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Title: Effect and Uptake of Cadmium and Lead Mixtures on Selected Vegetables: Environmental and Public Health Implications.

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Heavy metal pollution in the environment has toxicological importance, because heavy metals have been linked to food chain contamination which has resulted in acute and chronic toxic effects on humans.

Plants such as lettuce and radish have been shown to respond to heavy metals in soils and are valuable in assessing the toxicity and uptake of heavy metals in soils. These toxicity and uptake studies may also determine potential human exposure through a diet including these food crops. This study measured the effect and uptake of cadmium and lead mixtures in soil with lettuce (*Lactuca sativa* L., cv. buttercrunch), and radish (*Raphanus sativus* L., cv. cherry belle). Cadmium as $CdCl_2$, and Lead as $Pb(NO_3)_2$ were added to a Willamette Valley soil as mixtures ranging from

100 mg to 1000 mg cadmium and lead kg^{-1} soil. Untreated soil served as the experimental control for the study. Each test pot contained twenty pregraded seeds, and was monitored over a 30-day period (the complete growth cycles for lettuce and radish).

The study was conducted in three parts. First, germinated seeds were counted after five days, and percent germination values were calculated for each seed species for the entire test range. Second, plants were harvested after thirty days and total biomass measurements were taken. Third, chemical analysis of dried plant tissue samples were performed using the ICP analysis technique to measure cadmium and lead uptake.

Increased concentration of cadmium and lead mixtures in the soil resulted in reduced germination for both lettuce and radish. Statistically significant differences in toxicity values were observed when lettuce and radish were exposed to soil mixtures of cadmium and lead ($p \leq .0001$). For example, lettuce was more sensitive to the mixture (EC_{50} = 362.57 mg kg^{-1} cadmium at 400 mg kg^{-1} lead), than radish (EC_{50} = 480 mg kg^{-1} cadmium at 400 mg kg^{-1} lead).

Biomass was significantly reduced in both lettuce and radish in cadmium and lead mixtures in the soil ($p \leq .0001$). Additionally, the mean biomass values for both lettuce and radish indicated no interaction between cadmium and lead, even with increased levels of the two metals in the soil (lettuce, $p = .17$, $R^2 = 0.73$; radish, $p = .87$, $R^2 = 0.83$). The mean biomass for lettuce was lower than the mean biomass for radish. For example, the mean biomass for lettuce was 2.3 g and the mean biomass for radish was 17.5 g when treated with 200 mg kg^{-1} cadmium and 1000 mg kg^{-1} lead.

Analyses of chemical data from plant tissue indicated that cadmium uptake by lettuce was statistically significant ($p = .005$), but lead uptake by lettuce was not statistically significant ($p \geq .05$). The mean uptake of cadmium and lead by lettuce was 470.1 μg g^{-1} and 35.0 μg g^{-1}, respectively from soil mixtures containing 200 mg kg^{-1} cadmium and 1000 mg kg^{-1} lead. Cadmium and Lead levels in lettuce were highest at 200 mg kg^{-1} cadmium and 800 mg kg^{-1} lead whereas, in radish, cadmium levels were highest at 400 mg kg^{-1} cadmium and 1000 mg kg^{-1} lead. Lead levels were highest at 100 mg kg^{-1} cadmium and 1000 mg kg^{-1} lead in radish.

Cadmium and lead uptake by both lettuce and radish were relatively high, suggesting these metals be considered a potential source for human exposure through diet.

Effect and Uptake of Cadmium and Lead Mixtures on Selected Vegetables: Environmental and Public Health Implications

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Chapter 1

INTRODUCTION

The term 'heavy metal' is commonly found in the literature of environmental pollution (Martin and Coughtrey, 1982), and has various definitions. In this study, heavy metals were defined as the group of metals in the periodic table with densities greater than 5 g cm^{-3} (Passow et al., 1961). This definition is based on the physical and chemical properties of the elements in this group.

There are three major sources of heavy metals in most terrestrial ecosystems: the natural underlying bedrock materials, the atmosphere (human-related) and the biosphere (Friedland, 1989). Some of the studies demonstrating the presence of heavy metals in the ecosystem include those of Osibanjo et al. (1980), Kapu et al. (1991), Ho et al. (1988), Andrews et al. (1989), Yassoglou et al. (1987), Hussain et al. (1990), and Lagerwerff and Specht (1970).

Heavy metal pollution in the environment has toxicological importance, because heavy metals have been linked to food chain contamination which has resulted in acute and chronic toxic effects on humans (Ryan, 1982).

There is increasing concern about the health effects from ingestion of cadmium and lead. For example, a wide range of metabolic disorders and neuro-psychological deficits have been associated with environmental exposures to low levels of lead in children (NAS 1980; EPA 1986; Nriagu 1988). Additionally, chronic exposure to cadmium has been associated with kidney failure (Ryan et al., 1982) and bone disease (Kido et al., 1991). This information has prompted investigations about the behavior of these pollutants within the ecosystem (Andrew et al., 1989; Chaney, 1973).

Uptake of heavy metals by vegetable crops have been well documented (Chaney, 1973; Chaney et al., 1975; Chambers and Sidle, 1991; Granato et al., 1991). Although it is known that crops will take up heavy metals, Roberts and Johnson (1978) reported that the ecological significance of heavy metal pollution depends not only on the metals' abundance, but upon the toxicity, mobility and bioaccumulation of these metals.

Typically, heavy metals enter the food chain through the soil, because the soil serves as a "sink" for numerous contaminants in the environment. The ability of the soil to retain metals depend on several factors; pH, cation exchange capacity (CEC), organic matter content, oxides and carbonates (Yassoglou et al., 1987). For example, numerous studies have demonstrated that soil pH has a significant effect on heavy metal uptake (Chaney, 1973; Chaney et al., 1975), because it influences metal solubility in the soil and subsequently the concentration of the metal in the plants.

In the United States, contamination of garden soils and plants by cadmium and lead is well documented. Other primary sources of cadmium and lead include deteriorating paint from older homes, automobile emissions, and mining and smelting of metalliferous ores (Boon and Soltanpour, 1992; Chambers et al., 1991; Alloway et al., 1990).

The use of municipal sewage sludge to increase crop yield is a widespread practice (Schauer et al., 1980), which also contributes to cadmium and lead contamination of plants. Furthermore, the contaminant burden of a crop is usually a combination of (a) uptake via the root system, (b) direct foliar uptake and translocation within the plant, and (c) surface deposition of particulate matter (Jones, 1991).

Numerous investigations about heavy metal accumulation in soils and plant tissues from sewage sludge have been conducted. Most of these investigations, however, were conducted with agronomic crops. Only a few studies dealt exclusively with heavy metal uptake in vegetables (Schauer et al., 1980).

The public health concern about environmental pollution and the potential contamination of the food chain by heavy metals makes it imperative that new methods for the evaluation of toxicity be developed. The purpose of this study was threefold: (1) to determine the effect of the mixture of cadmium and lead on germination and biomass in lettuce and radish (2) to analyze the uptake of cadmium and lead by lettuce and radish (3) discuss the public health implications related to the uptake of cadmium and lead by lettuce and radish especially to humans, and the potential health risks associated with food chain contamination by cadmium and lead. The Null hypotheses for this study were as follows: (1) there are no significant differences in germination slopes between the controls and the various mixtures of cadmium and lead in the soil (2) there are no significant differences in biomass production rates between the controls and the various mixtures of cadmium and lead in the soil.(3) there are no significant differences in mean uptake levels of cadmium and lead by lettuce and radish at the various concentrations of cadmium and lead mixtures in the soil. (4) there are no significant interactions between cadmium and lead in the soil in germination, biomass production and uptake.

Lettuce and radish have been used in this study because they are among the two groups of (leafy and root) vegetable crops consumed by humans. Also, earlier studies have reported that lettuce and radish bioaccumulate cadmium and lead from heavy metal polluted soils (Schauer et al., 1980; Boon and Soltanpour, 1992).

Chapter 2

LITERATURE REVIEW

The topics in this Chapter will be discussed in this order: cycling of heavy metals in soils and ecosystems, toxicity of heavy metals, uptake of heavy metals, risk assessment and health risks associated with food chain contamination.

Cycling of Heavy Metals in Soils and Ecosystems

The cycling of heavy metals in soils and ecosystems has received a great deal of attention in recent decades due to concerns about the toxicity of these metals to humans. In a natural, undisturbed ecosystem, the primary sources of heavy metals are in either the underlying bedrock (Adriano, 1986; Peterson, 1978), or in surface material transported via the atmosphere from one location to another (Davidson et al., 1985). Weathering of bedrock in the terrestrial environment is a naturally occurring process by which heavy metals from the bedrock are incorporated into newly formed soil. With the exception of areas with ore deposits and unusually high concentrations of metals, heavy metal levels from the weathering of bedrock are quite low (Friedland, 1989). Heavy metals are emitted as particulates and gases from volcanoes. In addition, forest fires contribute to natural input to soils and ecosystems (Salomons, 1984; Verkliej, 1986).

Deposition of heavy metals in the ecosystem occurs primarily either as precipitation or dry deposition. In some ecosystems, particularly at high elevations, cloudwater may be a significant input (Lovett et al., 1982; Weathers et al., 1988). The importance of distinguishing between the two phases is that the amount of moisture available influences the relative solubility of metals. In general, metals are more apt to be soluble in wet than in dry deposition (Gatz et al., 1984).

In addition, the relative contributions of other nonhuman and human sources of metal pollution in the environment have been documented. For example, data are available for the deposition of atmospheric mercury in specific geographic areas (Lindberg, 1987; Pacyna, 1987), and to a lesser extent for the entire globe (Galloway et al, 1982), but these are contributions from global estimates of mercury cycling (Lantzy et al., 1979). In some global estimates, the anthropogenic to natural ratio for a number of heavy metals, such as, mercury is quite high. However, when natural vapor emissions are included, the anthropogenic to natural ratio for mercury is considerably lower (Lindberg, 1987; Pacyna, 1987; Galloway et al, 1982).

The biosphere is the second major source of heavy metals in soils and the ecosystem. The contributions from this source have been reported to occur in several ways; input from above-ground biomass, input from roots and other below-ground biomass; and leaching and washoff from leaf surfaces (Parker, 1983). The contributions from these sources are not necessarily equal. For example, the amounts of lead, cadmium, and nickel found in vegetation in forests in the United States and Europe are lower than the amounts of the same metals in soil (Smith et al., 1981; Friedland et al., 1985; Turner et al., 1985; Heinrichs et al., 1980). An exception to this is that, the levels of most metals are quite high in the vegetation near smelters that emit heavy metals in the combustion process (Jackson et al., 1977; Buchauer, 1973).

Human related activities is the third major source of heavy metal in soils and the environment. Lead is the primary heavy metal released into the environment as a result of the combustion of fossil fuels, and has perhaps the highest anthropogenic to natural emission ratios (Friedland, 1989). Automobile emissions and smelting are the two major sources of lead in the atmosphere (Murozumi et al., 1969). Lead has also been known to occur in agricultural soils, where the lead content is a function of cumulative inputs from atmospheric deposition, local geochemistry and soil type (Jones, 1991). Chamberlain (1983), has estimated that an elevation in the level of lead found in selective soils in England may be attributed to lead emission from fuel additives which has been occurring since 1946. In other words, it may be possible to speculate that all soils in industrialized nations of the world are contaminated with certain heavy metals above their pre-industrial levels (Jones, 1991).

Application of sewage sludge to agricultural soils is another important human-related source of heavy metals released into the environment. Sewage sludge is the primary source of cadmium contamination in soil, and represents a valuable source of plant nutrients (Chaney, 1988). Also, the application of domestic livestock manure is one significant means of introducing heavy metals such as arsenic, zinc and copper into the soil (Adriano, 1986).

Crop yields have increased dramatically in the latter part of this century because of the use of fertilizers and other chemicals to control plant pathogens. Heavy metals can also enter the soil and ecosystem from the application of pesticides and fertilizers to agricultural lands and forests. Pesticides used in protecting plants and trees from diseases contain some amounts of heavy metals such as copper, mercury, arsenic and lead (Lepp, 1981). Soil contamination from pesticides has been of particular concern in fruit-growing areas, where sprays containing chemicals such as cadmium and lead, calcium arsenate or copper sulphate, have been used

(Lepp, 1981). Also, the use of metal-contaminated sewage sludge, solid wastes or fertilizers as soil amendments may cause significant contamination of agricultural soils and crops under some conditions. Rock phosphates used in commercial fertilizers production often contain cadmium, which may be carried through to the fertilizers. For example, rock phosphates from the Pacific Island of Nauru contain cadmium, and may be carried through to the superphosphates which are used in fertilizer production (Lepp, 1981). Fortunately, modern fertilizer production has resulted in products which contain small quantities of impurities. Trace metals can still be detected in fertilizer products today, due to low application rates, do not seem to present hazard to humans and animals (Lepp, 1981).

Industrial activities have also contributed to heavy metal contamination of soil. For example, some agricultural soils in Britain contain about 0.3 mg/kg cadmium, and 50% or more of this burden, which may have anthropogenic origins (Jones, 1991). In addition, heavy metal contamination of sediments from mining wastes also contribute to soil pollution. For example, bottom sediments from the Milltown Reservoir, Montana, have been reported to contain high levels of arsenic, copper, zinc, cadmium and lead (Woessner and Moore, 1984). The application of dredged sediment on garden soils resulted in heavy metal contamination of vegetable crops (ETI, 1991).

Toxicity of Cadmium and Lead to Plants

Heavy metals occur in soils as inorganic compounds or as compounds bound to organic matter, clays or hydrous oxides of iron, manganese and aluminum (Alloway, 1968; Ellis et al., 1972; Hodgson, 1963; Jenne, 1968; Keeney et al., 1977; and Lindsay, 1972). Heavy metals can be toxic to plants at some level of solubility, but only a few have been observed to cause toxicity to plants in the soil. Precipitation and sorption of most metals by soils reduces the availability of these metals to plants. Zinc, copper and nickel toxicities have occurred frequently because these metals remain in equilibrium for extended period rather than forming crystalline insoluble compounds, thus are readily available to plants (Foy et al., 1978).

Many researchers have studied the toxicity of lead to plants after adding $PbCl_2$ to soil (Hassett et al., 1976; Hahne et al., 1973; Koeppe et al., 1975; Miller et al., 1975; Miller et al., 1975). The work of Hahne et al (1973) reported that when $PbCl_2$ is added to soil, the lead forms a soluble chloride complex which influences soil lead concentration and alters the distribution of the chemical species of lead in the soil. In short-term experiments, this altered state should be expected to increase lead toxicity to plants (Foy et al., 1978).

The effects of lead on other biological processes have been noted by various authors. For example, Hampp et al. (1973) reported on the effects of lead on enzymes of the reductive penthose phosphate pathway. Other authors have reported on the effects of lead on transpiration, nodulation, gas exchange, photosynthesis and respiration (Bazzaz et al. 1974; Bazzaz et al. 1974; Bittell et al. 1974; Carlson et al. 1977; Huang et al. 1974). Although these researchers found that lead affects enzymes of the reductive penthose phosphate pathway, transpiration, nodulation, gas exchange, photosynthesis and respiration, it is not certain if the effects of lead on these processes will be observed under environmental and physiological conditions (Foy et al., 1978).

Cadmium toxicity to plants is also influenced by a number factors. The availability of Cadmium is influenced by soil pH, Eh (electron availability), presence of chelators, and chloride ion (Garcia-Miragaya et al., 1976; Hahne et al., 1973; Street et al., 1977; Takijima et al., 1973). Cadmium is often found in combination with zinc and lead in naturally occurring ore deposits (Simon, 1977). Heavy metal toxicity to plants may be due primarily to competition between heavy metals and other essential elements in the soil. For example, Schmid et al. (1965), reported that copper strongly inhibited the absorption of zinc whereas manganese had no effect on zinc absorption. Phytotoxicity which means toxicity to plants, occurs because metals interfere with metabolic processes in the plant. Apparently, cadmium is toxic to plants because cadmium interferes with zinc in a zinc-dependent metabolic process (Falchuk et al., 1975; Rorison, 1965).

When plants assimilate phytotoxic amounts of cadmium and lead, growth is inhibited and biomass production decreases (Lepp, 1981). Stunted growth, leaf epinasty and chlorosis are visible symptoms of strong phytotoxicity. These symptoms may be less evident when soil pollution levels are low; however, reduction of plant quality and biomass production persists even at lower levels of toxicity (Van Assche et al., 1990).

Also, when plants accumulate toxic amounts of zinc, copper, cadmium or lead, the activity of some enzymes, such as, peroxidases, malic enzyme, isocitrate dehydrogenase, glutamic dehydrogenase, increase in leaves and or in roots (Mukherji and Das Gupta, 1972; Lee et al., 1976a,b; Weigel and Jager, 1980; Van Assche et al., 1988). This phenomenon, known as, 'enzyme induction', is strongly correlated to the inhibition of shoot growth and has been observed as a result of copper, zinc and cadmium phytotoxicity (Mukherji and Das Gupta, 1972; Van Assche et al., 1988).

Soil Factors Affecting Metal Toxicity to Plants

The primary soil factors that affect metal toxicity to plants are: pH, cation exchange capacity, organic matter and phosphorus content of the soil. These factors affect the mobility of heavy metals in the soil. Soil pH determines the availability of metals in the soil. Most metals are more available in the soil under acidic conditions (pH 4.2 to 6.6). In acidic soils , cadmium, nickel mercury and zinc are relatively soluble and are mobile. Lead, copper and selenium are moderately soluble at this pH range, but and are not as mobile as cadmium, nickel, mercury and zinc.

