Intraspecific differences in heavy metal accumulaton, distribution and uptake kinetics in the metallophyte, Deschampsia caespitosa
by Richard Stuart Cahoon

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University

Abstract:
The objective of this research is the investigation of intraspecific differences in heavy metal accumulation and distribution in a higher plant species which is known to exhibit heavy metal tolerance. By studying isogenic lines of a higher plant species which differ only in their response to heavy metals, the fundamental nature of metal tolerance and ion transport in a biological system is elucidated.

Two races of the grass, Deschampsia caespitosa were compared under a variety of metal-stress (Cu, Cd) conditions. One race, "Tailings", was collected on a metalliferous waste site. It exhibits heavy metal tolerance. The other race, "Agricultural", was grown from commercial seed and appeared to be heavy metal sensitive.

Three experimental systems were used to compare races: (1) Sand-solution culture - a defined sand-nutrient solutipn technique was used to determine the influence of (a) race, (b) organ, (c) ion, (d) time and, (e) metal loading rate on tissue metal concentration.

(2) Liquid-batch culture - was used to determine the accumulation and biochemical distribution of Cd109 in races of D. caespitosa as a function of time.

(3) Macrophyte Reactor - reactor engineering principles were used to develop a novel technique for measuring ion uptake kinetics of tissues of higher plants. Using a continuous stirred tank reactor (CSTR) Cd uptake kinetics of the races were compared.

These experiments lead to the conclusion that metal-tolerant "Tailings" plants accumulate less Cu or Cd than "Agricultural" plants. This difference is more significant with Cu than Cd. Acropetal (root to shoot) transport of Cu or Cd is much higher in "Agricultural" than "Tailings". Similar differences in Cd uptake kinetics (i.e., "Agricultural" greater than "Tailings") Were observed.

Two hypotheses are posited to explain the phenomenon: "Dying Osmometer" theory - Accumulation and distribution differences can be explained on the basis of the death of "Agricultural" root cells in heavy metal solutions. "Tailings" cells do not die under these conditions.

"Altered Carrier" theory - Racial differences in metal accumulation and distribution can be explained by the ion "carrier" model. Conformational alterations in specific ion "carrier" proteins in cell membranes result in ion uptake kinetic differences.

Other biological mechanisms which may play a role in the observed intraspecific differences are discussed.

INTRASPECIFIC DIFFERENCES IN HEAVY METAL ACCUMULATION, DISTRIBUTION AND UPTAKE KINETICS IN THE METALLOPHYTE, *Deschampsia caespitosa*

by

Richard Stuart Cahoon

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Rehabilitation

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1983

APPROVAL

of a thesis submitted by

Richard Stuart Cahoon

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

12/2/83
Date

Frank F. Munshower
Chairperson, Graduate Committee

Approved for the Major Department

12-2-83
Date

Head, Major Department

Approved for the College of Graduate Studies

12-2-83
Date

Michael Malone
Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his/her absence, by the Director of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Richard S. Cameron

Date 12/2/83

TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

LIST OF TABLES

LIST OF TABLES--Continued

LIST OF FIGURES

LIST OF FIGURES--Continued

LIST OF FIGURES--Continued

Abstract

The objective of this research is the investigation of intraspecific differences in heavy metal accumulation and distribution in a higher plant species which is known to exhibit heavy metal tolerance. By studying isogenic lines of a higher plant species which differ only in their response to heavy metals, the fundamental nature of metal tolerance and ion transport in a biological system is elucidated.

Two races of the grass, *Deschampsia caespitosa* were compared under a variety of metal-stress (Cu, Cd) conditions. One race, "Tailings", was collected on a metalliferous waste site. It exhibits heavy metal tolerance. The other race, "Agricultural", was grown from commercial seed and appeared to be heavy metal sensitive.

Three experimental systems were used to compare races:

(1) Sand-solution culture - a defined sand-nutrient solution technique was used to determine the influence of (a) race, (b) organ, (c) ion, (d) time and, (e) metal loading rate on tissue metal concentration.

(2) Liquid-batch culture - was used to determine the accumulation and biochemical distribution of Cd^{109} in races of *D. caespitosa* as a function of time.

(3) Macrophyte Reactor - reactor engineering principles were used to develop a novel technique for measuring ion uptake kinetics of tissues of higher plants. Using a continuous stirred tank reactor (CSTR) Cd uptake kinetics of the races were compared.

These experiments lead to the conclusion that metal-tolerant "Tailings" plants accumulate less Cu or Cd than "Agricultural" plants. This difference is more significant with Cu than Cd. Acropetal (root to shoot) transport of Cu or Cd is much higher in "Agricultural" than "Tailings". Similar differences in Cd uptake kinetics (i.e., "Agricultural" greater than "Tailings") were observed.

Two hypotheses are posited to explain the phenomenon:

"Dying Osmometer" theory - Accumulation and distribution differences can be explained on the basis of the death of "Agricultural" root cells in heavy metal solutions. "Tailings" cells do not die under these conditions.

"Altered Carrier" theory - Racial differences in metal accumulation and distribution can be explained by the ion "carrier" model. Conformational alterations in specific ion "carrier" proteins in cell membranes result in ion uptake kinetic differences.

Other biological mechanisms which may play a role in the observed intraspecific differences are discussed.

INTRODUCTION

Increasingly sophisticated methods of detection as well as heightened environmental awareness have led to the realization that heavy metal ions pose a serious environmental problem. These heavy metal ions, toxic to most biologic systems in relatively small concentrations, appear to be an ubiquitous by-product of a technological society. Industrial process effluents, atmospheric emissions, urban runoff, municipal sewage and wastewaters, mining and large scale construction projects are all associated with heavy metal contamination of soil, air and water (Bradshaw & Chadwick, 1981; Buchauer, 1973; Burkitt et al., 1972; Lagerwerff & Specht, 1970).

Heavy metal contamination by anthropogenic activity has given rise to an intriguing phenomenon: resilient biological systems which have adapted to these contaminants.

The existence of metallophytes, higher plants which exhibit the capacity to tolerate/accumulate normally toxic heavy metal concentrations in the environment, provides an opportunity to investigate the nature of the mechanisms of ion uptake in plants and the nature of tolerance to soil ionic stresses. Tolerant species often "hyperaccumulate" essential and nonessential elements providing more research questions of biologic, environmental, and

agricultural importance. This study seeks to characterize the nature of the metallophyte phenomenon via physiological and biochemical comparisons of metal tolerant and metal sensitive ecotypes of a single species under metal stress conditions. This research also develops several techniques for the analysis of ion accumulation by higher plants. These techniques include a novel reactor design capable of yielding information on ion uptake kinetics by higher plants.

Research Goals

The goals of this research are several fold:

(1) To determine whether intraspecific differences in heavy metal ion accumulation and transport exist in a known higher metallophyte species.

(2) To characterize those differences if they exist.

(3) To establish a data base for further research into the nature and possible technological application of metallophytism in higher plants.

(4) To determine the applicability of chemical engineering principles to the evaluation and exploitation of higher plant physiological phenomena.

To attain these goals, the following research objectives have been established:

Research Objectives

(1) To evaluate intraspecific differences in heavy metal accumulation and transport with traditional experimental techniques of sand-solution culture.

(2) To evaluate possible intraspecific biochemical difference in metal accumulation and transport using radioisotope techniques.

(3) To develop a technique to measure metal ion uptake kinetics in a higher plant and to use the method to evaluate possible differences between physiological races of the selected experimental species.

The Metallophyte Phenomenon

Biosorption of Elements by Higher Plants

The concept of plant uptake of nutrients from the soil by roots is just over three hundred years old. Prior to the published work of Glauber (1656), in which he stated that saltpetre was taken from manure by plants, water and air were considered the source of all plant constituents. In subsequent experiments de Saussure (1804) unequivocally demonstrated that plants take up a portion of their constituents from the soil. For the next fifty years, workers like Boussingault and Liebig continued to develop the science of plant nutrient uptake. During this period, confusion arose over the molecular nature of the nutrients taken up from the soil by the plant. The development of nutrient solution culture techniques in the 1860's confirmed that, with rare exception, (HBO_3, MoO_4, $HMoO_4$), elements were taken up by plants in ionic form. This technical development also lead to the search for essential micronutrients and to the distinction between biologically active vs. passive ion uptake.

In the 1950's, American workers developed purification techniques that allowed them to demonstrate that chlorine is an essential plant nutrient. In so doing, they completed a list of ions which are considered as essential for higher plant life. The list of

ions which are essential to all higher plants is comprised of C, O, H, P, K, N, S, Ca, Fe, Mg, Mn, Cu, Mo, Zn, B, Cl (Steward, 1963). Elements which are not required by all plants but which are essential to particular species: Co, I, V, Na, Se, Al, Si, Cr, F, (Mortvedt et al., 1972).

Higher plants tend to absorb all elements present in root zone (rhizosphere) solution but exhibit uptake rate selectivity (Hewitt, 1975) (Table 1).

Table 1. Typical Concentrations of Mineral Elements in Foliage of Normal Plants (from Hewitt, 1975)

Element	PPM Dry Matter	Total mM in Cells	Nutrient Solution (mM)
N	15,000-35,000	150-350	15.0
P	1,500-3,000	7-14	1.0
S	1,000-3,000	4.5-140	1.5
Ca	10,000-50,000	35-175	5.0
Mg	2,500-10,000	15-60	1.5
K	15,000-50,000	55-180	5.0
Na	200-2,000	1-12	1.0
Fe	50-300	0.15-0.75	0.1
Mn	25-250	0.06-0.6	0.01
Cu	5-15	0.01-0.03	0.001
Zn	15-75	0.03-0.15	0.002
Co	0.2-29	0.005-0.05	0.0002
B	15-100	0.2-1.3	0.05
Mo	0.5-5	0.004-0.075	0.0005
Cl	100-1,000	0.4-4	0.1

As research data gathers on nutrient sorption, the evidence increasingly points towards wide species variation in selectivity of quantity and type of ion taken up (Rorison et al., 1968). Furthermore, workers have shown ion uptake variations within species. Earley (1943) demonstrated varietal differences in rate of Zn

uptake by soybean cultivars. This intraspecific variation suggests a genetic basis of ion uptake selectivity. This subject is reviewed by Epstein and Jeffries (1964) and is thoroughly discussed in Epstein's pioneering work on the biochemical genetics of nutrient uptake by higher plants (Epstein, 1972). The intraspecific variation in mineral nutrition of plants from different habitats shown by Goodman (1968) with *Lolium perenne* may have its basis in mechanisms similar to those elucidated by Woolhouse (1968), Jeffries et al. (1968) and Brown and Jones (1979). These workers describe the alteration of molecular ion transport mechanisms by ecotypes in order to adapt to particular ionic regimes in the rhizosphere.

Higher Plants and Heavy Metal Soil Ions

Higher plant species characteristic of metal contaminated soils have been recognized for several centuries. Thalius, in 1588, noted *Minuartia verna* as a metal indicator (Ernst, 1965). Others (Williams, 1830; Henwood, 1857; Baumann, 1885) mention species found consistently on metal contaminated soils. Studies of "metallophytes" (plants which tolerate/accumulate inimical metal ions) remained descriptive until the work of Prat (1934), in which he demonstrated the physiological differences between metalliferous mine colonizing and non-mine colonizing populations of *Melandrium silvestre*. This study was unique until the 1950's when workers in the United Kingdom and Germany began studying higher plants found growing on areas contaminated with heavy metals due to mining disturbance. Bradshaw (1952) described lead and zinc tolerant

populations of *Agrostis tenuis*. Later, Wilkins (1957, 1960) used rooting techniques to show tolerance of mine populations of *Festuca ovina* to lead. Workers in Germany (Schwanitz and Hahn, 1954; Repp, 1963; Baumeister, 1954; Baumeister & Brughardt, 1956; Wachsmann, 1961; Broker, 1963) added to the list of metal tolerant species.

Metal Tolerant Plants and Hyperaccumulation. A large body of literature exists which describes the use of metallophytes as tools of geologic prospecting (Wild, 1970; Cole, 1965; Cannon, 1957, 1960). These "geobotanical indicator" species, as well as other metallophytes, exhibit an interesting biological phenomenon; they appear to extract large amounts of certain elements from the soil (Table 2). The kind and quantity of ion accumulated depends upon plant species and soil conditions. These species accumulate tissue concentrations of ions many times greater than those found in the soil solution and often in excess of levels considered adequate for biological function. Table 2, though not exhaustive, has been broken down into two categories: (1) hyperaccumulation of essential elements (2) hyperaccumulation of non-essential elements and compounds.

The hyperaccumulation of essential elements by biological systems is, perhaps, to be expected. However, the selective accumulation of nonessential elements and compounds poses intriguing questions.

Mechanisms of Heavy Metal Ion Regulation by Metallophytes

Critical concentrations of heavy metal ions may interfere with biological systems via several actions:

(1) competition with essential elements for uptake or for

Table 2. Elements Hyperaccumulated by Plant Species

ION	PLANT SPECIES	REFERENCE
Zn *	*Agrostis tenuis*	Turner & Marshall, 1972
	Silene inflata	Baumeister, 1967
	Thlaspi alpestre	Ernst, 1968
Cu *	*Agrostis alba*	Prat & Komarek, 1934
	Melandrium sylvestre	Prat & Komarek, 1934
	Minuartia verna	Ernst, 1968
	Agrostis tenuis	Bradshaw et al., 1965
	Aeolanthus biformifolius	Malaisse et al., 1978
Mn *	*Caultheria hispidula*	Gerloff et al., 1966
	Vaccinium myrtilloides	Gerloff et al., 1966
	Triticum aestivum	Foy et al., 1973
	Glycine max	Heenan & Carter, 1976
	Lactuca sativa	Sonneveldt & Voogt, 1975
Mo *	*Fabaceae sp.*	Dye & O'Hara, 1959
	Lycopersicon esculentum	Johnson, 1966
	Fabaceae sp.	Barshad, 1948
B *	various species	Oertli & Kohl, 1961
		Cook 1916
Co *	*Haumaniastrum robertii*	Morrison et al., 1979
	Pimelea suteri	Lyon et al., 1968
	Nyssa sylvatica	Beeson et al., 1955
Cl *	*Agrostis tenuis*	Cordukes & Parups, 1971
	Atriplex pelycarpa	Chatterton et al., 1970
Cd	*Pinus taeda*	Kelly et al., 1979
	Liriodendron tulipifera	Kelly et al., 1979
	Lactuca sativa	Haghiri, 1973
	Raphanus sativus	Haghiri, 1973
	Apim graveolens	Haghiri, 1973
	Hordeum vulgare	Cutler & Rains, 1974
Se	*Astragalus bisulcatus*	Shrift, 1969
	Astragalus sp.	Robinson & Edgington, 1945
Si	*Equisitaceae sp.*	Robinson & Edgington, 1945
F	*Lycopersicon sp.*	Gurirtsmon et al., 1957
	Rosa sp.	Gurirtsmon et al., 1957
	Festuca rubra	Johnson et al., 1976
Cr	*Pimelea suteri*	Lyon et al., 1965

Table 2. (cont.)

ION	PLANT SPECIES	REFERENCE
Sr	*Ulmus sp.*	Vaneslow, 1966
	Atriplex sp.	Wallace et al., 1972
Pb	*Lolium perenne*	Jarvis et al., 1977
	Pinus taeda	Roffe, 1973
	Hordeum vulgare	Dowdy & Larson, 1975
	Raphanus sativus	Alloway, 1968
Ni	*Alyssum serpyllifolium*	Brooks et al., 1981
	Alyssum bertolonii	Lee et al., 1978
	Pearsonia metallifera	Lee et al., 1978
As	*Agrostis tenuis*	Porter & Peterson, 1977
Al	*Camelia sp.*	Matsumoto et al., 1976
	Sporobolus caperesis	Moomaw et al., 1959
	Pinus sp.	Suchting, 1948
Ba	Brazil nut	Wagner, 1936
I	*Spinacea oleracea*	Beeson, 1941
	Brassica sp.	Beeson, 1941
V	Various species	Beath, 1943
Ag	*Eriogonum ovalifolium*	Henwood, 1857
Hg	*Alsine setaceae*	Linstow, 1929
Sn	*Trientalis europeae*	Linstow, 1929
Ra	*Bertholetia excelsa*	Hewitt, 1975
Li	*Thalictrum sp.*	Hewitt, 1975
Br	*Cucurbitaceae sp.*	Hewitt, 1975
U	*Sarcobatus sp.*	Hewitt, 1975
Au	*Equisetum palustre*	Hewitt, 1975
Rare Earths	*Castanea sp.*	Milton et al., 1944
	Carya sp.	Robinson & Edgington, 1945
Polychloro-biphenyls	*Spartina alternivolia*	Mrozek et al., 1982

*essential elements

functional sites, competitive inhibition

(2) inactivation of enzymes due to irreversible binding or denaturation non-competitive inhibition

(3) alteration of nucleic acid organization

(4) alteration of membrane structure

(5) alteration of various macromolecular structures (e.g., spindle apparatus)

(6) alteration of cytoplasmic water structure

(7) alteration of cytoplasmic colloid structure

Those species which exhibit hyperaccumulation of particular ions (Table 2) must, obviously, avoid these deleterious effects. Higher plants have several possible tactics at their disposal with which to deal with elevated metal concentrations. These tactics can be divided into two types: external and internal.

External - mechanisms which affect the adsorption and absorption of ions onto the root surface. These mechanisms involve alteration of the chemistry of the rhizosphere in order to make ions more or less available. Foy et al. (1978) have demonstrated that crop species alter the rhizosphere pH, rendering Al ions insoluble and thus unavailable for uptake by the plant. Levitt (1980) terms this, "metal ion stress avoidance". Ernst (1976) states that there is no evidence that higher plants can exclude ions in the soil solution, though exclusion has been demonstrated in unicellular *Chlorella* (Foster, 1977).

Internal - mechanisms which effectively reduce toxic cellular levels of heavy metals or otherwise mitigate their toxic effects upon

cellular components. A list of these theoretically possible mechanisms and the experimental evidence, or lack thereof, which supports or fails to support their validity follows:

(1) Differential uptake of ion - demonstrated in fungi (Okamoto and Fuwa, 1974), algae (Foster, 1977) and higher plants (Ernst, 1972)

(2) Removal of ion via deposition - in cell walls; demonstrated for Zn, Cu, Pb, Al (Ernst, 1972; Peterson, 1969; Turner & Marshall, 1972; Malone et al., 1974). In vacuoles; not demonstrated for heavy metals, however evidence for deposition in vacuole by halophytic species (Greenway & Munns, 1980).

(3) Removal of ion via extrusion - no evidence for this mech ism in metallophytes; demonstrated in halophytes (Greenway & Munns, 1980).

(4) Chelation or complexation of ion by organic compound - demonstrated in higher plants for Cu (Rauser & Curvetto, 1980), Ni (Thompson & Tiffin, 1974), Zn (Rauser, 1981), Cd (Wagner & Trotter, 1982).

(5) Selective translocation to non-vital areas of the plant - demonstrated in higher plants for Al (Sivasabramaniam & Talibudeen, 1972), Ni (Gambi, 1967), Pb (Roffe, 1973), Cu (Reilly & Reilly, 1973; Bradshaw et al., 1965), Zn (Turner & Marshall, 1972).

(6) Alternate metabolic pathways which by-pass inhibited enzymes - not experimentally verified in higher plants.

(7) Increased production of inhibited enzyme(s) - not experimentally verified.

(8) Production of organic antagonist - not experimentally verified, however, Mathys (1975) has shown a correlation between metal ion tolerance and production of glycosides in Cruciferae.

(9) Decreased requirements for products of inhibited system - not experimentally verified.

(10) Production of altered enzyme such that enzyme is rendered insensitive to metal ion effects - in-vivo activity of some enzymes of Cu tolerant ecotypes is less inhibited by elevated solution copper than enzymes of Cu-sensitive (Mathys, 1975); stages of the TCA (Krebs) cycle of Cu tolerant plants are not inhibited to the same extent as in Cu sensitive (Ernst,

1976); root acid phosphatases of Cu-tolerant ecotypes are less sensitive to Cu ions than non-tolerant (Woolhouse, 1970).

(11) Increased uptake of H_2O, increased growth, dilution of ion - not experimentally verified.

Significance of Research into Metal Tolerance and Hyperaccumulation by Metallophytes

Investigations into the nature of the metallophyte phenomenon are an important and fertile area of research. These studies have implications for various fields:

Biology

The bulk of evidence for the elucidation of the process of organic evolution is based upon phylogenetic studies of fossil records. However, some biological systems have exhibited evolutionary development over relatively short time periods. A classic example of this process is the development of "industrial melanism" in lepidopterous species near factories in the United Kingdom.

The unique significance of soil chemical composition as a factor in the selective complex of plant evolution is based upon the fact that there is, at the membrane surfaces of root cells bounding the apoplast, a direct confrontation between the environment (i.e., soil ions) and the structural and catalytic components of the biological system. Upon radicle extension into the soil, there exists an interface of protein-mediated activity at the plasmalemma with the external environment. As a result, the study of biochemical and physiological adaptation to excess metal concentrations in the soil and the plant offers a unique opportunity to investigate the process of plant evolution.

Given the genetic basis for the phenomenon of heavy metal tolerance and accumulation in higher plants, it seems logical to explore the mechanisms by which "metallophytes" exhibit these characters. The characterization of the ecological, physiological, and biochemical basis of the phenomenon presents the possibility of integrating those factors involved in genome-environment interaction.

Investigations into heavy metal uptake by metallophytes can provide insight into the basic biological processes of ion uptake. These processes can be characterized via the physiological and biochemical comparison of metal sensitive and metal tolerant, as well as metal accumulating and non-accumulating populations of the same species. This approach can provide data to elucidate the biochemical and genetic nuance of ion uptake, translocation, and assimilation.

Agriculture

Studies of metallophytic mechanisms provide a basis for a more thorough understanding of ion uptake. Such studies will lead to a more thorough understanding of the relationship of plants and soil chemistry. Such an understanding of the nature of ion-related physiology may lead to a refinement of current agricultural technology. Describing the molecular nature of metallophytic mechanisms will lead to the development of plant species which are suited to less favorable soil environments. This concept runs counter to the traditional approach of manipulating the environment via irrigation, fertilization, amendments, etc. to suit the plant. By investigating the nature of

the mechanisms by which higher plants tolerate and accumulate potentially inimical ions in the rhizosphere, the foundation will be laid for the use of crop species which ameliorate their own soil environment rather than relying upon traditional methods of soil manipulation. This concept, discussed in some recent literature (Christiansen and Lewis, 1982; Wright, 1976) provides much opportunity for research and could revolutionize agricultural science, leading to developments in the manner in which we use technology in agricultural systems.

Environmental Engineering and Land Reclamation

The prospect of developing a technology wherein selected higher plant metallophytes are utilized to ameliorate environmental contamination problems is a distinct possibility. Metallophyte species have already proven useful in the reclamation of metalliferous waste areas (Gadgil, 1968; Johnson et al., 1977; Smith and Bradshaw, 1970).

The use of higher plants in biosorptive water treatment systems (Bouwer and Chaney, 1974) suggests that metallophytes could be utilized to ameliorate metal contamination of mine drainage, urban runoff, and industrial effluents, etc. The demonstrated ability of higher plants to accumulate organic contaminants from soils (Mrozek et al., 1982) and to metabolize these compounds into benign substances (Harborne, 1977), suggests the possibility of utilizing selected species to reclaim contaminated land and to cleanup some industrial effluents and toxic soil residues remaining after hazardous material spills.

Biogeochemical Prospecting

The use of metallophytic species as indicators of ore bodies is not a novel practice. Agricola in his "De re Metallica" of 1556, described the anomalous growth and coloration of plants associated with veins and outcrops of metal ores.

This concept, though not thoroughly developed in this country (Cannon, 1957, 1960), has been utilized extensively in Australia, New Zealand, South Africa, and the Soviet Union (Brooks, 1972).

Recent advances in the field of biogeochemical prospecting utilize aerial photography and other remote sensing techniques to identify areas of metal accumulation. These techniques often rely upon the unique spectral reflectance of metallophytes under conditions of metal hyperaccumulation (Homer et al., 1980).

HEAVY METAL IONS AND HIGHER PLANTS

The heavy metals as defined by Passow et al. (1961), comprise thirty-eight elements. All these elements share one thing in common, they are all toxic to biological systems at relatively low concentrations. Fortunately not all of the thirty-eight elements exist in concentrations that pose environmental health hazards. In fact, increasingly sophisticated analytical techniques have revealed that of the thirty-eight heavy metals, relatively few exist at critical levels in soil or water; the elements of concern, and those emphasized in this report are Cu, Zn, Pb, Ni, Cd, Al, Mn.

Zinc - This element, an essential nutrient, is required in small quantities. Its presence is required in several enzyme systems (Clarkson & Hanson, 1980). Because of its small size, Zn tends to form tetrahedral complexes. For unknown reasons, the plant cell has need for stable metalloenzyme complexes in which the coordination is tetrahedral, thus, Zn best fits this need. Zinc appears to play the role of enzymatic cofactor and is absolutely essential for various metabolic pathways, i.e., the synthesis of tryptophan. All plants contain some level of Zn.

The occurence of high Zn concentrations in plant tissues grown on Zn-contaminated soils was first established in the 1800's. More

recent work on the phenomenon of Zn accumulation has revealed the following:

(1) Different species, growing in the same Zn-contaminated soil, differ in the degree to which they take up Zn (Ernst, 1965).

(2) Different plant organs accumulate different quantities of Zn. Generally, roots accumulate most of the Zn. In some species, however, the inflorescence seems to be the prime accumulating organ. While most Zn-accumulators retain the largest portion of Zn in root tissues, the pattern of distribution depends upon the species (Nicolls et al., 1965; Cole et al., 1968).

(3) The quantity of Zn in plant tissues varies with change in season and often shows an increase as season progresses, Zn uptake being correlated with growth (Ernst, 1965).

(4) Levels of Zn in tissues vary within species, implicating genetic control of uptake (White, 1976).

(5) No experimental evidence exists for an active Zn-carrier.

(6) Uptake of Zn appears to be independent of metabolic activity as it is unaffected by metabolic inhibitors and temperature; dead tissues take up more Zn than living tissue (Rathore et al., 1970). However, subsequent translocation does seem to be metabolically dependent (Chandel & Saxena, 1980).

(7) Zinc is involved in cell wall synthesis (Hewitt et al., 1954); Zn accumulating species appear to incorporate Zn into the cell wall matrix (Turner & Marshall, 1971, 1972). The amount of ^{65}Zn incorporated in the cell wall is correlated with tolerance of high Zn levels in the soil by the plant (Peterson, 1969); Zn tolerant *Agrostis tenuis* accumulate more cell wall ^{65}Zn than Zn sensitive *A. tenuis*. Furthermore, the amount of Zn in the shoot/amount of Zn in the root (shoot/root ratio) is highest in Zn tolerant species. The greatest proportion of ^{65}Zn taken up is incorporated in the cell walls of the root and at stem nodes (Peterson, 1969).

(8) Low CEC (cation exchange capacity) of roots of tolerant species appears to significantly reduce Zn absorption rate (Ernst, 1972a).

(9) Tolerance to high soil Zn can be conferred by way of reciprocal grafts of root and shoot tissue (White, 1976). Similar results have been found with uptake of B (Brown & Jones, 1971).

Copper - This element, an essential nutrient, is a bound moiety in many redox enzymes. The role of Cu as a strong competitor for ligands makes it vital for much enzymatic activity and toxic in any but low concentrations.

