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I am submitting herewith a thesis written by Tommy Allen Bowers entitled "Uptake of Selected Heavy Metal from Municipal Sewage Sludge by Tall Fescue, Lettuce and Radish Plants." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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A

UPTAKE OF SELECTED HEAVY METAL FROM MUNICIPAL SEWAGE SLUDGE BY TALL FESCUE, LETTUCE AND RADISH PLANTS

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

The objective of this greenhouse study was to determine the effects of a municipal sewage sludge on the uptake of Cd, Cu, Ni, Pb, and Zn by lettuce (Lactuca sativa L., cv. "Black Seeded Simpson"), radish (Raphanus sativus L. cv. "Early Scarlet Globe") and fescue (Festuca arundinacea) grown on a Decatur silt loam, a Rhodic Paleudult. Two lime levels, zero and 6700 kg/ha, were employed to ascertain the extent of soil pH effect on metal uptake. Three soil extractants, 0.005 M DTPA, 1N NH_4OAc, and 0.5 N acetic acid, were used to determine their suitability for predicting metal uptake by each of the three crops.

Plant uptake was affected to the greatest extent by the level of sludge applied to the soil. Generally, the 20% and 30% sludge application levels (by weight) caused all plants to increase their metal uptake over the zero rate. At the 10% level, the uptake differences were not as consistent. It was found that liming the soil did not always have a depressing effect on metal uptake of any crop. It was found that Cd was accumulated into lettuce and radish tops to a much greater extent than in radish roots or in tall fescue. Copper was found in higher concentrations in both radish tops and roots than lettuce or tall fescue. Tall fescue, on the other hand, accumulated more Ni than radish or lettuce plants. Lead was found to be present in highest amounts in radish plants while both radish and lettuce crops accumulated more Zn than tall fescue.

All three soil extractants were found to correlate well with Zn uptake for all plant species. DTPA was found to show the highest correlation with plant uptake of metals of the extracts tested. Liming generally reduced r values as compared to unlimed treatments.

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CHAPTER I

LITERATURE REVIEW

The disposal of liquid and solid wastes has rapidly become a major problem for industries and municipalities in the United States. The many different types of wastes and different constituents of these wastes make a universal method of waste disposal impossible. Regardless of the method of disposal, the end product is usually placed into the environment for final deposition by either burying, dumping, or spreading on the land. The land plays a very important role in the disposal, and being a non-renewable resource proper land management is essential.

One industry or process which shares in the problem of waste disposal is the sewage treatment facilities which supply secondary and tertiary waste treatment. The waste from these facilities must be disposed of in an economical and environmentally acceptable manner. One method of sludge disposal which is economical and may also be environmentally acceptable is the application of the sludge to agricultural land as a soil conditioner and fertilizer for the production of crops. Sewage sludge has a potential for supplying part of the nutrient requirements for plants. At a time when fertilizer is becoming costly, approximately 335,000 tons of potential fertilizer each year (19) is being wasted. This total could provide 200,000 tons of N, 120,000 tons of P, and 15,000 tons of K (19). These quantities account for approximately 2.5% of the N, 6.0% of the P, and 0.5% of the K that was sold as commercial fertilizer in the United States in 1973 (19). At the present, only 25% of the sewage sludge produced in the United States is applied on the land (25). If all the sludge currently being produced was applied to agricultural land for fertilization, it would require only 1% of the present available cropland and increase to 2% by 1985 (25).

There are several benefits from applying sewage sludge to the land; however, there are some adverse effects which can be encountered from this practice. The most severe effect is the accumulation in plants of heavy metals which are translocated from the soil in concentrations that may be detrimental to human and animal health. The range of heavy metal concentrations in dry sewage sludge varies widely among different wastewater facilities. Usually the sludges from industrial areas contain higher concentrations of heavy metals. The metals which possess the greatest potential for plant toxicities are zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), and lead (Pb) (19). Residues from sewage treatment plants may also contain pathogenic organisms which survive the treatment process (24).

Crops vary in their reaction to sludge enriched soils. Most crops benefit from the nutrients such as N, P, and K. However, the metals may accumulate in the plant and reach levels that cause adverse effects in the plant. A limiting factor in sludge application to agricultural lands is excessive accumulation of heavy metals and the resultant phytotoxicity to plants. Heavy metal phytotoxicity has been demonstrated with plants grown in solution cultures in greenhouse pots and in field experiments (23).