Page et al. (1978) reported that in neutral to alkaline soils (pH 6.7 to 7.8), cadmium was relatively soluble and mobile.However, under similar conditions, lead was classified as less soluble and less mobile. Andersson and Nilsson (1974) have found that the bioavailability of metals to plants from sludge treated soil decreases as the pH of the soil is artificially increased by adding liming. The bioavailability might have occurred because of the pH effect and/or an increase in calcium ions.

The cation exchange capacity (CEC) of the soil is the second important soil factor that influence the availability of heavy metals in soils. CEC is a measure of the net negative charge of the soil and it is expressed as milligram equivalent (meq) per 100 g soil. Although CEC has been reported to influence the absorption of heavy metals by plants, the effects of CEC are not as consistent and straightforward as those of soil pH (Page et al., 1981). Hagiri (1974) reported that the amount of cadmium absorbed by oat shoots (Avena sativa) decreased as the CEC of the soil was increased by adding organic matter. However, data published by Mahler et al. (1978) did not show any consistent pattern in cadmium absorption by lettuce (Lactuca sativa) and Swiss chard (Beta vulgaris var. cicla) in relation to soil CEC. Adjusting the CEC of a particular soil through the introduction of organic matter, may cause a decrease in the absorption of heavy metals by plants, because the introduced substance reacts with the metals.

Soil organic matter content (OM) is the third factor that has been reported to influence the absorption of metals in the soil. Organic matter content of a soil is a very important adsorptive medium for trace metals in all soils (Alloway and Jackson, 1991). King and Dunlop (1982) found that soil organic matter controlled as did pH the bioavailability of metals from sludge. They concluded, therefore that sludge could be applied to more acidic organic soils, because the organic matter component of the soil will control the availability of the metals, even though the pH may be lower than the specified pH of 6.5.

In addition to organic matter acting as a sink for metals in sludge-soil mixture, soluble low molecular weight organic molecules produced during the microbial decomposition of sludge in the soil form soluble complexes with the heavy metals. These complexes are more mobile, less readily absorbed and, possibly, more readily taken up by plants than are free metal ions (Yamada et al., 1984; Neal and Sposito, 1986).

Phosphorus content of the soil is the fourth factor that influences the availability of metals in the soil. High phosphate concentration in the soil has been associated with elimination of metal toxicity to plants (Spencer, 1966). Also, Smilde et al. (1974) studied additions of phosphorus to soil which contained phytotoxic levels of zinc. They discovered that the addition of the phosphorus reduced the toxicity of zinc to the plants in the soil.

Also, the phosphorus status of a soil has been shown to influence the accumulation of lead by plants. If the phosphorus level of a soil is adequate for normal plant growth, plants do not accumulate lead (Baumhardt et al., 1972). Phosphorus forms insoluble lead compounds in the soil that become unavailable to plant roots (Foy et al., 1978). Santillian-Medrano et al. (1975) reported that lead is strongly sorbed by soils to form insoluble crystalline compounds,which are also and hence are not available to the plant roots. An exception is in phosphorus deficient lead mine soils, where lead is more readily available to the plant roots (Johnson et al., 1977).

In addition to soil pH, CEC, organic matter, and phosphorus content of the soil, there are other factors affect metal availability in the soil. These factors include: soil metal concentration and soil absorptive strength, soil bulk density, soil moisture content, chelators, soil texture, and the concentration of other nutrients and cations in the soil (Foy et al., 1978).

Page et al.(1981) reported that absorption from soil increased with increased soil metal concentration, if the soils were similar in chemical, physical, biological and mineral properties. However, the increase in absorption rate was not linearly related to the increased concentration in the soil.

The availability of metals in soil also depends on soil adsorptive strength as well as plant effectors, such as root exudates for metal chelation or reduction. Plant effectors, such as secretions from plant cells alter the chemical nature of the metal, making it more available to the plants. Several investigators have developed models for the chemical activity of metals in soil (Lindsay, 1972; Santillian-Medrano, 1975; Street et al., 1977; Hodgson, 1963; Hodgson, 1969).

Furthermore, plant root growth may cause changes in the local pH and bulk density, these changes affect metal convection and diffusion rates, which may in turn affect the movement of metals to the plant root (Foy et al., 1978).

Soil water content influences the absorptive capacity for toxic metals and other toxic chemicals, because biologic oxidation reduction reactions occur between these toxic chemicals and soil moisture (Page et al., 1978). Because saturated soils are deficient in oxygen, one chemical reactions that occur under these conditions form sulfides. The sulfides are quite insoluble, and results in their lower levels in the soil (Page et al., 1978).

Also, Foy et al. (1978) reported that crystalline inorganic compounds are formed from the addition of heavy metals to soil, and that these metals would be unavailable to plants. Metals in soil solution (free ions, ion pairs, or chelates) can move with the soil water (convection or mass-flow) as the plant absorbs water for transpiration. Hodgson (1969), Lindsay (1974) and Elgawharry et al. (1970) noted that chelating compounds of low molecular weight are important in both convection and diffusion of metals in the soil. Chelating compounds in the soil are formed during the decomposition of organic material released by growing roots. In addition, most soluble heavy metals such as copper, zinc and cadmium are chelated up to 99 percent in the soil (Geering, 1969; Hodgson et al., 1965; Hodgson et al., 1966; Street et al., 1977).

Soil texture influences cation exchange and therefore, metal absorption in soils. Soil texture refers to the percentage of sand , silt, and clay in the mineral fraction of the soil. Woolsen et al. (1971b) reported that clay loam soils adsorbed arsenic ions to a greater degree than did sandy loam soils. This means that arsenic toxicity would be more likely to occur in plants grown in sandy loam soil.

Heavy metals may interact with nutrients and other metals in both adsorption equilibria and in the diffusion and convection processes (Foy et al., 1978). For example, plants growing in nutrient deficient lead mine spoils and waste heaps can have extremely high foliar lead. The translocation of lead from the soil to the leaves reveal a complicated interaction among nutrients and plant metabolic processes (Johnson et al., 1977; Johnson and Proctor, 1977).

Uptake of Heavy Metals by Plants

Much concern has been raised about the interaction between plants and heavy metals in soils. Plant uptake of heavy metals is a function of interrelated processes which include availability of the elements in the soil, movement of the elements to the roots, absorption by the roots, and translocation in the plant (Ryan, 1976).

Metals must be availability in the soil before plant uptake can occur. Availability is determined by soil pH which influences the solubility of metals in the soil. For example, plant tissue cadmium concentration decreased as the pH of the soil increased (Page et al., 1981). Chaney et al. (1975) also reported that cadmium concentration in soybean (Glycine soja) leaves decreased as the pH of the soil increased from 5.3 to 7.0. Also, Chaney et al. (1975) reported that cadmium concentration of soybean leaves was reduced from 33 to 5 µg cadmium per gram (dry weight basis) when the pH of the soil was increased from 5.3 to 7.

The movement of the metals to the roots is second factor that affects their uptake by plants. Metals move in the soil by mass-flow and diffusion (Barber, 1984). The modification of the soil rhizosphere (soil zone of increased microbial growth and activity around the roots of a plant) by the roots results in changes in the availability of metals to the plant roots. These modifications may cause changes in the availability of metals by both mass flow and diffusion. The ability of plant roots to modify the soil rhizosphere contributes to changes in pH in the rooting zone, which affects the solubility of metals in the surrounding soil (Youssef et al., 1991).

Absorption of metals by plant roots is the third factor that affects heavy metals uptake by plants. Jarvis et al. (1976), demonstrated that there is a considerable difference between the ability of various plants to absorb cadmium from solution. Little (1974), also demonstrated that differences in uptake of cadmium, lead and zinc can be achieved in greenhouse conditions with contaminated soils. Hinesly et al. (1978) grew 20 corn (Zea maize) inbreds on a sewage sludge amended soil and reported that the concentration of cadmium in the leaves varied from 3 to 63 μg/g. Also, Petersson (1977) similarly reported that different varieties of wheat (Triticum spp) and barley (Hordeum vulgare) differed in their cadmium absorption characteristics.

Translocation of heavy metals from the soil through root to above plant parts is the fourth factor that affects heavy metal uptake by plants. For example, cadmium appears to be absorbed passively and translocated freely in the soil (Cutler et al., 1974; Jarvis et al., 1976). This means that when compared to other metals such as lead, cadmium is not tied up as much in the soil. Also, pronounced interactions have been reported to occur between zinc and cadmium, and between calcium and cadmium, in processes involving cadmium uptake and translocation (John et al., 1976; Lagerwerff et al., 1972).

Methods of Heavy Metals Uptake by Plants

Plant uptake transfers heavy metals such as cadmium and lead into the food chain (Mahaffey et al., 1975; Vallee et al., 1972). However, plants will not grow under toxic conditions and therefore, are not available for consumption for animals and humans. Cadmium and lead are exceptions, because they are transferred into plants consumed by humans and have been found to be a health hazard to both humans and animals (Friberg et al., 1971; Ikeda et al., 1989).

Three mechanisms of uptake of heavy metals by plants that have been proposed are: Passive, facilitated and active uptake (Phipp, 1981). Passive uptake relies heavily upon concentration differences which are based on diffusion processes; they require no specific carriers. The capture of metals by roots and leaves is preceded by diffusion of the metal ions through the bulk phase of solution and membrane interface. Also, passive uptake is often subject to severe competition between ions of the same charge, and this competition is often used in desorption processes. For example, this method has been used in the desorption of copper by lead (Harrison et al., 1979).

Facilitated uptake requires metabolic activity that is not directly linked to any specific energy process. Typically, facilitated uptake is brought about by the secretions from the cells. These secretions alter the chemical structure of the metal, thus giving it a more favorable form for uptake (Phipps, 1981).

Active uptake usually shows the highest degree of specificity. It is controlled by metabolic inhibitors such as altered temperature that affect metabolic activity. Active uptake has sometimes been associated with the ability of plants to accumulate metals. This means that the plant may continue to take up the metal, even when the internal concentration exceeds the available concentration. In such cases, the process requires both high affinity and high specificity. As a result, saturation is often easily reached (Phipps, 1981).

Selective Uptake of Heavy Metals by Plants

Plants and animals show a remarkable degree of selectivity toward metal uptake. The three important factors that influence how plants selectively choose metals for uptake are: size (ionic radii), charge, and the redox behavior of the metals involved.

If a metal ion has a choice of sites onto which it may bind, binding of the metal will be controlled by the appropriate free energy charges. Several studies, including those of Andersson (1977c) suggested that a relationship existed between soil forming processes, such as weathering and disintegration of the underlying bedrock materials and the separation of metals in the soil. They found that these soil forming processes were responsible for the separation of metals between various soil components, due to the differences in ionic radius of the various metal ions. For example, divalent metal ions, such as Mn^{+2}, Zn^{+2}, and Cr^{+2}, show a close relationship between their ionic radii and their correlation coefficients in clay soils. Cadmium and lead do not fit the relationship because they have coordination numbers greater than 6 (Martin and Coughtrey, 1982). The coordination number of a metal refers to the number of donor atoms attached to the metal (Brown and LeMay, 1977).

Selectivity by charge is the second factor that influences how plants select metals for uptake. It might be expected that divalent metal ions (M^{+2}) would always displace a monovalent metal ion (M^{+}), if both ions are bound to the same site (Phipp, 1981). This does not always occur, and in some cases monovalent ions are preferred to divalent species. Diamond and Wright (1969) reported that the most critical factor that governs selectivity is the relative spacing of the binding sites.

Selectivity by redox potential is the third factor that influences how plants select metals for uptake. The majority of heavy metals have different oxidation states and as such, these different oxidation states may be the most important effect controlling biological counteractions (Phipps, 1981). For example, lead (IV) and lead(II) are both readily available oxidation states for the metal under biological conditions, but their chemistry are substantially different.

Soil Factors that Affect Heavy Metal Uptake by Plants

One of the main difficulties encountered when looking for long-term changes in soils is that soils vary in theirchemical and physical properties. The complexity of soils make the task of identifying the fundamental processes that determine contaminant behavior in a particular soil system difficult. For example, different chemicals may be subject to different degrees of leaching, degradation, volatilization and plant uptake. All these processes will influence the chemical's long-term persistence in the soil (Jones, 1991).

Variables that influence metal toxicity to plants are also involved in the subsequent uptake of these metals (Chaney, 1973). These variables include soil pH, metal concentration in the soil, cation exchange capacity of soil, and soil organic matter content. For example, the transfer of cadmium from soil into the edible components of food crops is affected by soil pH (Alloway et al., 1990), Andersson and Nilsson (1974), also have outlined that the application of lime to a soil raises the pH and represents a means by which the transfer of cadmium from soil to plant may be reduced.

Also, the work of John et al. (1972) demonstrated that liming of soil decreased lead uptake by lettuce, however the effect did not occur to the same extent in oats, nor was it clear whether or not it was the effect of lead precipitation in soil or a restriction of lead translocation to shoots (or a combination of both).

A large percentage of the studies that compare cadmium accumulation in plants to cadmium concentration in soil, have used soil amended with municipal sewage sludge. Field and greenhouse studies conducted by Page and Cheng (1978) and by De Vries and Tiller (1978) have demonstrated that plants grown in cadmium-enriched soils in containers in the greenhouse absorb more cadmium than the same plants grown on the same soil amended with identical amounts in the field. Chang et al.(1979) and Giordano et al.(1977), also have studied the relationship between amounts of cadmium added in soil and the corresponding cadmium concentration in variety of vegetables (including lettuce and radish).

Cation exchange capacity of soil has been shown to affect plant uptake of heavy metals. For example, a study by Miller et al. (1977), where different concentrations of lead and cadmium were incorporated into a sandy loam soil with relatively low cation-exchange capacity. The study indicated that there was an increase in the plant cadmium concentration attributed to the level of lead added to the soil. Also, the study indicated that the amount of cadmium added to the soil reduced the total lead uptake, and lead levels on the shoots.

Plant uptake of heavy metals from the soil is affected by the organic matter content of the soil, because the organic matter content of a soil provides adsorptive medium for trace metals in all soils (Alloway and Jackson, 1991). Organic matter in soil binds to insoluble metal complexes, reducing their availability to plants (Hodgson et al., 1966). Also, some studies have demonstrated that soil organic matter can substitute for pH in controlling the availability of metals from sludge (King and Dunlop, 1982).

Risk Assessment of Cadmium and Lead Contamination of Food Crops

Risk is defined as the expected frequency of undesirable effects arising from exposure to a pollutant (Lu, 1991). This section examines the potential risks associated with cadmium and lead contamination of food crops (vegetables).

Each year considerable amount of cadmium is deposited on agricultural soils by the application of phosphate fertilizers, and other chemicals (Ryan et al. 1982). Additionally, the occurrence of acid rain, in recent years has contributed to the increased availability of cadmium in the soil, and the resultant increase in cadmium levels in agricultural products (Piscator, 1985).

Lead additives in gasoline used in automobiles have also served to increase lead levels in the soil, air, water and crops located near major highways (Harrison et al. 1979; Chamberlin, 1983; Ho et al. 1988; Hussain et al. 1990; Onyari et al. 1991).

Another important source of cadmium and lead in the environment previously mentioned is due to atmospheric fallout from metal smelters (Pacyna, 1987). Majority of these smelters have been in operation for decades, and have resulted in substantial contamination of surrounding soils. Towns located near these smelters are subject to contamination from fallout containing cadmium and lead (Jennett et al. 1977).

The uptake process transfers heavy metals such as cadmium and lead from soils into the food chain (Mahaffey et al., 1975; Vallee et al., 1972). Information related to dietary intake of cadmium and lead are important in assessing risks to humans from these metals. In most cases, for example, the main source of exposure to cadmium is through a diet containing leafy vegetables such as spinach and lettuce. Leafy vegetables represent a potential source for high rate of intake by humans, because they have been shown to accumulate high levels of cadmium when grown in soils treated with municipal sludge (Ryan et al., 1982). World Health Organization established that the provisional weekly intake levels for cadmium and lead for adults are 430 µg per day and 60-70 µg per day respectively (WHO, 1972; WHO, 1985).

Because cadmium accumulates in the kidney, there is considerable interest in monitoring the general population's dietary intake of cadmium (Ryan et al., 1982). For example, studies in Sweden have shown a slow but steady increase in cadmium content of vegetables over the years. Increase in body burden has been determined from historic autopsy study (Friberg et al., 1986a). In the general population, people with diets which include more than normal amounts of leafy vegetables, visceral meats, fish and shellfish may be at increased risk, because these food groups can contain rather higher levels of cadmium (Ryan et al., 1982). Also, studies conducted on animals revealed that age is a significant factor in cadmium metabolism, with younger animals showing increased intestinal absorption (Engstrom and Nordberg, 1979; Kello and Kostial; Kostial et al., 1978). Lactating women may also have increased absorption due to their negative calcium balance (Ryan et al., 1982). In humans, the lethal oral dose for cadmium resulting from ingestion of contaminated foods or fluid is about 20 to 130 mg/kg in a 70-kg adult (CEC, 1978).

Dietary intake of lead has decreased since the 1940s when estimates were 400 to 500 μg per day for U. S. populations to present levels of under 100 μg per day for adults (EPA, 1986). Mahaffey et al. (1982) reported that national estimates of human exposure to lead are based on blood lead levels collected from the National Health and Nutrition Examination Survey of 1976-80. The data estimated blood lead levels in the general U. S. population from age 6 months to 74 years. The data indicated that children aged 6 months to 5 years and adults of 25 to 54 years showed the highest blood lead levels. The mean blood lead level pattern was generally similar for males and females.