Investigations into the aspects of Cu accumulation have not received the attention that has accompanied Zn uptake. Workers have reported the following findings on Cu accumulation:

(1) Species grown on identical soil Cu concentrations, differ in the quantity of copper which they accumulate in their tissues (Bateman & Wells, 1917).

(2) Copper uptake mechanisms are apparently different from Zn uptake mechanisms (Peterson, 1969).

(3) Copper levels in aerial parts remain constant with increasing soil copper concentration until a "threshold" concentration is reached at which time the Cu content in above-ground parts rises dramatically. Species differ in the level at which this "threshold" uptake occurs (Nicolls et al., 1965).

(4) Roots consistently contain more Cu than aerial parts (Ernst, 1968).

(5) In solution culture, tolerant and non-tolerant races of *Agrostis tenuis* exhibit very slight increases in shoot tissue Cu with increases in soil Cu concentration. Roots, however, show highly significant increases (Bradshaw et al., 1965).

(6) Species show a remarkable ability to "hyperaccumulate" tissue Cu from low soil Cu concentrations (Bradshaw et al., 1965).

(7) Cu appears to be translocated via a specific chelator (Thompson & Tiffin, 1974).

(8) Copper is selectively translocated from sensitive roots to tolerant aerial parts in *Becium homblei* (Reilly & Reilly, 1973).

(9) High copper concentrations (16 micromoles $CuSO_4$) in nutrient solution apparently triggers the production of copper-thioneins (low molecular weight proteins that bind metals in mercaptide complexes (Rauser & Curvetto, 1980) in copper tolerant *Agrostis gigantea* (see also Morrison et al., 1979; Malaisse & Gregoire, 1978).

Lead - This element exhibits no known function in plant cells. Not being an essential element, its pattern of uptake should differ substantially from the previously discussed metals.

Although Pb contamination is an environmental problem, little work has been done to characterize its uptake by higher plants. A review of this literature reveals:

(1) The pattern of Pb uptake resembles that of Cu, initially static tissue levels in aerial parts with increasing soil concentration and subsequent rapid uptake at a threshold level (Nicolls et al., 1963);

(2) Uptake of Pb is not influenced by metabolic inhibitors or low temperatures but is dependent upon soil pH (in *Phaseolus, Zea, Glycine*) Arvik & Zimdahl, 1974);

(3) Plant species show variability in degree of Pb uptake as well as in localization of deposition (Rolfe, 1973).

(4) Pb which is taken into the cell is concentrated in the dictyosomes (of corn root cells); lead appears to be subsequently incorporated into the cell wall matrix (Malone et al., 1974);

(5) Roots typically accumulate more Pb than shoots (in *Lolium perenne*) (Jarvis et al., 1977).

Nickel - A review of the literature reveals scant details on the nature of Ni uptake. Wild (1970) has demonstrated that the pattern of uptake of Ni resembles that of Cu. Similarly, some reports indicate

that Ni "hyperaccumulators" exist (Antonovics & Bradshaw, 1971). The following details of Ni uptake have been reported:

(1) Ni appears to accumulate in the epidermis of leaves and in the schlerenchyma between vascular bundles (in *Alyssum bertoloni*, a Ni accumulator) (Gambi, 1967).

(2) Ni is translocated via a specific chelator (Thompson & Tiffin, 1974).

Cadmium - This element has received attention because of its toxic effects in low concentrations and accumulation in the food chain. Cd has no known role in metabolic function. However, the accumulation of Cd by higher plants has been observed by several workers. Some crop species appear to be such "hyperaccumulators" of Cd that health concerns have been voiced. This is particularly evident in the use of municipal sewage sludges as fertilizer (Hinesly et al., 1978; Turner, 1973). The following details of Cd accumulation have been published:

(1) The pattern of Cd uptake is similar to Pb. Initial uptake is via diffusion and exchange adsorption; subsequent translocation and sequestering involves metabolic activity (Cutler & Rains, 1974);

(2) There exists no experimental evidence for an active carrier of Cd;

(3) Cd concentrations (in soybean cultivars) is in the order stems > leaves > pods > beans (Haghiri, 1973);

(4) Cd is accumulated against a concentration gradient (in Barley) and is retained primarily in roots and the lower stem. Cd appears to be "irreversibly sequestered" once taken up by the cell (Cutler & Rains, 1974). Dead root tissues accumulate more Cd than living tissue (Jarvis et al., 1977);

(5) Cd accumulation is under genetic control (Hinesly et al., 1978);

(6) Species differ in the degree to which they take up Cd (Haghiri, 1973; Miles & Parker, 1979).

(7) All species tested exhibit increased tissue Cd levels with increasing soil Cd concentrations; uptake rate varying between species (Kelly et al., 1979).

Aluminum - This element is not an essential plant nutrient. Aluminum toxicity is rare in temperate climates but common in tropical soils.

The work by Foy et al. (1978) is tangentially related to metal uptake in that it involves plant mechanisms which inhibit Al uptake. These avoidance mechanisms exhibit a unique survival tactic, manipulation of the chemistry of the rhizosphere by the plant root. Olsen et al. (1981) have investigated similar tactics which involve decreasing the soil pH in order to render Fe more available.

Work on the accumulation of Al has shown that:

(1) Al tolerance (in barley) appears to be controlled by one major, dominant gene (Reid, 1971);

(2) Al tolerance may be due to a chelating mechanism (Grine & Hodgson, 1969);

(3) Al-sensitive wheat and barley varieties accumulate higher Al concentrations in aerial parts than Al-tolerant (Foy & Fleming, 1978);

(4) Tea plant (*Camellia* spp.) selectively translocates Al to non-sensitive areas (older leaves), avoiding more sensitive areas (new leaves and buds) (Sivasabramaniam & Talibudeen, 1972);

(5) Al is located in leaf epidermis cells which exhibit distinctly thickened walls (Matsumoto, 1976);

(6) Some Al-tolerant species have the ability to increase the rhizosphere pH, rendering Al less available (Chamura & Koike, 1960; Adams & Pearson, 1970; Clark & Brown, 1974).

Manganese - Ionic Mn is an essential nutrient. As such, one can expect Mn uptake mechanisms to exist in all species. Excessive uptake

occurs when the divalent form Mn^{++} is present in high soil concentrations. This situation typically arises under reducing conditions.

The scant reports on Mn accumulation have demonstrated the following:

(1) Plant species and cultivars differ greatly in their ability to tolerate excess soluble Mn (Foy, 1973);

(2) Mn tolerance is associated with greater retention in the root (Andrew & Hegarty, 1969);

(3) Mn-tolerant rice plants apparently oxidize Mn^{++} (available) to Mn^{+3} (less available) (Engler & Patrick, 1975).

BIOLOGY OF ION TRANSPORT IN HIGHER PLANTS

The transport of ions from aqueous solutions into root and shoot cells of higher plants is vital to life on earth. The difficulty in analyzing the processes which are involved in ion accumulation and assimilation in higher plants is reflected in the general lack of basic data in this area. A review of the pertinent literature (Lüttge & Pitman, 1976; Pitman, 1977; Nissen, 1974; Anderson, 1972; Clarkson & Hanson, 1980; Briggs et al., 1961; Wardlow & Passioura, 1976; Lüttge & Higinbotham, 1979; Nye & Tinker, 1977) reveals the complexity of the various interactive processes involved in higher plant-ion transport phenomena. A synthesis of this literature suggests the following general mechanisms of ion transport into the xylary stream.

Once ions are at the root surface they may move through the root tissues via either of two pathways, (1) the apoplasm and (2) the symplasm (Figure 1). The apoplast is that zone which includes all extracellular wall material and intercellular spaces. Ions may be transported in the apoplast up to a layer of suberized material (casparian strip) which separates root cortical cells from vascular tissue. A combination of exchange sorption and diffusion allows ions to move in the apoplast. The symplasm is that zone which is

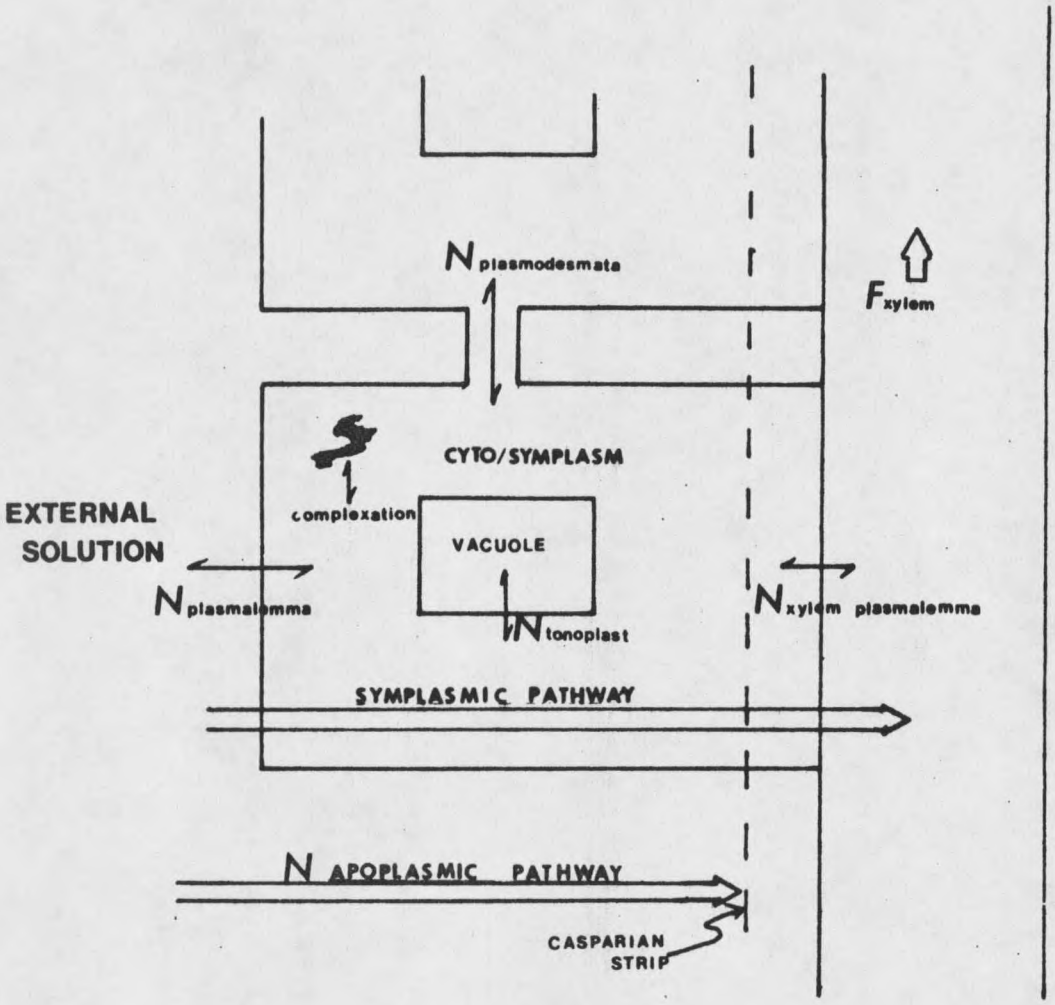


Figure 1. Schematic diagram of higher plant root ion transport system. N = diffusive transport processes. F = convective transport processes. (Adapted from Pitman [1971]).

bounded by the external surface of the cell membrane (plasmalemma). Considerable evidence (Gunning & Robards, 1976) indicates that the cytoplasm in the root is connected by channels (plasmodesmata) making the symplasm a contiguous system. Movement by ions through the symplasm is not understood though plasmodesmata and cytoplasmic streaming have been implicated.

The existence of the casparian strip necessitates ion transport across the plasmalemma prior to transfer to the xylary stream. Here, at the plasmalemma/apoplast interface, lies the crucial transport mechanism debate. Is ion transport through the membrane a purely physico-chemical process or is it mediated by metabolic activity? Though individuals ascribe various levels of importance to each mechanism, the available evidence (i.e., ion selectivity, transport against diffusion and electrical potential differences) suggests a large role for biological structures in ion transport.

Of the various models which have been put forth to describe the empirical results, the one which is widely accepted by physiologists and which elicits much debate is termed the "carrier" hypothesis (Epstein, 1972; Laüchli, 1976). This hypothesis is based upon the concept of enzymatic saturation (Nissen, 1974) which implicates protein "carriers" as the mode of ion transport across the plasmalemma. Like enzymes, these carriers may be more or less specific for particular substrates, and will exhibit saturation kinetics described by Michaelis-Menten type equations. Using this approach, Epstein (1972) stated that there are two distinct mechanisms of potassium

uptake by barley roots. Nissen (1983) alleges that there are several distinct saturation kinetics phases in ion uptake as a function of external ion activity. The following evidence corroborates the existence of protein "carriers" which mediate ion transport:

(1) Sulfate and phosphate binding protein can be isolated from a variety of cell membrane systems (Rothfield, 1971)

(2) Potassium transport in barley and sunflower is regulated by allosteric control of K^+-carrier activity by cytoplasmic potassium concentration (Glass, 1977; Nye & Tinker, 1969; Pettersson, & Jensen, 1978)

(3) Existence of a constitutive phosphate uptake mechanism in *Neurospora* (Lowendorff et al., 1974)

The review articles and texts on biological transport mentioned above generally agree that the "carrier" hypothesis at least partially explains a wide variety of ion transport phenomena.

A corollary to the "carrier" hypothesis is that differences in uptake characters, especially within species (Laüchli, 1976) may be explained on the basis of protein conformational alterations. In fact, nutritional ecotypes do exhibit enzymatic differences (Laüchli, 1976). Cox and Thurman (1978) demonstrated that zinc-tolerant *Anthoxanthum* forms less stable metal-enzyme complexes (lower Km) than zinc-sensitive clones. Wainwright and Woolhouse (1975) demonstrated similar enzyme differences between Cu-tolerant and Cu-sensitive races of *Agrostis tenuis*. The *in-vivo* activity of several enzymes of Cu-tolerant ecotypes is less inhibited by elevated solution copper than enzymes of Cu-sensitive plants (Mathys, 1975).

Laüchli (1976) has stated that ions are "carried" across the plasmalemma from the apoplasm, transported to xylary parenchyma by unknown mechanisms and then actively secreted into the xylem stream by "carriers" located at the plasmalemma of the cells lining the xylem. Pitman (1977) concludes that while the apoplast to symplast and symplast to xylem ion transfer processes are similarly mediated by "carriers", they are distinctly different systems based on metabolic inhibitor studies.

The implication of the carrier hypothesis is that ion uptake characteristics are genetically controlled. This fits with the few studies which attempt to correlate ion uptake parameters with genetics. In 1934, Weiss stated that iron "utilization" in soybean was under monogenic control. Similarly, Pope and Munger (1953a, 1953b) demonstrated that boron and magnesium uptake and/or translocation in a celery variety is controlled by a single allele. Boron deficiency in tomato has been linked to a single, recessive gene (Wall & Andrus, 1962). Bernard and Howell (1964) showed that phosphorus accumulation in a soybean is also governed by a single gene. Epstein (1972) discusses much of this type of work. Other workers (Brown & Devine, 1980; Brown et al., 1971; Jones, 1974; Clark, 1975; Wright, 1976; Clarkson & Hanson, 1980) have shown significant ion transport differences between genotypes, suggesting genetic control at one or several loci. Most of the above reports discuss the nature of the ion "carrier". However, other processes are involved in ion uptake phenomena. For example, removal of an

ion from the symplasm directly affects uptake rate and may be accomplished by extracellular secretion, or complexation in the cytoplasm or cell wall. Several investigators have suggested that cell wall composition (i.e. CEC) (Ernst, 1972) may play an important role in uptake processes. Complexation of metal within the wall matrix has been demonstrated for Zn, Cu, Pb, and Al (Peterson, 1967; Ernst, 1972; Turner & Marshall, 1972; Malone et al., 1974). Complexation of ions in cytoplasmic chelates has been demonstrated in several plant-ion systems (Rauser & Corvetto, 1980; Wagner & Trotter, 1982). These intracellular chelates are relatively low molecular weight proteins that have the peculiar characteristics of low aromatic amino acid moieties and high cysteinyl residue content. In mammalian systems, metals can induce the transcription of genes directing the synthesis of proteinaceous chelates (Hamer & Walling, 1982). Deposition in the vacuole also represents another process by which the intracellular ion concentration and ion uptake may be affected. Depositional processes have been implicated in biological mechanisms of tolerance to Na^+ and Cl^- accumulation (Greenway & Munns, 1980).

The various transport systems in the higher plant root are diagrammed in Figure 1. Control of the uptake process can take place at any of the sites (N) shown. Genetic control of these various processes is implicit even though the rate limiting step per se may be a strictly physical process (i.e., passive adsorption to wall matrix). In other words, the rate-limiting step in ion transport in a particular species for a particular ion may be under

monogenic (i.e., cytoplasmic chelates) or polygenic (i.e., altered membrane and wall structure) control.

RESEARCH STRATEGY

General

The focus of this research is a characterization of the biological mechanisms of heavy metal accumulation in a higher plant which exhibits unusual metal-tolerance capacity. The primary tool of this investigation is the comparison of a known metal-tolerant species with a metal-sensitive race of the same species under various metal-stress conditions. Assuming that the races are essentially identical, differences in physiological response to rhizosphere heavy metals allow for an investigation of the basic mechanisms which differentiate these races. Indeed, the study of physiological "mutants" led to Garrod's (1909) "one gene-one enzyme" hypothesis of biochemical genetics. The use of intraspecific, isogenic comparisons of physiologic response to heavy metals is a first step towards an understanding of the fundamental biological mechanism underlying ion transport.

The primary means of comparing these races is by determining the accumulation and distribution of heavy metals in plant tissues under different culture conditions of metal stress. From differences in metal accumulation and distribution under identical conditions one may infer differences in transport characteristics. Distribution of metals in organs (root vs. shoot) and biochemical fractions

allows the investigator to draw conclusions concerning metal accumulation (and thus transport) mechanisms. Some consideration is also given to a comparison of growth rates in the experimental races.

A review of the literature shows that ion accumulation characteristics vary widely between ions. It seems useful, therefore, to compare accumulation patterns of two dissimilar heavy metals in these two physiological races.

Finally, this research develops a technique for precise control over rhizosphere conditions which permits accurate determination of ion uptake kinetics and other physiological responses under tightly controlled rhizosphere conditions.

Using these approaches, the following questions have been posed:

(1) Are there heavy metal accumulation differences between metal-tolerant and metal-sensitive races of the same species?

(2) Are there differences in heavy metal distribution in organs (root/shoot) between races?

(3) Are there differences in heavy metal distribution in biochemical fractions of the two races?

(4) Are there differences in heavy metal accumulation/distribution relative to the species of ion?

(5) Are there differences in heavy metal accumulation/distribution relative to species of ion and genotype?

(6) What are the general magnitudes of accumulation/distribution differences, if they exist?

(7) Can established methods of reactor design engineering be applied to a higher plant root-ion system?

(8) Can a "macrophyte reactor" be designed to yield reliable ion uptake kinetic data for the heavy metal-metallophyte system?

(9) Are heavy metal accumulation differences (if they exist) reflected in differences in ion uptake kinetics?

(10) What are the general magnitudes of ion uptake kinetics differences, if they exist?

(11) How do differences in heavy metal accumulation, distribution, and uptake kinetics relate to established and purported biological mechanisms?

(12) Do the patterns of differences in heavy metal accumulation, distribution and kinetics suggest fundamental mechanisms of ion transport in biological systems?

(13) Do these differences imply biological mechanisms of heavy metal tolerance and accumulation in higher plant systems?

Experimental Species *Deschampsia caespitosa*

Selection

A suspected metal tolerant grass species was collected on the Anaconda Reduction Works tailings ponds in the fall of 1981. Analysis of the tailings material (Table 3) showed elevated levels of several metals and low pH. Determination of metal concentration in tissues of this grass (Table 4) indicated accumulation of several metals well above that considered adequate or normal (Mortvedt, 1972). The collected species was taxonomically determined as *Deschampsia caespitosa* (L.) Beauv. commonly referred as "tufted hairgrass".

Table 3. Data from analysis of tailings material collected near *Deschampsia caespitosa* collection site at Anaconda, MT. All elements in $\mu g\ ml^{-1}$ of a cold-water extraction of saturated paste.

SAR*	1.68
pH	3.37
EC (mmhos)	6.10
Ca	411.0 $\mu g\ ml^{-1}$
Mg	350.0 $\mu g\ ml^{-1}$
Na	191.2 $\mu g\ ml^{-1}$
Fe	3.32 $\mu g\ ml^{-1}$
Zn	340.0 $\mu g\ ml^{-1}$
Cu	364.0 $\mu g\ ml^{-1}$
Mn	1000.0 $\mu g\ ml^{-1}$
Pb	0.10 $\mu g\ ml^{-1}$
Cd	4.10 $\mu g\ ml^{-1}$
Al	155.0 $\mu g\ ml^{-1}$

*Sodium adsorption ratio

Table 4. Concentrations of metals in samples of *D. caespitosa* collected in October 1981 on the Anaconda Reduction Works tailings ponds. Samples were decomposed by perchloric acid digestion. The residue was analyzed for metals by atomic absorption flame spectrophotometer.

Deschampsia caespitosa samples	Metals in $\mu g\ g^{-1}$ dry tissue							
	Cu	Cd	Zn	Mn	Mg	Fe	Pb	Ni
Standing Dead Litter	238	19.8	419	682	930	1838	59.0	--
Living Roots & Shoots	1000	25.5	550	670	1150	9750	231	3.57

This species is catholic in distribution in the northern hemisphere. It is a "bunch" grass which reproduces vegetatively by tillering. Metal tolerance in this species has been previously reported (Cox & Hutchinson, 1979; Surrbrug, 1982).

The *D. caespitosa* collected at the metal tailings site is referred to throughout this report as the "TAILINGS" race or population.

Seed of *Deschampsia caespitosa* from agricultural-field grown plants was obtained from the Oregon State University Seed Laboratory. This population is referred to as the "AGRICULTURAL" race or population.

Collection of *Deschampsia caespitosa* "TAILINGS"

Fifteen to twenty individual clumps of *D. caespitosa* were collected randomly across a several acre grassed site on the tailings ponds. Individual clumps were selected at least five meters apart. The plants were dormant when collected in October and were transported in tailings to the greenhouse.

Preparation of *D. Caespitosa* "TAILINGS" and "AGRICULTURAL"

Two months after field collection (December, 1981) "Tailings" plants were washed numerous times with double distilled water to remove adhering tailings from the root system. One hundred and fifty smaller clumps were removed from the 15 larger clumps, rinsed with distilled H_2O and were placed in 125 ml plastic beakers which contained acid-washed (0.1N HNO_3) sand which was saturated with Arnon and Hoagland's (Hewitt, 1975) (1X) solution (Table 5).

Plants were watered daily with nutrient solution and double distilled water only, until the initiation of: EXPERIMENT 1, five months later; EXPERIMENT 2, fifteen months later; EXPERIMENT 3, seventeen months later.

Table 5. Modified 1X Hoagland and Arnon nutrient solution used in heavy metal uptake experiments.

SALT	CONCENTRATION
KNO_3	1.03 g ℓ^{-1}
$Ca(NO_3)_2 \cdot 4H_2O$	0.708 g ℓ^{-1}
$NH_4H_2PO_4$	0.231 g ℓ^{-1}
$Mg\ SO_4 \cdot 7H_2O$	0.466 g ℓ^{-1}
H_3BO_3	2.86 mg ℓ^{-1}
$Mn\ Cl_2 \cdot 4H_2O$	1.80 mg ℓ^{-1}
$Cu\ SO_4 \cdot 5H_2O$	0.077 mg ℓ^{-1}
$Zn\ SO_4 \cdot 7H_2O$	0.218 mg ℓ^{-1}
$H_2MoO_4 \cdot H_2O$	0.078 mg ℓ^{-1}
Fe Na EDTA	0.164 g ℓ^{-1}

Seeds of the "AGRICULTURAL" race were germinated in December 1981 in identical acid-washed sand, nutrient solution culture. These plants were watered identically to the tailings population until the onset of experimentation. All specimens were kept in the greenhouse during the preparation phase.

Experimental Heavy Metals

Two heavy metal ions were chosen for experimentation because of their similarities and differences. Copper and Cd were

selected because of their importance as environmental contaminants. Both induce toxicity in living systems at relatively low concentrations. These metals are essentially different in their functional relationships to biological systems. Each exhibits different coordination chemistry. Copper is a required element for biological function whereas Cd assumes no known role in metabolic processes. This essential difference could result in different patterns of accumulation in the two races of metallophyte.

Copper. Like all members of the transition metals, this element has an incompletely filled d-orbital. Copper is commonly found in the II oxidation state though I and III oxidation states are also relatively stable. Like other transition metals, Cu forms numerous inorganic and organic complexes. The chemistry of Cu is complex and a source of intense interest and debate (Leckie & Davis, 1979).

The physiological role of Cu is also a complex problem. It provides organisms with a metal which in its reduced state readily binds and reduces O_2. In its oxidized form, Cu is readily reduced. In protein complexes Cu has a high redox potential. These properties have been exploited by use in enzymes that hydroxylate monophenols (oxidizing them to create complex polymers), terminate electron transport chains, detoxify superoxides, oxidize amines, and act generally as cytoplasmic oxidases. Only Cu fills this vital biochemical niche. Although the primary biological role of Cu lies in its relationship with proteins in metal-enzyme complexes,

it can act in other ways (Peisach, 1966):

(1) in trigger and control mechanisms;

(2) in structural forms (i.e., elastin, collagen)

(3) as a Lewis acid (i.e., Cu^{++} accepts electron pairs);

(4) as redox catalyst.

The copper requirement of plants is quite low relative to other nutrients. It is essential in amounts near 3-10 ppm dry weight but toxic in amounts over 50 ppm (Clarkson & Hanson, 1980; Nriagu, 1979).

Cadmium. This metal belongs to the IIb group of elements and is almost always found in the II oxidation state. Although Cd exhibits no known vital role in biological processes, it is accumulated by higher plants. (Nriagu, 1979). Growth reduction due to Cd has been shown in both soil and solution studies (Jastrow and Koeppe, 1980). Cadmium is known to complex with certain proteins, to substitute for other metals in metalloenzymes. Ernst (1980) has reviewed the data on cadmium effects on plants and has stated the following:

(1) Cd inhibits *in vitro* activity of several enzymes;

(2) Cd has a high affinity for sulfhydryl groups;

(3) Nitrate reductase is very sensitive to low concentration of Cd;

(4) Cd reduces the amount of chlorophyll in leaf mesophyll cells;

(5) Cd inhibits photosynthesis;

(6) Cd induces chromosomal aberrations.

EXPERIMENTAL TECHNIQUES

Sand-Solution Culture - Experiment 1

Acid-washed silica oxide wetted with a defined nutrient solution has been widely applied in plant-ion investigations (Hewitt & Smith, 1975). It permits a certain level of control to the investigator but does not allow direct evaluation of uptake kinetics.

The sand-solution system was used in Experiment 1 to determine if any heavy metal accumulation or distribution differences exist between the experimental races. The system was also used to evaluate differences arising due to different metal ions (Cu, Cd). Finally, Experiment 1 describes accumulation and distribution differences due to metal loading rate and in time (plant age). This experiment was carried out in the greenhouse which adds environmental variables to the system. However, if one assumes that each race responds identically to environmental factors other than rhizosphere metals, the system may be used to make comparisons between races. Response variable in this experiment is metal concentration in harvested plant tissue as determined by atomic absorption flame spectrophotometry.

