saps can be identified by using ^{113}Cd-Nuclear Magnetic Resonance Spectroscopy (Cd-NMR) (Grassi and Mingazzini, 2001). This analysis is carried out by combining the ligand with a stable isotope of Cd (^{113}Cd). When the metal is coordinated with various chelating ligands the chemical shift of ^{113}Cd differs according to the polarity of complexed ligand (Larive et al., 1996). Therefore, the Cd-NMR technique is able to differentiate free ionic Cd from complexed Cd in plant saps based on their chemical shifts. Ueno et al. (2008) reported that Cd is transported as free Cd^{2+} in rockcress xylem sap and they observed a Cd-NMR peak corresponding to Cd^{2+} at a chemical shift of 0.27 ppm. Similarly, Ueno et al. (2005) found that Cd complexed with malate in the leaf sap of alpine penny grass was defined by a Cd-NMR peak at a chemical shift of 16.9 ppm (Table 1). This method has been widely recognised for its high performance in selectivity, reproducibility and sample recovery. However, low sensitivity and high maintenance costs have been noted as a key limitation (Emwas, 2005).

X-ray Absorption Spectroscopy (XAS) is another widely used element specific method to analyse Cd complexes in plant saps where the chemical nature of the Cd bound atom in an organic complex is directly analysed. Huguet et al. (2012) reported that 80% of Cd^{2+} in rockcress leaf sap was coordinated with oxygen-containing ligand molecules and 20% of Cd^{2+} was coordinated with sulphur-containing ligands (Table 1).) Vogel-Mikuš et al. (2010) in XAS analysis identified that 43% and 57% Cd in seed sap of wild thyme was complexed with oxygen atom contain ligand and sulphur atom contain ligand, respectively. The XAS methodology is preferred over Cd-NMR for its high sensitivity and capability to measure Cd complexes in intact frozen plant tissues. However, the limitation of XAS is its high cost and difficulties associated with handling samples (Salt et al., 1995).

Development of a Cd^{2+} ion specific electrode

Cadmium ion analysis can be carried out with various methods such as atomic absorption (AAS), inductively-coupled plasma atomic emission spectrometry (ICP-AES), atomic fluorescence spectrometry and inductively-coupled plasma mass spectrometry (ICP-MS) (Barbosa Jr et al., 1999). Even though these methods provide accurate and precise results, they also involve low sensitivity and high maintenance costs (Hu et al., 2003). In addition, analysing a small sample volume such as that obtained from plant xylem saps is not possible with these instruments. Therefore, electrochemical methods have become promising tools for Cd analysis due to their speed, low-cost, simplicity and high sensitivity for small size samples. Stripping voltammetry (SV) is a very sensitive and selective electrochemical method to determine the concertation of metal ions in biological samples (Kounaves, 1997). The development of a Cd electrode based on stripping voltammetry techniques could therefore be a promising frontier for the determination of Cd ions in plant saps.

Stripping voltammetry consists of a three electrode system: working electrode, reference electrode and a counter electrode (Pramanik et al., 2013). In the past, a mercury electrode has been widely used as the working electrode with Ag/AgCl as the reference electrode and a platinum counter electrode (Shams and Torabi, 2006). However, the toxicity of mercury excludes its application as the working electrode in stripping analysis and attempts have been made to introduce modified carbon paste electrodes in stripping analysis of metal ions (Afkhami et al., 2013). Afkhami et al., (2012) reported that phosphorusylide N-BDMP is a suitable modifier for constructing a chemically modified carbon electrode. With this combination they successfully measured Cd^{2+} with a lower detection limit of 6.6 µg/L in biological samples. In addition, Gayathri et al. (2017) developed a carbon-nanotube/SABA (Salicylidene-2-Aminobenzylalcohol) fabricated carbon electrode for the determination of Cd^{2+} in biological samples. This modified electrode showed excellent selectivity and sensitivity for the determination of Cd^{2+} ions with a lower detection limit of 0.13 µg/L Cd. A diacetyldioxime modified carbon paste electrode was developed by Hu et al. (2003) which showed a 101% Cd^{2+} recovery in water samples. Considering these references, an effort is ongoing at FLRC, Massey University to develop a chemically modified carbon paste Cd electrode to measure the free Cd^{2+} in plant saps.

Conclusion

This review has discussed the physiological variations of plant species in the uptake and transportation of Cd from the rhizosphere to xylem. Further, this literature survey has also critically analysed various analytical methods to accurately determine the forms and levels of Cd in plant sap.

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