It appears that children are at most risk if lead is ingested through diet, because they are known to have more efficient absorption of ingested lead than adults. Casarett and Doull (1991) reported that some studies in the United States have found an average absorption rate of 41.5 percent and a net retention rate of 31.8 percent in infants on regular diets.

Health Risks Associated With Food Chain Contamination by Heavy Metals

Heavy metals are one of the oldest toxic substances known to humans. Evidence of the deleterious effects of lead on human health dates back to the ancient Roman and Greek civilizations (Gilfillan, 1965). Human contact with lead may have begun as far back as 2000 B.C. when abundant supplies were obtained from ores as by-products of smelting silver (Casarett and Doull, 1991). Today, the presence of lead in the storage vessels for water or wine, in pipes that carry water, and in cooking and eating utensils, provide increased potential for lead ingestion and poisoning (Schuhmacher et al., 1991).

Cadmium was first recognized in ores containing zinc carbonate in 1817 (Casarett and Doull, 1991). Although the majority of humans are exposed to cadmium through diet, cigarette smoking is an additional source of non-occupational exposure to this metal (Watanabe et al. 1987; Ikeda et al. 1989).

Cadmium Toxicity

Cadmium in virtually all chemical forms is toxic to human and other organisms (Sandstead et al., 1974). Cadmium is a nonessential, nonbeneficial element which cause acute and chronic illness in humans (James, 1976). Cadmium accumulates in the renal cortex, and may lead to renal dysfunction (Ryan et al., 1982). The acute effects of the ingestion of cadmium are nausea, vomiting and diarrhea. Chronic effects include the eventual disintegration of the bones, and damage to the liver, testes, immune system, kidneys and the cardiovascular system (CEC, 1978).

Humans consume cadmium in plants fertilized with cadmium containing municipal sludge (CAST, 1976). Cadmium is selectively concentrated in certain food crops, and in such as root and leafy vegetables (Ryan et al., 1982). Also, other food items like meat, fish and fruits contain about 1 to 50 μg per kilogram, grains contain 10 to 150 μg per kilogram, and the greatest concentrations are found in liver and kidney of animals (Casarett and Doull, 1991).

To date, there are no documented occurrences of cadmium toxicity in animals or humans attributed to direct consumption of vegetation grown on land treated with municipal sludge (Garrigan, 1977). However, the most serious case of cadmium poisoning known as Itai-Itai disease occurred in a small Japanese farming and fishing village (Kato, 1978).

In this situation, the victims consumed rice contaminated by irrigation water from a river that had previously received industrial cadmium waste.

Itai-Itai disease is characterized by osteomalacia and multiple renal tubular failure (Friberg et al., 1974). Osteomalacia is another disease related to cadmium toxicity, and it is a condition marked by softening of the bone with pain, tenderness, muscular weakness, anorexia and loss of weight due to lack of calcium and vitamin D (Friberg et al., 1974). Cadmium has been reported to depress the chemical conversion of vitamin D in humans and has resulted in a decrease in calcium absorption and mineralization of bone, which in turn may lead to osteomalacia (Casarett and Doull, 1991). Because of these observation, some researchers consider it likely that nutritional deficiency plays an important role in the etiology of the disease (Kato, 1978).

Because of it known toxicity, cadmium is stringently regulated in drinking water standards (Koren, 1991). Cadmium has a half-life (time required for the body to eliminate 50% of the amount absorbed) of 16 to 33 years (Koren, 1991). WHO guidelines for cadmium in drinking water is 0.005 mg per liter (WHO, 1984), and maximum safe level of cadmium intake is 0.026 mg per day (James, 1976). Also, the maximum contaminant level (maximum allowable level of contaminants in water) for cadmium in drinking water in the U. S. is 0.01 mg per liter (EPA, 1980; ATSDR, 1989).

Lead Toxicity

Lead is a toxic metal that tends to accumulate in the bones, liver, kidney, lungs, spleen and hair of humans and other animals. It has no beneficial nutritional effects. Lead intoxication most often result from eating lead-contaminated paint still present in older homes, and has been linked to irreversible brain damage in children. Chronic lead poisoning symptoms are: loss of appetite, weakness, lesions, neuromuscular damage, brain damage and circulatory damage (CDC, 1991).

Lead is found in large number of foods (Mahaffey et al., 1975). However, fruits and vegetables are the most important sources. The daily intake from water is usually about 10 µg and unlikely to be more than 20 µg. Adults absorb 5 to 15 percent of ingested lead and usually retain less than 5 percent of what is absorbed, absorption and retention rates are higher in children (Casarett and Doull, 1991). The total body burden for lead are found in the skeleton, kidney and in the central nervous system. The gross lifetime accumulation of lead is about 200 mg and over 500 mg from occupational exposure, with a half-life of about 20 years (Casarett and Doull, 1991). The daily lead intake is about 100 µg for adults in the United States (EPA,1986). The maximum contaminant level in drinking water is 0.05 mg/l (Casarett and Doull, 1991).

Numerous studies have documented the effects of low and high lead levels on humans. For example, Smith et al. (1983) conducted a large scale study of blood and tooth lead, behavior, intelligence and a variety of other developmental defects in a population of 4000 children aged six to seven years in three London boroughs. Initial results of the study did not show any statistically significant association between lead levels and IQ (academic performance) of the children. However, further analysis of the data by Pocock et al. (1985), revealed that the IQ of the group of children with average blood lead levels of 12 to 15 µg per deciliter was below average for the children in the control group.

Rabinowitz et al. (1988) conducted a study on soil lead-blood lead relationship on 249 infants aged 1 to 24 months in Boston, Massachusetts. Samples were collected by dusting furniture, living room floors and windowsills. Also indoor aerosol, paint, drinking water and soil (around the homes) samples were collected. The study found that there was a strong correlation between blood lead level and soil lead level. The correlation was stronger at ages 18 through 24 months than the first year.

Goldberg (1974) studied a population of British citizens exposed to elevated lead levels in the domestic drinking water resulting from storage in lead lined tanks and the use of lead pipes. The average level in the contaminated water was 943 µg per liter and some samples were as high as 2000 -

3000 μg per liter. The average blood lead level in the population was 28 μg per liter. Several people in the study population suffered from clinical lead toxicosis with symptoms that included acute abdominal pain, tremor, blood abnormalities, and hyperuremia (excess blood in urea).

Chapter 3

METHODS AND MATERIALS

Introduction

This study was conducted in the greenhouse at the EPA facility (ERL-Corvallis, OR). This study is a modification of the EPA test method used to assess the toxicity of soils from Superfund hazardous waste sites on plants (EPA, 1988). This method was selected because it enabled the researcher to investigate the effects of mixing these two metals in the soil, and also to determine if any interactions occurred between the two metals. Other studies have looked at the effect of zinc, copper, nickel and sulphur dioxide on the absorption of cadmium and lead in the soil by plants (Miller et al., 1977; Mitchell et al., 1981; Krause and Keiser, 1977), but none has been able to ascertain if these metals interact with each other in the soil, and pose a danger to food crops cultivated in contaminated soils.

Soil Characteristics

The soil used for the study was the Newberg silt loam common to Benton County of Oregon. The Newberg soil series consist of deep, somewhat excessively drained soils that formed in mixed sandy alluvium. These soils are found on recent flood plains along major rivers and streams of the Willamette valley. The soil is used for the cultivation of cereal grains, hay, grass seed, orchard and specialty crops. The soil is also used for recreation and wildlife habitat (Knezevich, 1975).

Soil samples were collected from a depth of about 0-15 cm. along the banks of the Willamette river (Willamette Park, Corvallis, OR). The soil was then screened through a 1/4 inch (6.35 mm) mesh stainless steel screen, and then stored in a plastic bag at room temperature until use. The screening procedure was necessary to remove oversized materials such as rocks, sticks and plant debris from the soil. This process also contributes to uniformity in particle size in the experimental soil. Soil moisture content was measured using the gravimetric method (Gardner, 1986) immediately after screening.

Chemical analysis of soil was performed by the Oregon State University Soil Testing Laboratory and the results are indicated in Table 1.

Table 1.

Chemical Analysis of Newberg Soil

pH	6.2
Cation Exchange Capacity (CEC)	29.7 meq/100 g **
Total Nitrogen (TN)	0.169 %
Organic Matter (OM)	4.31 %
Sand (2mm-50μ)	32.2 %
Silt (50-2μm)	50.2 %
Coarse Silt (50-20μm)	16.0 %
Fine+Medium Silt (20-2μm)	34.2 %
Clay (<2μm)	16.7 %
Moisture Content	25.8 %

** meq = milliequivalents

The amount of soil used per pot was determined by a mathematical calculation, which included dividing the total dry weight amount by the moisture fraction of the soil. Untreated soil served as the experimental control for the study. Soil samples were weighed into plastic ziploc bags, and each bag contained 100 grams of soil based on dry weight.

Preparation of Metal Solutions

Stock solution of cadmium was prepared by weighing 65.24 g. analytical grade of cadmium chloride (J. T. Baker, Phillipsburg, NJ), into a 1-liter volumetric flask; cadmium constitutes 61.3% of the molecular weight (183.3 g) of cadmium chloride. Equally, stock solution of lead was prepared by weighing 64.0 g. analytical grade of lead nitrate (Mallinckrodt, St Louis, MO), into a separate 1-liter volumetric flask; lead constitutes 62.6% of the molecular weight (331.2 g) of lead nitrate. These volumetric flasks were brought to 1-liter volumes with RO water. The final concentrations of cadmium and lead in each of these flasks were 40,000 parts per million (ppm).

The initial test range for individual metal concentrations was determined for both plant species by using a logarithmic scale (0, 1, 10, 100, 1000 mg kg^{-1} in soil). A definitive test was conducted with cadmium and lead mixtures using concentrations ranging from 100 to 1000 mg kg^{-1} in soil (see Table 2). Test soils were hydrated to 45% moisture on a dry weight basis by adding 20.5 ml. of solution to the soil. The solution consisted of a mixture of reverse osmosis (RO) water and calculated amounts in ml. of cadmium and lead from two separate 40 g per liter (40,000 ppm) stock solutions of cadmium and lead.

The concentrations of the metals used for the definitive test were prepared as follows (See Table 2): for example, the 100/100 mg kg^{-1} cadmium and lead treatment; 0.725 ml. from each of the stock solutions were combined with 19.05 ml. of RO water in a 100 ml. beaker and mixed thoroughly on a stir plate and then poured into the ziploc bag containing 100 grams of soil. Subsequent test concentrations were prepared similarly. The soils in the ziploc bag were hand-mixed to ensure uniform distribution of test solution. Test controls received 20.5 ml. of RO water treatment.

The mixed soils from each bag were placed in 4 inch plastic pots and twenty pregraded seeds were planted in each pot. The seeds were covered with 25 grams of 16 mesh silica sand. Six pots from the same treatment were placed in one 18 x 20 inches plastic plant tray, and were irrigated daily with approximately 250 ml. of nutrient solution (North Carolina State University phytotron nutrient solution, EPA, Corvallis, OR, 1987).

Table 2.

Dilution Profile for Test

	Concentrations of Lead (mg/kg)					
	0	100	200	400	800	1000
	100	100	100	100	100	100
Concentrations of	200	200	200	200	200	200
Cadmium	400	400	400	400	400	400
(mg/kg)	800	800	800	800	800	800
	1000	1000	1000	1000	1000	1000

Test Seed Screening and Grading

Seeds of lettuce (Lactuca sativa; cultivar butter crunch) and radish (Raphanus sativus; cultivar cherry belle) were obtained from a local seed company (Nichols Garden Nursery, Albany, Oregon). The lettuce seeds were screened through a series of wire mesh screens of sizes ranging from 1/6 x 1/28 to 1/6 x 1/34; these are fractions of an inch. The radish seeds were also screened through a series of wire mesh screens of sizes ranging from 6.5 to 8; these are 64ths of an inch. The seeds were visually graded after screening to remove trash, empty hulls and damaged seeds and stored in plastic bags in a refrigerator at 5 C, before testing (Porcella, 1983).

Endpoint Measurements

The primary endpoints measured in the study are germination, biomass and uptake in both lettuce and radish. Germination and biomass are critical in assessing the toxicity of the metal mixtures on the two plant species, whereas uptake assesses the potential contamination of the food chain by these metals.

After five days, effect on seeds were evaluated by the rate of germination in both seeds (lettuce and radish). The germinated seeds were defined as the shoots above the soil surface (EPA, 1988). Toxicity to plants were quantified by percent seed germination in each pot at each treatment level.

Uptake measurements were initiated by thinning down each test pot (test pots with more than five germinated plants) to five plants per pot; this minimized crowding. The remaining plants were monitored over a 30-day period for subacute physiological effects such as chlorosis, stunting and shoot and leaf deformities. After 30 days, plants were harvested, weighed for total biomass, washed with RO water and dried in an oven at 100 C for 24 hours.

Dried plant tissues were digested using the modified EPA method (EPA, revision # 2.1, 1983), which consisted of nitric acid digestion at 150 C, followed by perchloric acid digestion at 250 C. Soil samples were also digested using the EPA test methods for evaluating solid waste (EPA method 3050- Acid digestion of sediments, sludge and soils, 1986), which consisted of nitric acid digestion at 95 C, followed by hydrogen peroxide treatment. The digested plant tissues and soil samples were analyzed for total cadmium and lead contents using the ICP (Inductively Coupled Plasma) technique (McQuaker et al., 1979).

Statistical Analysis

Multiple regression analyses were performed using STATGRAPHICS™ (Statgraphics, Rockville, MD) on germination, biomass and uptake results. Tests of significance of regression slope estimates were performed (α=.05), to test the null hypotheses that there is no interaction between cadmium and lead in the soil in germination, biomass production and uptake. Also tests of significance of regression slope estimates were performed to test the null hypotheses that there are no significant differences in rates of germination and mean biomass production between the control groups and the groups with varying mixtures of cadmium and lead in the soil. Finally, tests of significance of regression slope estimates were performed to test the null hypotheses that there are no significant differences in the mean uptake of cadmium and lead by lettuce and radish at various concentrations of the metal mixtures.

Quality Assurance

The quality assurance and quality control (QA/QC) procedures for the test were in accordance with the EPA Site Assessment Program (ERL-Corvallis, Ecotoxicology Branch), guidelines (EPA, 1988). Prior to testing, seeds were graded and percent germination for each plant species was determined by germinating them in uncontaminated silica sand. The seed batch used for this study met the EPA QA/QC germination criteria; a minimum of 80 percent germination in each replicate.

Test temperature and humidity in the greenhouse were measured using a hygrothermograph (Belfort Instrument Co., Baltimore, MD), light intensity was measured using a light meter (LI-COR, Model LI-185B, Lincoln, Ne). Soil pH was measured using the ORION™ pH meter. All seeds used were not chemically pretreated, since fumigants used in seed treatment may bias test results. The uncontaminated soils were analyzed using the ICP technique (McQuaker et al., 1979), before the study to establish baseline levels of cadmium and lead (See Table 3).

Table 3.

Mean Concentrations of Cd and Pb in Newberg Soil

Cadmium	Lead
1.866 mg/kg	0.545 mg/kg

Chapter 4

RESULTS

The results will be presented as follows: Effect of cadmium and lead mixtures on germination, effect of cadmium and lead mixtures on plant biomass production and uptake of cadmium and lead by lettuce and radish.

Effect of Cadmium and Lead Mixtures on Plant Germination

Overall, results indicated that cadmium and lead mixtures had a gradient toxic effect on both lettuce and radish (See Figures 1-5). This means that increasing concentrations of mixtures of cadmium or lead in the soil, resulted in differences in rates of germination. The results also indicated that lettuce was slightly more sensitive to the mixture (EC_{50} = 362.57 mg kg^{-1} cadmium at 400 mg kg^{-1} lead), than radish (EC_{50} = 480 mg kg^{-1} cadmium at 400 mg kg^{-1} lead).

Statistical Interpretation of Germination Results

The statistical analysis indicated that the addition of cadmium alone caused significant differences in mean germination in lettuce in the soil ($p \leq .0001$) (See Appendix Table 1). Also lead alone in the soil caused significant differences in germination rate in lettuce ($p = .023$). Furthermore, statistical results indicated that increasing the concentration of cadmium in the mixture caused significant differences in germination for lettuce at fixed levels of lead in the soil ($p =.0001$). There were no significant differences in germination in lettuce when the concentration of lead was increased at various levels of cadmium in the soil ($p = .12$).

There were no significant differences in germination in lettuce attributed to the interaction of cadmium and lead in the mixture at any given concentration of the mixture in the soil ($p = 0.60$).

Lead alone in the soil did not result in any significant differences in mean germination rates in radish ($p = .54$), but cadmium alone caused significant differences in mean germination rates in radish ($p \leq .0001$) (See Appendix Table 1). Increasing the concentration of cadmium at fixed levels of lead in the soil caused significant differences in mean germination rates for radish ($p = .002$). There were no statistically significant differences in germination for radish when the concentration of lead was increased at fixed levels of cadmium in the soil ($p = .26$). Furthermore, significant differences in mean germination rates resulted in radish due to interaction between cadmium and lead at any given concentration of the mixture in the soil ($p = 0.073$). (See Appendix Table 1).