Batch Solution-Cd Isotope-Experiment II

Batch solution culture is a common technique to investigate plant-ion relations (Nye & Tinker, 1969). Determination of ion uptake kinetics in batch systems is very difficult, if not impossible, owing to the changing concentrations of ionic species in the reactor vessel over time. Batch culture was used in Experiment II for one purpose: to determine the biochemical distribution of Cd ions in the two experimental races of *D. caespitosa*.

Experimental use of radio isotopes has, perhaps more than any other technique, lead to a greater understanding of the nature of ion transport in plants. Its utility lies in the investigator's ability to detect minute ion concentration differences over short time periods. Cadmium was selected for experimentation because of a commercially available source of Cd^{109} (half life = 456 $\pm$ 10 days) and the fact that isotopes of Cu have half lives which preclude feasible use. Response variable in this experiment was concentration of Cd based upon measurements of gamma radiation from Cd^{109}. Though gamma emitters pose safety problems, they are experimentally convenient due to the fact that sample preparation is very simple (i.e. quenching is not a factor).

Macrophyte Reactor - Experiment III

Nye and Tinker (1979) have pointed out the conceptual failure in the use of batch (solid and liquid media) culture systems to obtain ion uptake data. They state that continuous-flow systems are required for accurate evaluation of the kinetic characteristics

of ion accumulation by higher plants. With this motivation and the existing body of literature on the modeling of environmental processes (Waite & Freeman, 1977) and reactor kinetics (Smith, 1970), Experiment III was designed to determine the feasibility of constructing a "macrophyte reactor" suitable for obtaining ion uptake kinetic data. The objectives of this experiment are:

(1) To construct a reactor which would provide control of a continuous-flow nutrient solution stream around the roots of *D. caespitosa*.

(2) To obtain ion uptake kinetics data from the *Deschampsia*-heavy metal system.

(3) To compare heavy metal accumulation kinetics between the two experimental races of *D. caespitosa*.

Several investigators have used flowing solutions to determine ion uptake in plants (Clement et al., 1974; Asher et al., 1965; Edwards & Asher, 1974; Van de Dijk, 1981; Veen, 1977; Wild et al., 1974) and more recent systems have employed elaborate control of particular ions in the nutrient solution (Breeze et al., 1982). All of these systems are batch or semi-batch reactors which means that ambient nutrient concentrations around roots are changing over time. Accurate kinetic data requires constant nutrient composition at the root surface. Bloom and Chapin (1981) have presented the only system which actually employs a continuous-flow reactor. None of the reports in the literature uses reactor kinetics theory to develop a means of describing the biological processes involved in ion accumulation in higher plants.

The development of a "macrophyte reactor" represents a novel scientific activity. It is important, therefore, to present a theoretical treatment of the system.

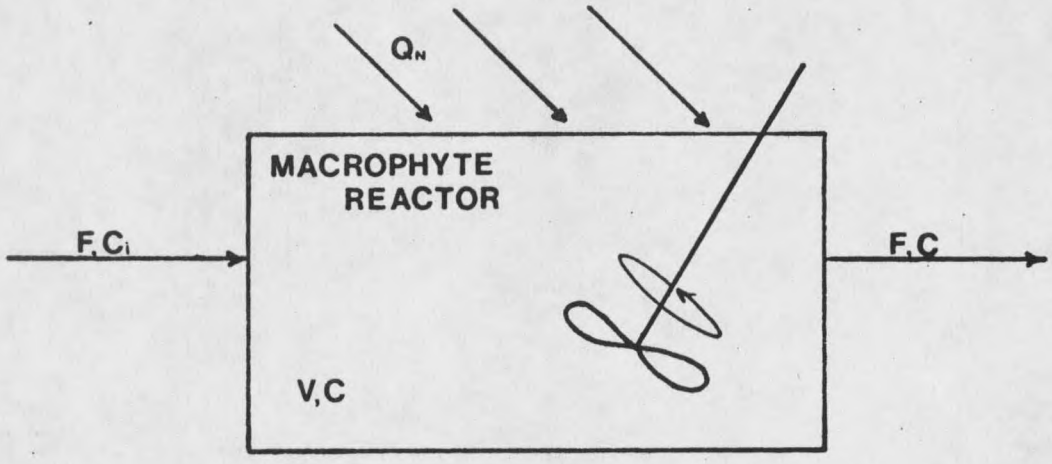
The Macrophyte Reactor: Theoretical Basis

Development of the macrophyte reactor is based upon the law of conservation of mass. Simply stated, this law means that mass influx into a system minus accumulation must equal mass efflux. Figure 2 depicts the basic flow of material in the macrophyte reactor system. The reactor system contains a finite volume, V which has the units of cubic length (L^3). Volumetric fluid flow through the system, F has units of volume per time (L^3t^{-1}). Mass concentration of a component in the fluid entering the reactor is termed C_i and has the units of mass per volume (ML^{-3}). Concentration of C leaving the reactor also has units of mass per volume (ML^{-3}). The macrophyte system is modeled as a continuous stirred tank reactor (CSTR) and, as such, complete mixing of the reactor fluid is assumed. This means that the concentration of C in the reactor effluent is identical to the concentration in the reactor. Input of radiant energy to the reactor is denoted as Q_N Mass balances in a CSTR are described by the following:

ACCUMULATION = TRANSPORT + TRANSFORMATION

Mathematically, this equation is:

$$V\frac{dC}{dt} = FC_i - FC + RV \qquad (1)$$



NET ACCUMULATION = NET TRANSPORT + TRANSFORMATION

$$V\frac{dC}{dt} = FC_i - FC + RV$$

Figure 2. Schematic diagram of theoretical continuous stirred tank macrophyte reactor (CSTMR) of volume V containing constituent C with throughput flow F. Radiant energy input is denoted as Qn. Mass balance equation for C across the reactor is shown where R is a composite process rate term.

where the left hand side of the equation is a differential expressing the rate of change or rate of accumulation of C in the reactor. The right hand side of the equation is comprised of a rate of change component due to mass transport $[F(C_i-C)]$ and a process rate (R) due to transformation. The rate term R has the units of mass per volume per time ($ML^{-3}\ t^{-1}$). R may take on positive or negative values depending upon the reaction taking place within the reactor.

The CSTR establishes a steady-state condition which eliminates the left hand side of equation (2):

$$\frac{dC}{dt} = \frac{F}{V}(C_i - C) + R \qquad (2)$$

Thus, in a CSTR, mass balance equations can be reduced to an algebraic expression:

$$\frac{F}{V}(C_i-C) = R \qquad (3)$$

The term $\frac{F}{V}$ is important as it gives the dilution rate (D) of reactor contents:

$$\frac{F(L^3t^{-1})}{V(L^3)} = D(t^{-1}) \qquad (4)$$

The reciprocal of dilution rate, $(\frac{V}{F})$ is detention time "Θ" which gives the average time spent by a particle in the reactor. It has the units of t. It is important to note that all terms on the left hand side of equation (3) can be easily measured or controlled.

The utility of this approach in ion uptake studies is apparent. By constructing a reactor of known volume in which the fluid contents

are close to ideally mixed and by measuring the influent and effluent concentrations of an ion, one may describe an inferred process rate for ion accumulation by root tissues.

It is important to keep in mind that the process rate R may be composed of several other process rates. That is, the process rate R is probably composed of at least two distinct processes: (1) ion influx into tissues and (2) ion efflux from tissues. R is, therefore, a net process term.

Statistical Analysis

The major objective in this research is the determination of differences, if they exist, in heavy metal accumulation, distribution and kinetics between the two races of *D. caespitosa*. Comparison of sample means using t-and paired t-tests is the primary statistical tool for making these determinations. In addition, Analysis of Variance is used in EXPERIMENT I to evaluate the significance of MAIN effects (e.g. type of metal ion, organ, loading rate, etc.) and factor interactions. Regression analysis is used in EXPERIMENT III to determine differences in slopes of uptake rate over time between the two races.

The null hypothesis in all comparisons of sample means is:

$$H_o : \mu_1 = \mu_2$$

That is, there are no differences in metal accumulation, distribution, or uptake rates between means of "TAILINGS" and

of "AGRICULTURAL" races. The alternative hypothesis is:

$$H_A: \mu_1 \neq \mu_2 .$$

EXPERIMENTATION

Experiment I - Sand-Solution Culture

Materials and Methods

Plants of the two races ("TAILINGS" and "AGRICULTURAL") of *Deschampsia caespitosa* were removed from acid-washed sand medium, rinsed with double distilled water and transplanted into 125 ml, plastic beakers with drainage holes and filled with 100 g of clean acid-washed sand. Five individual clumps of plant material were placed in each beaker, watered with 1X Arnon & Hoagland's (A & H) solution and placed in a 10°C chamber for 3 days. The beakers were subsequently transferred to a greenhouse and were watered daily with 5 ml/beaker of 1X A & H solution. Four weeks later plants were arranged in a completely randomized factorial design.

Three heavy metal loading rates were selected on the basis of the literature. Reports (Mortvedt et al., 1975) indicate that the selected ranges should yield non-toxic, moderately toxic, and highly toxic responses to Cu and Cd. Cadmium loading rates were lower than Cu (at the HIGH loading rate) because of reports of toxic responses in plants at quite low concentrations of Cd.

Copper was applied to the plastic beakers daily, in

the following amounts:

LOADING RATE	ml/beaker/day	Cu CONCENTRATION	Mass of Cu Applied/beaker/day
low	3.0	0.019 μg ml^{-1}	0.06 μg/beaker/day
med	3.0	10.0 μg ml^{-1}	30.0 μg/beaker/day
high	3.0	20.0 μg ml^{-1}	60.0 μg/beaker/day

Cadmium as aqueous $Cd(NO_3)_2$ solution was applied to beakers in the following amounts:

LOADING RATE	ml/beaker/day	Cd CONCENTRATION	Mass of Cd Applied/beaker/day
low	3.0	<0.001 μg ml^{-1}	<0.003 μg/beaker/day
med	3.0	5.0 μg ml^{-1}	15.0 μg/beaker/day
high	3.0	10.0 μg ml^{-1}	30 μg/beaker/day

Plants were watered every morning with unmeasured amounts of double-distilled water and every evening with (1) 1X nutrient solution (controls) or (2) nutrient solution with metal spike at 3.0 ml per day. In order to determine total metal in roots and shoots three of four replicate beakers per treatment were selected randomly at each sampling period. Plants were harvested at 0,3,6, and 9 weeks after initiation of metal application. They were removed from sand, rinsed thoroughly with double distilled water to remove all sand, rinsed with 0.1 N HNO_3, rinsed again with double distilled water, blotted dry and oven dried at 70°C. Samples were then ground with a stainless steel Wiley Mill to pass a 40 mesh screen. The samples were weighed, then digested in a mixture of nitric and perchloric acids and the metal concentration determined by atomic

absorption spectrometry (Munshower & Neuman, 1978;

In order to obtain preliminary data on the gross biochemical location of accumulated copper or cadmium, three beakers of each treatment (15 plants) were harvested as above and were fractionated as follows:

Plant samples were separated into root and shoot and were frozen at -70°C after the HNO_3 rinse. Samples were cut up with stainless steel scissors and were homogenized in 25 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.4) with a chilled Sorvall tissue homogenizer for 10 minutes at high speed. Volume of the homogenate was determined and an aliquot removed for dry weight analysis. The homogenate was centrifuged at 10,000 g for 20 minutes, the supernatant solution collected and labeled as "cytoplasmic" fraction. The pellet debris was resuspended in 0.1 N HNO_3 and recentrifuged at 10,000 g for 20 minutes. The supernatant was collected and added to the "cytoplasmic" fraction. The pellet was collected and labeled "wall" fraction. Prepared "wall" samples were digested as above and metal concentration measured by atomic absorption spectrometry. Liquid samples were analyzed directly by atomic absorption spectrometry.

Results and Discussion

The mean concentration of metals in the two races as a function of metal ion, loading rate and harvest time are tabulated in Tables 6 and 7. The accumulation of Cd and Cu over time in the two races is shown in Figures 3,4 and 5,6. One-way analysis of variance

Table 6. Concentration of Cd in tissues of races of *Deschampsia caespitosa* grown in acid-washed sand-nutrient solution culture. Data are in µg Cd/g. tissue dry weight as determined by acid digestion and atomic absorption spectrophotometry. Data are for four sample times (0,3,6,9 weeks), three Cd loading rates (low = < 0.001 µg/day; med = 15 µg/day; high = 30 µg/day Data are average of three independent samples ± standard error of the mean.

	TAILINGS RACE			AGRICULTURAL RACE		
Week	Loading Rate	Root	Shoot	Loading Rate	Root	Shoot
0	low	0.20 ± 0.0	0.20 ± 0.0	low	0.20 ± 0.0	0.20 ± 0.0
	med	0.20 ± 0.0	0.20 ± 0.0	med	0.20 ± 0.0	0.20 ± 0.0
	high	0.20 ± 010	0.20 ± 0.0	high	0.20 ± 0.0	0.20 ± 0.0
3	low	0.20 ± 0.0	0.20 ± 0.0	low	0.20 ± 0.0	0.20 ± 0.0
	med	18.5 ± 0.20	20.4 ± 8.5	med	19.2 ± 0.81	24.1 ± 2.6
	high	34.9 ± 2.6	38.6 ± 8.3	high	34.8 ± 0.26	53.4 ± 4.7
6	low	0.30 ± 0.10	0.18 ± 0.02	low	0.20 ± 0.0	0.20 ± 0.0
	med	32.0 ± 4.6	13.1 ± 2.3	med	29.7 ± 0.38	41.8 ± 4.7
	high	62.3 ± 1.5	26.7 ± 3.3	high	66.6 ± 2.5	65.3 ± 5.8
9	low	0.20 ± 0.0	0.30 ± 0.05	low	1.4 ± 0.12	0.20 ± 0.0
	med	28.2 ± 6.2	15.1 ± 2.4	med	34.5 ± 2.1	22.0 ± 0.64
	high	67.3 ± 3.2	30.3 ± 5.2	high	158.7 ± 18.8	58.3 ± 8.3

Table 7. Concentration of Cu in tissues of races of *Deschampsia caespitosa* grown in acid-washed sand-nutrient solution culture. Data are in µg Cu/g tissue dry weight as determined by atomic absorption spectrophotometry. Data are for four sample times (0,3,6,9 weeks), three Cu loading rates (low = < 0.06 µg/day; med = 30 µg/day; high = 60 µg/day). Data are average of three independent samples ± standard error of the mean.

	"TAILINGS"			"AGRICULTURAL"		
Week	Loading Rate	Root	Shoot	Loading Rate	Root	Shoot
0	low	4.7 ± 0.94	8.8 ± 1.9	low	16.2 ± 1.9	12.7 ± 2.6
	med	4.7 ± 0.94	8.8 ± 1.9	med	16.2 ± 1.9	12.7 ± 2.6
	high	4.7 ± 0.94	8.8 ± 1.9	high	16.2 ± 1.9	12.7 ± 2.6
3	low	7.6 ± 0.47	8.8 ± 0.12	low	12.7 ± 0.69	13.1 ± 2.1
	med	37.5 ± 4.5	20.4 ± 0.57	med	74.4 ± 3.0	30.7 ± 3.0
	high	91.4 ± 4.7	34.4 ± 1.7	high	114.7 ± 8.1	85.2 ± 1.2
6	low	7.4 ± 1.7	6.4 ± 0.37	low	17.3 ± 2.8	5.7 ± 1.2
	med	69.4 ± 1.4	19.3 ± 1.8	med	96.7 ± 3.8	26.4 ± 1.0
	high	125.7 ± 16.4	35.8 ± 4.8	high	196.7 ± 2.0	79.3 ± 1.5
9	low	5.7 ± 0.55	5.8 ± 0.09	low	3.7 ± 0.27	7.5 ± 0.24
	med	28.1 ± 1.2	21.9 ± 2.4	med	93.2 ± 4.7	20.5 ± 2.5
	high	82.4 ± 1.3	42.5 ± 4.6	high	202.5 ± 34	109.3 ± 11.7

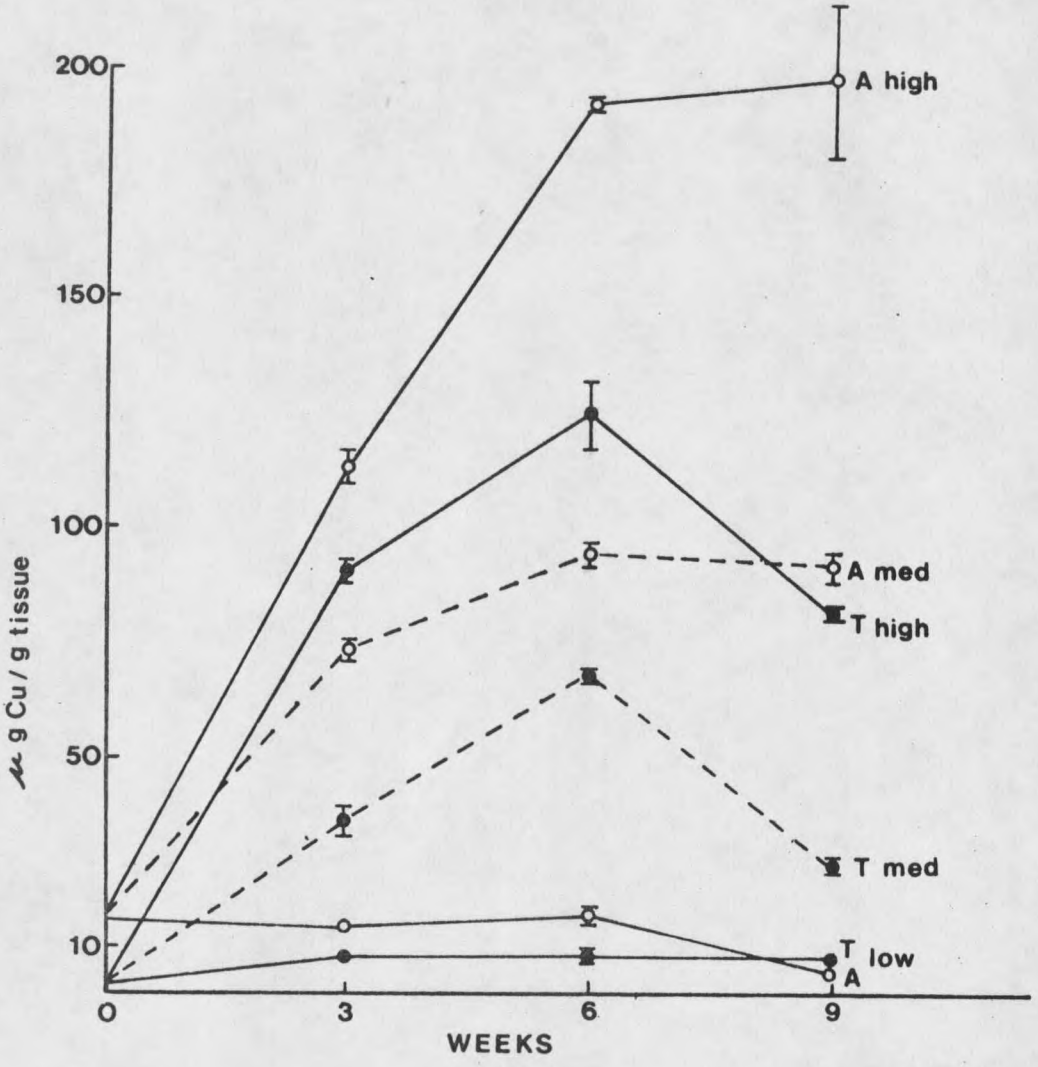


Figure 3. Concentration of Cu in ROOTS of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in sand-solution culture at three Cu loading rates (low = 0.06 μg beaker^{-1} day^{-1}; med = 30 μg beaker^{-1} day^{-1}; high = 60 μg beaker^{-1} day^{-1}). Data are means of three independent determinations. Cu measured by atomic absorption flame spectrophotometry.

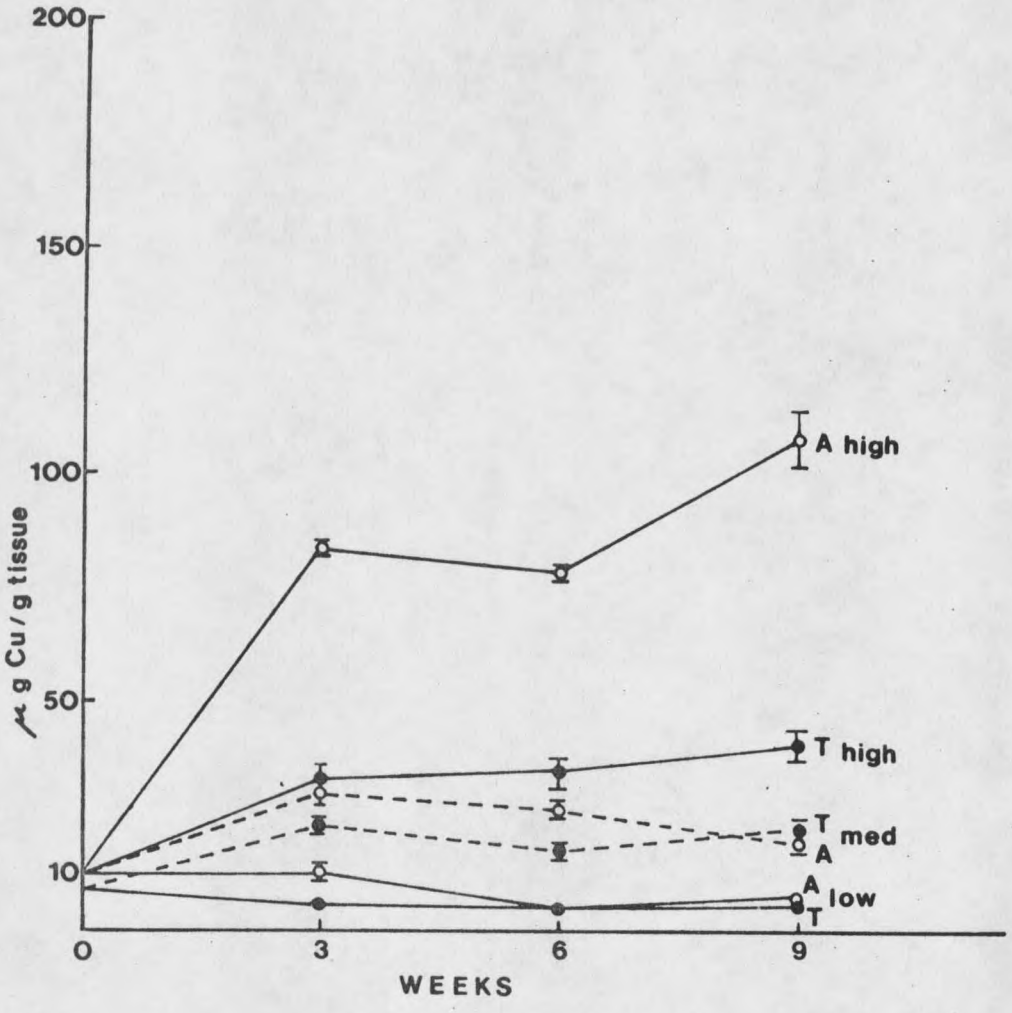


Figure 4. Concentration of Cu in SHOOTS of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in sand-solution culture at three Cu loading rates (low = 0.06 μg beaker^{-1} day^{-1}; med = 30 μg beaker^{-1} day^{-1}; high = 60 μg beaker^{-1} day^{-1}). Data are means of three independent determinations. Cu measured by atomic absorption flame spectrophotometry.

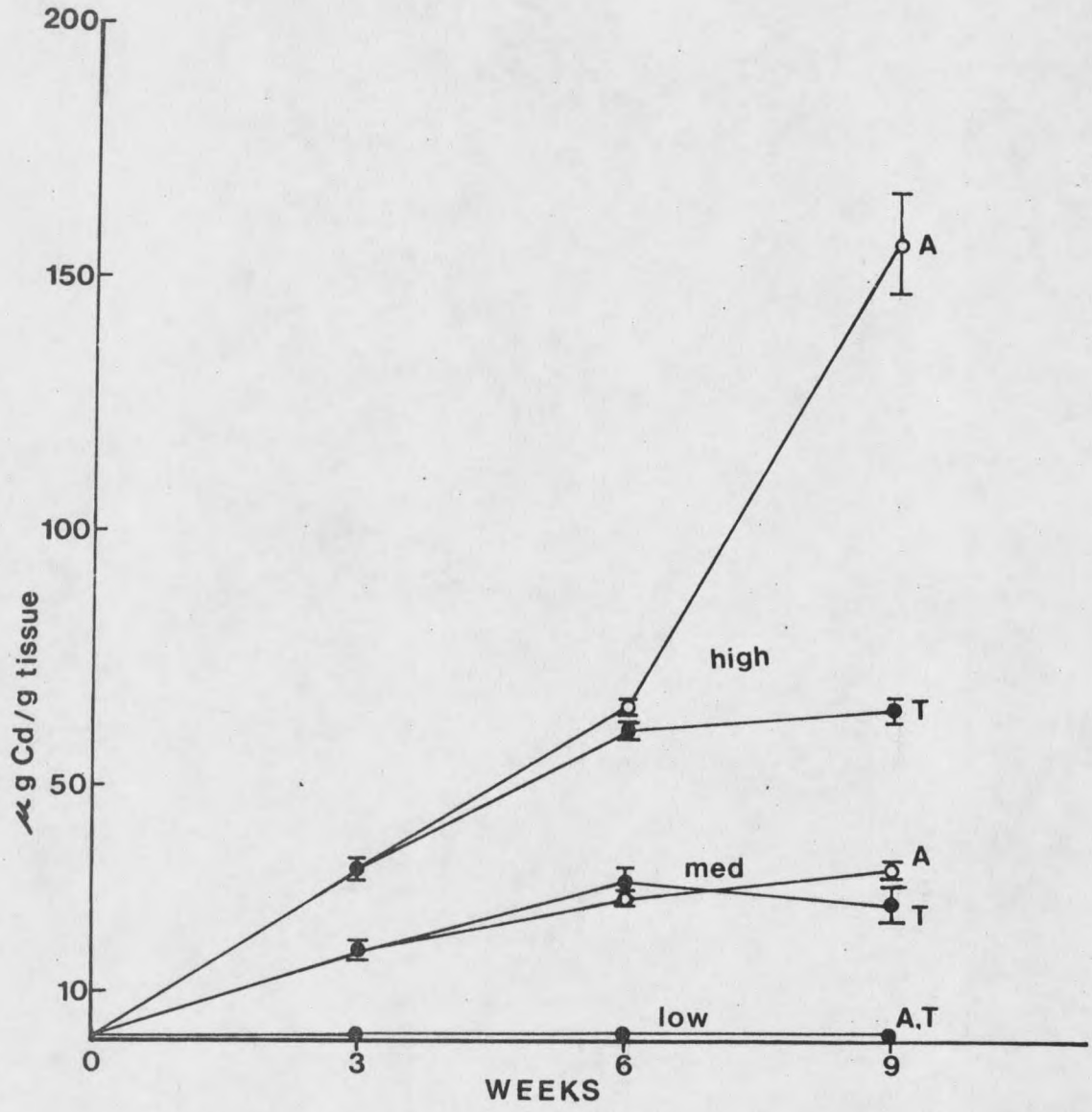


Figure 5. Concentration of Cd in ROOTS of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in sand-solution culture at three Cd loading rates (low = <0.001 μg beaker^{-1} day^{-1}; med = 15 μg beaker^{-1} day^{-1}; high = 30 μg beaker^{-1} day^{-1}). Data are means of three independent determinations. Cd measured by atomic absorption flame spectrophotometry.