With the recent increase in ecological concern by the public, there has been a demand for better measures of pollution control which has stopped or reduced incineration, ocean dumping, landfilling, and other disposal practices. Legislation has forced the restriction of these practices and has supported the utilization of wastes for recycling. Regulations such as the Resource Conservation and Recovery Act (RCRA) specify the types of materials and quantities which can be applied to the land which is used for crop production. The act recognizes the need for environmentally sound means for sludge utilization or disposal. The requirements of the act will ultimately result in the production of greater quantities of sludge (24). The act also controls the level of mercury (Hg) and persistent organic chemicals such as pesticides and PCBs in certain components of the human food chain (24). These laws and regulations were developed to insure that the application of sludge to agricultural land be accomplished in a manner which insures cropland resources are protected and that harmful contaminants are not accumulated in the food chain which could result in a risk to public health.

The degree of metal uptake by different crops and specific plant tissue is highly variable and depends upon several soil chemical and physical properties. These properties include pH, organic matter content, crop species, and land management and affect the rate at which metals are taken up and accumulated by plants (26). An excess of certain salts, persistent organic compounds, radionuclides, and other materials in sludges may also influence plant uptake of heavy metals (24). Each of these major factors should be considered individually before an environmentally safe sludge application rate can be determined.

<u>Soil pH</u>

The pH of the soil/sludge mixture should be controlled to keep heavy metal uptake by plants at a minimum. A soil pH of 6.5 or greater will minimize uptake of most heavy metals by most crops (24). As the pH decreases, the solubility of heavy metals increases making these metals more available to crops (17). Under certain conditions, availability of metals present in the soil before sludge application can actually be reduced by the addition of sludge to the soil. This is because the addition of sewage sludge may tend to raise the pH of an acid soil resulting in the binding of heavy metals into organic complexes. However, it is unlikely that the final pH will be greater than 6.5 (24).

Organic Matter and CEC

The soil organic matter content also affects metal uptake by plants. Applications of sewage sludge which are high in organic matter affects metal uptake of crops directly by serving as a source of heavy metals and by altering the soil chemistry which in turn changes the solubility and mobility of metals (7). The chemical change created by additions of organic matter is primarily because of the high CEC of the organic matter and its high chelating ability (19). These properties bind the metals into an organic complex which is maintained at a neutral pH making the metals unavailable for plant uptake. The effect of organic matter on the metal uptake of plants is not permanent. Should the pH of the soil decrease, the organic complexes will be broken making the metals more available to the plants. The organic matter level of the soil will also be changed depending upon the decomposition rate. Dowdy and Larson (8) showed that after a period of sludge incubation, the metal uptake increased. Incubation of soil/sludge mixtures for one growing year prior to cropping resulted in greater removal of metals from an acid soil. This indicates that fallowing acid soils after sludge application and before cropping will increase the availability of metals for plant removal. Metals released by decomposition of sludge in alkaline soil are generally not as available for plant uptake as those in an acid soil (8). Haghiri (9) showed that after the initial additions of sewage sludge, a net concentration of available heavy metals would increase over a number of years. Initially the metals in the sludge will not be available to plants; however, as the sludge decomposes, the organic matter content of the soil will decrease allowing the availability of metals to increase. When the addition of organic matter is discontinued, the organic content of the soil will decrease while the concentration of available heavy metals will increase and eventually may exceed the toxic threshold for crop production (9).

Crop Species

The degree of metal uptake can also be controlled by selecting crop species and/or varieties which are tolerant to metal uptake. Different crop species and varieties differ in their ability to absorb heavy metals. Crops also differ in the location where the metals will be concentrated whether it be in the roots, stems, leaves, or fruit. Vegetables such as lettuce, spinach, chard and tobacco are plants which characteristically accumulate the highest amounts of heavy metals (24). In a study performed by Haghiri (10) using Cd, it was shown that the Cd concentration in descending order for various vegetables was lettuce > radish tops > celery stalks > celery leaves > green peppers > radish roots. The concentration of radish tops was more than two times greater than that of the roots.