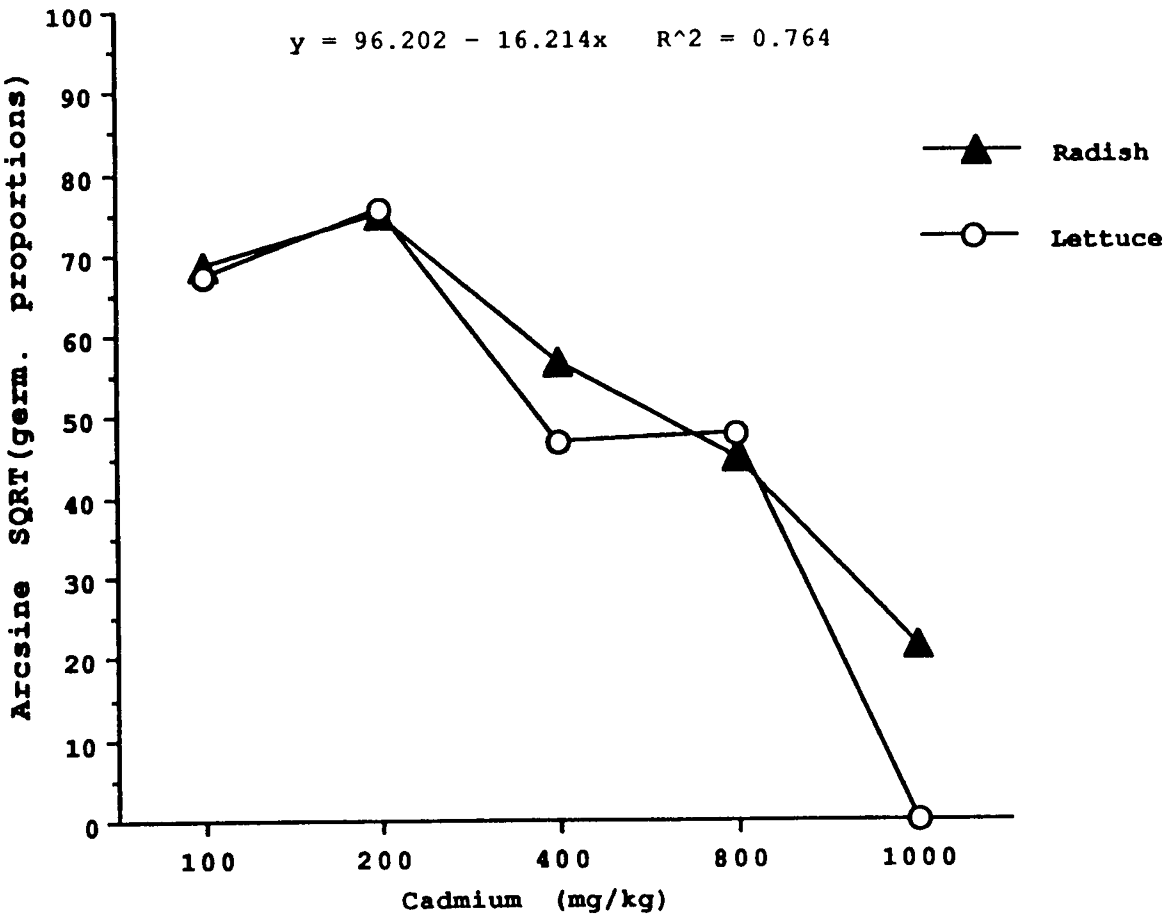

Figure 1. Germination rates for lettuce and radish at various concs of Cd when conc. of Pb = 100 mg/kg.

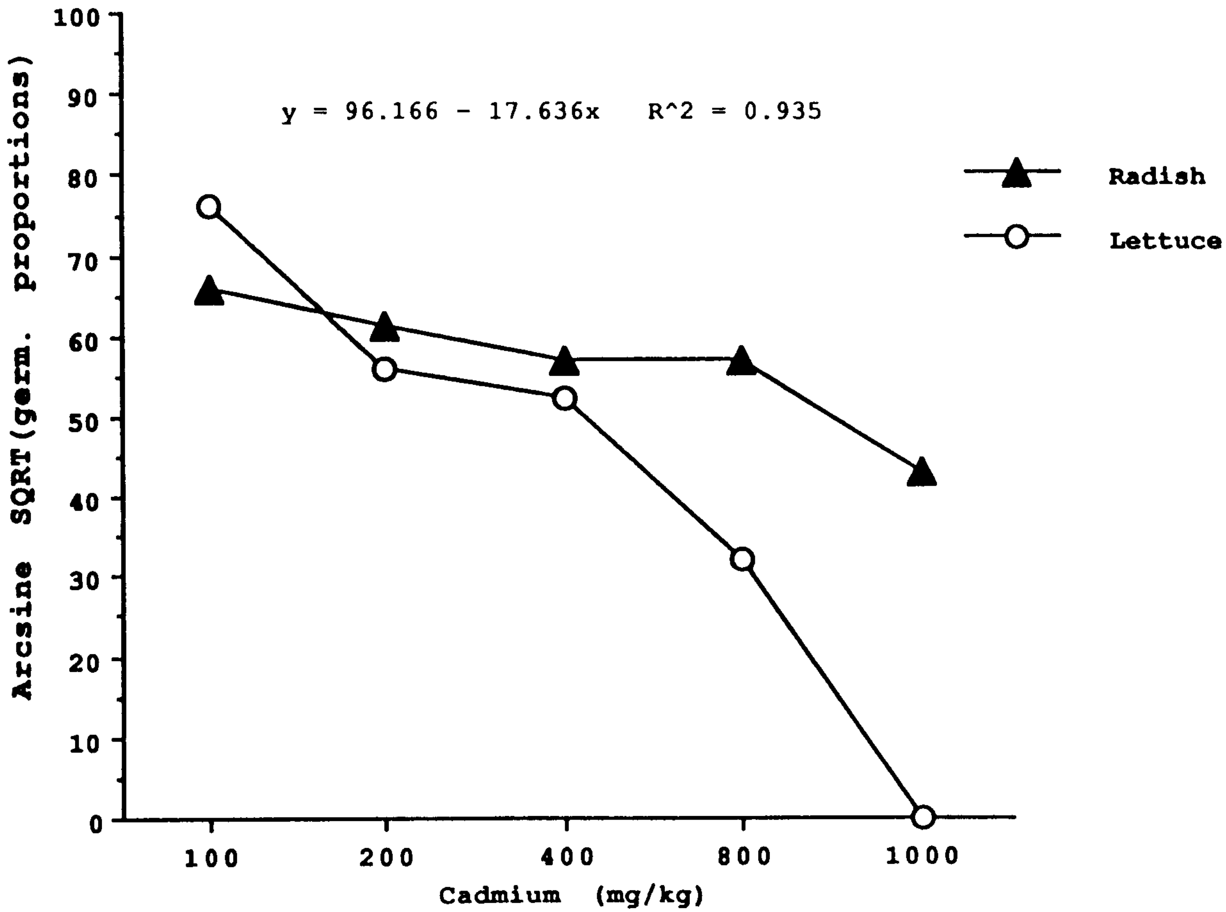

Figure 2. Germination rates for lettuce and radish at various concs. of Cd when conc. of Pb = 200 mg/kg.

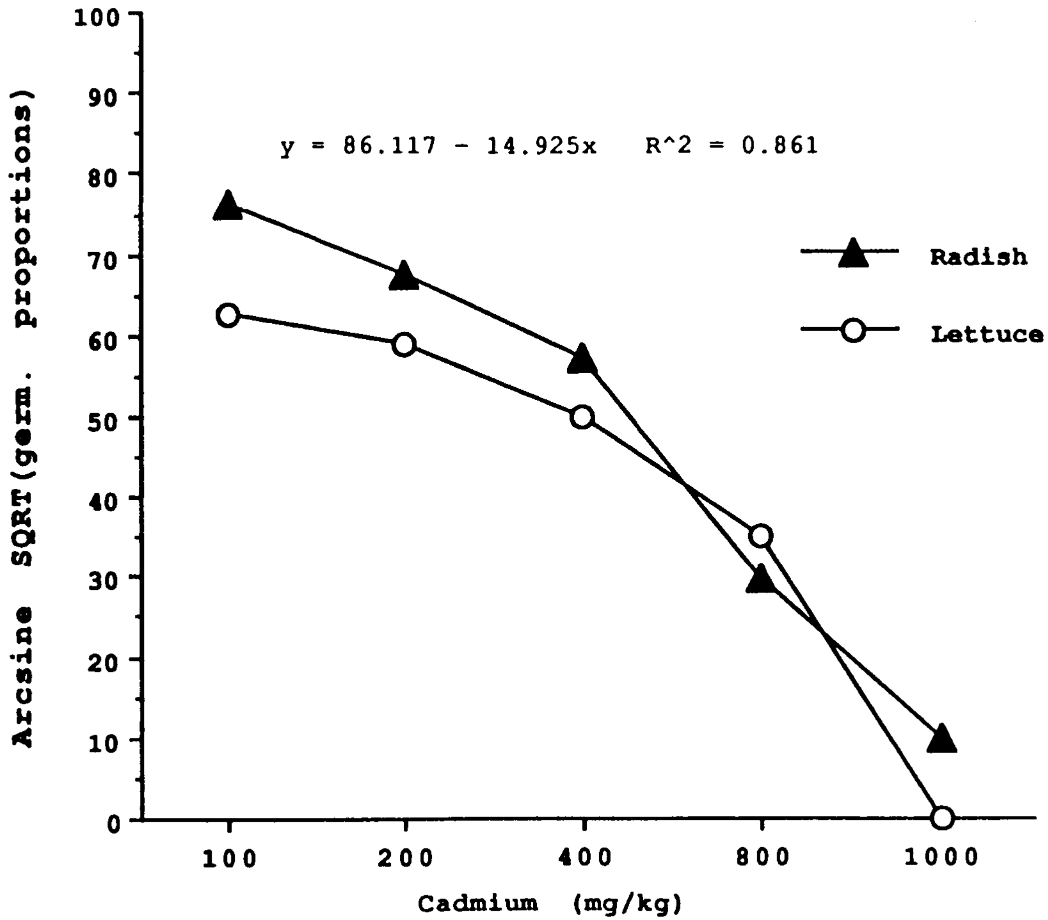

Figure 3. Germination rates for lettuce and radish at various concs of Cd when conc. of Pb = 400 mg/kg.

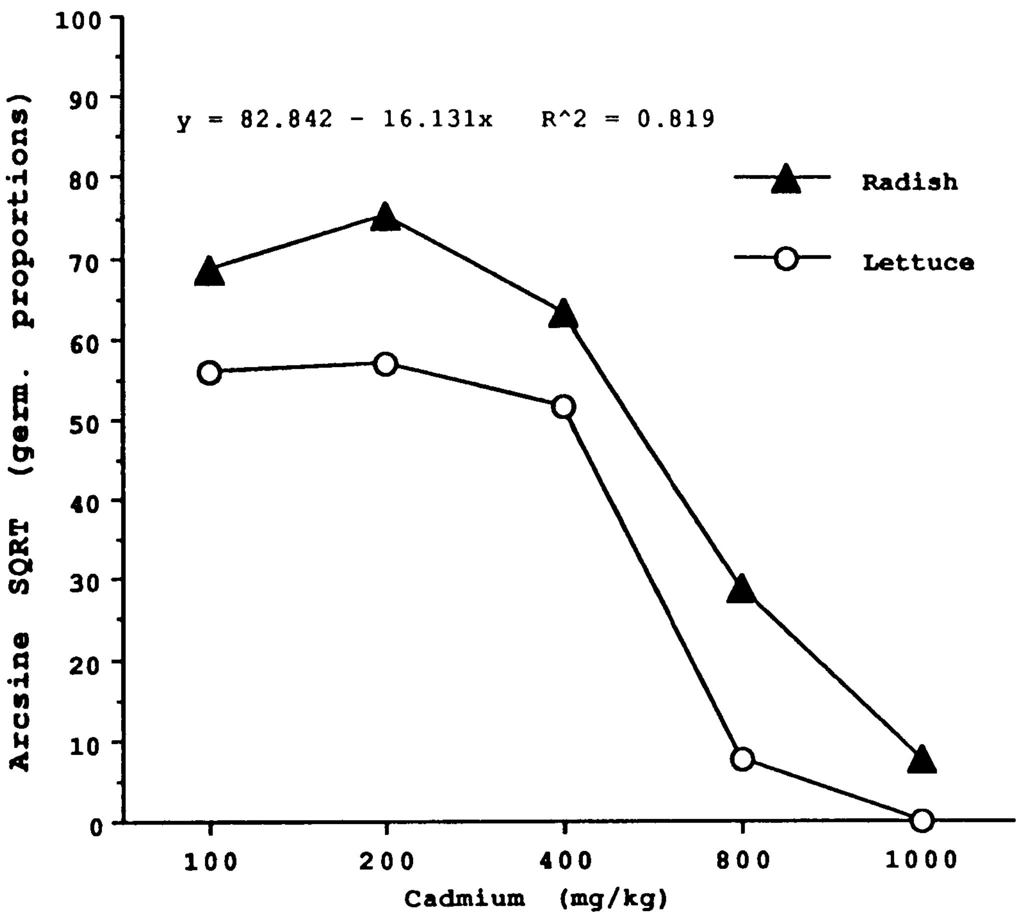

Figure 4. Germination rates for lettuce and radish at various concs of Cd when conc. of Pb = 800 mg/kg

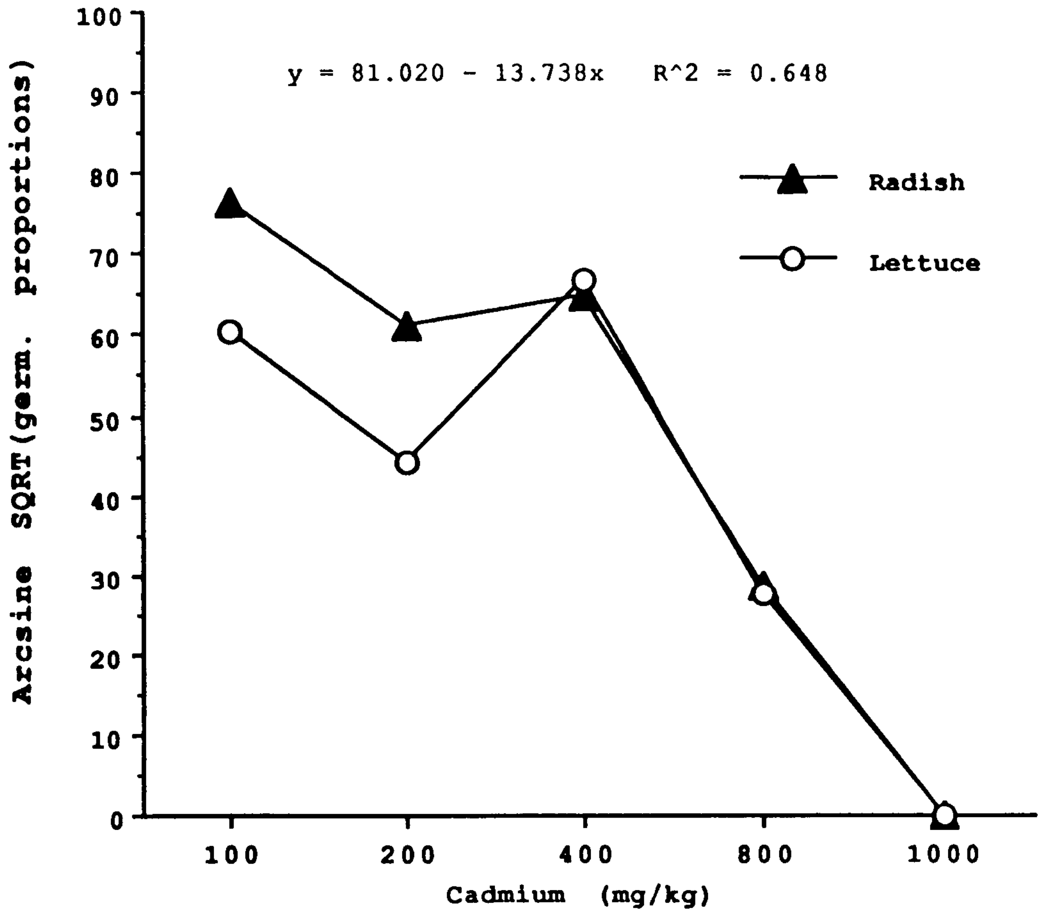

Figure 5. Germination rates for lettuce and radish at various concs of Cd when conc. of Pb = 1000 mg/kg.

Effect of Cadmium and Lead Mixtures on Plant Biomass

Biomass results indicated that the mean plant biomass production decreased in general in both lettuce and radish, as the concentration of cadmium and lead in the soil increased. Plant biomass production decreased gradually, but biomass declined sharply when the concentration of cadmium reached 400 mg kg^{-1} in the soil at various concentrations of lead. However, the mean biomass values for lettuce were significantly lower than the mean values biomass for radish (See Figures 6-10).

Statistical Interpretation of Biomass Results.

The statistical analysis indicated that cadmium alone caused significant reductions in mean biomass values in lettuce ($p \leq .0001$) (See Appendix Table 2). Also analysis indicated that there were no significant reductions in biomass in lettuce due to lead alone ($p =.07$). Furthermore, there was no statistical evidence of any interaction between the two metals on mean biomass reduction ($p = .17$) (See Appendix Table 2).

Increasing the concentration of cadmium in the soil caused significant reductions in mean biomass values in lettuce ($p \leq .0001$) and radish ($p \leq .0001$) (See Appendix

Table 2). However, increasing the concentration of lead in the soil did not cause any significant reductions in mean biomass values in lettuce ($p = 0.70$) and radish ($p = 0.69$) (See Appendix Table 2).

Furthermore, there were no significant reductions in mean biomass values attributed to interaction between cadmium and lead in the soil in lettuce ($p = 0.17$) and radish ($p = 0.87$) (See Appendix Table 2).