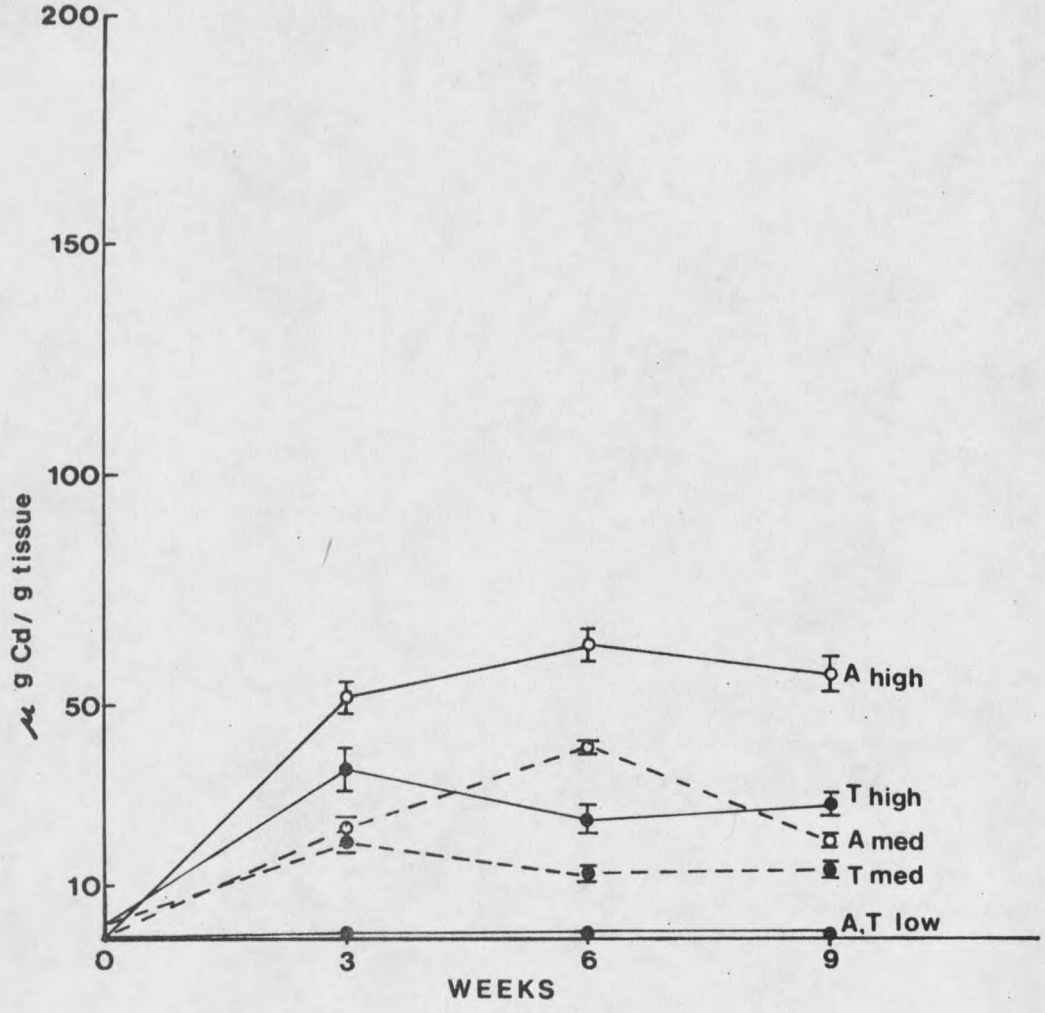


Figure 6. Concentration of Cd in SHOOTS of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in sand-solution culture at three Cd loading rates (low = <0.001 μg beaker^{-1} day^{-1}; med = 15 μ g beaker^{-1} day^{-1}; high = 30 μg beaker^{-1} day^{-1}). Data are means of three independent determinations. Cd measured by atomic absorption flame spectrophotometry.

indicates that replicates of samples are homogeneous (F = 0.01), eliminating replicate to replicate variability as a factor.

Figures 3,4 and 5,6 show that under identical conditions of metal loading rate and harvest time the "Agricultural" race accumulates more metal than the "Tailings" race. The difference is exacerbated with time and appears to be more significant with Cu than with Cd.

Analysis of variance indicates that race, ("Agricultural" vs "Tailings"), organ (root, shoot), metal (Cu, Cd), loading rate (low, med, high), and harvest time (0,3,6,9 weeks) all elicit significant (P = 0.001) effects of metal concentration in tissues (Appendix 1). Analysis of the data using a t-statistic reveals several significant differences (Table 8).

"Tailings" vs. "Agricultural" differences. Averaged over all treatments, the "Agricultural" race accumulates almost twice as much heavy metal as the "Tailings" race. This basic difference is more pronounced with Cu than Cd and with shoots more than roots. Visual assessment of the races indicated that Cu had a more dramatic effect on the "Agricultural" race than did cadmium. The "Tailings" race did not appear to be adversely affected by either metal treatment.

Copper vs. Cadmium differences. Averaged over all treatments, at identical loading rates there is a significantly higher (p = 0.001) amount of Cu in plant tissues than Cd (1.94 times more). Differences in metal concentration between races and organs are less significant

Table 8. T-statistic of comparisons of means of metal concentrations in tissues of races of *Deschampsia caespitosa* ("Agricultural" ("A"), "Tailings" ("T")). Data from Experiment I (sand-solution culture).

		µg metal/g dry tissue				Statistical Comparison		
TREATMENT	AVERAGED OVER	n	$\bar{x}$	min	max	calculated t-value	tabled t-value	level of significance
"A" Race "T" Race	Roots & shoots; Cu & Cd; all sample times, loading rates	144 144	45.75 26.33	0.20 0.12	270.6 158.5	3.52	3.291 (df=286)	0.001
Cu Cd	"A" & "T" races; roots & shoots all sample times med loading rate only	48 48	36.3 18.7	3.2 0.12	270.6 156.3	3.75	3.402 (df=94)	0.001
Roots Shoots	"A" & "T" races; Cu & Cd; all sample times, loading rates	144 144	40.0 21.8	0.20 0.12	270.6 129.2	3.48	3.291 (df=286)	0.001
Roots Shoots Cu	"A" & "T" races; all sample times, loading rates	60 60	64.4 29.7	3.2 3.6	270.6 129.2	3.92	3.291 (df=118)	0.001
Roots Shoots Cd	"A" & "T" races; all sample times, loading rates	60 60	29.5 20.5	0.20 0.12	192.4 74.9	1.57	1.282 (df=118)	0.20
"A" Race-Cu "T" Race-Cu	Roots & shoots; all sample times, loading rates	60 60	60.9 33.2	3.2 3.3	270.6 158.5	3.07	2.86 (df=118)	0.005

Table 8. (cont)

TREATMENT	AVERAGED OVER	µg metal/g dry tissue n	$\bar{x}$	min	max	Statistical Comparison calculated t-value	tabled t-value	level of significance
"A" - Cd "T" - Cd	Roots & shoots; all sample times, loading rates	60 60	30.5 19.4	0.20 0.12	192.4 73.7	1.96	1.658 (df=118)	0.10
"A" roots "T" roots	Cu & Cd; all sample times. loading rates	72 72	50.2 29.7	0.20 0.20	270.6 158.5	2.41	2.24 (df=142)	0.025
"A" shoots "T" shoots	Cu & Cd; all sample times, loading rates	72 72	28.4 15.3	0.20 0.12	129.2 52.7	3.26	2.807 (df=142)	0.005
"A" Root-Cu "T" Root-Cu	All sample times, loading rates	36 36	73.8 39.1	3.2 3.3	270.6 158.5	2.38	2.29 (df=70)	0.025
"A" Root-Cd "T" Root-Cd	All sample times, loading rates	36 36	28.8 20.3	0.20 0.20	192.4 73.7	0.98	0.847 (df=70)	0.40
"A" Shoot-Cu "T" Shoot-Cu	All sample times, loading rates	36 36	34.0 18.4	3.6 5.7	129.2 51.4	2.60	2.29 (df=70)	0.025
"A" Shoot-Cd "T" Shoot-Cd	All sample times, loading rates	36 36	22.2 12.1	0.20 0.12	24.9 52.7	2.05	1.994 (df=70)	0.050
"A" Shoot-Cd "A" Shoot-Cd	All sample times, loading rates	36 36	28.8 22.2	0.20 0.20	192.4 74.9	0.80	0.674 (df=70)	0.50
"T" Root-Cd "T" Shoot-Cd	All sample times, loading times	36 36	20.3 12.1	0.20 0.12	73.7 52.7	1.86	1.667 (df=70)	0.10

Table 8. (cont)

TREATMENT	AVERAGED OVER	µg metal/g dry tissue n	$\bar{x}$	min	max	Statistical Comparison calculated t-value	tabled t-value	level of significance
"A" Root-Cu	All sample times,	36	73.8	3.2	270.6	2.94	2.899 (df=70)	0.005
"A" Shoot-Cu	loading rates	36	34.0	3.6	129.2			
"T" Root-Cu	All sample times	36	39.1	3.3	158.5	3.13	2.899 (df=70)	0.005
"T" Shoot-Cu	loading rates	36	18.4	5.7	51.4			

with Cd whereas these differences are very significant with Cu.

Root vs. Shoot differences. Averaged over all treatments, the differences between metal concentration in roots and shoots is highly significant ($p = 0.001$). This difference is high for Cu ($p = 0.001$) but diminishes with Cd ($p = 0.20$). In all cases, there is higher metal concentration in roots than shoots. This is not surprising given the difficulty in removing particles attached to root surfaces and the high ion adsorptive capacity of root cell wall material indicated in the literature (Nye & Tinker, 1977).

Loading rate differences. Table 9 shows differences in average concentration of metals in tissues of both races. Comparison using a t-statistic (Table 10) shows significant differences between races at the low and medium loading rate of copper in roots and significant differences with copper at high loading rate for shoots. Cadmium does not exhibit significant differences at any loading rate and organ except for the high rate in shoots.

Figures 7 and 8 show the relationship between average metal concentration in roots and shoots in the two races and loading rate. It can be seen that generally, metal concentration in tissues of "Tailings" is lower than metal concentration in "Agricultural" tissues at most loading rates. The differences are more pronounced as loading rate increases. Figure 7, showing copper effects, demonstrates a significant point about these races. The differences between root and shoot concentration are an indication of the level

Table 9. Analysis of tissue metal concentrations as a function of metal loading rate-comparison of two races (i.e. "Tailings", "Agricultural"). (Data are μg metal/g dry wt.). n = 12
Data from Experiment I (sand-solution culture).
Phyiological races of *Deschampsia caespitosa*

	"TAILINGS"		"AGRICULTURAL"	
		COPPER		
	Loading Rate	$\bar{x}$	Loading Rate	$\bar{x}$
ROOT	low	6.4	low	12.8
	med	34.9	med	70.1
	high	76.0	high	132.5
SHOOT	low	7.4	low	9.8
	med	17.6	med	22.6
	high	30.4	high	124.3
		CADMIUM		
ROOT	low	0.23	low	0.50
	med	19.7	med	20.9
	high	41.0	high	65.0
SHOOT	low	0.22	low	0.29
	med	12.0	med	22.0
	high	24.0	high	44.0

Table 10. T-statistic of "Tailings" ("T") vs. "Agricultural" ("A") race comparisons at different loading rates (low, med, high) for Cd and Cu, roots and shoots (n=12; df for comparisons=22).

COMPARISON	METAL	ORGAN	METAL LOADING RATE	CALCULATED t-value	TABLED t-value	LEVEL OF SIGNIFIANCE
"T" vs "A"	Cu	Root	low	2.20	3.119	0.005
"T" vs "A"	Cu	Root	med	2.91	2.819	0.01
"T" vs "A"	Cu	Root	high	2.04	1.717	0.10
"T" vs "A"	Cu	Shoot	low	1.71	1.717	0.10
"T" vs "A"	Cu	Shoot	med	1.73	1.717	0.10
"T" vs "A"	Cu	Shoot	high	3.47	3.119	0.005
"T" vs "A"	Cd	Root	low	1.59	1.321	0.20
"T" vs "A"	Cd	Root	med	0.13	0.686	ns
"T" vs "A"	Cd	Root	high	1.20	0.858	0.40
"T" vs "A"	Cd	Shoot	low	1.12	0.858	0.40
"T" vs "A"	Cd	Shoot	med	1.82	1.717	0.10
"T" vs "A"	Cd	Shoot	high	2.16	2.074	0.05

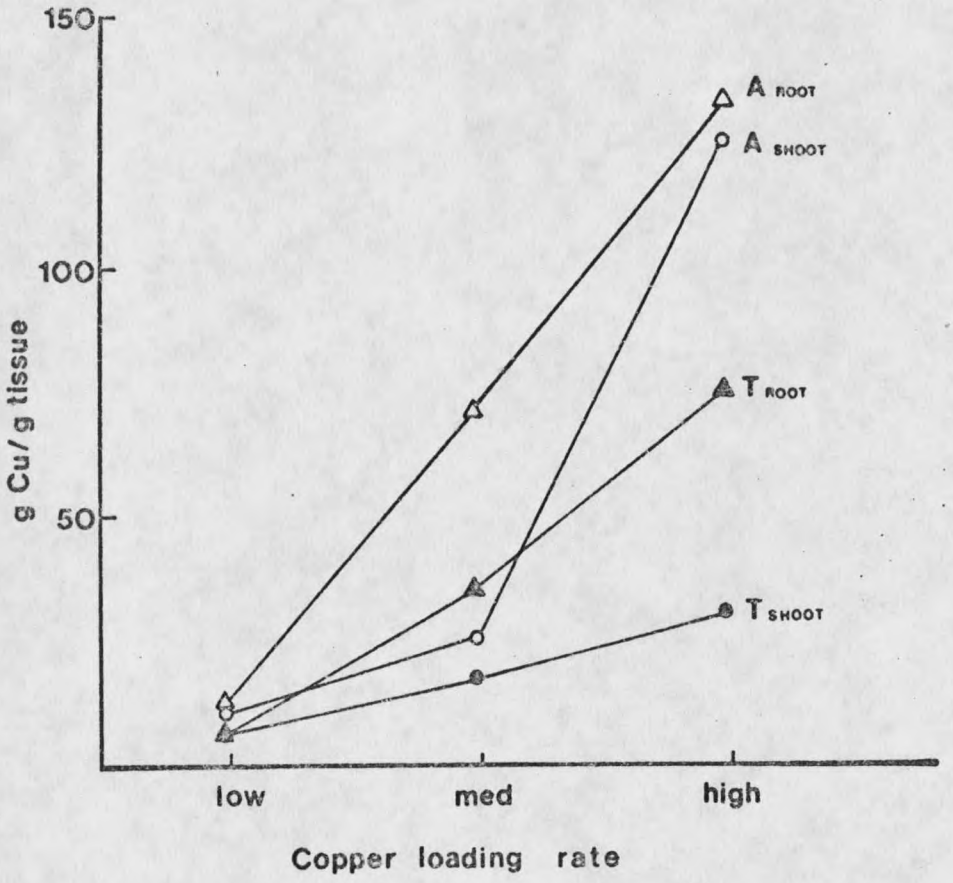


Figure 7. Average concentration of Cu in tissues of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* as a function of Cu loading rate (low = 0.06 µg beaker^{-1} day^{-1}; med = 30 µg beaker^{-1} day^{-1}; high = 60 µg beaker^{-1} day^{-1}). Data are means of 12 independent samples averaged over all times, replications. Cu concentration measured by atomic absorption spectrophotometry.

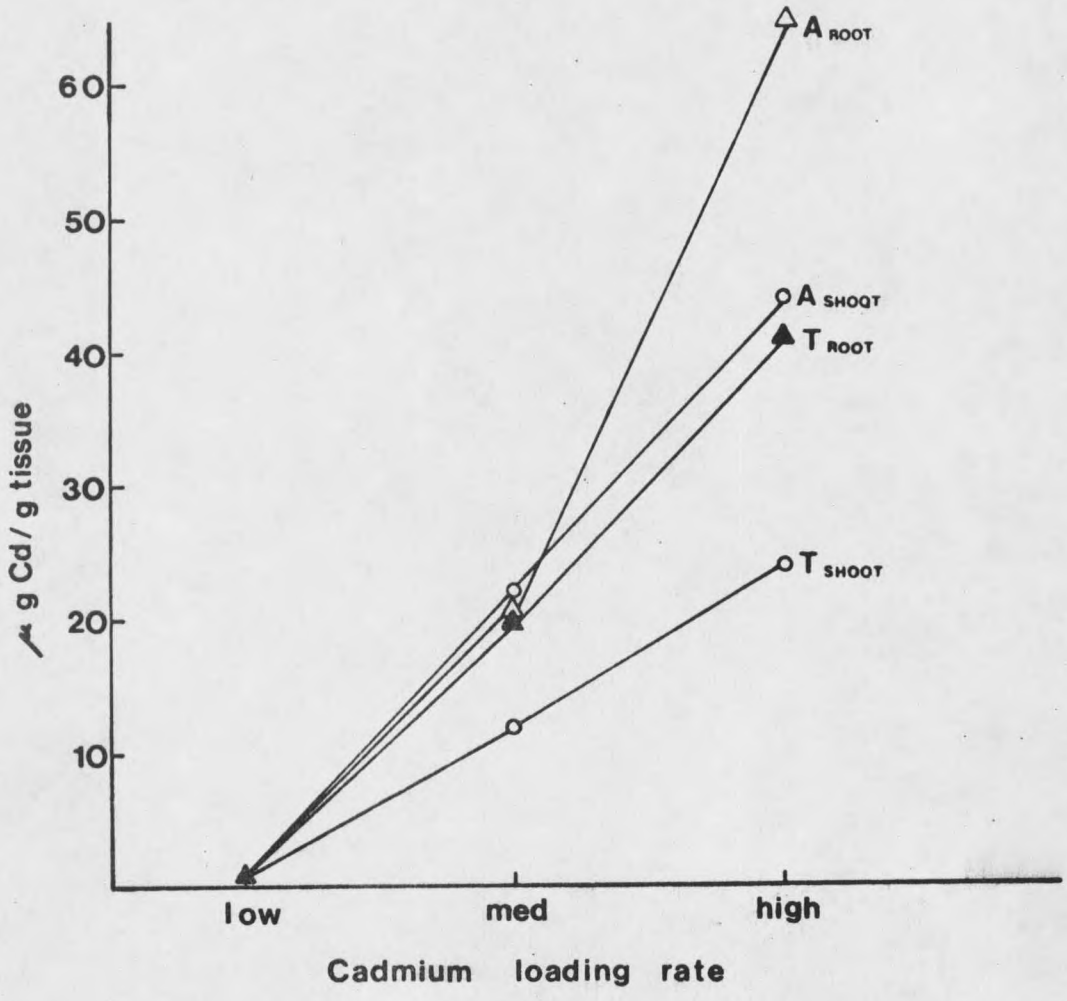


Figure 8. Average concentration of Cd in tissues of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* as a function of Cd loading rate (low = <0.003 μg beaker^{-1} day^{-1}; med = 15 μg beaker^{-1} day^{-1}; high = 30 μg beaker^{-1} day^{-1}). Data are means of 12 independent samples averaged over all times, replications. Cd concentration measured by atomic absorption spectrophotometry.

of control exercised over transport. In the "Tailings" race, shoot copper concentration increases gradually as loading rate increases and the difference between root and shoot gradually increases. In the "Agricultural" race the copper concentration in the shoot increases greatly as loading rate increases such that there is little difference between root and shoot concentration at the high loading rate. The difference between races with Cd at various loading rates is not as significant though Figure 8 does reflect the general trend of higher tissue concentration of Cd in the "Agricultural" race at any particular loading rate.

Harvest time differences. Figures 3,4,5,6 show the trend of metal accumulation over time. A striking feature is the general leveling off or decline in metal concentration as time progresses. This response is shown in Table 11 which tabulates the average metal concentration (Cu and Cd) in all tissues of both races over time.

TABLE 11

TIME	n	$\bar{x}$ (µg metal/g dry tissue)
0 weeks	72	5.4
3 weeks	72	32.3
6 weeks	72	42.7
9 weeks	72	43.3

Table 11. Average metal concentration of both *D. caespitosa* races, whole plant; all loading rates, over time.

Figures 3, 4, 5, and 6 show that the decline in accumulation of metal is most significant for the "Tailings" race and Cu treatments.

The fact that both races exhibit significant differences in their accumulation of copper relative to cadmium may be explained by the fact that copper is a nutrient ion and cadmium is not. One could expect an organism to readily accumulate a nutrient species while excluding a non-nutrient. This may fit with a selective protein "ion carrier" hypothesis. The significant differences between the races relative to metal accumulation suggest that the "Tailings" population is capable of avoiding or excluding excess metal ions. This is contrary to Ernst's (1975) statement that metal ion exclusion is not possible in higher plant systems. The difference in metal accumulation between races could be explained on the basis of loss of membrane integrity and lysis of "Agricultural" cells. Jarvis et al. (1976) have shown that freshly killed plant tissue accumulates Cd at a higher rate than living material until saturation of adsorption sites on the cell wall matrix. One would expect that if gradual death of root cells was the cause of increased metal accumulation in the "Agricultural" race, transport to the shoots would decline. However, the data show that metal concentrations in shoots of the "Agricultural" race continued to increase over time. This may indicate a passive leakage of ions into the xylem stream by slowly dying root xylem parenchyma cells.

The data indicates that the "Tailings" race is capable of excluding metals from the roots but, more significantly, it apparently restricts metal ion transport to the shoot. This may implicate an

altered transport mechanism at the xylem parenchyma plasmalemma. Overall differences in root vs. shoot can be easily explained by the fact that the root apoplast represents an ion exchange matrix capable of adsorbing large amounts of ions. It is assumed that transport to the shoots is an active biological process and the ratio of root/shoot concentration differences reflect a mechanism of control of metal transport. These data would suggest that the "Tailings" population has the capacity to restrict ion entry into root tissues and transport to the xylary stream while the "Agricultural" race does not possess such a capacity.

Variation in metal concentration in tissues over time implicates growth processes as well as transport processes. This is because metals are not known to leach out of tissues in significant amounts once translocated. Growth rate differences may explain the decrease in metal concentration over time. Significantly lower tissue metal concentration exhibited by the "Tailings" race suggests that this genotype can tolerate high intracellular metal levels as well as restrict transport. By the same reasoning, the "Agricultural" race may exhibit both reduced growth rate and lack of metal transport control.

Biochemical fractionation. Table 12 lists the concentration of Cu and Cd in "cytoplamic" and "wall" fractions of the "Tailings" and "Agricultural" races. Figure 9 shows the gross biochemical distribution of Cd and Cu. Small sample size precludes any comparisons between genotypes; however, one may conclude that heavy metals

Table 12. Cu and Cd concentrations in "wall" and "cytoplasmic" fractions of races of *D. caespitosa* grown in sand-solution culture with applied metals. Analysis by acid digestion-atomic absorption spectrophotometry. Data are from single independent determinations.

RACE	ORGAN	METAL	FRACTION	Concentration of Cu in sample (µg Cd/g dry tissue)	concentration of Cd in sample (µg Cd/g dry tissue)	% of total metal in tissue
"T"	Root	Cu	"wall"	18.0	-	86.5
"T"	Root	Cu	"cytoplasm"	2.8	-	13.5
"A"	Root	Cu	"wall"	44.1	-	89.5
"A"	Root	Cu	"cytoplasm"	5.2	-	10.5
"T"	Shoot	Cu	"wall"	5.0	-	70.4
"T"	Shoot	Cu	"cytoplasm"	2.1	-	29.6
"A"	Shoot	Cu	"wall"	8.5	-	90.3
"A"	Shoot	Cu	"wall"	0.9	-	9.7
"T"	Root	Cd	"wall"	-	5.6	34.2
"T"	Root	Cd	"cytoplasm"	-	5.9	36.0
"A"	Root	Cd	"wall"	-	22.3	92.8
"A"	Root	Cd	"cytoplasm"	-	1.7	7.2
"T"	Shoot	Cd	"wall"	-	1.2	81.8
"T"	Shoot	Cd	"cytoplasm"	-	0.27	18.2
"A"	Shoot	Cd	"wall"	-	2.4	71.7
"A"	Shoot	Cd	"cytoplasm"	-	0.96	28.3
"T"	Root	control	"wall"	4.1	<1.00	*96.0 ** -
"T"	Root	control	"cytoplasm"	0.17	<0.10	4.0 -
"A"	Root	control	"wall"	6.8	<0.01	83.0 -
"A"	Root	control	"cytoplasm"	1.4	<0.01	17.0 -

Table 12. (cont)

RACE	ORGAN	METAL	FRACTION	Concentration of Cu in sample (µg Cd/g dry tissue)	concentration of Cd in sample (µg Cd/g dry tissue)	% of total metal in tissue	
"T"	Shoot	control	"wall"	1.5	1.47	96.0	97.4
"T"	Shoot	control	"cytoplasm"	0.6	0.04	4.0	2.6
"A"	Shoot	control	"wall"	3.2	<0.01	89.0	-
"A"	Shoot	control	"cytoplasm"	0.4	<0.01	11.0	-

* = % Cu in tissue fraction; ** = % Cd in tissue fraction

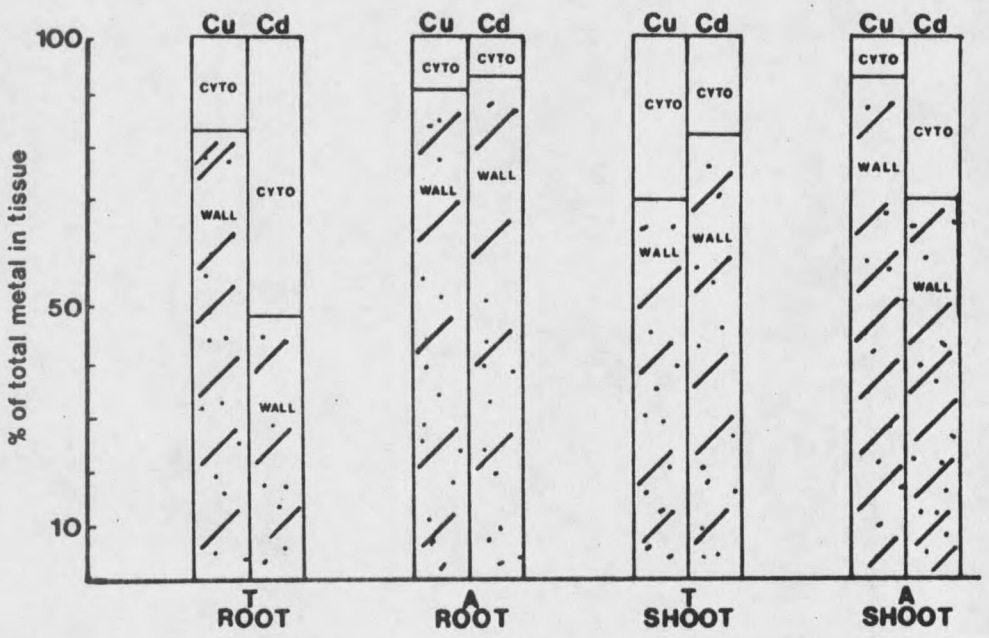


Figure 9. Percent distribution of total Cu and Cd in tissues of "Agricultural" (A) and "Tailings" (T) plants grown in sand-solution culture. Samples taken from high Cd (30 µg beaker^{-1} day^{-1}) and med Cu (30 µg beaker^{-1} day^{-1}) treatments 9 weeks after initiation of metal loading. Tissues fractionated into soluble "cytoplasmic" (cyto) and insoluble "wall" (wall) fractions; Cu and Cd concentration determined by atomic absorption spectrophotometry.

accumulated by genotypes of *D. caespitosa* are primarily incorporated within or irreversibly adsorbed to the cell wall matrix.