Grasses have been reported to accumulate higher levels of heavy metals in the vegetative portions rather than the seeds or roots (24). John and VanLaerhaven (18) reported that Cd concentrations in lettuce tissue varied significantly as a function of both the Cd level in the soil and the different lettuce varieties. These variatal differences could be beneficial in reducing metal uptake by plants by selecting species and varieties that are tolerant to high levels of metals.

Metal Contents of Sludges

There are many trace elements found in sewage sludge; but the heavy metals including Pb, Cu, Zn, Ni, and Cd are generally recognized as possessing the greatest potential to be in toxic concentrations in soil and plants. Table 1 shows the range of concentration of these elements that was found in a composite sample of 42 different sewage sludges (20).

TABLE 1

TYPICAL CONCENTRATIONS OF HEAVY METALS IN SEWAGE SLUDGE[a]
(Dry Weight Basis)

Element	Range (ppm)
Cd	1-1500
Cu	200-8000
N	20-5300
Pb	120-3000
Zn	70-49000

[a]Reference 20.

If these elements are applied to the soil, it is important that management practices be incorporated to minimize the potential of introducing toxic levels of these elements into the food chain. These elements are considered individually in order to determine how plants respond to the addition of these elements into their growing media.

Lead

Lead (Pb) is a non-essential element for plant growth. High levels of Pb are generally found in industrial sewage sludges and along roadsides as a result of burning gasoline containing tetraethyl lead (6). Typical background and sewage sludge concentrations of Pb are 10 and 120-3000 ppm respectively (20). Concentrations of Pb in plant tissues typically range from 0.1 to 10.0 ppm (26).

Lead normally remains in a profile in greatest amounts at the surface adsorbed on organic complexes. It also remains at the surface because some plants absorb it in substantial amounts, and it is also very strongly fixed by the soil (20). The fixation by soils is due to hydrolyzation and polymerization and is inactivated as the pH rises (20).

Lead is generally not translocated to the leaf, stems, or seed except when extremely high concentrations are in the soil. Lead can be taken up by plants in acid soils; however, most of it is retained in the roots (8). Dowdy and Larson (8) found that Pb uptake increased over 50% (6.2 to 9.9 ppm) with the addition of 30 ton/ha sludge to acid soils in barley seedlings and with a sludge containing 410 ppm of lead. In the same experiment, it was shown that Pb uptake from calcareous soils did not increase with sludge applications up to 30 ton/ha. This was probably due to the insolubility of Pb at higher pH levels (8). Bradford et al. (5) found Pb concentrations in beans, tomatoes, and barley at 27.0, 11.0, and 5.4 ppm respectively using an aqueous sludge solution having a Pb concentration of 1120 ppm.

John et al. (16) found that Pb uptake by lettuce was not significantly increased with an application of 25 g sludge/kg soil containing 490 ppm Pb. Using limed and unlimed solutions, the Pb concentration in lettuce leaves were 7.3 and 7.2 ppm respectively. John et al. (16) also showed that 200 mg of extractable Pb/kg soil did not increase Pb concentrations in leaf lettuce. However, it was also reported that an application rate of 1000 mg of extractable Pb/kg soil increased leaf lettuce Pb content to 59 ppm.

Dowdy and Larson (7) using a soil with a pH of 5.3 and a sludge loading rate of 0, 112, 225, and 450 ton/ha and a Pb content of 515 ppm, found that Pb concentration in lettuce leaves at 1.1, 1.4, 0.7, and 0.8 ppm respectively. In the same experiment radish roots were reported to contain 0.5, < 0.4, 0.4, and 0.7 ppm using the same sludge and application rates.

Plants do have a tendency to take up Pb from the soil but at relatively low levels. Since most of the Pb is retained in the roots, it does not represent a threat in contaminating food crops if non-tuberous crops are selected for growing on the contaminated soil. However, since Pb is immobile in soil, long term continuous sludge applications could cause excessive Pb accumulations in surface soil and subsequently could enhance Pb concentrations in food crops (16).

Copper

Copper (Cu) is an essential element to plants and can become phytotoxic at high concentrations. Plants typically have been found to contain 4 to 15 ppm and soils normally contain 20 ppm of Cu (26, 20). When the Cu content reaches 30 ppm in the plant, it is considered to cause toxic effects (20). Sewage sludge normally contains 200 to 8000 ppm Cu (20). The addition of these sludges to the soil creates a potential for plants to absorb Cu at toxic levels.