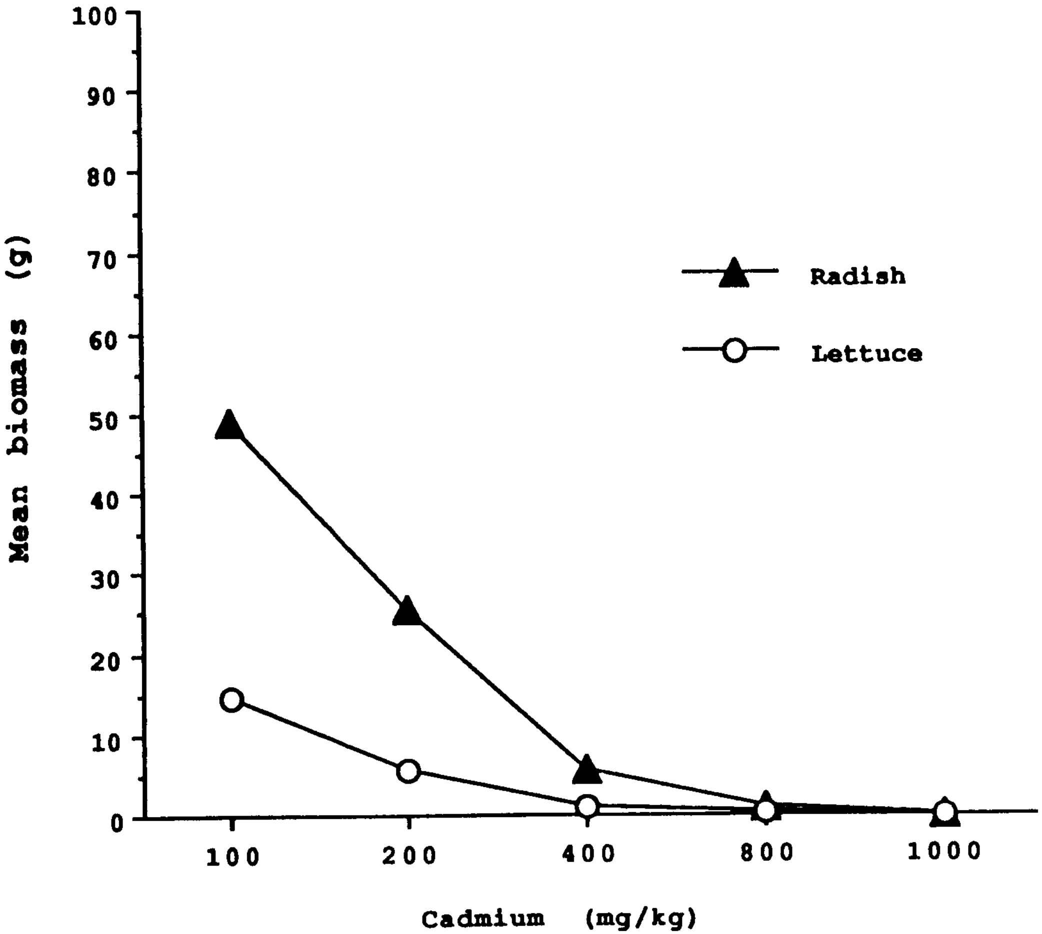

Figure 6. Mean biomass for lettuce and radish at various concs of Cd when conc. of Pb = 100 mg/kg

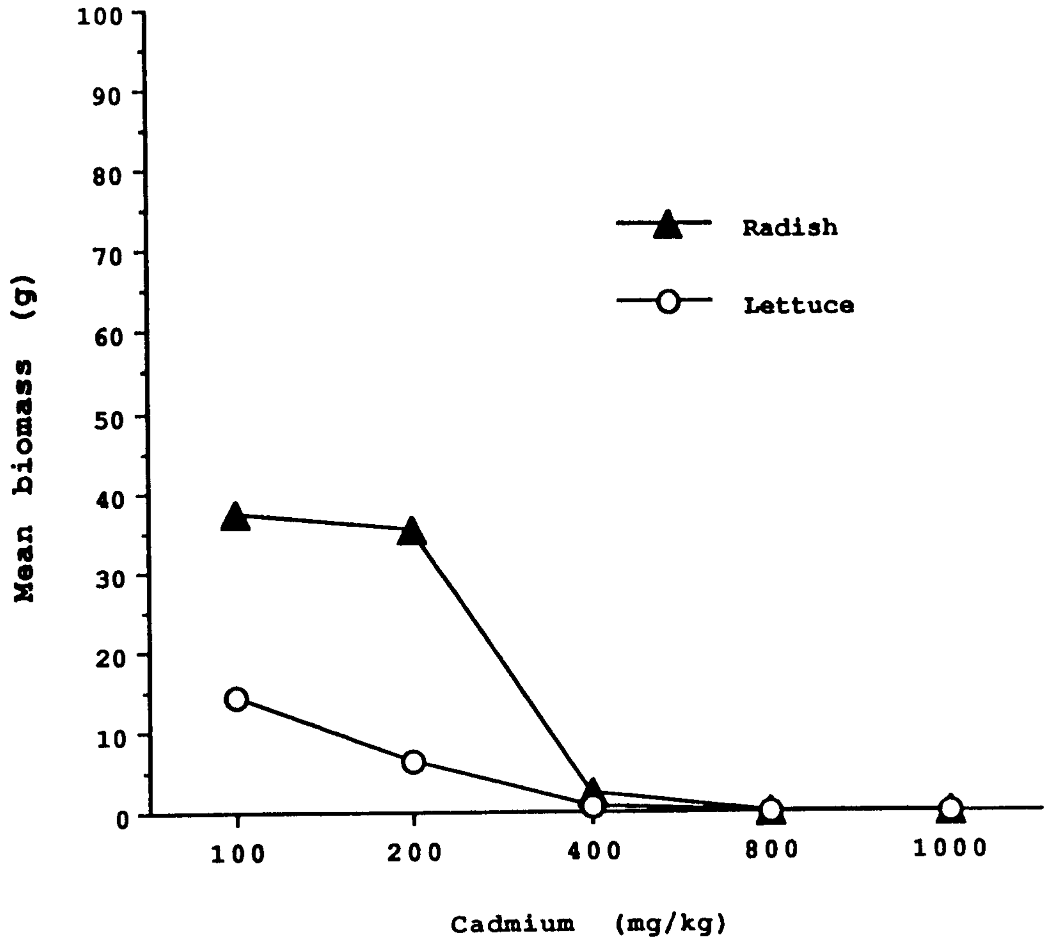

Figure 7. Mean biomass for lettuce and radish at various concs of Cd when conc. of Pb = 200 mg/kg

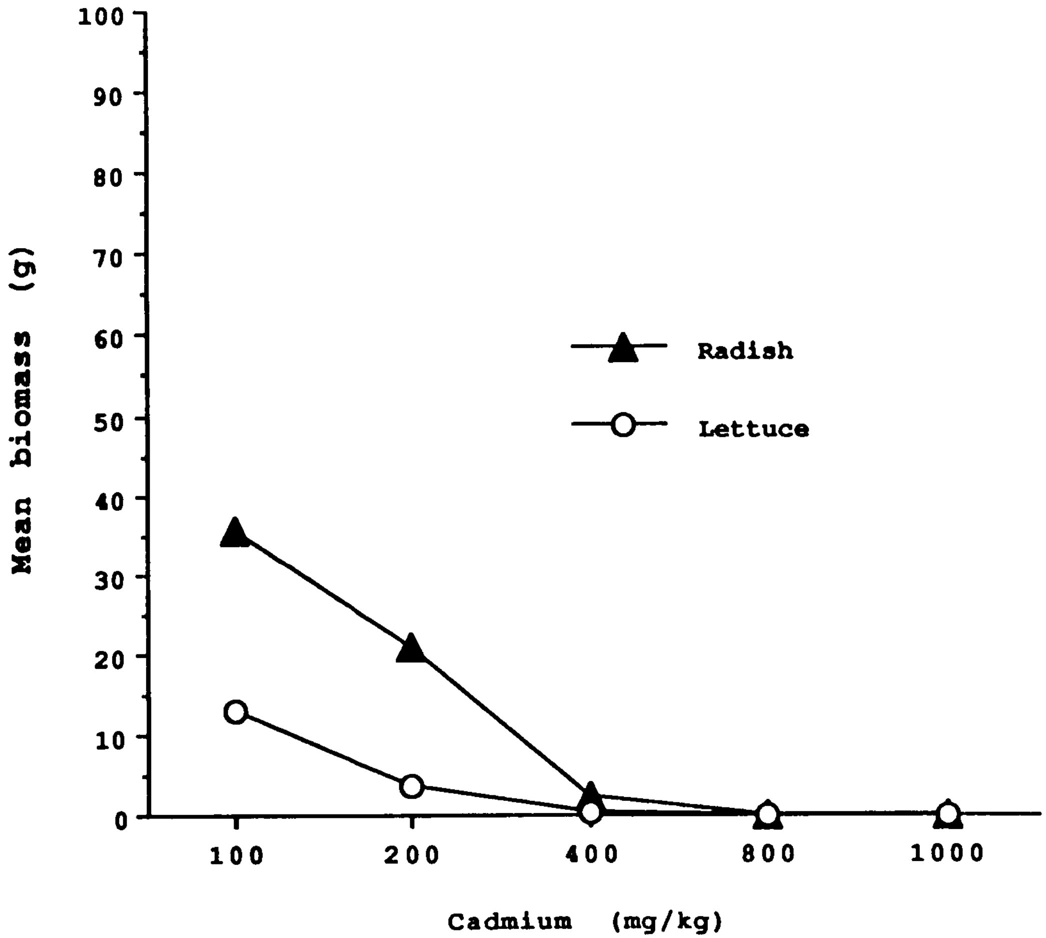

Figure 8. Mean biomass for lettuce and radish at various concs of Cd when conc. of Pb = 400 mg/kg

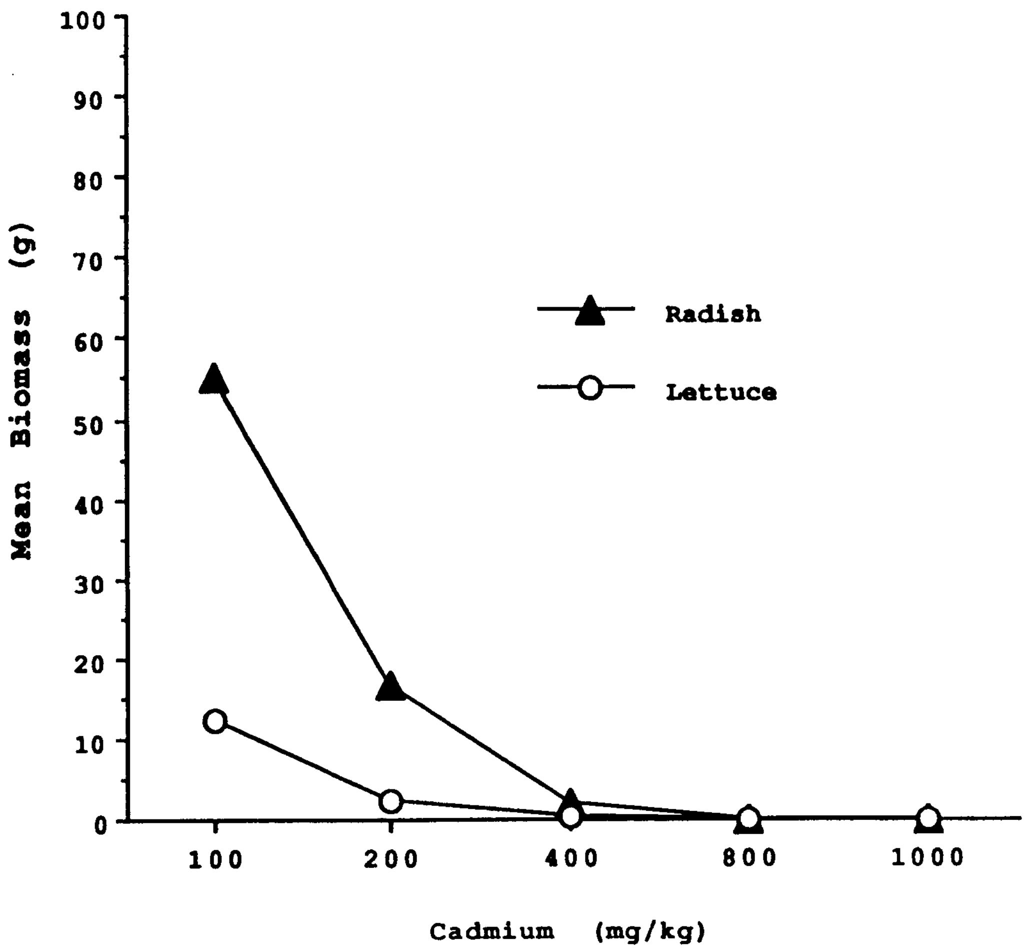

Figure 9. Mean biomass for lettuce and radish at various concs of Cd when conc. of Pb = 800 mg/kg.

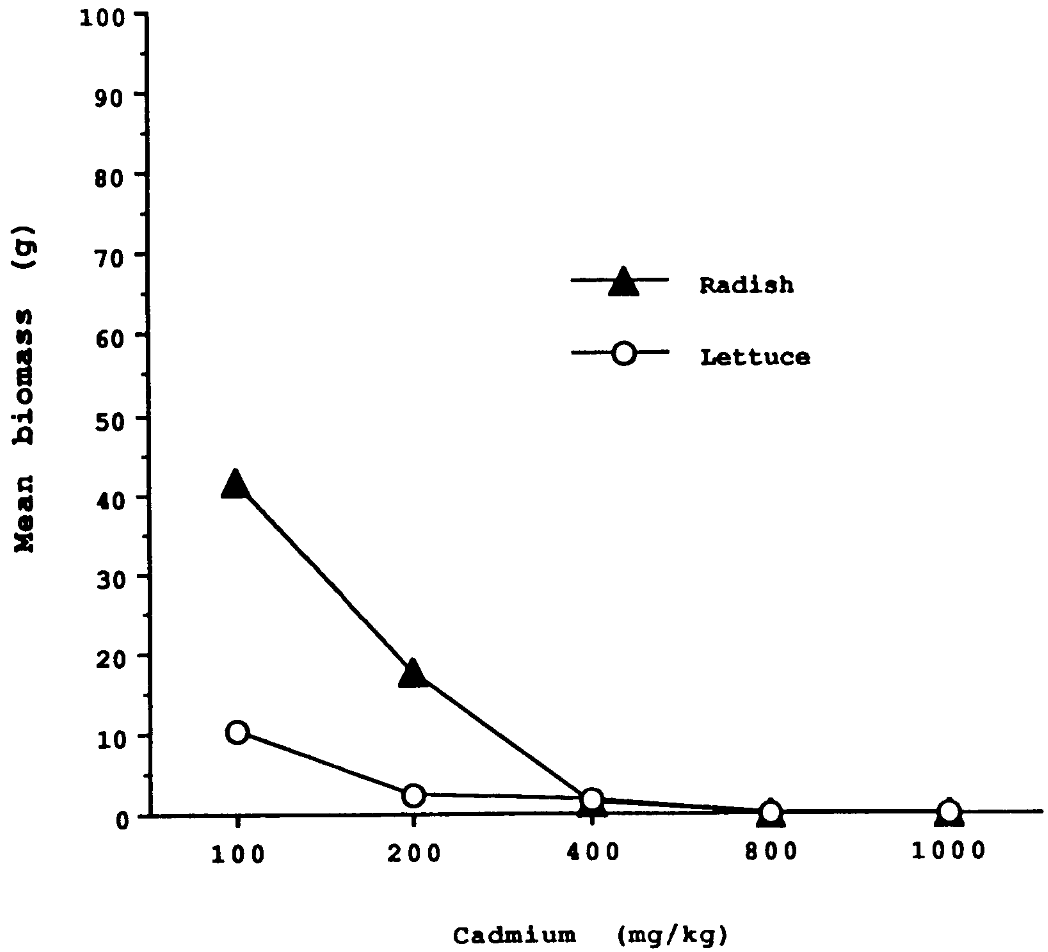

Figure 10. Mean biomass for lettuce and radish at various concs of Cd when conc. of Pb = 1000 mg/kg

Uptake of Cadmium and Lead by Lettuce and Radish

Cadmium Uptake

Test results indicated that the mean uptake of cadmium by lettuce and radish increased as the concentrations of cadmium and lead in the soil increased. In lettuce, cadmium uptake peaked when the concentration of cadmium reached 400 mg kg^{-1} at 100 and 200 mg kg^{-1} lead in the soil. (See Figures 11 and 12).

There was moderate uptake of cadmium by lettuce when the concentration of lead reached 400 mg kg^{-1} in the soil at a cadmium concentration of 200 mg kg^{-1} (See Figure 13). However, the uptake of cadmium by lettuce peaked again at 200 mg kg^{-1} cadmium when the concentrations of lead in the soil increased to 800 and 1000 mg kg kg^{-1} respectively (See Figures 14 and 15).

The uptake of cadmium by radish peaked at 200 mg kg^{-1} of cadmium when the concentrations of lead in the soil were 100 and 200 mg kg^{-1} respectively, but declined as the concentration of cadmium increased. However, cadmium uptake by radish increased as the concentrations of cadmium and lead in the soil increased, and peaked when the concentration of cadmium was 400 mg kg^{-1} (See Figure 14).

In both lettuce and radish, there was no uptake of cadmium beyond 800 mg kg^{-1} cadmium.

Statistical Interpretation of Uptake Results.