Conclusions from Experiment I

(1) "Tailings" race accumulates less copper or cadmium in both roots and shoots than "Agricultural" race.

(2) Differences in metal accumulation between "Tailings" and "Agricultural" are much more significant with Cu than with Cd treatments.

(3) *Deschampsia caespitosa* accumulates more Cu than Cd under identical treatment conditions.

(4) Roots of *D. caespitosa* have higher metal concentration than shoots, the difference being much more pronounced with Cu than with Cd.

(5) Most (50-90%) of the Cu and Cd accumulated by *D. caespitosa* is irreversibly bound to or (complexed) within the cell wall matrix.

These conclusions must be viewed with some caution. Throughout the experimental period random fluctuations in environmental conditions (i.e., temperature, relative humidity, and insecticide applications) took place in the greenhouse. The possibility exists that these races do not respond identically to these parameters. This possibility casts doubt on the above conclusions. Rigorous physiological evaluation of this species is required to confirm or refute these conclusions. It can be stated, however, that the two experimental ecotypes originated in similar mesic conditions. Coupling this fact with the fact that the ecotypes were grown in similar temperature regimes suggests that the stated conclusions can be initially accepted.

Experiment II - Cd^{109} Batch System

Materials and Methods

Approximately 25 g. each of "Tailings" and "Agricultural" plants were collected from plant materials prepared as discussed previously. Necrotic roots were removed from each plant before placing specimens into separate 300 ml narrow-neck flasks containing 200 ml of 1X Arnon and Hoagland's (A & H) solution. Flasks were aerated with compressed air and stirred continuously with a magnetic stir bar. Plants were allowed to equilibrate one week in this system prior to addition of metal spike. After equilibration the nutrient solution was replaced with fresh A & H solution spiked with Cd. Stable Cd as $Cd(NO_3)_2$ was added to the solution to make a concentration of 9.55 µg ml^{-1}. The gamma emitter (88Kev) Cd^{109} was added to the solutions to 0.45 µg/ml such that the Cd concentration in each flask was 10.0 µg/ml and was comprised of the ratio 0.45 µg Cd^{109}/9.55. µg Cd^{112} per ml. This ratio was assumed constant throughout the experiment which allowed the calculation of cadmium concentration in fractions based upon the counts per minute (CPM) of samples measured in a Beckman Biogamma counter.

Plants were placed in a growth chamber with 16 hour days at approximately 25°C and 8 hour nights at approximately 10°C. Whole plants were removed for processing at 2, 12, 24, 48 hours, and one

week after initiation of Cd^{109}/Cd treatment.

Harvested plants were separated into root and shoot and frozen at -70°C. Samples were subsequently thawed, cut into small segments with stainless steel scissors and homogenized in 25 mM phosphate buffer at high speed in a chilled Sorvall tissue homogenizer. Homogenate was centrifuged at 10,000 g for 20 minutes. Resulting supernatant was collected, labeled "cytosol", frozen, and stored until counting. The pellet was resuspended in 1.0% TRITON X-100 and vortexed for two minutes. The homogenate was centrifuged at 10,000 g for 20 minutes. The resulting supernatant was collected, labeled soluble "membrane" fraction and stored. The pellet was collected, labeled "wall" fraction and stored.

Gamma degradations are powerful enough to eliminate the problem of quenching, therefore, samples prepared as above were analyzed directly in a Beckman Biogamma counter. The CPM data was converted to total mass of Cd by using the specific activity of the Cd^{109} (1.55×10^7 CPM/1.0 µg Cd^{109}) and the known ratio of Cd^{109}/Cd in the reaction flasks. Mass of Cd was divided by the dry weight of tissue homogenate to give Cd concentration in µg/g.

Results and Discussion

Means of two independent determinations of Cd concentration of each fraction are given in Table 13. The concentrations of Cd in biochemical fractions of the two races over time are shown in Figures 10,11,12. These figures are difficult to interpret except for the obvious higher Cd levels in root fractions than in shoots.

Table 13. Data are concentration of Cd in plant tissue (μg/g) of "Tailings" and "Agricultural" races of *D. caespitosa* grown in aerated, batch material culture spiked with Cd^{109}. Data are calculated from CPM of two independent determinations ± SD. CPM measured in a Beckman BioGamma Counter.

TAILINGS						
	ROOT			SHOOT		
SAMPLE TIME	"CYTO"	"MEMB"	"WALL"	"CYTO"	"MEMB"	"WALL"
2 hours	42 ± 2.5	14.2 ± 0.1	65.3 ± 6.1	1.4 ± 0.01	1.4 ± 0.3	6.1 ± 0.8
12 hours	82 ± 5.2	22 ± 0.6	131 ± 17.9	4.8 ± 0.3	6.1 ± 0.6	15.1 ± 1.2
24 hours	41.3 ± 4.0	13.3 ± 0.5	86 ± 8.2	4.5 ± 0.4	3.4 ± 0.6	13.9 ± 4.3
48 hours	46.4 ± 4.1	16.9 ± 0.4	89.8 ± 9.3	3.0 ± 0.02	2.6 ± 0.8	9.1 ± 6.7
1 week	76.4 ± 3.0	43.2 ± 0.6	298 ± 13.4	14.2 ± 1.0	13.4 ± 0.2	57.8 ± 4.1

AGRICULTURAL						
	ROOT			SHOOT		
SAMPLE TIME	"CYTO"	"MEMB"	"WALL"	"CYTO"	"MEMB"	"WALL"
2 hours	20.3 ± 2.4	14.2 ± 0.2	86.1 ± 4.5	2.2 ± 0.01	1.6 ± 0.01	8.9 ± 0.3
12 hours	33 ± 3.0	21.5 ± 1.1	88.9 ± 5.80	4.5 ± 0.01	11.1 ± 0.2	13.9 ± 1.0
24 hours	92 ± 6.7	29.3 ± 0.3	260.7 ± 18.3	20.7 ± 0.8	20.7 ± 1.3	61.3 ± 5.7
48 hours	98.6 ± 14.0	22.1 ± 0.8	235 ± 20.4	9.9 ± 0.9	6.9 ± 0.2	32.9 ± 4.5
1 week	35.8 ± 18.2	15.1 ± 0.9	209.2 ± 13.6	21.9 ± 1.1	15.1 ± 0.10	164 ± 18.8

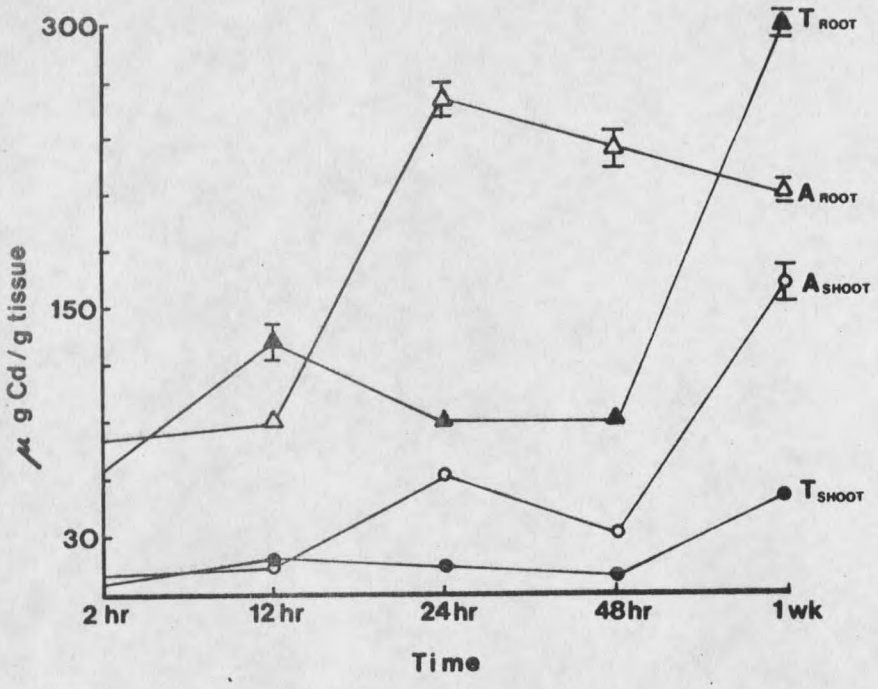


Figure 10. Average concentration of Cd in root and shoot "WALL" fractions overtime of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in aerated, liquid batch culture spiked with Cd^{109}. Cd concentration calculated from CPM of Cd^{109} (gamma, 88 Kev) incorporated in tissue fractions. Data are average of two independent sample determinations. Error bars omitted where S.E.M. smaller than symbol.

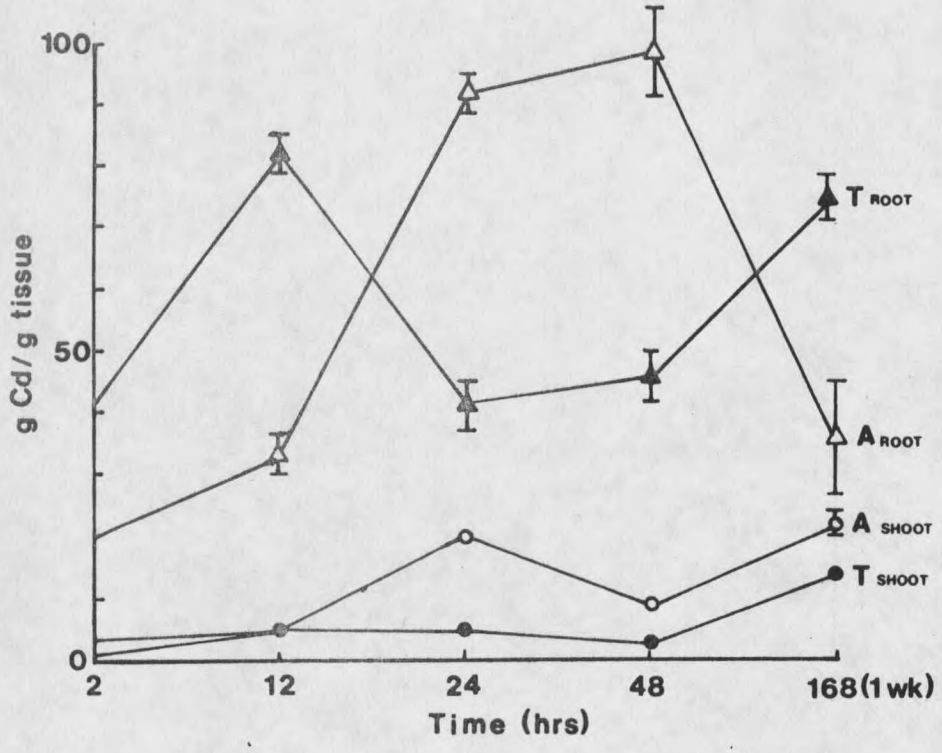


Figure 11. Average concentration of Cd in root and shoot "CYTOPLASMIC" fractions over time of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in aerated, liquid batch culture spiked with Cd^{109}. Cd concentration calculated from CPM of Cd^{109} (gamma, 88 Kev) incorporated in tissue fractions. Data are average of two independent sample determinations. Error bars omitted where S.E.M. smaller than data symbol.

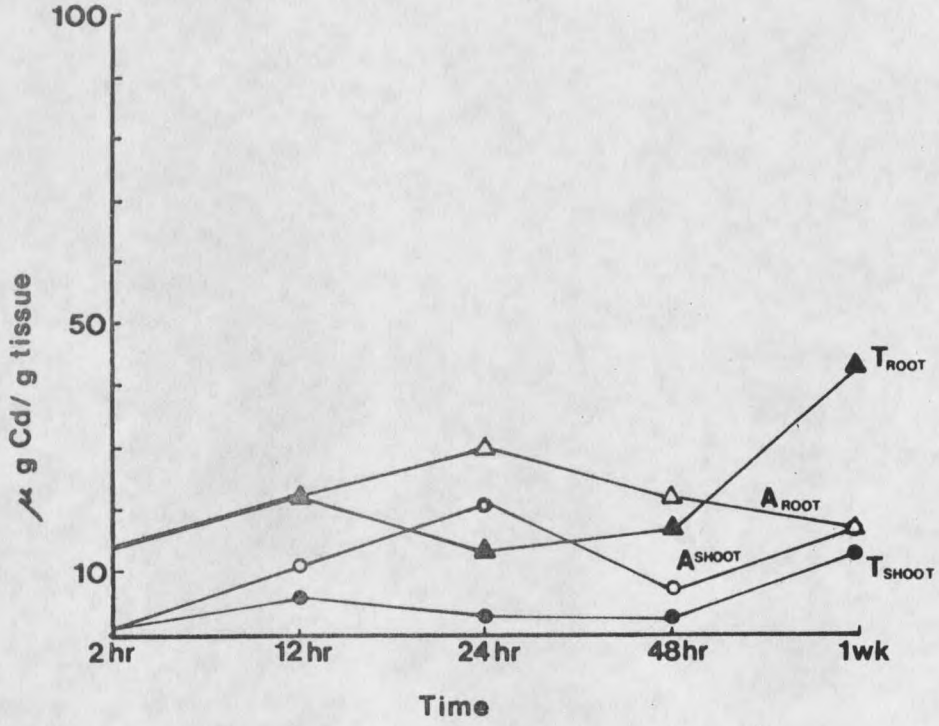


Figure 12. Average concentration of Cd in root and shoot "MEMBRANE" fractions over time of "Agricultural" (A) and "Tailings) (T) races of *D. caespitosa* grown in aerated, liquid batch culture spiked with Cd^{109}. Cd concentration calculated from CPM of Cd^{109} (gamma, 88 Kev) incorporated in tissue fractions. Data are average of two independent sample determinations. Error bars omitted where S.E.M. smaller than data symbol.

Inspection suggests that these data may reflect periodicity of physiological function.

Summary of statistical analysis of the data is given in Table 14. Paired and unpaired t-tests were used to evaluate differences between groups. The differences can be summarized as follows: Differences in Cd accumulation between the two races are only slightly significant when averaged over roots, shoots and all biochemical fractions. This result is in keeping with the finding of Experiment I in which racial differences were highly significant with Cu but only slightly significant with Cd. As in the previous experiment, roots accumulate considerably more Cd than shoots averaged over both races, fractions, and times. There are no differences between the Cd concentration in roots of "Tailings" and that in "Agricultural". Averaged over all sample tissues, there are no differences in Cd concentration between any of the root biochemical fractions of the two races. Very significant differences do appear between shoot fractions, however. Averaged over all fractions and all times, there are significant differences between the Cd accumulation in shoots of the races. This suggests that a difference between these genotypes may lie in those processes which transport ions from the xylary symplast into vascular vessels. Comparison of "cytoplasmic", "wall" and "membrane" fractions from shoot tissue shows them all different between races. In every case, the "Tailings" population has accumulated lower concentrations of Cd in the shoot tissue. Of some significance is the fact that

Table 14. Comparisons of groups using paired t-test. Data is concentration of Cd in fractions ($\mu g\ g^{-1}$) of tissues of "Tailings" ("T") and "Agricultural" ("A") races of *Deschampsia caespitosa* grown in batch, nutrient solution spiked with Cd^{109}. Concentrations of Cd are calculated from measurement of degradations in a Beckman Biogamma counter.

COMPARISON OF MEANS	AVERAGED OVER	n	µg Cd/g dry tissue $\bar{x}$	min	max	t-STATISTIC OF COMPARISON calculated t-value	tabled t-value	level of significance
"T"	All times; all	30	40.8	1.4	298	1.49	1.31	0.20
"A"	fractions; roots and shoots	30	55.2	1.6	260.7		(df=29)	
Root	Both races; all	30	77.6	14.2	298	3.91	3.476	0.001
Shoot	fractions; all times	30	18.4	1.4	164		(df=58)	
"T" Root	All fractions;	30	71.3	14.2	278	1.00	0.854	0.40
"A" Root	all times	30	84.1	14.2	260.7		(df=29)	
"T" Shoot	All fractions;	30	10.8	1.2	57.8	3.09	3.038	0.005
"A" Shoot	all times	30	26.3	1.5	164		(df=29)	
"T" Root wall	All times	10	134	61.0	328.8	1.21	0.883	0.20
"A" Root wall		10	176	83.0	273.7		(df=9)	
"T" Shoot wall	All times	10	20.4	5.5	60.7	2.72	2.634	0.025
"A" Shoot wall		10	56.2	8.7	177		(df=9)	
"T" Root cyto	All times	10	58.0	40.2	78.5	0.14	0.703	NS
"A" Root cyto		10	56.0	18.6	108.5		(df=9)	
"T" Shoot cyto	All times	10	5.6	1.4	14.9	3.18	2.684	0.025
"A" Shoot cyto		10	11.8	2.2	22.7		(df=9)	
"T" Root membrane	All times	10	21.9	14.1	43.6	0.30	0.703	NS
"A" Root membrane		10	20.4	14.0	19.5		(df=9)	

Table 14. (cont)

COMPARISON OF MEANS	AVERAGED OVER	n	μ Cd/g dry tissue $\bar{x}$	min	max	t-STATISTIC OF COMPARISON calculated t-value	tabled t-value	level of significance
"T" Shoot membrane	All times	10	5.4	1.2	13.5	2.82	2.685	0.025
"A" Shoot membrane		10	11.1	1.5	21.6		(df=9)	
"T" 2h	All fractions;	12	21.7			0.13	0.703	NS
"A" 2h	roots & shoots	12	22.2				(df=11)	
"T" 12h	All fractions;	12	43.5			2.18	1.796	0.10
"A" 12h	roots & shoots	12	28.8				(df=11)	
"T" 24h	All fractions;	12	27.1			3.17	3.106	0.010
"A" 24h	roots & shoots	12	80.8				(df=11)	
"T" 48h	All fractions:	12	27.1			2.60	2.593	0.025
"A" 48h	roots & shoots	12	67.5				(df=11)	
"T" 1 wk	All fractions;	12	83.8			0.38	0.697	NS
"A" 1 wk	roots & shoots	12	76.8				(df=11)	
"T" membrane	All times;	20	13.8	1.2	43.6	t=0.688	0.688	0.50
"A" membrane	roots & shoots	20	15.2	1.5	29.5		(df=19)	
"T" wall	All times;	20	77.2	5.5	328.8	t=2.16	2.093	0.05
"A" wall	roots & shoots	20	116	8.7	273.7		(df=19)	
"T" cyto	All times;	20	31.8	1.4	78.5	t=0.29	0.688	NS
"A" cyto	roots & shoots	20	33.9	2.1	108.5		(DF=19)	
Root cyto	All times; both	20	57.0	18.6	108.5	t=7.83	3.646	0.001
Shoot cyto	races roots & shoots	20	7.71	1.4	22.7		(df=38)	

Table 14. (cont)

COMPARISON OF MEANS	AVERAGED OVER	n	µg Cd/g dry tissue $\bar{x}$	min	max	t-STATISTIC OF COMPARISON calculated t-value	tabled t-value	level of significance
Root wall	All times; both	20	155	61.0	328.8	t=5.34	3.64	0.001
Shoot wall	races roots & shoots	20	38	5.5	177.0		(df=38)	
Root membrane	All times; both	20	21.2	12.1	43.6	t=5.24	3.646	0.001
Shoot membrane	races roots &	20	8.2	1.2	21.6		(df=38)	

there is a significantly higher concentration of Cd in the wall fraction of the "Agricultural" race. This difference is more pronounced in the shoot "wall" fraction. A simple explanation for this phenomenon of increased "wall" concentration in the "Agricultural" race is that death of cells renders more wall sites available for adsorption. Increased cell death in the "Agricultural" race would lead to increased Cd accumulation according to the work of Jarvis et al. (1976). Analysis of variance (Appendix 2) and least-significant-difference analysis show that there are significant differences in Cd concentration between the biochemical fractions when averaged over all times and races (Table 15). This difference is in the order "wall" > "cytosol" > "membrane".

Table 15. Average of Cd concentration ($\mu g\ g^{-1}$) in biochemical fraction of *D. caespitosa* grown in batch nutrient culture spiked with Cd^{109}. Data is calculated from measurements of tissue gamma degradations in a Beckman Biogamma counter and is averaged over all sample times, roots and shoots and both races.

Biochemical fraction	averaged over	n	µg Cd/g. dry tissue $\bar{x}$	min	max
"cytosol"	Both races; all times roots & shoots	40	32.8	1.4	108.5
"wall"	Both races; all time roots & shoots	40	96.6	5.5	328.8
"membrane"	Both races; all time roots & shoots	40	14.7	1.2	43.6

The above relationship is shown in Figures 13 and 14 which depicts per cent Cd distribution in roots and shoots of these races. Comparisons of relative distribution within plants (root vs. shoot) are shown in Figure 15. The differences in root/shoot ratio are shown in Figure 16. It is apparent that the two races exhibit similar dynamic patterns of Cd distribution. The figures also suggest that the root/shoot ratio is always higher in the "Tailings" race. This would be expected given the lowered levels of Cd in the "Tailings" shoots and again suggests an acropetal translocation mechanism difference. The per cent distribution of Cd in fractions over time is listed in Table 16 . A comparison of these distribution patterns using a paired t-test is given in Table 17. This data shows that there is a significantly higher per cent of accumulated Cd in the "Agricultural" root "wall" fraction than in the corresponding "wall" fraction of the "Tailings". This relationship is reversed in the cytoplamic fraction. The per cent of total root Cd in the cytoplasmic fraction is higher in the "Tailings" race. This fact may be explained in several ways. For example, the higher Cd level in the root cytoplasm of the "Tailings" could implicate a cytoplasmic "tolerance" factor such as a chelator. These differences could also be explained by an increased death rate of root cells in the "Agricultural" race. Lysis of cells would release metals to be adsorbed onto wall exchange sites.

The fluctuations in differences in whole-plant Cd concentrations (i.e., no difference at 2 hr; "Tailings" higher at 12 hr; "Agricultural"

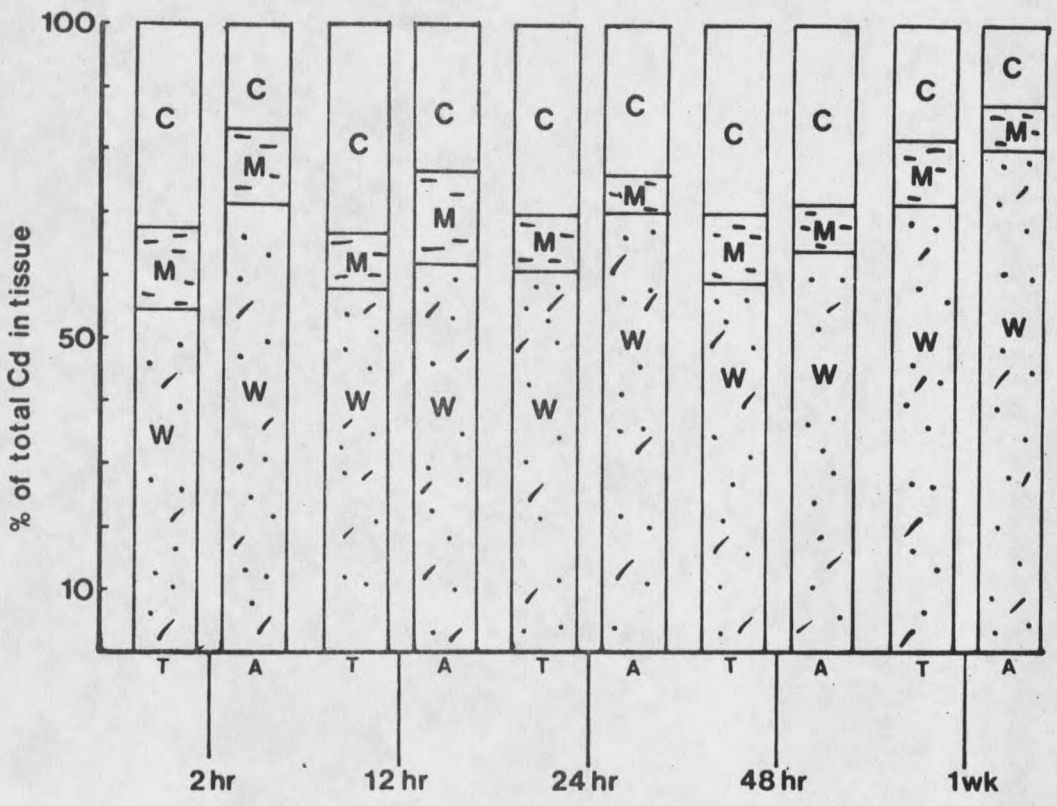


Figure 13. Average per cent distribution of total ROOT Cd in cellular fractions of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* (n=2). (C = "cytoplasmic" fraction; M = "membrane" fraction; W = "wall" fraction). Cd ratios (i.e., $\frac{\text{mass fraction Cd}}{\text{mass total tissue Cd}}$) calculated from CPM of incorporated Cd^{109} (gamma, 88 Kev). Plants grown in aerated, liquid batch culture spiked with Cd^{109}.

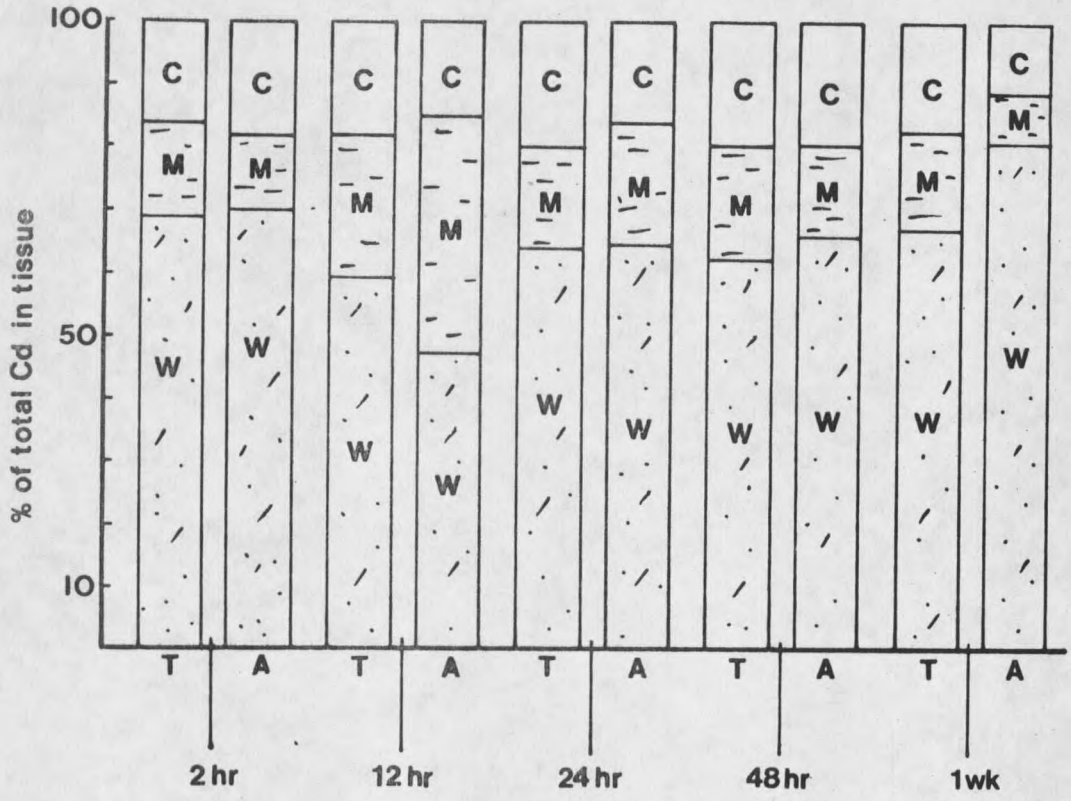


Figure 14. Average per cent distribution of total SHOOT Cd in cellular fractions of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* (n=2). (C = "cytoplasmic" fraction; M = "membrane" fraction; W = "wall" fraction). Cd ratios (i.e., $\frac{\text{mass fraction Cd}}{\text{mass total tissue Cd}}$) calculated from CPM of incorporated Cd^{109} (gamma, 88 Kev). Plants grown in aerated, liquid batch culture spiked with Cd^{109}.

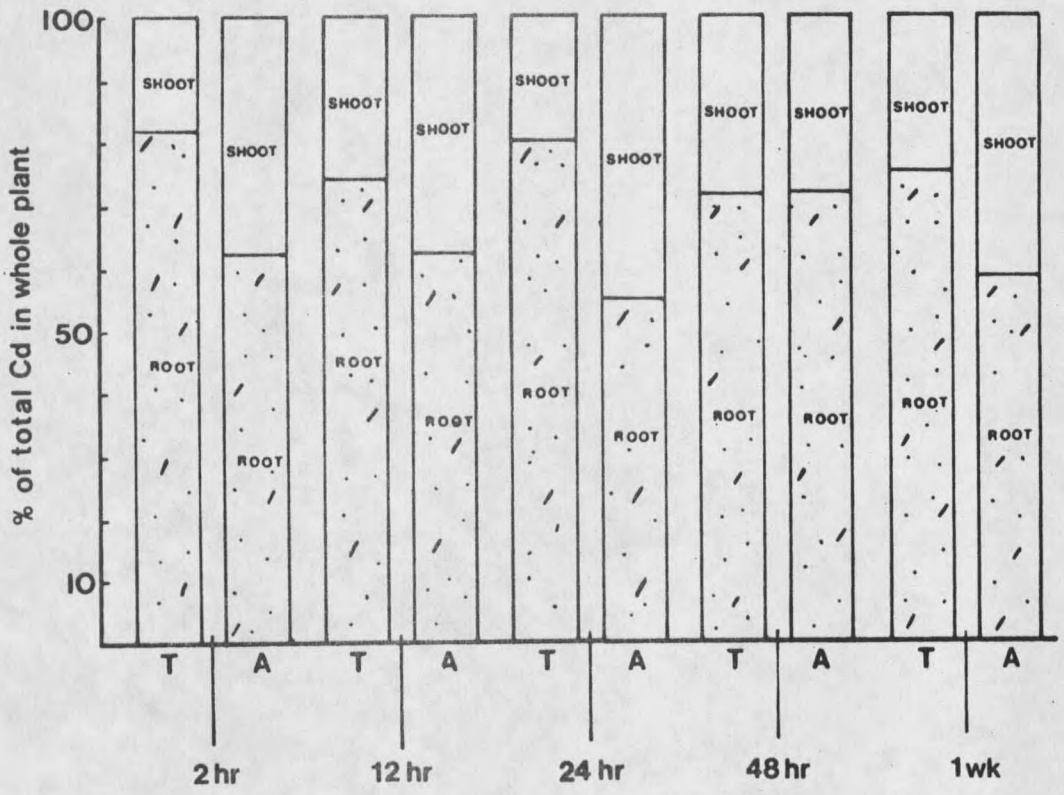


Figure 15. Average ROOT/SHOOT Cd distribution (as per cent of total Cd accumulated) in "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in aerated, liquid batch culture spiked with Cd^{109} (n=2). Tissue Cd calculated from CPM of incorporated Cd^{109} (gamma, 88 Kev).

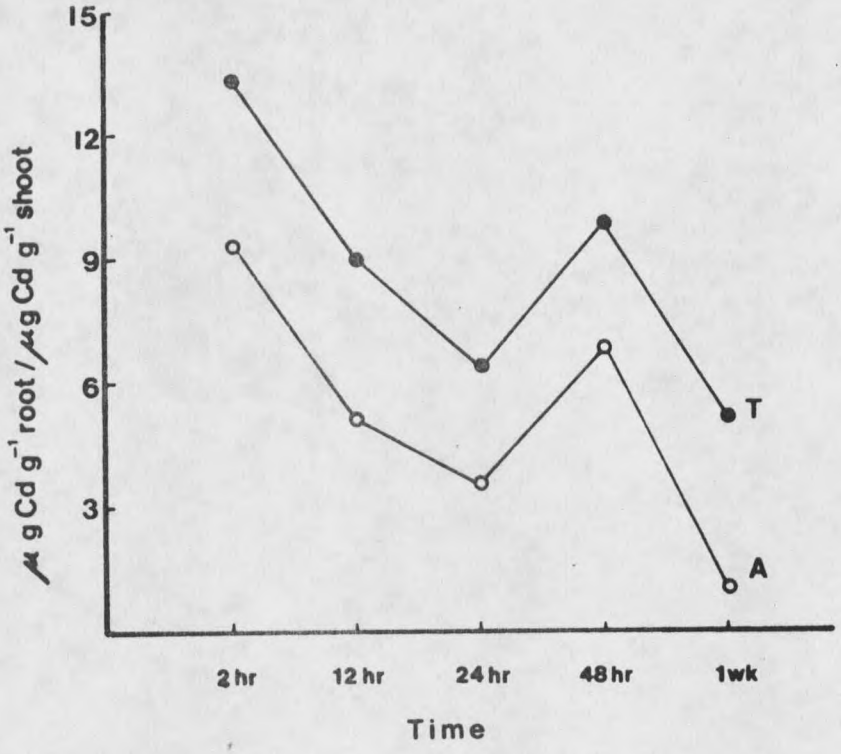


Figure 16. Average ROOT/SHOOT Cd distribution (Root Cd concentration/ shoot Cd concentration) over time in "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in aerated, liquid batch culture spiked with Cd^{109}. Data are average (n=2) Cd total of "cytoplasmic", "membrane", and "wall" fractions as determined by CPM of incorporated Cd^{109} (gamma, 88 Kev).

Table 16. Percent distribution of Cd in fractions, over time, of two races of *D. caespitosa* grown in aerated, batch culture spiked with Cd^{109}. Data are calculated from CPM measured in a Beckman BioGamma counter. Data are average of two independent samples. "T" = "Tailings" race, "A" = "Agricultural" race

RACE	ORGAN	FRACTION	SAMPLE TIME				
			2 hr	12 hr	24 hr	48 hr	1 wk
"T"	ROOT	WALL	54	56	61	59	71
		MEMB	12	9	9	11	10
		CYTO	34	35	30	30	18
	SHOOT	WALL	68	58	64	62	68
		MEMB	16	23	16	18	16
		CYTO	16	18	20	20	17
"A"	ROOT	WALL	71	62	68	66	80
		MEMB	12	15	8	6	6
		CYTO	17	23	24	28	14
	SHOOT	WALL	70	47	60	66	82
		MEMB	12	38	20	14	8
		CYTO	18	15	15	20	11

Table 17. Comparisons of percent Cd distribution in fractions of tissues of *D. caespitosa* races grown in batch, Cd^{109} solution culture. Cd distribution calculated from CPM obtained in Beckman BioGamma counter. "T" = "Tailings" "A" = "Agricultural"

COMPARISON OF MEANS	n	Average Percent Cd In Tissue	Calculated t-value	Table t-value	Level of Significance
"T" Root "WALL"	5	60.2	4.58	4.604 (df=4.00)	0.01
"A" Root "WALL"	5	69.4			
"T" Root "CYTO"	5	29.4	2.97	2.776 (df=4.00)	0.05
"A" Root "CYTO"	5	21.2			
"T" Root "MEMB"	5	10.2	0.41	0.741 (df=4.00)	NS
"A" Root "MEMB"	5	9.4			
"T" Shoot "WALL"	5	64.0	0.24	0.741 (df=4.00)	NS
"A" Shoot "WALL"	5	65.0			
"T" Shoot "CYTO"	5	18.2	1.60	1.533 (df=4.00)	0.20
"A" Shoot "CYTO"	5	15.8			
"T" Shoot "MEMB"	5	17.8	0.15	0.741 (df=4.00)	NS
"A" Shoot "MEMB"	5	18.4			

higher at 24 and 48 hr; no difference at 1 week) may reflect different ion uptake-growth periodicites as suggested by Figures 10,11 and 12. Data on growth rate is critical to the elucidation of this point, unfortunately, the difficulty in handling Cd^{109} precluded the measurement of changes in biomass.

The use of batch, Cd^{109} solution culture has lead to the following conclusions of the *D. caespitosa* Cd interaction.

Conclusions

(1) More Cd is accumulated in root fractions than shoot fractions.

(2) Less Cd is accumulated by "Tailings" shoots than "Agricultural" shoots (all fractions).

(3) There are no differences in Cd accumulation between "Tailings" roots and "Agricultural" roots.

(4) Root/shoot ratio (µg Cd per g. root/µg Cd per g. shoot) is higher in "Tailings" than in "Agricultural".

(5) Dynamic root/shoot distribution patterns of Cd accumulation are similar.

(6) "Tailings" root "wall" has a lower per cent of Cd than the corresponding fraction of "Agricultural".

(7) "Tailings" root "cytosol" has a higher per cent of Cd than corresponding fraction of "Agricultural".

Experiment III - "Macrophyte Reactor" - Cd uptake Kinetics

Materials and Methods

A continuous stirred-tank reactor (CSTR) was designed to allow nutrient solution to flow continuously through the root zone of *D. caespitosa*. Figure 17 depicts the reactor system which permitted control of flow rate and detention times of the nutrient solution.

Briefly, clumps of *D. caespitosa* are inserted into stoppers and placed in 125 ml Erlenmeyer flasks with overflow ports (Figure 18). Nutrient solution is piped into the flask and is continuously stirred by magnetic stir bar. Flow into each flask is controlled by a screw clamp. Overflow from the flask is collected and recycled into the influent stream. Use of recycle provides an easily adjusted system variable and allows fluid detention times sufficient to measure uptake rate processes. The system includes six separate flasks each fed by a common recycle reservoir. Theoretically, these six flasks can be considered as a single reactor volume. Each flask contained approximately 5.0 g of plant material and is covered (as is all tubing) with an opaque shield. All flasks are contained in the same constant-temperature bath. Light was provided by a single 4' Sylvania cool-white flourescent bulb. Temperatures were maintained at ambient laboratory levels (i.e., day = 25 °C; night = 22 °C). Photoperiod of 16 hour day, 8 hour night was controlled by

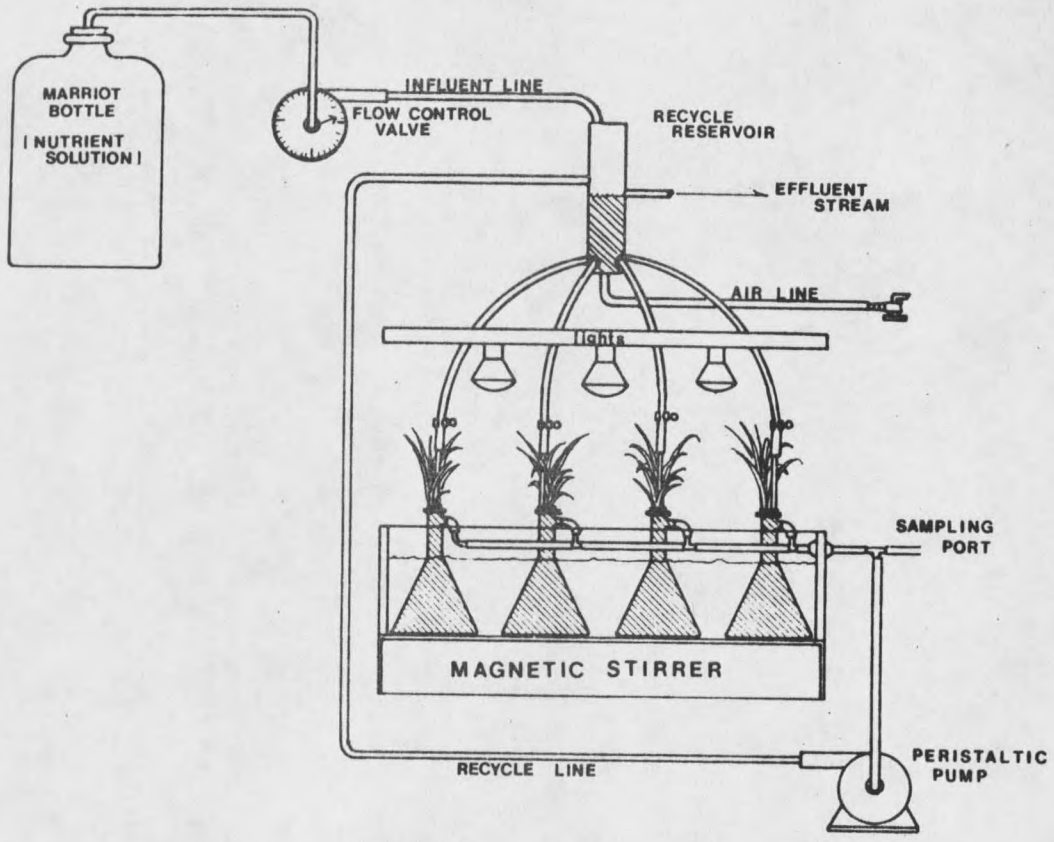


Figure 17. Diagram of continuous stirred tank macrophyte reactor (CSTMR) used to evaluate Cd uptake kinetics of *D. caespitosa*.

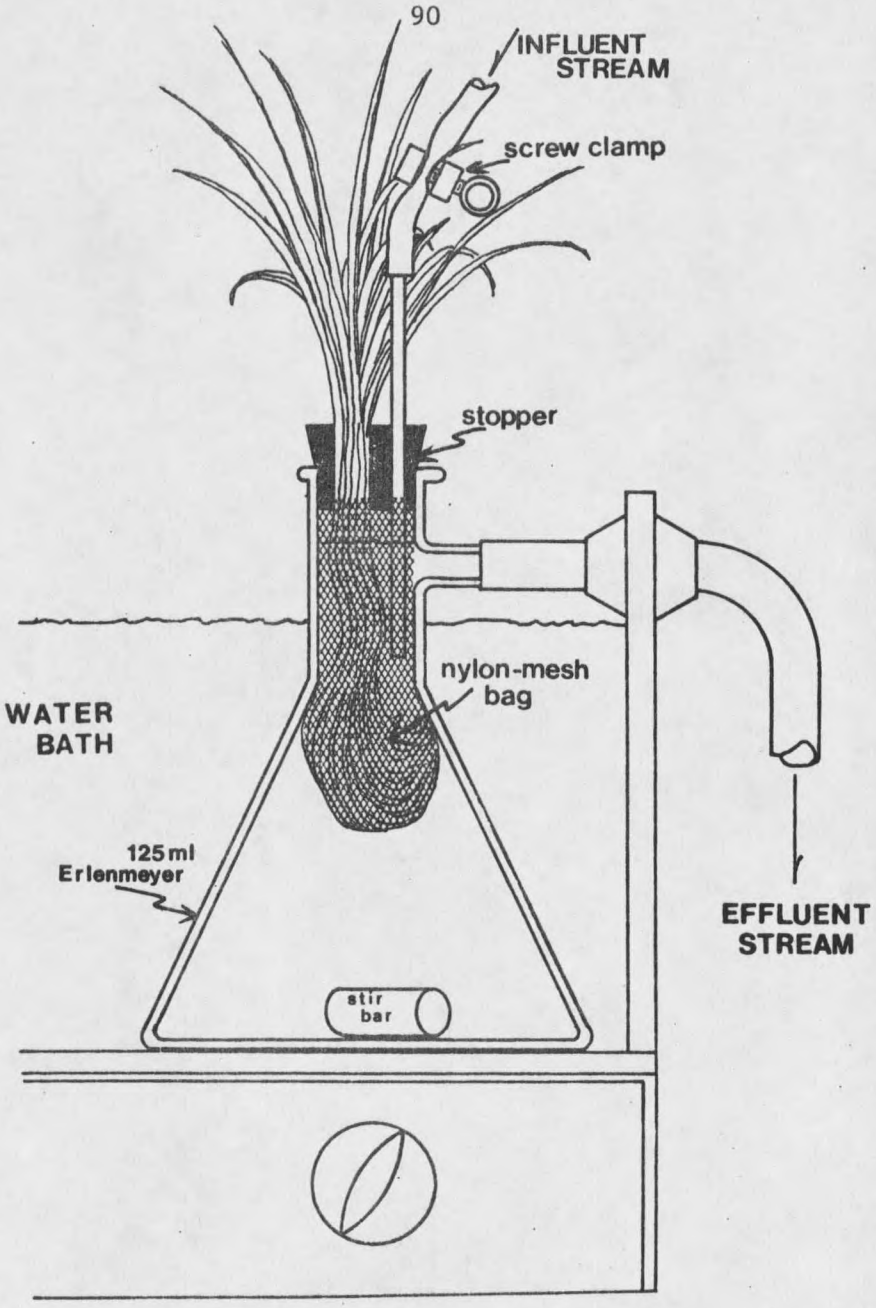


Figure 18. Diagram of one of six flasks in the continuous stirred tank macrophyte reactor (CSTMR).

electric timer. Influent solution was maintained by a 10 gallon marriot bottle and controlled by a small valve. Volume of the reactor was at 1150 ml. A dye study was used to evaluate the reactor's mixing characteristics. Figure 19 shows measured concentration of dye in the reactor after a pulse input of dye and the theoretical dilution curve for the reactor based upon the following calculations:

$$V\frac{dC}{dt} = -FC$$

$$\frac{dC}{C} = -\frac{F}{V}\,dt$$

$$\ln\frac{C}{C_o} = -\frac{F}{V}(t_1 - t_o)$$

$$\ln C = \ln C_o - Dt$$

$$C = C_o e^{-Dt}$$

where: C = concentration of dye in reactor x [=] ML^{-3}
C_o = initial input concentration of dye [=] ML^{-3}
F = flow rate through reactor [=] L^3t^{-1}
αF = recycle flow rate [=] $ML^{-3}t^{-1}$
V = volume of reactor [=] L^3
D = dilution rate = $\frac{F}{V}$ [=] t^{-1}

For the dye study, 0.01 M $KMnO_4$ was pulsed to the system with a flow rate of 1500 ml hr^{-1} and a recycle rate of 7,200 ml hr^{-1}. Aliquots were removed from the reactor periodically and concentration of $KMnO_4$ determined by colorimetry. Figure 19 shows that the macrophyte reactor does not exhibit ideal mixing at a recycle ratio ($\alpha F/F$) of

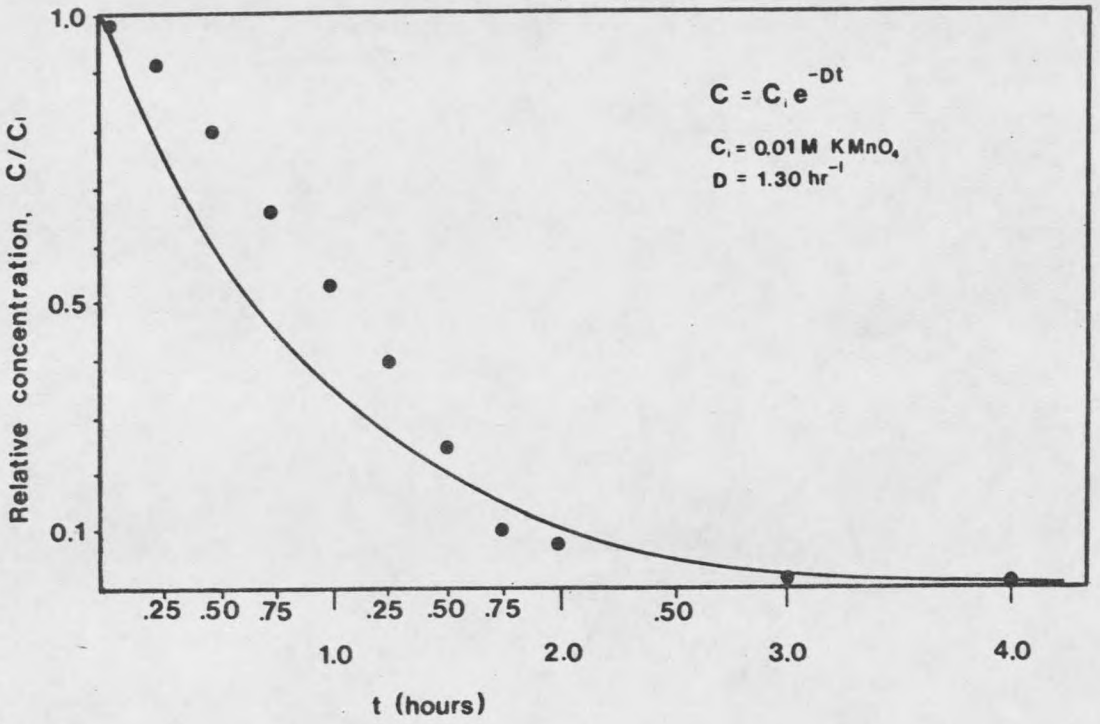


Figure 19. Theoretical dilution curve of continuous stirred tank reactor (CSTR) for a conserved substance pulsed into system. Data points are observed concentrations of in reactor effluent as determined by colorimetric analysis. Reactor mixing experiment carried out under following conditions: Reactor volume (V) = 1150 ml; Flow (F) = 1500 ml min^{-1} Recycle ratio ($\alpha F/F$) = 4.8

4.8. A higher recycle ratio of 80 was used in experimental runs. It is presumed that the higher $\alpha F/F$ resulted in a more nearly ideal dilution curve than that observed in the dye study. For the Cd uptake experiment, system parameters were set as follows:

V = Volume = 1150 ml

F = Flow = 1.5 ml min^{-1}

θ_R = (REACTOR Fluid detention time) = $\frac{V}{F}$ = 12.8 hours

θ_F = (FLASK Fluid detention time) = $\frac{100 \text{ ml}}{20 \text{ ml min}^{-1}}$ = 5.0 minutes

αF = (Recycle rate) = 120 ml min^{-1}

$\alpha F/F$ = (Recycle ratio) = 80

C_i = Influent concentration of Cd = 1.0 µg ml^{-1} in 0.25 X Arnon & Hoagland's solution

Each race was treated in the reactor in separate experimental runs. Plants were collected from prepared (as above) stock specimens and were placed in fresh .25 X Arnon & Hoagland's solution for one week. Plants were transferred to reactor flasks and were allowed to equilibrate in the system without Cd for two days. The system was drained, solution replaced with the 1.0 µg ml^{-1} Cd solution, and the reactor started. Plants were maintained in the reactor for three weeks. Samples of the mixed reactor fluid were collected daily and analyzed for Cd concentration by atomic absorption spectrometry. Samples of the reactor influent were taken every three days and analyzed similarly. Plants were removed four times throughout the three week experiment and measured for volume displacement by immersion in a graduated cylinder. Weights of plants were calculated from volume displacement and by calculating density of root and shoot tissues for each race. Daily pH measurements of the reactor fluid

were also taken. Samples of plant biomass were taken upon experiment termination but, due to an accidental acid spill, an entire set of samples was destroyed. No tissue analysis was used in interpreting the reactor results.

Results and Discussion

The calculated daily specific Cd uptake rate (k_{Cd}) for both races is tabulated in Table 19. Positive values indicate that more Cd is flowing out than flowing into the reactor at sampling time. Cd uptake rate is calculated as a specific uptake rate, that is, mass of Cd accumulated per mass of plant tissue per time (μg Cd g^{-1} dry tissue t^{-1}). Three possible specific uptake rates can be calculated (i.e., whole plant, shoot, and root). Table 18 lists the comparisons between races of these specific uptake rates.

Table 18. Comparison of specific Cd uptake rates for whole plant, root, and shoot tissues of two races of *D. caespitosa* using t-test.

	(μg Cd/g/min)				
COMPARISON	k_{Cd} $\bar{x}$	k_{Cd} min	k_{Cd} max	calculated t-value	tabled t-value
"T" whole plant k_{Cd}	0.29	+0.22	0.68	3.14	2.750 **
"A" whole plant k_{Cd}	0.59	0.04	0.98		(df=36)
"T" root k_{Cd}	0.80	+0.68	1.94	4.65	2.750 **
"A" root k_{Cd}	2.28	0.16	4.18		(df=36)
"T" shoot k_{Cd}	0.48	+0.32	1.90	2.10	2.042 *
"A" shoot k_{Cd}	0.80	0.05	1.30		(df=36)

* = p .025; ** = p .005

Table 19. Net specific Cd accumulation rates (k_{Cd}) of two races of *D. caespitosa* grown in continuous-flow solution culture. Rates based on whole plant weight (k_{Cd} WHOLE), root weight (k_{Cd} ROOT) and shoot weight (k_{Cd} SHOOT). Data calculated from mass balance equation of Cd across reactor and is based on measurement of Cd concentration in reactor effluent by atomic absorption spectrophotometry. Positive values are recorded when the concentration of Cd in the effluent is greater than the concentration of Cd in the influent.