Statistical analysis indicated significant differences in the mean uptake levels of cadmium by lettuce ($p = .005$) (See Appendix Table 3). The uptake of cadmium occurred at lower concentrations in the mixture, however, and the uptake declined sharply when cadmium concentration in the mixture reached 400 mg kg^{-1}, at different concentrations of lead in the soil. Data also indicated that there was no significant differences in mean uptake levels of cadmium by radish ($p = 0.43$). There was no interaction between cadmium and lead on cadmium uptake by both lettuce and radish ($p = 0.17$) (See Appendix Table 3).

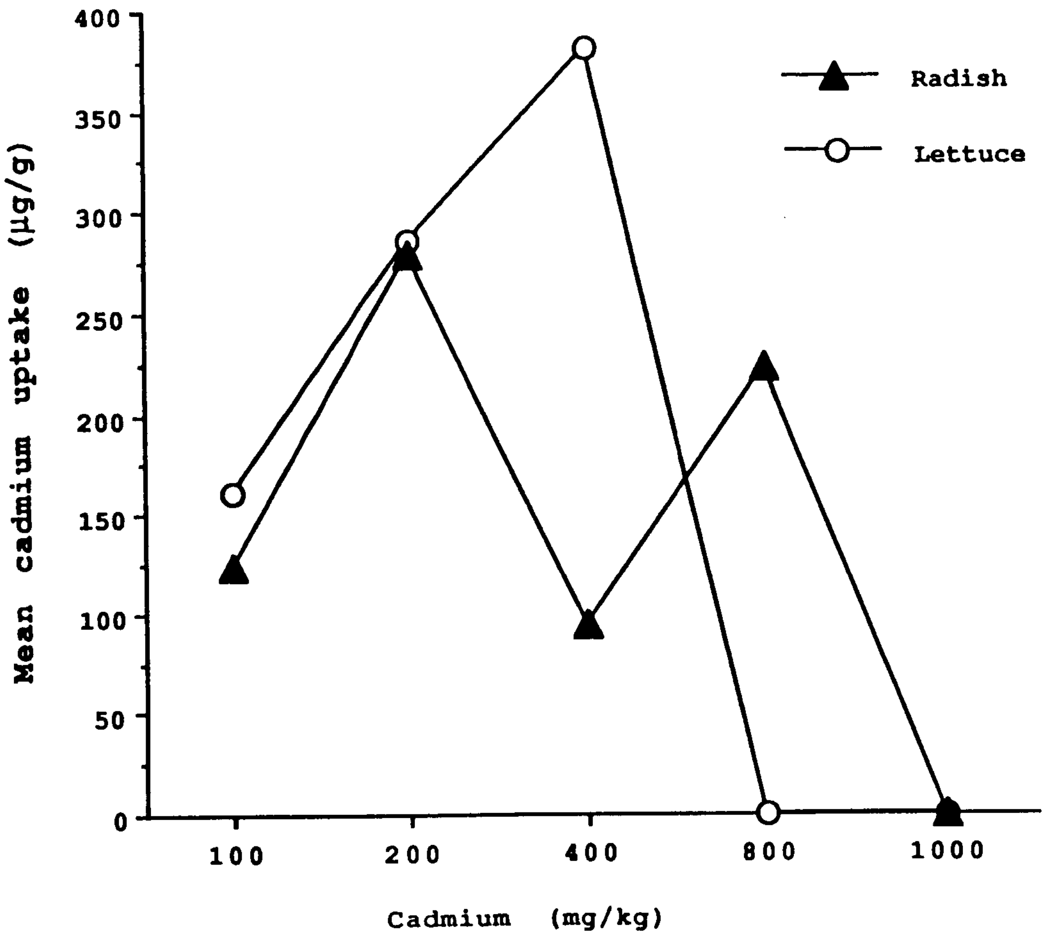

Figure 11. Mean cadmium uptake by lettuce and radish at various concs of Cd when conc. of Pb = 100 mg/kg.

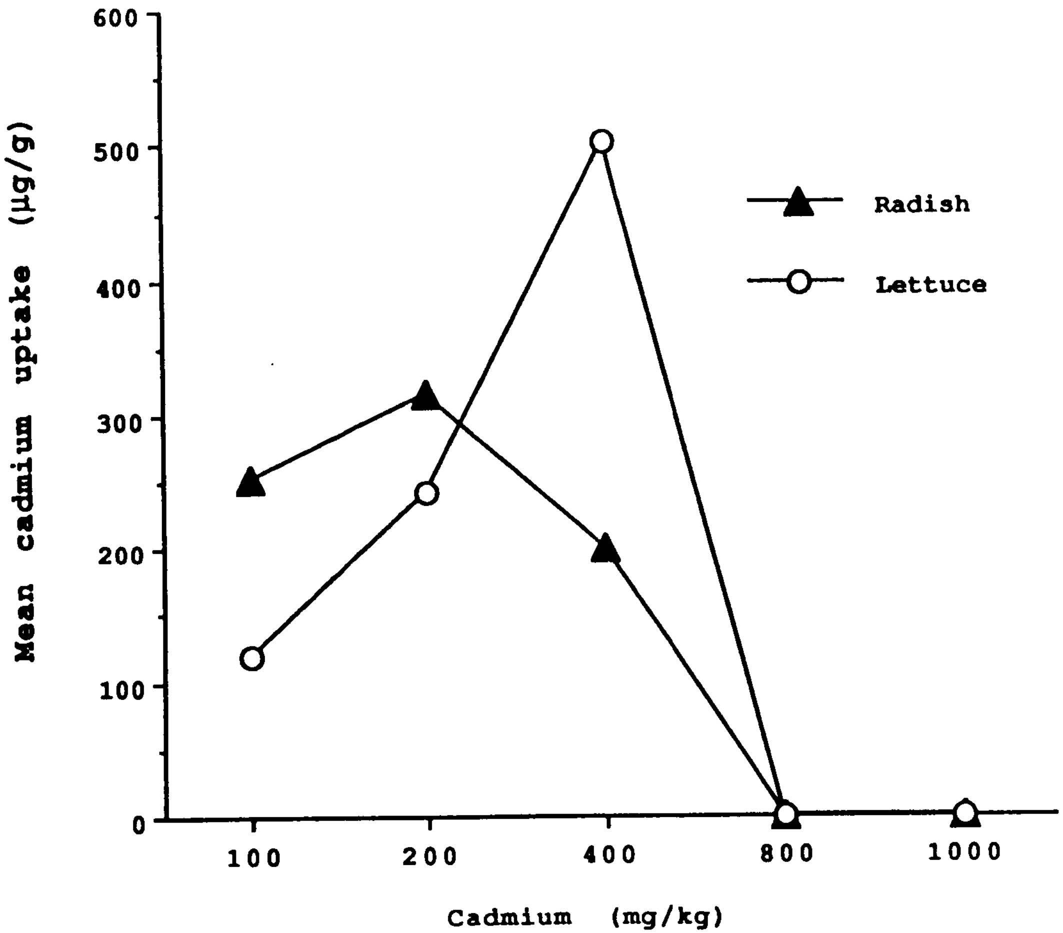

Figure 12. Mean cadmium uptake by lettuce and radish at various concs of Cd when conc. of Pb = 200 mg/kg

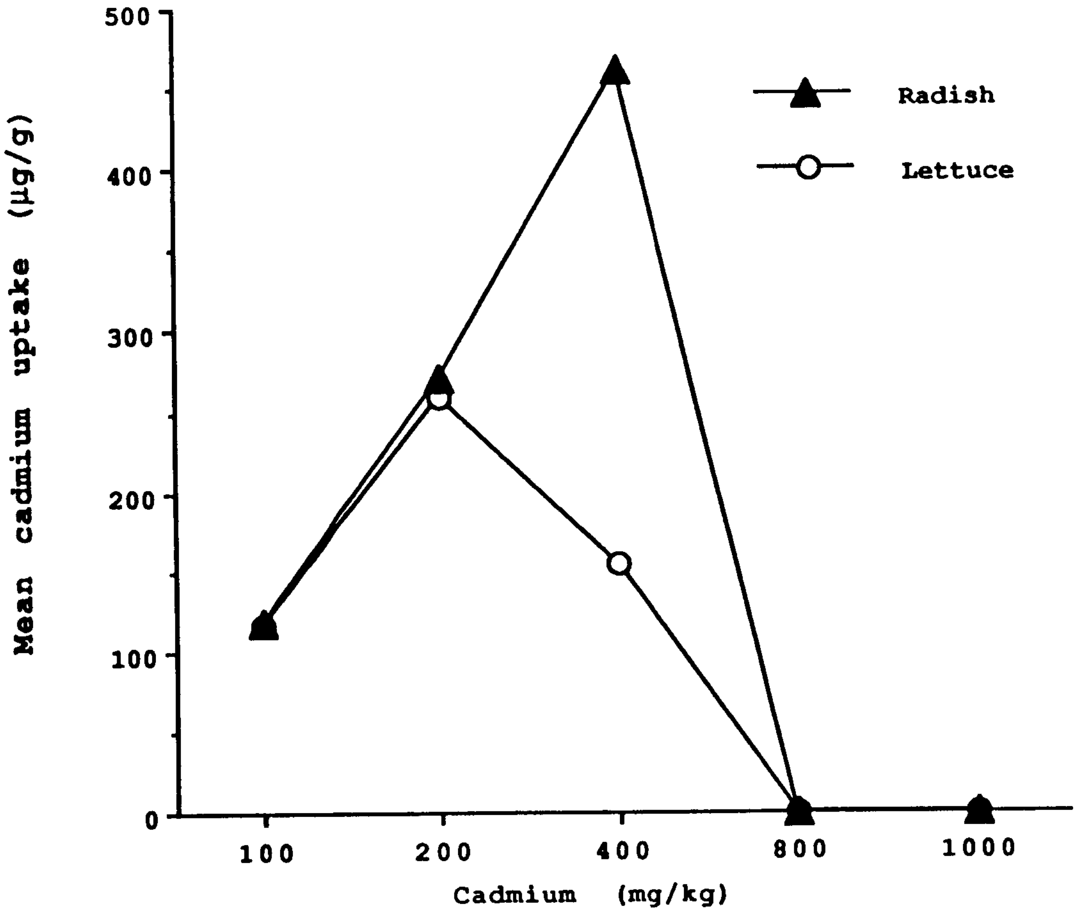

Figure 13. Mean cadmium uptake by lettuce and radish at various concs Cd when conc. of Pb = 400 mg/kg.

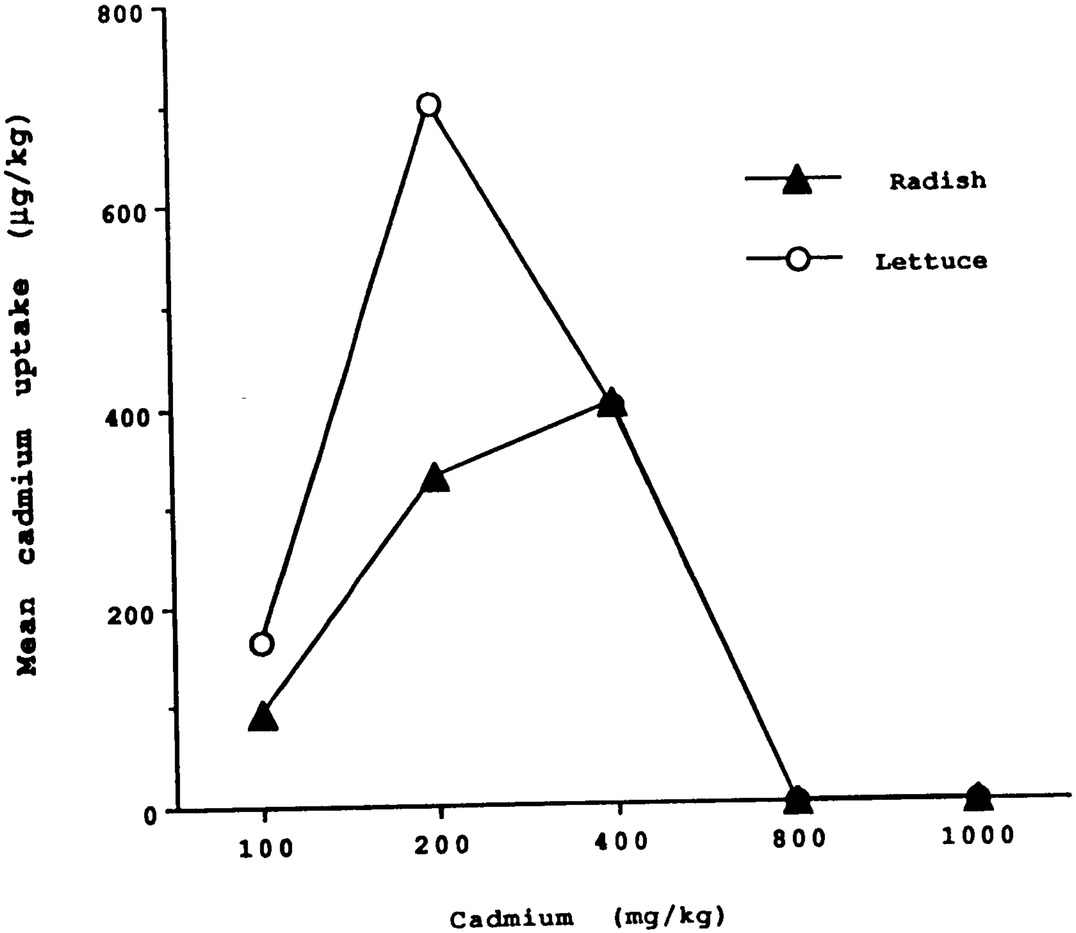

Figure 14. Mean cadmium uptake by lettuce and radish at various concs of Cd when conc. of Pb = 800 mg/kg.

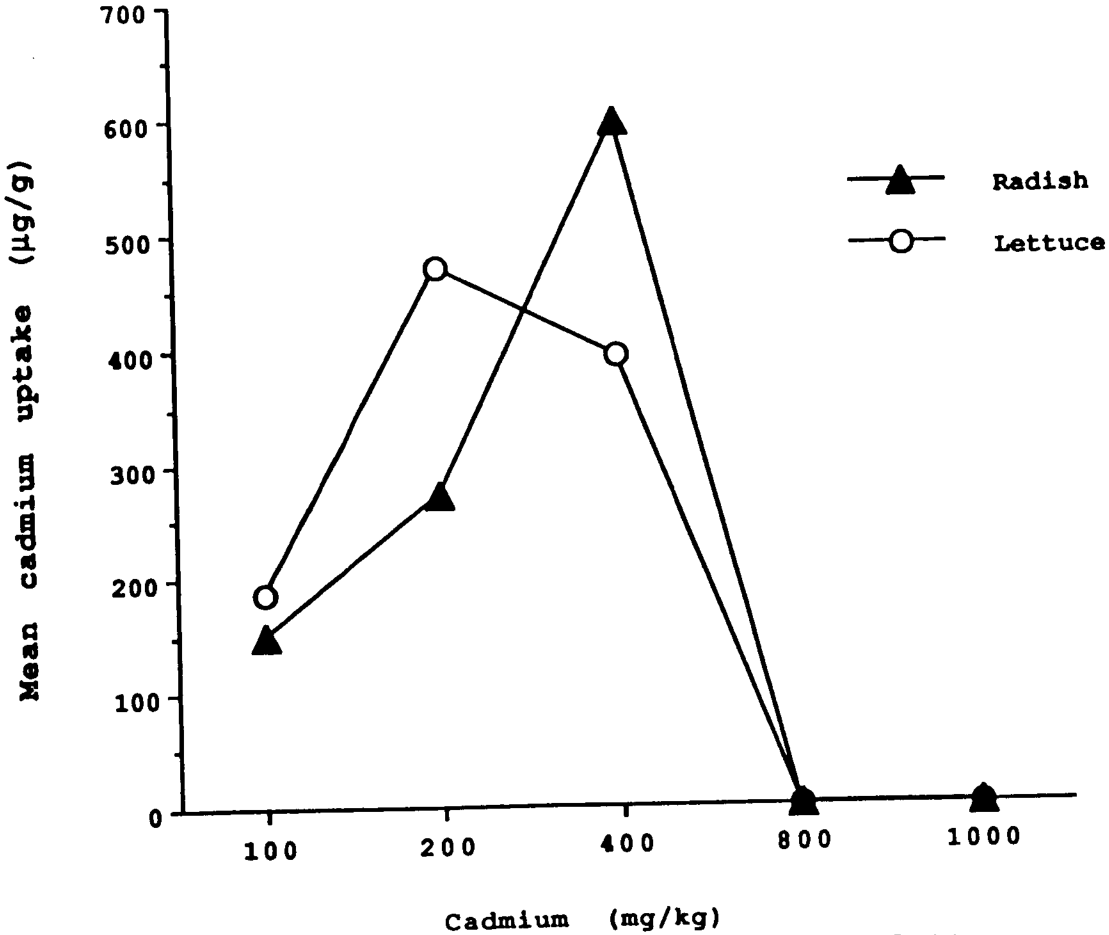

Figure 15. Mean cadmium uptake by lettuce and radish at various concs of Cd when conc. of Pb = 1000 mg/kg.

Lead Uptake

Data indicated that the mean uptake levels of lead by lettuce increased as the concentrations of lead increased in the mixture. However, uptake was more pronounced when the concentration of lead in the mixture reached 800 mg kg^{-1}, but declined sharply when the concentration of cadmium in the mixture exceeded 200 mg kg^{-1} (See Figures 16-20).

The uptake of lead by radish increased as the concentration of lead in the mixture increased, however, lead uptake declined when the concentration of cadmium exceeded 400 mg kg^{-1}. Also, there was no uptake of lead by both radish and lettuce, as cadmium levels in the soil mixture exceeded 400 mg kg^{-1} (See Figures 16-20).