	"TAILINGS" (all data in μg Cd g^{-1} tissue min^{-1})			"AGRICULTURAL"		
DAY	k_{Cd} WHOLE	k_{Cd} ROOT	k_{Cd} SHOOT	k_{Cd} WHOLE	k_{Cd} ROOT	k_{Cd} SHOOT
1	0.32	0.94	0.48	0.52	2.65	0.64
2	0.18	0.52	0.27	0.12	0.67	0.18
3	0.16	0.45	0.24	0.25	1.07	0.32
4	0.27	0.76	0.42	0.19	0.77	0.25
5	0.68	1.88	1.90	0.04	0.16	0.05
6	0.62	1.67	0.97	0.33	1.21	0.45
7	0.58	1.53	0.90	0.50	1.74	0.69
8	0.25	0.64	0.39	0.24	2.11	0.34
9	0.20	0.53	0.31	0.62	1.98	0.88
10	0.08	0.21	0.11	0.65	2.10	0.92
11	0.38	1.03	0.55	0.68	2.26	0.90
12	0.57	1.61	0.84	0.72	2.41	1.02
13	0.67	1.92	0.98	0.92	3.12	1.30
14	0.66	1.94	0.96	0.86	2.94	1.20
15	+0.22	+0.68	+0.32	0.86	3.06	1.20
16	+0.01	+0.04	+0.02	0.91	3.25	1.26
17	+0.04	+0.14	+0.06	0.91	3.73	1.23
18	0.11	0.36	0.16	0.98	4.18	1.28
19	0.04	0.14	0.06	0.84	3.88	1.06
$\bar{x}$	0.29	0.80	0.48	0.59	2.28	0.80
S.E.M.	0.06	0.18	0.12	0.07	0.26	0.10

The most significant difference is in root specific uptake rate, "Tailings" exhibiting a much lower uptake rate. On the other hand, racial differences in shoot specific uptake rate are less significant. Racial differences in whole plant uptake rate mediate in significance. In all cases, the "Tailings" race exhibits a lower specific Cd uptake rate in the reactor system. The pH data in Figure 20 indicates significant differences between the races. Both solutions begin at pH 5.8. The "Tailings" reactor fluid remains quite stable around that value for the duration of the experiment. The drop in pH from 5.8 to 4.0 in the "Agricultural" fluid is dramatic. The climb in "Agricultural" reactor fluid pH to a final level higher than the "Tailings" fluid is quite different the stability exhibited by the "Tailings" population. This could suggest that the "Tailings" race has the capacity to buffer the pH of rhizosphere conditions while the "Agricultural" is incapable of such regulation. Figure 21 shows the significant difference in root specific Cd uptake (k_{Cd} root) dynamics. The "Agricultural" race exhibits a very linear increase in uptake rate over time. The "Tailings" k_{Cd} is more complex but certainly quite different from that exhibited by the "Agricultural" race. The k_{Cd} values of the "Tailings" are positive in some instances. This can be explained by remembering that the reported uptake rate k_{Cd} is a <u>net</u> rate composed of influx and efflux components. Cadmium adsorbed at early stages of the experiment may be effluxed from the tissue or, more likely, sloughed off with sheared root tissue. A low specific

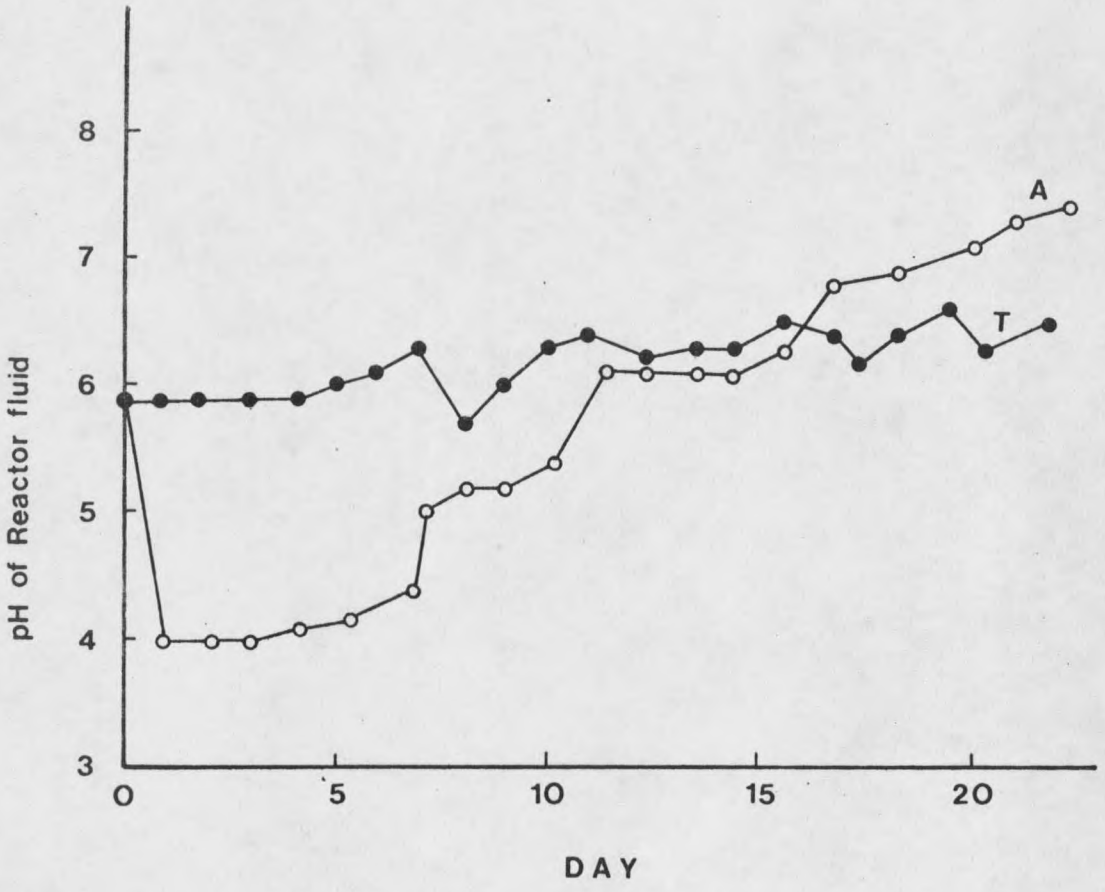


Figure 20. Daily pH measurements of effluent stream from continuous stirred tank macrophyte reactor (CSTMR) containing either "Agricultural" (A) or "TAILINGS" (T) races of *D. caespitosa*. Influent stream composition is 0.25 X Arnon and Hoagland's solution spiked with 1.0 µg Cd ml^{-1} as $CdSO_4$ (pH 5.85).

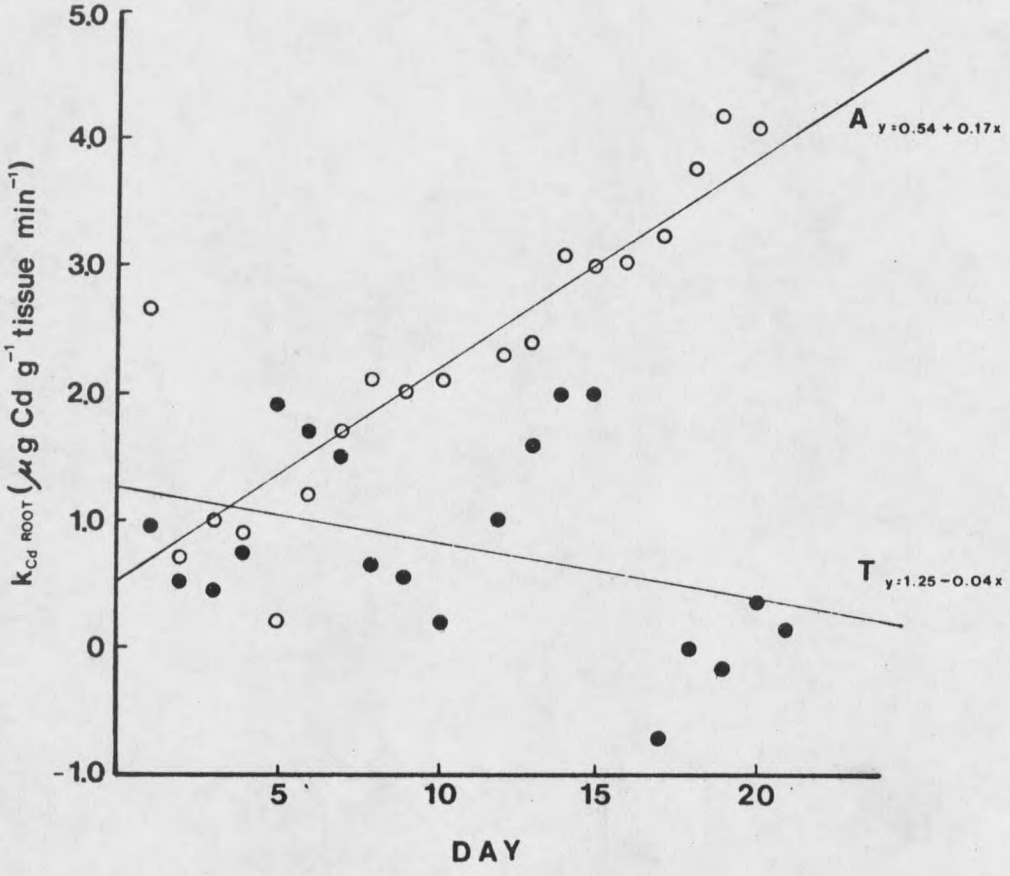


Figure 21. Daily net specific Cd uptake rate for ROOT tissues (k_{Cd} ROOT [=] μg Cd g^{-1} tissue min^{-1}) of "Agricultural" (A; open circles) and "Tailings" (T; closed circles) races of *D. caespitosa* grown separately in continuous stirred tank macrophyte reactor (CSTMR). k_{Cd} calculated from measurements of Cd concentration in influent and effluent streams. Cd levels determined by atomic absorption flame spectrophotometry.

uptake rate could be easily masked by such sloughing of Cd containing tissue, yielding a positive, net flux of Cd out of the system. The root specific uptake rate in the "Agricultural" race appears to be related to its pH curve. In fact, the two variables are highly correlated (r^2 = 0.94). There is low correlation between pH and k_{Cd} in "Tailing" (r^2 = .56). However, the increase in uptake with increasing pH is counter intuitive given the increasing solubility of Cd with decreasing pH. Investigators have suggested that Cu (which exhibits pH-solubility relations similar to Cd) increases its toxic effect with increasing pH. They explained this by stating that at higher pH, Cu will complex with cell components more readily than if the Cu is more soluble (Lexmond and Van der Vorm, 1981). The correlation between pH and k_{Cd} in the "Agricultural" race may reflect the fact that k_{Cd} in the "Agricultural" race is primarily a passive-adsorption process caused by a gradual death of cells. The death hypothesis is supported by Figures 22 and 23 and Table 19 which show the gradual decrease in "Agricultural" growth rate and biomass over time compared to a biomass increase in the "Tailings" race. Table 19 shows the differences in relative growth rate (RGR), the "Tailings" root reaching a steady state while the "Agricultural" eventually exhibits a negative growth rate (i.e., decay). The inverse correlation in shoot RGR between the two races is also apparent. The death hypothesis does not fully explain the differences in shoot Cd concentrations, unless death of cells somehow abolishes Cd transport control at the xylem plasmalemma. This line of reasoning

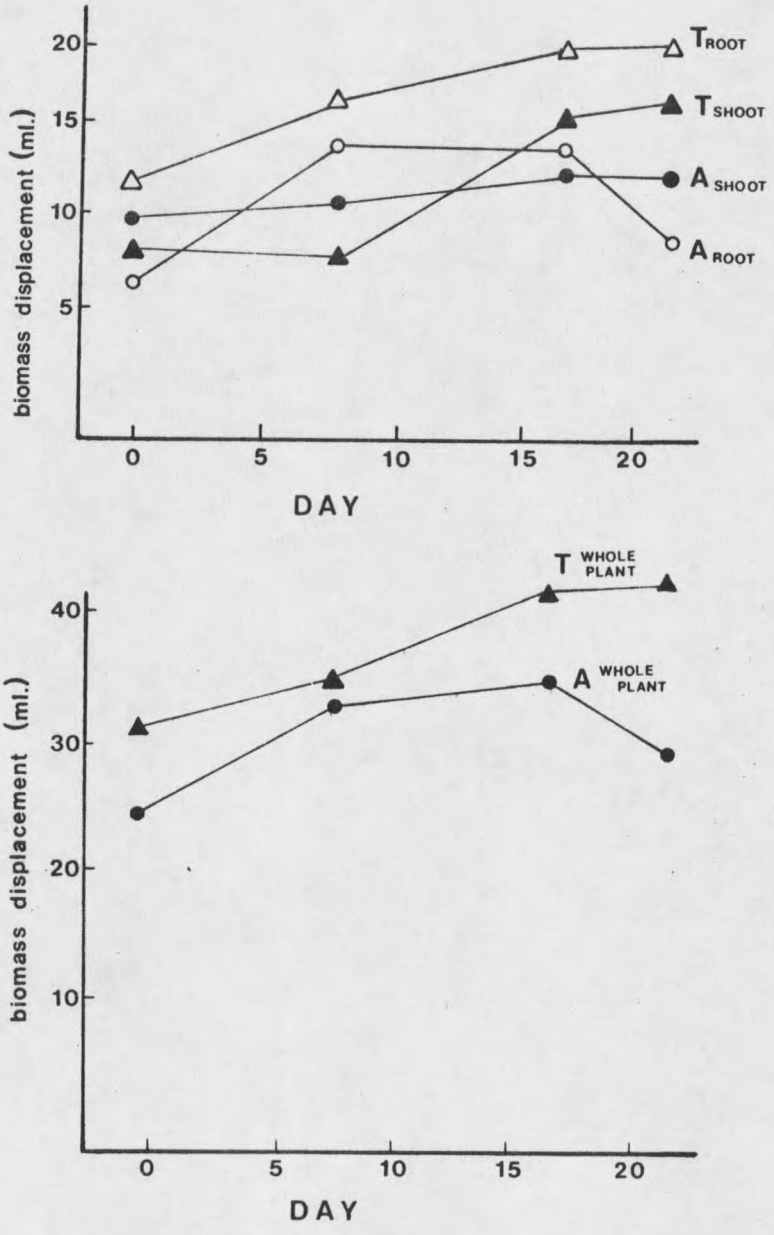


Figure 22. Volume displacement of ROOTS, SHOOTS, and WHOLE PLANT of "Agricultural" (A) and "Tailings" (T) races of *D. caespitosa* grown in continuous stirred tank macrophyte reactor (CSTMR). Each data point is a total of six independent measurements.

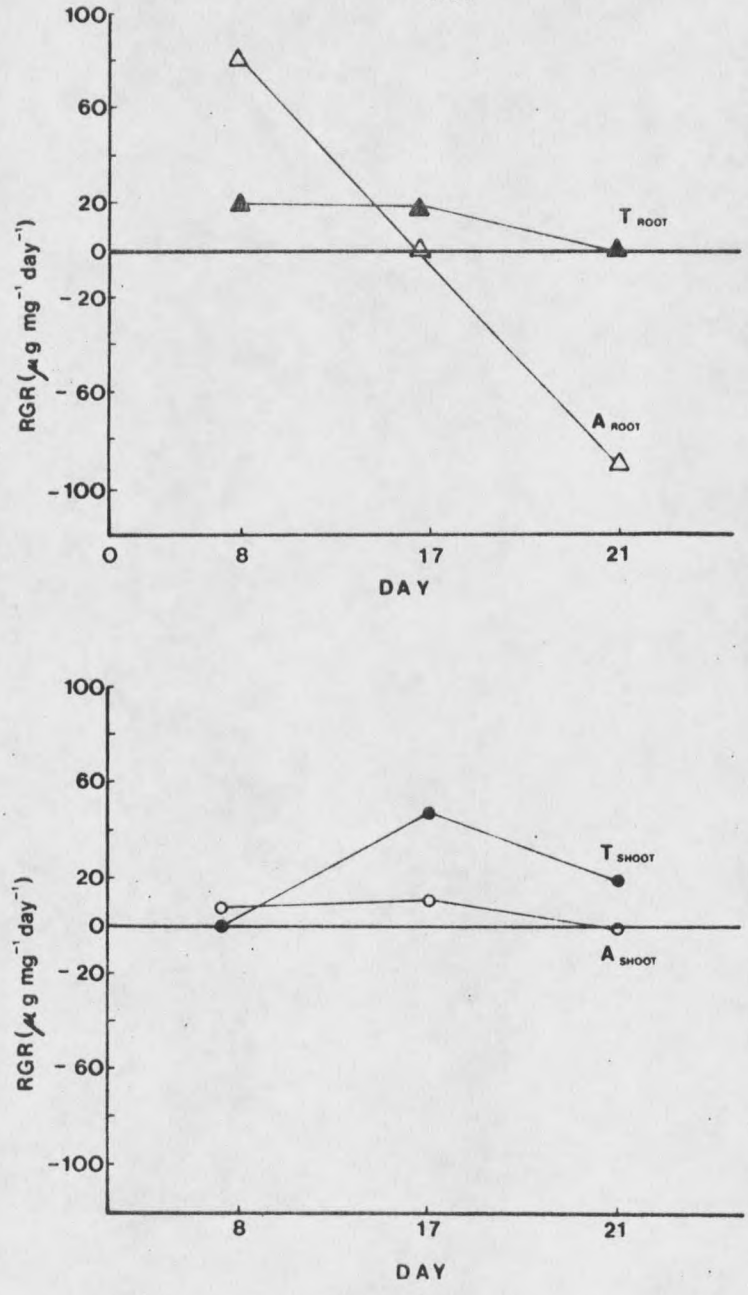


Figure 23. Relative growth rate ($\frac{1}{W} \cdot \frac{dw}{dt}$) of ROOTS and SHOOTS of "Agricultural" (A) and Tailings (T) races of *D. caespitosa* grown in continuous culture (CSTMR) contining 0.25 X Arnon and Hoagland's solution spiked with 1.0 μg ml^{-1} Cd as $CdNO_3$. Mass of tissues (W) calculated from volume displacement of fresh, blotter dried tissues and determination of tissue density.

brings up two questions.

(1) Is the symplast a contiguous structure in the root/shoot continuum?

(2) Is the apoplast a contiguous structure in the root/shoot continuum allowing for passive diffusion from root to shoot tissues?

The importance of these pathways of root-shoot ion transport processes must be evaluated to ascertain the mechanism of acropetal transport.

Table 20. Relative growth rate of root and shoot of "Agricultural" and Tailings races of *D. caespitosa* grown in continuous stirred tank reactor.

TABLE 20

RACE	ORGAN	Relative Growth Rate (RGR) in $\mu g\ mg^{-1}\ day^{-1}$		
		Day 8	Day 17	Day 21
Tailings	Root	21	18	0.0
Agricultural	Root	80	2.0	-9.0
Tailings	Shoot	0.0	46	21
Agricultural	Shoot	5.0	12	0

Inspection of Figure 21 shows the dramatic difference in Cd uptake kinetics between these two races. It also shows that while the dynamics of root k_{Cd} in the "Agricultural" race is modeled quite well by a linear equation, the "Tailings" root k_{Cd} shows a more complex pattern. Although a linear regression line has been fit to the "Tailings" data in Figure 21, it is apparent that this phenomenon is not satisfactorily described by linear models.

The "Tailings" data appears to exhibit a cyclic trend with increasing periods and amplitudes over time. The phenomemon of non-diurnal cyclic ion flux in higher plants has been observed before (Hershey & Paul, 1983; Borchert, 1975). A statistical measure of trendiness in data, the Runs-test (Sokal & Rohlf, 1981) was applied to all the uptake kinetic data. No data set trends were found to depart from randomness, however, it is important to note that these tests depend upon a critical number of cycles in the data for statistical validity. Observation of the "Tailings" root k_{Cd} data suggests that there are 2.5 cycles (if they exist) in the data. This is not a statistically sufficient number of cycles. Clearly, further experimentation is required to determine the nature of these cycles if they are real. Interestingly, the pH data in Figure 20 also shows some hint of periodicity though this data is also random according to the Runs-test.

Attempts to fit the "Tailings" root k_{Cd} into suitable equations has not been successful for linear, exponential, logarithmic, power, or polynomial equations. Further research should be designed to obtain long term (several cycles) data in order to describe the metal uptake phenomenon in the tolerant "Tailings" race.

Conclusions

(1) The net specific uptake rate of Cd (k_{Cd}) of the "Tailings" race is significantly lower than k_{Cd} of "Agricultural". This difference is most pronounced for root k_{Cd} but is also significant for whole plant k_{Cd} and shoot k_{Cd}.

(2) Under the experimental conditions of 1.0 μg Cd ml^{-1} at 1.5 ml min^{-1}, the "Tailings" race exhibits steady-state

root growth (growth = decay rate) whereas the "Agricultural" race exhibits declining root growth rate leading to a net decay of root tissue. "Tailings" shoot growth rate increases to a plateau over time while "Agricultural" race exhibits steady shoot growth decline.

(3) Under the experimental reactor conditions, "Tailings" plants exhibit stable pH of rhizosphere solution over time. "Agricultural" race exhibits dramatic fluctuation of rhizosphere pH.

(4) The macrophyte reactor is capable of yielding ion uptake kinetic data and has potential for observing the dynamics of a variety of higher plant processes *in vivo*.

SUMMARY AND DISCUSSION

Summary of Conclusions

Experiment I (sand-solution culture) conclusions can be summarized as follows:

(1) The metal-sensitive, "Agricultural" race of *D. caespitosa* accumulates on average 1.7 times more Cu or Cd than the metal tolerant, "Tailings" race ($p=0.001$). This difference is more significant with Cu (i.e., "Agricultural" accumulates 1.8 times more Cu than "Tailings"; $p=0.005$) than with Cd (i.e., "Agricultural" accumulates 1.57 times more Cd than "Tailings"; $p=0.10$).

(2) The "Agricultural" root accumulates 1.7 times more Cu or Cd than "Tailings" root ($p=0.005$).

(3) The "Agricultural" shoot accumulates 1.85 times more Cu or Cd than "Tailings" shoot ($p=0.005$).

(4) *D. caespitosa* accumulates 1.9 times more Cu than Cd ($p=0.001$) under identical conditions.

(5) Root tissues of *D. caespitosa* accumulate 1.8 times more Cu or Cd than shoot tissues ($p=0.001$).

(6) Metal accumulation differences between "Agricultural" and "Tailings" (i.e., "Agricultural" > "Tailings") increase with increasing metal loading rate.

(7) Tissue concentrations of Cu and Cd increase over time in *D. caespitosa* up to six weeks after initial metal application. Between six and nine weeks after initial application, increases in Cu and Cd concentration plateaus.

(8) The major portion ($\geq$ 50%) of accumulated Cu or Cd is bound within or irreversibly adsorbed to cell wall material in *D. caespitosa*.

Experiment II (liquid, batch - Cd^{109}) conclusions may be summarized by the following:

(1) "Agricultural" shoots accumulate 2.4 times more Cd than "Tailings" shoots ($p=0.005$).

(2) There are no significant differences in Cd accumulation in roots of "Agricultural" and "Tailings".

(3) The root/shoot ratio (i.e., root Cd concentration/shoot Cd concentration) is higher in "Tailings".

(4) Root tissues of *D. caespitosa* accumulate 4.2 times more Cd than shoot tissues.

(5) Concentration levels of Cd in biochemical fractions of *D. caespitosa* are in the order: "wall" > "cytosol" > "membrane".

(6) Average per cent of tissue Cd is higher for "Agricultural" root "wall" (69.4%) than "Tailings" root "wall" (60.2%).

(7) Average per cent of tissue Cd is higher for "Tailings" root "cytoplasm" (29.4%) than "Agricultural" root "cytoplasm" (21.2%).

Experiment III (Macrophyte reactor) conclusions are summarized as follows:

(1) A continuous stirred tank reactor can be used to determine net ion uptake kinetics in *D. caespitosa*.

(2) Average specific Cd uptake rates (k_{Cd}) are higher in "Agricultural" than in "Tailings" race.

"A" k_{Cd} whole plant ($0.59\ \mu g\ g^{-1} min^{-1}$) > "T" k_{Cd} whole plant ($0.29\ \mu g\ g^{-1}\ min^{-1}$) ($p= 0.005$)

"A" k_{Cd} SHOOT ($0.80\ \mu g\ g^{-1}\ min^{-1}$) > "T" k_{Cd} SHOOT ($0.48\ \mu g\ g^{-1}\ min^{-1}$) ($p=0.05$)

"A" k_{Cd} ROOT($2.28\ \mu g\ g^{-1}\ min^{-1}$) > "T" k_{Cd} ($0.80\ \mu g\ g^{-1}\ min^{-1}$) ($p=0.0008$)

(3) "Agricultural" exhibits negative growth rates (decay) in the Cd solution reactor. "Tailings" exhibits positive or steady state growth rates.

Discussion

The summary of conclusions of this research leads to the overall thesis that the metal-sensitive "Agricultural" race accumulates more Cu or Cd than the metal-tolerant "Tailings" race under identical conditions. An assumption underlying this interpretation is that vegetatively propagated tillers are physiologically identical to recently germinated plants.

The correlation of increasing metal accumulation with increased metal sensitivity is in agreement with much of the literature (Woolhouse, 1983; Woolhouse & Walker, 1981) though there are reports of tolerance linked to hyperaccumulation (Wither & Brooks, 1977).

The finding that Cu is accumulated to a greater degree than Cd and that racial differences in accumulation are more significant with Cu than Cd lends support to the existence of a mechanism of ion absorption selectivity. If the ion "carrier" concept is real, one would expect a biological system to have evolved the capacity to selectively absorb Cu via a specific "carrier" whereas a Cd-specific carrier would not exist. This assumes that availability of Cu and Cd ions is identical at equal concentrations in the experimental systems used in this study.

A fundamental question concerning the nature of the observed

phenomena can be stated by the following:

> **Is the "Tailings" race metal tolerant because it accumulates less metal or does it accumulate less metal because it is metal tolerant?

In other words, are the racial differences in metal accumulation, distribution, and kinetics the cause of metal tolerance or a result of other tolerance mechanisms?

If one assumes that accumulation differences are the tolerance factor, a variety of mechanisms underlying that tolerance can be put forth. Differing uptake may be explained on the basis of the "carrier" concept. The experimental data would support the idea that these races differ by an altered "carrier" conformation. In enzymatic terms, the "Tailings" race may have metal "carriers" with higher K_m values than the "Agricultural" race. This would explain the root absorption, shoot transport and kinetic data differences.

The observed accumulation differences may have a basis in extracellular processes. For example, the "Tailings" race could render metal ions less available by pH adjustment or organic ligand extrusion into the rhizosphere (Dodge & Hiatt, 1972). The racial variability in rhizosphere pH observed in Experiment III may indicate that this mechanism is a factor in uptake differences. Cathala and Salsac (1975) have shown that the adsorption of ions to cell wall material affects the ion absorption process. Thus, it is conceivable that the ion exchange capacity of the "Tailings" cell wall material

may retard metal ion uptake (Darvill, 1983). This could be tested by intraspecific comparison of ion absorption by cell wall material. The existence of a bacterial factor in accumulation differences cannot be overlooked. The differential transport to shoot tissues suggests that rhizosphere solution mechanisms do not fully explain uptake differences.

The suggestion that selective vacuolar desposition plays a role in metal tolerance (Woolhouse, 1983) is not supported in these experiments on *D. caespitosa*. If such a mechanism were operative one would expect the "Tailings" roots to accumulate at least as much as the "Agricultural" roots. In fact the "Tailings" root contains much less Cu or Cd. The production of intracellular chelants may play a role in higher plant metal tolerance mechanisms (Casterline & Barnett, 1977; Bartolf et al., 1980) however, one might expect total metal levels to be similar in the races if such a mechanism were the primary tolerance factor. The fact that Experiment II showed a higher average cytoplasm metal content in the "Tailings" roots suggests that this mechanism can not be discounted entirely. This observation could also be explained by the fact that the "Agricultural" cells are lysing, leaving less metal in the extracted "cytoplamic" fraction. The role of plasmodesmatal transport in the *D. caespitosa* system has been ignored in this discussion largely because of the lack of basic information on the phenomenon (Spanswick, 1976) and the assumption that acropetal transport via the symplasm would be neglible relative to convective transport through vascular tissue. An interesting point in this context is the hazy characterization

in the literature of the extent of the symplasm in higher plants. It seems reasonable to assume that the symplasm is a contiguous structure throughout the plant and that therefore, a higher plant is composed of a single large membrane with many invaginations. This point may be important to questions of regulation of root ion uptake/shoot ion demand relations.

"Dying Osmometer" Theory

The theory which most simply explains the experimental observations is termed the "dying osmometer". This statement refers to the fact that all the observed data can be interpreted by the death of "Agricultural" root cells under metal ion stress conditions. According to this theory, the following sequence of events occurs in "Agricultural" root tissues in the presence of toxic metals in the rhizosphere. The presence of heavy metal ions induces a toxic effect on sensitive cell processes. This leads to loss of membrane integrity and lysis. Lysis renders more cell wall sites available for metal ion adsorption. Gradual necrosis of poisoned root cells results in a loss of control of ion secretion into vascular tissue. In summary, the death of root cells increases adsorption rate of metals to polysaccharides and leads to the "leakage" of ions from the symplast into xylem vessels. The kinetic and growth rate data from Experiment III support this theory. The k_{Cd} of "Agricultural" root increases linearly with time with a concomitant decrease in growth rate leading to eventual net decay of tissue.

The "dying osmometer" theory does not explain intraspecific differences in metal tolerance stating only that the "Tailings"

race accumulates less metal because it is tolerant. One hypothesis which does explain observed accumulation differences as well as differential metal tolerance is the "altered carrier" concept.

"Altered carrier" Theory

This theory, based on the ion carrier protein models of Laüchli and Epstein states that the two races differ in metal response because of a conformational alteration in those membrane proteins responsible for selective ion absorption. Wainwright and Woolhouse (1975) have shown membrane protein alteration in Cu tolerant plants relative to non-tolerant clones. Uptake kinetic differences, accumulation and acropetal transport differences could be a result of carrier protein conformational change that affects the K_m and/or V_{max} of the enzyme-like ion "carrier".

The fact that heavy metal ions affect a spectrum of metabolic processes suggests that the observed phenomena reflect a variety of specific processes. It is intuitive that a metal-tolerant race may have developed several defense mechanisms acting in concert.

LITERATURE CITED

LITERATURE CITED

Alloway, B. 1968. Anomalous levels of trace metals in Welsh soils. Welsh soils discussion group Report No. 9, Davies (Ed.) p. 87.

Anderson, W.P. 1972. Ion transport in the cells of higher plant tissues. Ann. Rev. Plant Physiol. 23:51-72.

Andrew, C.S. 1966. A kinetic study of phosphate absorption by excised roots... Aust. J. Agric. Res. 17:611-624.

Asher, C.J., et al. 1965. A method for controlling the ionic environment of plant roots. Soil Sci. 100(3):149-156.

Barshad, I. 1948. Molybdenum content of pasture plants in relation to toxicity to cattle. Soil Sci. 6:187:195.

Bartolf, M., et al. 1980. Partial characterization of cadmium-binding protein from the roots of cadmium-treated tomato. Plant Physiol. 66:438.

Bauman, A. 1885. Das Verhalten von Zinksälzen gegen Pflanzen und im Boden. Landun Verstnen 31:1-53.

Baumeister, W. 1967. Schwermetall-Pflänzengesellschaften und Zinkresistenz einiger Schwermetallpflanzen. Angew. Bot. 40:185.

_____. 1954. Uber den Einfluss des Zinks bei Silene inflata. Ber. dt. Bot. Ges. 67:205-213.

Baumeister, W. and H. Burghardt. 1956. Uber den Einfluss des Zinks bei Silene inflata. II CO_2 Assimilation und Pigmentgehalt. Ber. dt. Bot. Ges. 69:161-168.

Beath, O.A. 1943. Toxic vegetation growing on the salt wash sandstone member of the Morrison Formation. Amer. J. Bot. 30:698-707.

Beeson, K.C. 1941. The mineral composition of crops with particular reference to the soils in which they were grown. U.S.D.A. Misc. Pub. 369.

Beeson, K.C., et al. 1955. Some plant accumulators of the micronutrient elements. Ecology 36:155-156.

Bernard, R.L. and R.W. Howell. 1964. Inheritance of phosphorus sensitivity in soybeans. Crop Sci. 4:298-299.

Bloom, A.J. and F.S. Chapin. 1981. Differences in steady-state net ammonium and nitrate influx by cold and warm adapted barley varieties. Plant Physiol. 68:1064-1067.

Borchert, R. 1975. Endogenous shoot growth rhythms and indeterminate shoot growth in oak. Physiol. Plant. 34:152-157.

Bouwer, H. and R.L. Chaney. 1974. Land treatment of wastewater. Advan. Agron. 26:133.

Bradshaw, A.D. 1952. Populations of *Agrostis tenuis* resistant to lead and zinc poisoning. Nature, Lond. 169:1098.

Bradshaw, A.D., et al. 1965. Industrialization, evolution and the development of heavy metal tolerance in plants. in Ecology and the Industiral Society Brit. Ecol. Soc. Symp. 5:327-343.

Bradshaw, A.D. and R. Chadwick. 1981. Restoration of Land. Blackwell Sci. publ., London.

Breeze, B.G., et al. 1982. The uptake of phosphate by plants from flowing nutrient solution. I. Control of phosphate concentration in solution. J. Exp. Bot. 33:183-189.

Briggs, G.E., et al. 1961. Electrolytes and Plant Cells. F.A. Davis Co. Phila.

Broker, W. 1963. Genetisch-physiologische Untersuchungen über die Zinkvertraglichkeit von Silene inflata. Flora, Jena. B. 153:122-156.

Brooks, R.A. 1972. Geobotany and Biogeochemistry in Mineral Exploration. Harper & Row publ., Lond.

Brooks, R.A., et al. 1981. Some observations on the ecology, metal uptake and nickel tolerance of *Alyssum serphyllifolium* subspecies from the Iberian penninsula. Vegetatio 45:183-188.

Brown, J.C. and T.E. Devine. 1980. Inheritance of tolerance or resistence to manganese toxicity in soybeans. Agron. J. 72:898-904.

Brown, J.C. and W.E. Jones. 1971. Differential transport of boron in tomato. Physiol. Plant. 25:279.

Brown, J.C., et al. 1971. A new tomato mutant inefficient in the transport of iron. Physiol. Plant. 25:48-53.

Buchauer, M.J. 1973. Contamination of Soil and Vegetation near a zinc Smelter by Zn, Cd, Cu, and Pb. Envr. Sci. and Tech. 7(2):131.

Burkitt, E.B., et al. 1972. Distribution of heavy metals in the vicinity of an industrial complex. Nature 238:327-328.

Cannon, H.L. 1960. Botanical prospecting for ore deposits. Science, NY, 132:591-598.

_____. 1957. Description of indicator plants and methods of botanical prospecting for uranium deposits... Bull. USGS No. 1030-M:399516.

Casterline, J.L. and N.M. Barnett. 1977. Isolation and characteriztion of cadmium binding components in soybean plants. Plant Physiol. 59:115-124.

Chatterton, N.J., et al. 1970. Absorption of Na, Cl, and B by desert saltbrush in relation to composition of nutrient solution. Agron. J. 62:351.

Clark, R.B. 1975. Differential magnesium efficiency in corn inbreds... Soil Sci. Soc. Amer. Proc. 39:488-491.

Clarkson, D.T. and J.B. Hanson. 1980. The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31:239-298.

Clement, C.R., et al. 1974. A system for measuring the uptake of ions by roots from flowing solutions of controlled composition. J. Exp. Bot. 25:81-99.

Cook, F.C. 1916. Boron: its absorption and distribution in plants and its effect on growth. J. Agr. Res. 5:887-890.

Cordukes, W.E. and E.U. Parups. 1971. Cl uptake by various turfgrass species. Can. J. Plant Sci. 51:485.

Cox, R.M. and R. Hutchinson. 1979. Metal co-tolerances in the grass *D. caespitosa*, Nature 279:231.

Cox, R.M. and D.A. Thurman. 1978. Inhibition by Zn of soluble and cell wall acid phosphatases of Zn-tolerant and non-tolerant clones of *Anthoxanthum oderatum*. New Phytol. 80:17-22.

Cutler, J.M. and D.W. Rains. 1974. Characterization of cadmium uptake by plant tissue. Plant Physiol. 54:67-71.

Darvill, A. 1983. Personal communication. Dept. of chemistry, U. of Colorado, Boulder, CO.

de Saussure, T. 1804. Récherches chémiques sur la végétation. in Soil Conditions and Plant Growth. by E.W. Russell, Longman publ., Lond.

Dodge, C.D. and A.J. Hiatt. 1972. Relationship of ion uptake imbalance by variations of wheat. Agron. J. 64:476-477.

Dowdy, R.H. and W.E. Larson. 1975. Metal uptake by barley seedlings grown on soils amended with sewage sludge. J. Environ. Qual. 4(2): 229-232.

Dye, W.B. and J.L. O'Hara. 1959. Molybdenosis. Bull. Nev. Agr. Exp. Sta. 208:34p.

Earley, E.B. 1943. Minor element studies with soybeans. J. Amer. Soc. Agron. 35:1012.

Edwards, D.G. and C.J. Asher. 1974. The significance of solution flow rate in flowing culture experiments. Plant & Soil 41:161-175.

Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. J. Wiley & Sons, Inc. NY.

Epstein, E. and R.L. Jeffries. 1964. The genetic basis of selective ion transport in plants. Ann. Rev. Plant Physiol. 15:169.

Ernst. W.H.O. 1980. Biochemical aspects of cadmium in plants. in Cadmium in the Environment. J.O. Nriagu (Ed.) J. Wiley & Sons, NY.

_____. 1976. Physiologic and biochemical aspects of metal tolerance. in Effects of Air Pollution on Plants. T.A. Mansfield (Ed.) Columb. U. Press.

_____. 1972a. Ecophysiological studies on heavy metal plants in So. Central Aftica. Kirkia 8:125.

_____. 1972b. Schwemetallrediditenz und Mineralstoffhaushalt. Forschungsberichte des Landes Nordein-Westfalen. 2251:1.

_____. 1968a. Zur Kenntnis der Soziologie und Ökologie der Schwermetallvegetation Grossbritanniens. Ber. dt. Bot. Ges. 81:116.

_____. 1968b. Okologische Untersuchungen auf Pflanzengesellschaften unterschiedlich gestörter schwermetallreichen Boden in Grossbritannien. Flora, Jena B. 158:95-106.

_____. 1965. Okologische soziologische Untersuchungen der Schwermetall-Pflanzengesellschaften Mitteleuropas unter Einschluss der Alpen. Abh Landesmus. Naturk. Munster 27.

Foster, L. 1977. Cu exclusion as a mechanism of heavy metal tolerance in a green alga. Nature 269:322.

Foy, C.D., et al. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29:511566.

Foy, C.D., et al. 1973. Opposite Al and Mn tolerances in two wheat varieties. Agron. J. 65:123-126.

Gambi, O.V. 1967. First data on the histological localization of nickel... G. Bot. Ital. 101:59.

Garrod, A.E. 1909. in Inborn Errors of Metabolism. Oxford Univ. Press publ. Lond. 1963.

Gartside, D.W. and T. McNeilly. 1974. Genetic studies in heavy metal tolerant plants heredity. 32:287-297.

Gerloff, G.C., et al. 1966. Selective absorption of mineral elements by native plants of Wisconsin.. Plant & Soil 25:393-405.

Glass, A.D.M. 1977. Regulation of K^+ influx in barley roots: evidence for direct control by internal K^+. Aust. J. Plant Physiol. 4:313-318.

Glauber, J.R. 1656. Des Teutschlandts Wohlfart (Erster Teil), das dutte Capittel de Concentratione vegetabilium.

Gadgil, R.L. 1968. Tolerance of heavy metals and the reclamation of industrial waste.. J. of Appl. Ecol. 6:247-259.

Goodman, P.J. 1968. Intraspecific variation in mineral nutrition of plants from different habitats. in Plant Mineral Nutrition by E.J. Hewitt, J. Wiley & Sons, NY.

Gorsuch, T.T. 1970. The Destruction of Organic Matter. Pergamon Press, Oxford U.K. 152 pp.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol. 31:149.

Gunning, B.E.S. and A.W. Robards. 1970. Plasmodesmata and symplastic transport. in Transport and Transfer Processes in Plants. U. Luttge & N. Higinbotham Springer-Verlag, NY.

Gwirtsman, J.R., et al. 1957. Determination of flourides in plant tissue, air, and water. Anal. Chem. 29:887-892.

Haghiri, F. 1973. Cadmium uptake by plants. J. Environ. Qual. 2(1): 93-96.

Hamer, D. and M. Walling. 1982. Regulation in-vivo of a cloned mammalian gene: Cd induces the transcription of a mouse metallothionein gene in SV40 vectors. J. of Molecular and Appl. Genetics 1:273-288.

Harborne, J.B. (Ed.) 1982. Introduction of Ecological Biochemistry. Academic Press, NY.

Heenan, D.P. and O.G. Carter. 1976. Tolerance of soybean cultivars to Mn toxicity. Crop Sci. 16:389-391.

Henwood, W.J. 1857. Notice of the copper turf of Meironeth. Edin. New Phil. J. 5:61.

Hershey, D.R. and J.L. Paul. 1983. Ion absorption by a woody plant with episodic growth. Hort. Sci. 18(3):357-359.

Hewitt, E.J. 1975. Plant Mineral Nutrition. J. Wiley & Sons, NY.

Homer, J., et al. 1980. Whole leaf flourescence as a technique for measurement of tolerance of plants to heavy metals. Oecol. 45:88.

Jarvis, S.C., et al. 1977. Uptake and transport of Pb by perennial ryegrass... Plant & Soil 46:371-379.

_____. 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant & Soil 44:179-191.

Jefferies, R.L., et al. 1968. in Ecological Aspects of the Mineral Nutrition of Plants. I.H. Rorison (Ed.) Blackwell Sci. Publ., Oxford.

Johnson, C.M. 1966. Molybdenum. in Diagnostic Criteria for Plants and Soils. Chapman (Ed.) U. of CA Riverside Press.

Johnson, M.S., et al. 1977. Revegetation of metalliferous mine spoil contaminated by lead and zinc. Environ. Pollut. 12:261-277.

_____. 1976. Revegetation of metalliferous flourspar mine tailings. Trans. Inst. Min. Metall. 85:A32-37.

Jones, R.K. 1974. A study of the phosphorus responses of a wide range of accessions from the genus *Stylosanthes*. Aust. J. Agric. Res. 25:847-862.

Kelly, J.M., et al. 1979. Heavy metal accumulation and growth of seedlings of five forest species as influenced by soil cadmium level. J. Environ. Qual. 8(3):361-364.

Lagerwerff, J.V. and A.W. Specht. 1970. Contamination of roadside soil and vegetation with Cd, Ni, Pb, and Zn. Environ. Sci. & Tech. 4(7):583.

Laüchli, A. 1976. Genotypic variation in transport. in Encyclopedia of Plant Physiology Vol. 2B. U. Lütge, M.G. Pitman (Eds.) Springer-Verlag, Berlin.

Leckie, J.O. and J.A. Davis. 1979. Aqueous environmental chemistry of copper. in Copper in the Environment. J.O. Nriagu (Ed.), J. Wiley & Sons, Inc., NY.

Lee, J., et al. 1978. The relation between Ni and citric acid in some Ni-accumulating species. Phytochem. 17:1033-35.

Levitt, J. 1980. Responses of Plants to Environmental Stress. Vol. II. Academic Press, NY.

Lexmond, T.M. and P.D.J. van der Vorm. 1981. The effect of pH on copper toxicity to hydroponically grown maize. Neth. J. Agric. Sci. 29:209-230.

Lüttge. U. and N. Higinbotham, 1979. Transport in Plants. Springer-Verlag, NY.

Lüttge, U. and M.G. Pitman (Eds.). 1976. Transport in Plant II. Encyclopedia of Plant Physiology Vols. 2A, 2B, Springer-Verlag, Berlin.

Lowendorff, H., et al. 1974. Phosphate transport. in Neurospora, kinetic characterization of a constitutive, low affinity transport system. Biochem. Biophys. Acta. 373:369-382.

Lyon, G.L., et al. 1968. Trace elements in a New Zealand serpentine flora. Plant & Soil. 29:225-240.

Mailaisse, F., et al. 1978. *Aeolanthus biformifolius*: A hyperaccumulator of copper from Zaire. Science 199, 24 Feb.:887-888.

Malone, C., et al. 1974. Localization of lead accumulated by corn plants. Plant Physiol. 53:388-394.

Mathys, W. 1974. Enzymes of heavy metal resistant and non-resistant populations. Physiol. Plant. 33:161.

Matsumoto, H., et al. 1967. Localization of Al in tea leaves. Plant Cell Physio. 17:890-895.

McNeilly, T. 1968. Evolution in closely adjacent plant populations. Heredity 23:99-108.

McNeilly, T. and A.D. Bradshaw. 1968. Evolutionary processes in populations of copper tolerant *Agrostis tenuis* Sibth. Evolution 22:108-118.

Milton, C., et al. 1944. Weinshenkite, Yttrium phosphate dihydrate, from Virginia Amer. Min. 29:92-110.

Morrison, R.S., et al. 1979. Copper and cobalt uptake by metallophytes from Zaire. Plant & Soil 53:535-39.

Mortvedt, J.J., et al. (Eds.) 1972. Micronutrients in Agriculture. Soil Sci. Soc. Amer. Madison, WI.

Mrozek, E., et al. 1982. Polychlorinated biphenyl uptake and translocation by *Spartina alternifolia*. Water, Air and Soil Pollution 17:3-15.

Munshower, F.F. and D.D. Newman. 1978. Elemental concentration in native range grasses from the Northern Great Plains of Montana. J. Range Manage. 31:145-148.

Moomaw, J.C., et al. 1959. Aluminum in some Hawaiian plants. Pac. Sci. 13(1):335-41.

Nissen. P. 1974. Uptake mechanisms: inorganic and organic. Ann. Rev. Plant Physiol. 25:53-79.

Nissen, P. and O. Nissen. 1983. Validity of the multiphsic concept of ion absorption in plants. Physiol. Plant. 57:47-56.

Nriagu, J.O. (Ed.). 1979. Copper in the Environment. J. Wiley & Sons, Inc., NY.

Nye, P.H. and P.B. Tinker. 1977. Solute Movement in the Soil-Root System. U. of CA Press, Berekly.

Nye, P.H. and P.B. Tinker. 1969. The concept of a root demand coefficient. J. Appl. Ecol. 6:293-300.

Oertli, J.J. and H.C. Kohl. 1961. Some considerations about the tolerance of various plant species to excessive supplies of boron. Soil Sci. 92:243-247.

Okamoto, K. and K. Fuwa. 1974. Copper tolerance of a new strain of *Penicillium ochachloron*. Agric. Biol. Chem. 38:1405.

Peisach, J., et al. (Eds.). 1966. The Biochemistry of Copper. Academic Press, NY.

Peterson, D.J. 1969. The distribution of Zn-65 in *Agrostis tenuis* and *stolonifera*. J. Exp. Bot. 20:863.

Petterson, S. and P. Jenson. 1978. Allosteric and non-allosteric regulation of rubidium influx in barley roots. Physio. Plant. 44:110-114.

Pitman, M.G. 1977. Ion transport into the xylem. Ann. Rev. Plant Physiol. 28:71-88.

Pope, D.T. and H.M. Munger. 1953a. Heredity and nutrition in relation to magnesium deficiency chlorosis in celery. Proc. Am. Soc. Hort. Sci. 61:472-480.

Pope, D.T. and H.M. Munger. 1953b. The inheritance of susceptibility to boron deficiency in celery. Proc. Am. Soc. Hort. Sci. 61:481-486.

Porter, E.K. and P.J. Peterson. 1977. Arsenic tolerance in grasses growing on mine waste. Eviron. Pollut. 14:255-265.

Prat, S. 1934. Die Erblichkeit der Resistenz gegen Kupfer. Ber, dt. bot. Ges. 52:65-67.

Prat, S. and K. Komarek. 1934. Vegetace u medenych dolu. Zulastni otisk u sbornicku map.

Rauser, W.E. 1981. Occurence of heavy metal-binding proteins in plants. in Proc. of Intnatl. Conf. Heavy Metals in the Environment, Amsterdam.

Rauser, W.E. and N. Curvetto. 1980. Metallothioneins occur in roots of *Agrostis* tolerant to excess copper. Nature 287:563-564.

Reilly, A. and C. Reilly. 1973. Copper-induced chlorosis in *Becium homblei* (De Wild.) Duvign. & Plancke. Plant & Soil 38:671.

Robinson, W.O. and G. Edgington. 1945. Minor elements in plants and some accumulator plants. Soil Sci. 60:15-28.

Roffe, G. 1973. Lead uptake by selected tree seedlings. J. Environ. Qual. 2(1):153-157.

Repp, G. 1963. Die Kupferresistenz des Protopl mas höherer Pflanzen auf Kupfererzboden. Protoplasma 57:643-659.

Rorison, I.H., et al. (Eds.). 1968. Ecological Aspects of Biological Membranes. Academic Press, NY.

Rothfield, L.I. (Ed.) 1971. Structure and Function of Biological Membranes. Academic Press, NY.

Schwanitz, F. and H. Hahn. 1954. Genetischentwicklungs-physiologische Untersuchungen on Galmei Pflanzen... Z. Bot. 42:179-190.

Shrift, A. 1969. Selenium toxicity. Ann. Rev. Plant Physio. 20: 475-494.

Sivasubranamiam, S. and O. Talibudeen. 1972. Effects of Al on the growth of tea... Tea 43:4.

Smith, J.M. 1970. Chemical Engineering Kinetics. McGraw-Hill, NY.

Smith, R.H. and A.D. Bradshaw. 1970. Reclamation of toxic metalliferous wastes using tolerant populations of grass. Nature 227:376-377.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry 2nd ed. W.H. Freeman Publ., NY.

Sonneveldt, C. and dS.J. Voogt. 1975. Studies on the Mn uptake of lettuce on steam-sterilized glasshouse soils. Plant & Soil 42:49-64.

Steward, F.C.(Ed.). 1963. Plant Physiology A Treaties Vol.III., Academic Press, NY.

Suchting, H. 1948. Investigations on nutrition conditions in the forest. The effect of soluble Al on pines... Pflernahr. Dung 42:193-218.

Surbrugg, J.E. 1982. Copper and zinc tolerance in two Montana Grass species from copper mill tailings. MSC. Thesis, Montanta State University, Bozeman, MT.

Thompson, J.F. and L.O. Tiffin. 1974. Fractionation of nickel complexing compounds of peanut seeds with ion exchange resins. Plant Physio. 53; suppl. abstract 125.

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

Van de Dijk, S.J. 1981. Differences in nitrate uptake of species using a new growth technique. Plant & Soil 62:265-278.

Vaneslow, A.P. 1966. Strontium. in Diagnostic Criteria for Plants and soils. Chapman (Ed.) Div. Agr. Sci. U. of CA, Berkely.

Veen, B.W. 1977. The uptake of potassium nitrate, water, and oxygen by a maize root system in relation to its size. J. Exp. Bot. 28:1389-1398.

Wachsmann, C. 1961. Wasserkulturversuche zur Wirkung von Blei, Kupfer, und Zink auf die Gartenform und Schwermetallbiotypen von Silene inflata. Dissertation an der Mathematisch-Naturwissenschaftlichen

Fakultat der Westafalischen Wilhelms Universitat zu Munster in Referaten 19:35-46.

Wagner, G.J. and M.M. Trotter. 1982. Inducible cadmium binding complexes of cabbage and tobacco. Plant Physiol. 69:804-809.

Wagner, K. 1936. Uber den Barium Gehalt des Paras-Nuss. J. Prakt. Chem. 47:110-112.

Wainwright, S.J. and H.W. Woolhouse. 1975. Physiological mechanisms of heavy metal tolerance. in The Ecology of Resource Degradation and Renewal. Blackwell Sci. Publ., Oxford.

Waite, R.D. and N.J. Freeman. 1977. Mathematics of Environmental Processes. D.C. Heath & Co., Lexington, MA.

Wall, J.R. and C.F. Andrus. 1962. The inheritance and physiology of boron response in tomato. Am. J. Bot. 49:758-762.

Wallace, A., et al. 1972. Radioecology and Ecophysiology of Desert Plants at the Nevada Test Site. NTIS; U.S. Dept. of Commerce.

Wardlaw, I.F. and J.B. Passioura (Eds.). 1976. Transport and Transfer Processes in Plants. Academic Press, NY.

Weiss, M.G. 1943. Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics 28:253-268.

Wild, A., et al. 1974. Comparison of potassium uptake by four plant species grown in sand and in flowing solution culture. J. Appl. Ecol. 11:801-812.

Wild, H. 1970. Geobotanical anomalies in Rhodesia 3. The vegetation of nickel bearing soils. Kirkia 7 supplement:1-62.

Wilkins, D.A. 1960. The measurement and genetic analysis of lead tolerance in *Festuca ovina*. Rep. Scott. Pl. Breed. Stn. 1960:85.

Wilkins, D.A. 1957. A technique for the measurement of lead tolerance in plants. Nature, Lond., 180:37.

Williams, J. 1830. Faunula Grustensis, being an outline of the natural contents of the parish of Llanwst. Mem. Geol. Survey Sp. Rept. Min. Res. Gt. Brit. 23:59.

Wither, E.D. and R.R. Brooks. 1977. Hyperaccumulation of nickel by some plants of SE Asia. J. Geochem. Explor. 8:579-583.

Woolhouse, H.W. 1983. Toxicity and tolerance in the responses of plants to metals. in Encyclopedia of Plant Physiology Vol. 12C. Springer-Verlag, Berlin.

_____. 1970. Environment and enzyme evolution in plants. in Phytochemical Phylogeny. J.B. Harborne (Ed.) Academic Press Lond.

_____. 1968. Ecological Aspects of The Mineral Nutrition of Plants. Rorison, I.H., et al. (Eds.) Blackwell Sci. Publ., Oxford.

Woolhouse, H.W. and S. Walker. 1981. The physiological basis of copper toxicity and copper tolerance in higher plants. in Copper in Soils and Plants. Loneragan, J.F., et al. (Eds)., Academic Press, Sydney.

Wright, M.J. (Ed.). 1976. Plant Adaptation to Mineral Stress in Problem Soils. Cornell Univ. Press, Ithaca, NY.

Wu, L. and J. Antonovics. 1976. Experimental ecological genetics. in Plantago II. lead tolerance in Plantago lanceolata and *Cynodon dactylon*. Ecology 57:205-208.

APPENDICES

APPENDIX 1

Analysis of Variance Table - Experiment I

(3 replications)

FACTORS	RACE	ORGAN	METAL	LOADING RATE	TIME
LEVELS	AGRICULTURAL TAILINGS	ROOT SHOOT	Cu Cd	low med high	0 weeks 3 " " 6 " " 9 " "

MAIN EFFECTS	df	S.S.	M.S.	F-value	SIGNIFICANCE
RACE	1	20403.3	20403.3	75.6	.001
ORGAN	1	23687.2	23687.2	87.7	.001
METAL	1	29074.2	29074.2	107.7	.001
RATE	2	152137.0	76068.5	281.8	.001
TIME	3	67891.2	22630.4	83.8	.001
2-WAY INTERACTIONS					
RACE X ORGAN	1	982.4	982.4	3.64	
RACE X METAL	1	4102.0	4102.0	15.19	
RACE X RATE	2	13893.1	6946.5	25.73	
RACE X TIME	3	7553.4	2517.8	9.33	
ORGAN X METAL	1	8272.4	8272.4	30.64	
ORGAN X RATE	2	15269.3	7634.6	28.28	
ORGAN X TIME	3	13805.7	4601.9	17.04	
METAL X RATE	2	7893.6	3946.8	14.6	
METAL X TIME	3	3531.8	1177.2	4.36	
RATE X TIME	6	60158.7	10026.4	37.13	
RESIDUAL	255	68845.3	269.98		
TOTAL	287	497500.6	1733.4		

APPENDIX 2

ANOVA TABLE - EXPERIMENT II

SOURCE	df	S.S.	M.S.	F
BIOCHEMICAL FRACTIONS	2	147793	73896	24.01
ERROR	117	360148	3078	
TOTAL	119	507941		

$F_{.005}(2,117) = 5.54 < 24.01$

μ_1 = mean of Cd concentration in "CYTOSOL"

μ_2 = mean of Cd concentration in "WALL"

μ_3 = mean of Cd concentration in "MEMBRANE"

H_0: $\mu_1 = \mu_2 = \mu_3$

H_A: $\mu_1 \neq \mu_2 \neq \mu_3$

reject H_0

LEAST SIGNIFICANT DIFFERENCE (LSD)

$$LSD = t_\alpha \frac{2(\text{M.S. ERROR})}{r} = 2.871 \frac{2(3078)}{40} = 35.48$$

$\alpha = 0.005$

BIOCHEMICAL FRACTION	$\bar{x}$ (μg Cd g^{-1})
"MEMBRANE"	14.7 a
"CYTOSOL"	32.8 a
"WALL"	96.6 b

means followed by a different letter are significantly different at p = 0.005