Statistical Interpretation of Plant Uptake Results.

The statistical analysis indicated that the mean uptake levels of lead by lettuce was not significant ($p = 0.31$) (See Appendix Table 3). Data also indicated that the mean uptake levels of lead by radish were not statistically significant ($p = 0.40$) (See Appendix Table 3). Furthermore, there was no interaction between cadmium and lead on lead uptake by both lettuce and radish ($p = 0.22$) (See Appendix Table 3).

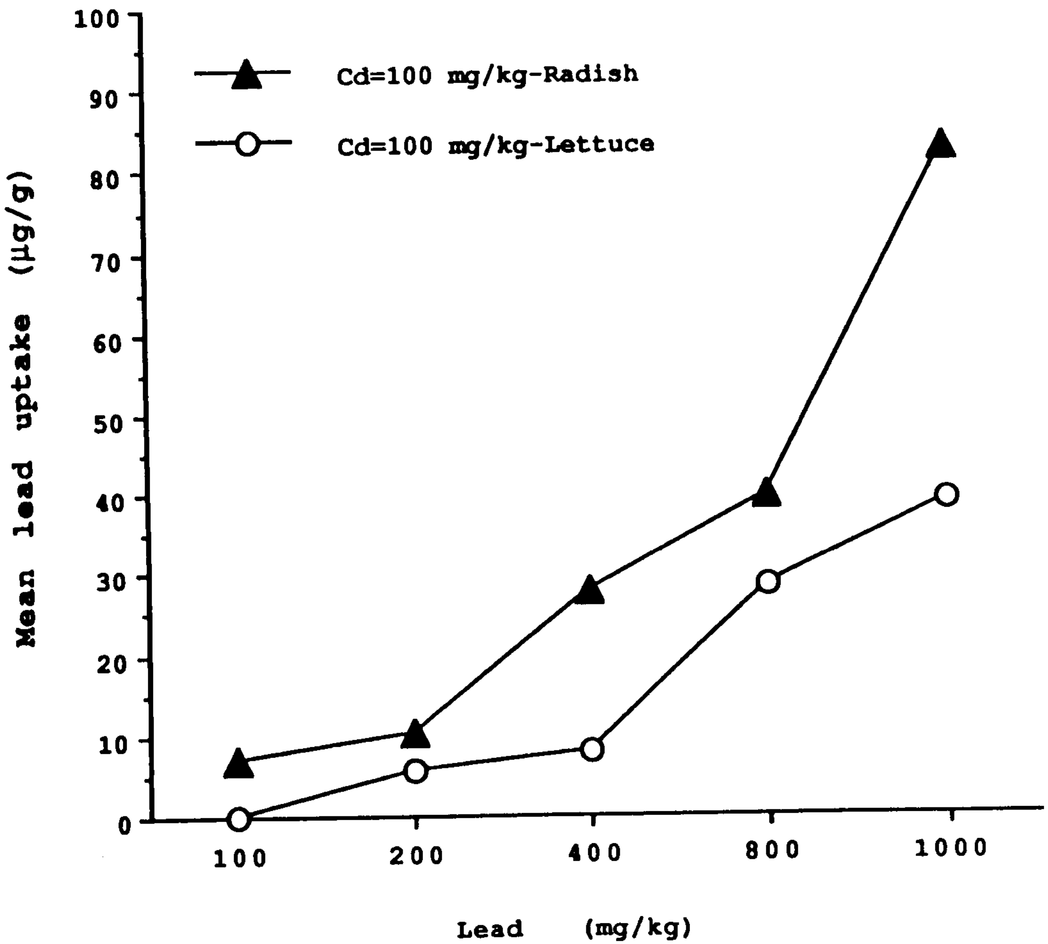

Figure 16. Mean lead uptake by lettuce and radish at various concs of Pb when conc. of Cd = 100 mg/kg.

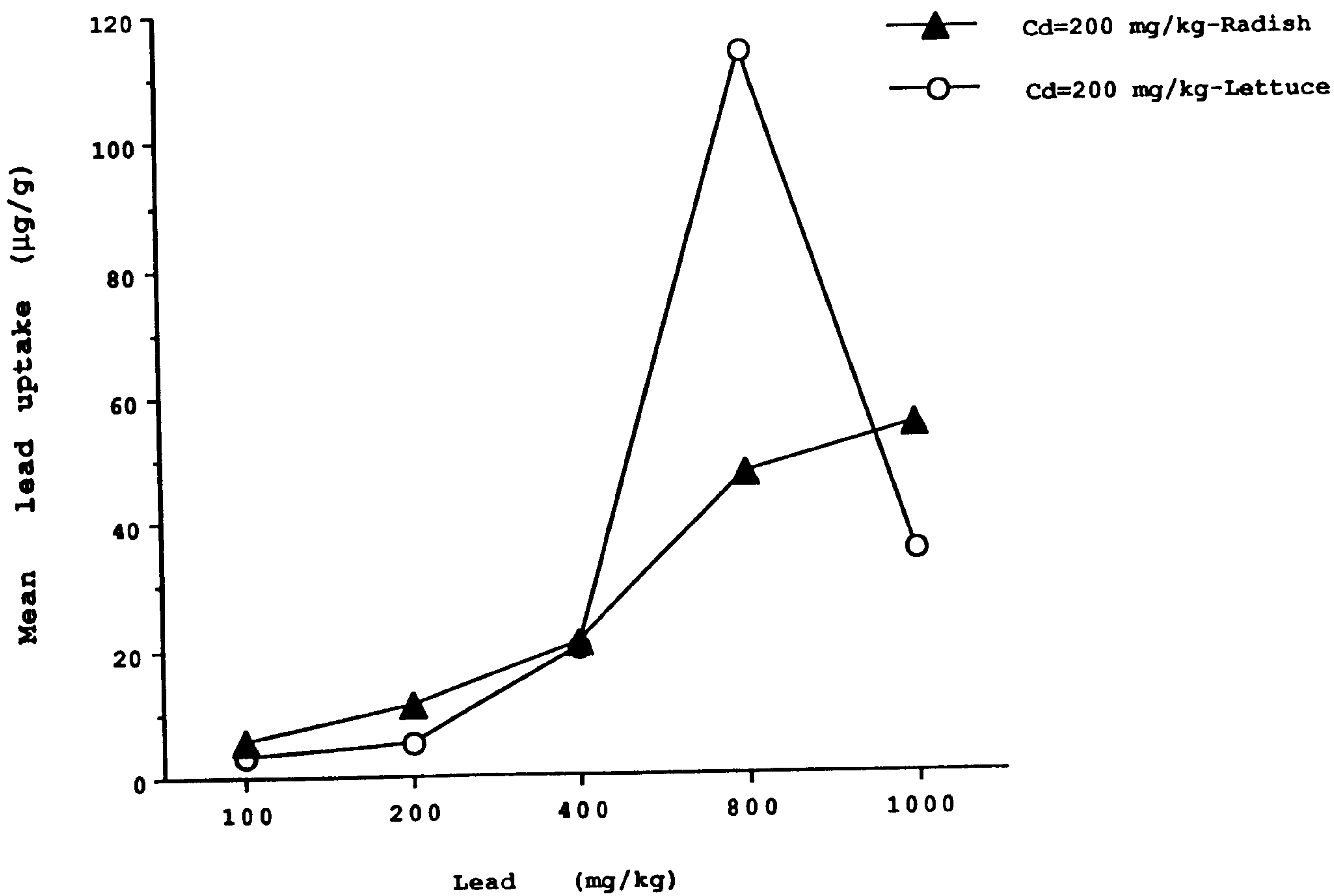

Figure 17. Mean lead uptake by lettuce and radish at various concs of Pb when conc. of Cd = 200 mg/kg.

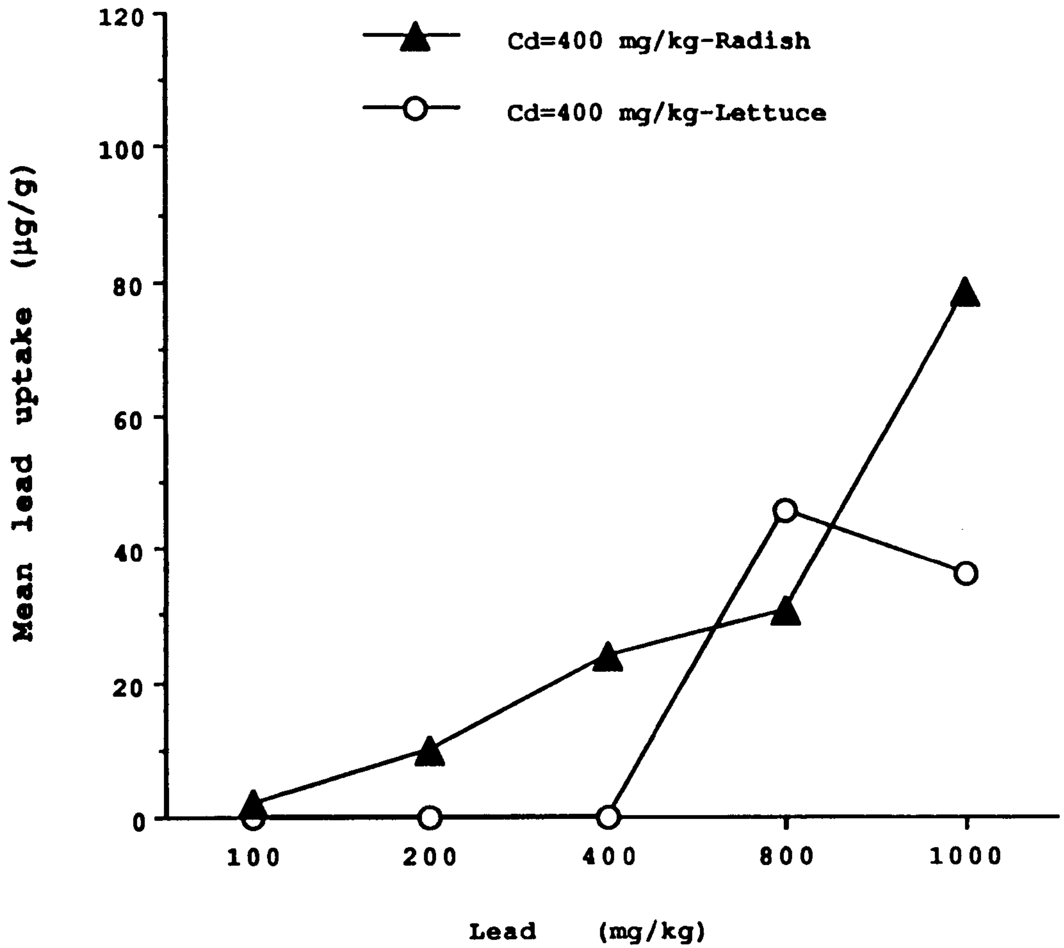

Figure 18. Mean lead uptake by lettuce and radish at various concs of Pb when conc. of Cd = 400 mg/kg.

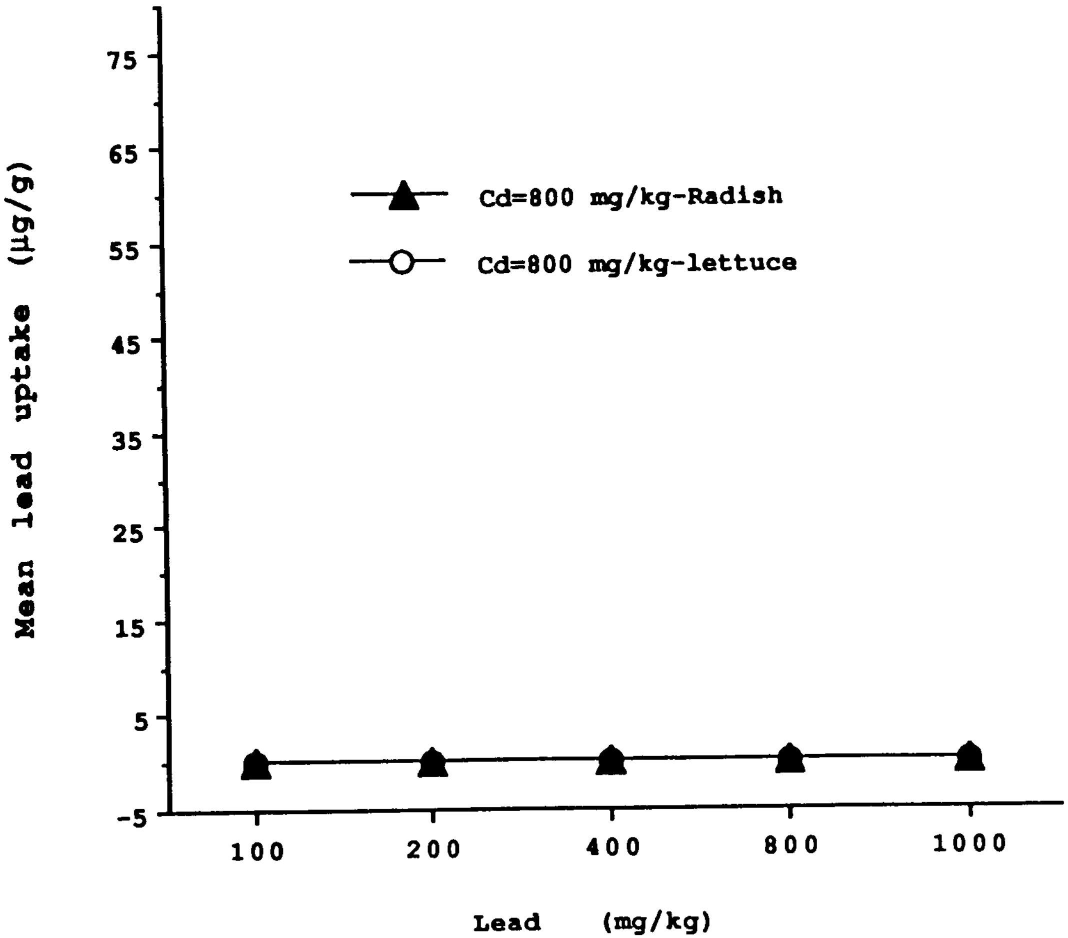

Figure 19. Mean lead uptake by lettuce and radish at various concs of Pb when conc. of Cd = 800 mg/kg.

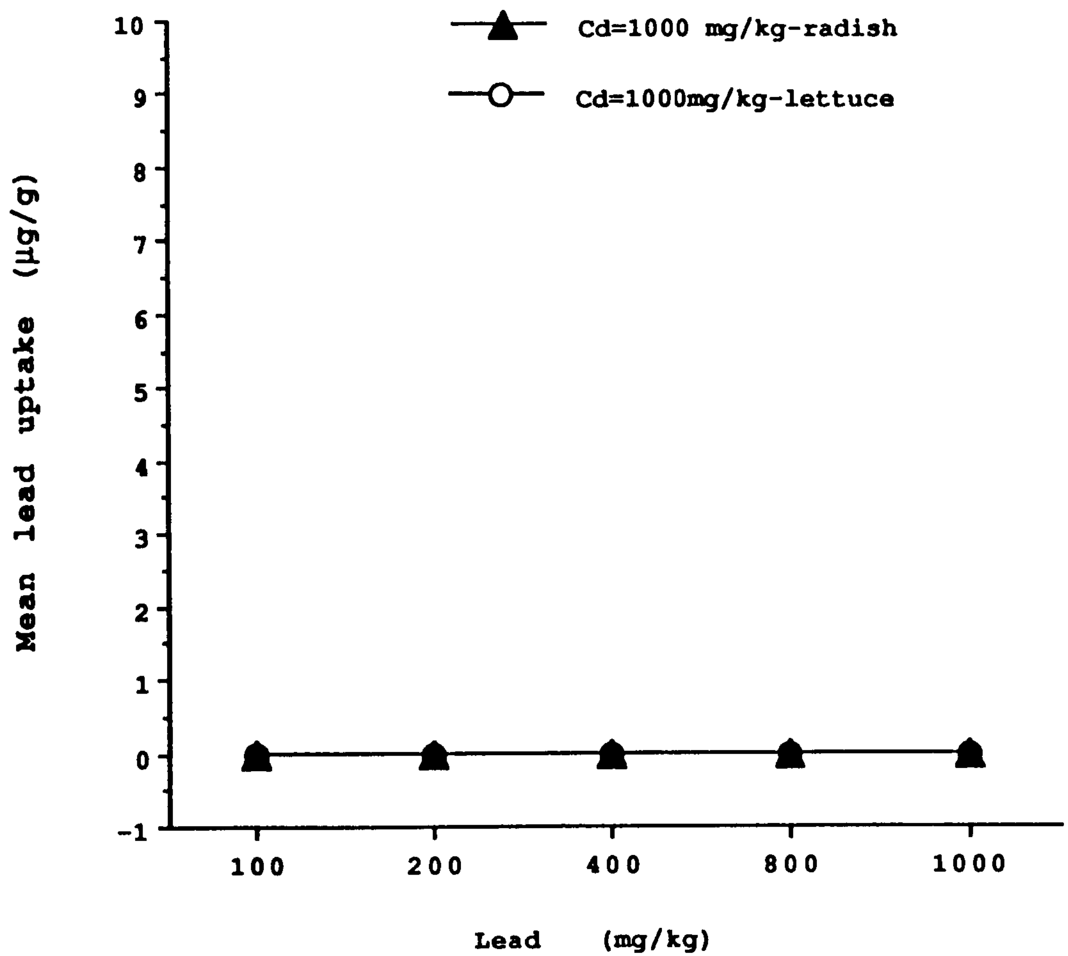

Figure 20. Mean lead uptake by lettuce and radish at various concs of Pb when conc. of Cd = 1000 mg/kg.

Chapter 5

DISCUSSION AND CONCLUSIONS

Discussion

The presence of cadmium and lead mixtures in the test soil resulted in differences in germination rates in both lettuce and radish seeds. The toxic effects of cadmium and lead, exhibited by the two plant species, are consistent with previously published reports (Page et al., 1981; Koeppe, 1981; Miller et al., 1977; Chaney, 1973; Chaney et al., 1975; Chambers and Sidle, 1991; Granato et al., 1991). However, the combined effects of the mixture in the soil on lettuce and radish did not differ from what was observed in the single chemical exposure studies (Cutler et al., 1974; Jarvis et al., 1976; John et al., 1976; Lagerwerff et al., 1972; Falchuk, 1975; Rorison, 1965).

The lack of interaction between cadmium and lead in the soil for lettuce, indicated that the presence of lead in the mixture did not affect the germination rates of lettuce when exposed to cadmium, nor did cadmium affect the germination rates of lettuce when exposed to lead. In the pre-emergence stage, it appeared that cadmium and lead were readily available to the seeds in the soil, and the differences germination rate observed in lettuce, would have been expected (Lagerwerff et al., 1972; Chaney, 1973; Chaney et al., 1975). The results observed in radish, showing interaction between the two metals, indicated that there was some competition between the two metals in the soil for available binding sites and/or anions. It was not quite clear if this competition was synergistic or antagonistic in nature, because both cadmium and lead are toxic to radish.

Lead toxicity to plants depends on biologically-available lead, and previous studies have reported that lead incorporated into soils is always tightly bound to organic matter and is precipitated out thus making it biologically unavailable and ineffective to plants (Zimdahl and Koeppe, 1977). It appears that because the Newberg silt loam soil used in this study was low in organic matter and relatively low in CEC, availability of metals was enhanced. Therefore, increased availability of lead resulted in increased lead uptake in the soil. The interaction observed was possibly due to competition between cadmium and lead for available anions in the soil.

In the post-emergence stage, it appeared that the severe physiologic effects observed on plants due to exposure to cadmium and lead were primarily due to the ability of the plants to absorb these metals from the soil. Because pH, CEC and organic matter content of the soil were relatively ideal (See Table 1), cadmium and lead were biologically available to the plant roots for absorption; this may have contributed considerably to the observed effects on germination and the uptake of cadmium by lettuce.

The results from the study also showed that EC_{50} estimates calculated for lettuce (362.57 mg kg^{-1} cadmium at 400 mg kg^{-1} lead), further support previously published reports about the sensitivity of lettuce to cadmium (Lagerwerff et al., 1972; Chaney, 1973; Chaney et al., 1975). Perhaps the adverse effect exhibited by lettuce to cadmium and lead mixtures in the soil may be linked to the plant's biochemical processes; toxic chemicals affect enzymatic processes in the plant that are essential to the plant's survival. For example, the activity of some enzymes like peroxidases, malic enzyme, isocitrate dehydrogenase, glutamic dehydrogenase, increase in plant leaves and roots, when toxic metals like cadmium accumulate in the plant (Mukherji and Das Gupta, 1972; Lee et al., 1976a,b; Weigel and Jager, 1980; Van Assche et al., 1988). Toxic metals such as cadmium and lead exert damaging effects on important enzymatic processes in plants. In this process, essential metals like iron and manganese are displaced. For example, if chlorosis (yellow color resulting from reduced chlorophyll synthesis) is observed on lettuce and radish leaves, this may be due to the interaction of cadmium and lead with foliar iron (Foy et al., 1978). Also, enzyme induction has been strongly linked to the inhibition of shoot growth as a result of copper, zinc and cadmium phytotoxicity (Mukherji and Das Gupta, 1972; Van Assche et al., 1988).

The slight increase in sensitivity of lettuce to the cadmium and lead treatments could be attributed to the ability of the plant to absorb these metals more readily than radish (see Figures 1-5). These results are in agreement with previously published reports that have indicated that cadmium is absorbed by passive diffusion and translocated freely in the soil (Cutler et al., 1974; Jarvis et al., 1976). In addition, lead has been reported to be strongly sorbed by soils, to form insoluble crystalline compounds (Santillian-Medrano et al., 1975), and such binding of lead reduces its activity in the soil or rooting-medium solution (Koeppe, 1981). This may have been responsible for the lack of significant differences in mean uptake levels for lead observed in this study. The results of this study strongly indicated that the toxic effects of cadmium and lead mixtures in the soil on lettuce was additive; the effects observed are attributed to the sum of the effects of the individual metals. Furthermore, the lack of interactions between the two metals in lettuce, also support this assumption.

The results of the study also showed that cadmium and lead mixtures in soil affect plant biomass production. Biomass production in plants is a secondary event, which undoubtedly is also influenced by those factors that affect the critical stage of a plant's life cycle (germination). As cadmium and lead were absorbed from the soil by the plant seedlings,lettuce and radish germination was inhibited and growth was subsequently decreased. These findings are consistent with previous reports by Lepp (1981), which reported that the assimilation of phytotoxic amounts of cadmium and lead, inhibited growth and decreased biomass production. Also, Van Assche et al.(1990) reported that at lower levels of pollutants such as cadmium and lead in the soil, that physical symptoms of phytotoxicity may be less evident, but reduction of plant quality and reduced biomass production persisted.

Cadmium and lead have been implicated in food chain contamination (Ryan et al., 1982; Wolnik et al., 1983; Boon et al., 1992). The results from this study are consistent with previously published reports that showed that lettuce accumulated significant amounts of cadmium when grown on contaminated soil (John et al., 1976; Lagerwerff et al., 1972) (See Appendix Table 3).

The decline in mean cadmium uptake by lettuce at 400 mg kg^{-1}, may be attributed to saturation of the active binding sites on the plant root system (See Figures 1-5). Also, the lack of interaction between cadmium and lead in the soil, indicated that the decline in mean cadmium uptake was not due to lead antagonism (See Appendix Table 3). The observed uptake of cadmium by radish from the mixture was consistent with previously documented reports, which showed that radish did not accumulate as much cadmium as lettuce (Santillian-Medrano et al., 1975).

The lack of significant differences in mean uptake values of lead by lettuce is not consistent with those reported by other researchers. For example, Roberts et al. (1974), Langerwerff and Brower (1974), and Preer (1980), reported that leafy portions of vegetable crops contained the highest levels of lead. The lack of uptake exhibited by lettuce in this study, may be due to the binding of lead to the plant roots, thus keeping it from being transported to the plant leaves. Furthermore, reports have linked the low availability of lead in the plant root zone for uptake to biochemical processes involved in lead binding, such as inactivation and precipitation. For example, Hammet (1928a) reported that lead was localized in cell walls and nuclei of absorbing roots. Also, Tandler and Solari (1969) found that lead was bound to orthophosphate ions within the nucleolus of onion root tips fixed in a lead solution. The lack of significant differences in mean uptake of lead by lettuce could be attributed to some of these factors. Furthermore, the low mean uptake of lead by lettuce may be attributed to the concentration of lead by the roots, making the below ground concentration of lead greater that the above ground tissues.

There are obvious differences between biological significance and statistical significance; biological significance does not imply statistical significance and vice versa. For example, uptake results indicated that lead was absorbed and taken up by radish, however, statistical analysis indicated otherwise. The lack of statistically significant mean uptake of lead by radish, have serious environmental and public health implications. The implications are twofolds: First, the uptake of toxic metals such as lead, by a root crop such as radish, contribute to potential food chain contamination. Statistical results do not always portray, for example, the possibility of bioaccumulation that occurs in human tissues over time. Secondly, when two metals like cadmium and lead are present in soil as a mixture, it is difficult to predict their behavior in the soil. In addition, test results from this study indicated that differences existed in the mean uptake of cadmium and lead among the plant species tested, and there was no evidence that one metal was preferred over the other.

Interactions that occur between metals and nutrients, and also between metals and soluble salts in the soil, vary with crop and soil types. The lack of significant interaction between cadmium and lead in this study exposed some of the drawbacks that may be apparent in using metal complexes as a tool to immobilize metals in polluted soils.

One confounding factor in this of study is that toxic metals such as lead are ubiquitously distributed in the soil and in the ecosystem. Aerial deposition of this metal from automobiles and other anthropogenic sources are important pollution source. The impaction on plant leaves of aerosol lead particles from automobile exhausts have been well documented (Zimdahl and Koeppe, 1977). Undoubtedly, plants growing near highways have elevated levels of lead associated with their leaves and stems (Koeppe, 1981). This may be a potential source of lead contamination of leafy vegetable crops such as lettuce.

Conclusions

The mixtures of cadmium and lead in the soil resulted in significant differences in mean germination rates in both lettuce and radish. In addition, differences in mean germination rates were evident in both radish and lettuce with increasing concentrations of cadmium in the soil, but increasing the concentration of lead in the soil did not result in any significant differences in mean germination rates in lettuce and radish.

Plant biomass production was affected by cadmium and lead mixtures in the soil. Mean biomass decreased as the concentration of cadmium and lead increased in both lettuce and radish. However, mean biomass production by lettuce and radish decreased significantly as the concentration of cadmium in the soil increased, but did not decrease as the concentration of lead increased in the soil.

The mean uptake of cadmium by lettuce was significant as the concentrations of cadmium and lead increased in the mixture, but was not significant in radish under similar conditions (mean cadmium uptake did increase as the concentration of cadmium increased).

The mean lead uptake by lettuce increased as the concentration of lead increased in the soil mixture. Although, lead was taken up by radish, however, the mean uptake by radish did not increase as the concentration of lead increased in the soil.

It is important to understand the behavior of these metals in the soil in order to prevent the potential for food chain contamination. These studies focussed on cadmium and lead uptake rates with radish roots and lettuce leaves because those are commonly consumed.

Cadmium accumulation in food crops such as lettuce and radish can lead to serious health problems, such as cadmium accumulation in the kidney, which may lead to kidney failure. In addition, cadmium ingestion may result in the destructions of the immune system, liver and the cardiovascular system (Casarett and Doull, 1991).

Ingestion of lead can result in the accumulation of lead in the kidney, central nervous system, bone, spleen, lungs and hair, thus may cause serious health effects. Children are especially susceptible to lead, because studies have shown that absorption and retention rates are higher in children than in adults (Casarett and Doull, 1991). Neurological deficits which is associated with learning disability in children, have been linked to blood lead levels (Smith et al., 1983).

Recommendations

The availability of cadmium and lead to plants in the soil environment is affected by soil variables such as pH, CEC and organic matter. For example, when sewage sludge amended soils are used for agricultural purposes, the soil pH should be critical, since the solubility of these metals in the soil is pH dependent.

Liming of contaminated soil elevates pH, and decreases the availability of these metals to plants. Chaney et al. (1975), demonstrated that cadmium concentrations of soybean leaves were reduced substantially when the pH of the soil on which the plants were grown was increased by liming from pH 5.3 to pH 7.0.

The CEC and organic matter content (OM) of the soil have been reported to affect the availability of metals in soil (Hagiri, 1973). For example, Stevenson et al.(1972) reported that the amount of cadmium absorbed by oats (Avena sativa) as CEC of the soil was increased by adding organic matter decreased. It is quite possible that adding substances like peat moss may decrease the absorption of heavy metals by plants, because they may exhibit strong reactions to these metals.

The clay constituents of soil play an important role in the soil's ability to bind cations of heavy metals (Korte et al., 1976). The cultivation of edible crops in soils with moderate amounts of clay (that will not impact essential minerals), will decrease the chances of heavy metal accumulation in plants.

In addition, several investigators have suggested that oxides of iron and manganese exhibit highly specific adsorption affinity for trace metals, including cadmium (Jenne, 1968; Forbes et al., 1976). Cultivating edible crops in iron and manganese rich soils may reduce the absorption and uptake of heavy metals such as cadmium and lead from polluted soils.

Finally, since the use of sewage sludge to fertilize agricultural soils is practiced in most parts of the world, it is quite possible that crops cultivated in these soils are imported to the U. S. Because these countries may not have adequate regulations controlling the application of sewage sludge to agricultural soils, produce imported from these countries may contain higher levels of toxic heavy metals. The United States should establish technology transfer programs with these countries to enable them acquire the necessary technology, which in turn would help improve standards in these countries.

Study Limitations

The use of pre-selected batch of seeds for the study may contribute to bias, because biological variability may exist between those exposed to higher levels of cadmium and lead mixtures and those exposed to lower levels. Also, it was obvious from the germination results that seeds exposed to higher levels of cadmium and lead mixtures had lower germination rates than those exposed to lower levels. Germinated plants from the the group exposed to higher levels of the chemical mixtures may not have been as healthy as the group exposed to lower levels, and therefore, biomass and uptake rates could have been affected in these plants.

Suggestions For Future Research

It is suggested that further research be directed toward understanding the chemistry and behavior of toxic metals in soils. Also, more research efforts are needed to develop new methods that may be used in the future to immobilize these metals in the soil. In addition, extensive research with a variety of soils and plants to evaluate the possibility of predicting the behavior of heavy metals in soils, is needed. Furthermore, research should be conducted with non-edible plants to identify those that are capable of accumulating heavy metals from contaminated soils.

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APPENDIX

DEFINITIONS

CEC: Cation Exchange Capacity. The ability to exchange available cations in the soil equally.

Chlorosis: Yellow color resulting from reduced chlorophyll synthesis in plants.

Desorption: Removal of metal from soil or root surface.

EC_{50}: Effective Concentration that will cause a 50 percent reduction in germination.

Endpoint: The process upon which conclusions can be made.

ICP: Inductively Coupled Plasma. A chemical analysis technique used to quantify metal concentrations in soils and animal and plant tissues.

µg/g: Microgram per gram-parts per million(ppm) 1 µg/g = 1ppm, ratio used for tissues, e.g., plant tissues.

mg/kg: Milligram per kilogram-(ppm), ratio used for chemicals in solids, e.g., soil.

Organic matter: Plant and animal residues in soil that provide essential nutrients in soil and also can complex heavy metals in soil, rendering them innocuous.

pH: The measure of acidity or alkilinity.

Phytotoxicity: Toxicity to plants.

Quality assurance(QA): A program designed and organized to provide accurate and precise results. This includes methods and procedures, sample collection, data evaluation and selection of limits for test.

Quality control(QC): Specific actions required to provide information for the quality assurance. These include standardization, calibration, replication and controls.

Rhizosphere: A zone of increased microbial activity around the root of a plant in the soil.

RO Water: Reverse Osmosis water produced by the MilliQ system.

Translocation: Movement of metals from the soil through the plant roots to the leaves.

Table 1.

ANOVA Table for Effects of Cd and Pb on Germination of Plants

Source	Sum of Squares	DF	F-Ratio	P-value
Lettuce				
Cd	34301.53	1	269.63	≤0.0001*
Pb	685.41	1	5.39	0.0234*
$(Cd)^2$	2250.74	1	17.69	0.0001*
$(Pb)^2$	303.21	1	2.38	0.1274
Cd*Pb	36.36	1	0.29	0.60
Error	8396.35	66	127.22	
Total	45973.6	71	R^2 = 0.82, SD = 127.22	
Radish				
Cd	30576.77	1	362.52	≤0.0001*
Pb	32.95	1	.39	0.5408
$(Cd)^2$	887.09	1	10.52	0.0019*
$(Pb)^2$	110.73	1	1.31	0.256
Cd*Pb	647.58	1	7.68	0.0073*
Error	9328.98	66	84.35	
Total	37821.9	71	R^2 = 0.75, SD = 84.35	

n = 72

*Significant at $p \leq .05$

Table 2.

ANOVA Table for Effects of Cd and Pb on Biomass Producton

Source	Sum of Squares	DF	F-Ratio	P-value
Lettuce				
Cd	778.3	1	74.0	≤0.0001*
Pb	36.26	1	3.45	0.07
$(Cd)^2$	422.90	1	40.21	≤0.0001*
$(Pb)^2$	1.76	1	.17	0.6887
Cd*Pb	20.61	1	1.96	0.1685
Error	462.789	44	10.518	
Total	1722.63	49	R^2 = 0.73, SD = 10.518	
Radish				
Cd	10825.73	1	159.49	≤0.0001*
Pb	62.66	1	.92	0.3521
$(Cd)^2$	4612.25	1	67.95	0.0001*
$(Pb)^2$	10.79	1	.16	0.6962
Cd*Pb	1.81	1	1.81	0.8729
Error	2986.50	44	67.87	
Total	18499.7	49	R^2 = 0.83, SD = 67.87	

n = 50

* Significant at $p \leq .05$

Table 3.

ANOVA Table for Cd and Pb uptake by Plants

Source	Sum of Squares	DF	F-Ratio	P-value
Lettuce				
Cd	17.49	1	8.52	0.005*
Pb	2.20	1	1.07	0.3070
$(Cd)^2$	17.85	1	8.70	0.0053*
$(Pb)^2$	9.02	1	4.39	0.0425*
Cd*Pb	3.888	1	1.89	0.1771
Error	82.11	40	2.05	
Total	132.535	45	R^2 = 0.38, SD = 2.05	
Radish				
Cd	2.60	1	.64	0.4348
Pb	3.03	1	.74	0.4042
$(Cd)^2$	3.89	1	.95	0.3454
$(Pb)^2$	0.94	1	.23	0.6400
Cd*Pb	6.45	1	1.58	0.2165
Error	163.62	40	4.09	
Total	180.52	45	R^2 = 0.32, SD = 4.09	

n = 46

* Significant at $P \leq .05$