Copper is not a hazard to the food chain since high levels of Cu will be phytotoxic. Dowdy and Larson (7) indicated that some crops are very effective in excluding Cu when it has been added to the soil at high concentrations. In an experiment using radishes, lettuce, carrots, corn, potatoes, and peas, it was discovered that only the potatoes accumulated increased amounts of Cu with increased additions of a sludge containing 245 ppm Cu. Mitchell et al. (22) also indicated that Cu concentrations in plant tissue were least affected by soil Cu additions, showing significant increases only at 320 and 640 mgCu/g soil. In the experiment, Cu was added to soil solutions ranging from 5 to 640 ugCu/g soil and reported Cu concentrations in lettuce leaves at 6.2 and 9.3 ppm respectively (7).

Several investigators have indicated that Cu uptake, unlike other heavy metals, is not increased by low soil pH. It has been shown that Cu uptake was greater from calcareous soils that acid soils. Dowdy and Larson (8) showed that sludge applied Cu was not absorbed by barley from either acid or calcareous soil containing 610 ppm total Cu. Hyde et al. (13), reported that Cu levels in plants increased primarily in proportion to the total amount of metal applied regardless of soil pH with concentrations of Cu in wheat leaves and grain, Hyde indicated that the plants grown on acid and calcareous soils were essentially the same. Mitchell et al. (23) found that Cu toxicity to lettuce was primarily concentration dependent on acid and calcareous soils. They discovered that at treatment levels of < 60 ugCu/g soil, Cu was more toxic to lettuce grown on the calcareous soil while at higher concentrations the toxic effects were more prominent on acid soils. Lettuce yield in the calcareous soil was significantly reduced at 160 ugCu/g soil while 320 ugCu/g soil was required for a significant yield reduction in the acid soil. Yield reductions in the 320 and 640 ugCu/g soil treatments were, however, much greater in the acid soil reflecting sharp increases in soluble Cu in these treatments (23).

From these experiments, it can be seen that Cu behavior in soils and plants differs from the other heavy metals. It has been found that Cu can be absorbed in calcareous soils as well as acid soils. Unlike other heavy metals, plant uptake of Cu is not always increased with subsequent increases of applied Cu.

Due to these different reactions of Cu in the plant-soil regime, it is difficult to predict the effect of this metal when managing the application of a Cu enriched waste to the soil. Should a Cu enriched sludge be applied to the soil for cropping purposes, the soil and plants should be sampled on a routine basis to insure that toxic concentrations of Cu are not being accumulated in the soil and absorbed by the plants.

Nickel

Nickel (Ni) is a non-essential element for plant growth. Soils normally contain 40 ppm, and plants usually contain 1.0 ppm. Sewage sludge contains from 20 to 5300 ppm Ni (20).

Little has been studied about the tolerance of different species for Ni except that the grasses are found to be more tolerant than other crop species. Nickel differs from other heavy metals in that it is more concentrated in the seeds than in vegetative portions of plants (20).

Nickel uptake of plants is dependent upon soil pH and concentration of the element in the soil. John et al. (16) reported a significant difference in plant tissue and soil Ni with a sludge-lime interaction. Applying a Ni enriched sludge containing 18 ppm Ni to an acid soil significantly increased Ni concentrations in lettuce (16). Mitchell et al. showed 25% yield reductions in lettuce when 60 ug Ni/g soil was added to an acid soil.

Halstead et al. (11) showed in an experiment growing oats in a Ni contaminated soil that Ni uptake was dependent on soil pH and concentrations of Ni added to the soil. There was a repressive effect of Ni on yields when the Ni concentrations reached 60 ppm in oat grain and 28 ppm in oat straw. These concentrations were reached in oat tissue when a total Ni concentration of 100 ppm was added to an acid soil (11). In calcareous soils, toxicity was reduced which may be attributed to its effect in reducing the level of soluble Ni in the soil.

Zinc

Zinc (Zn) is an essential element for plants which typically contain 8 to 15 ppm (26). Soils normally contain 50 ppm of Zn. Sludges normally contain from 500 to 50,000 ppm Zn and if applied to the soil could cause substantial increases of Zn uptake by plants. A toxic level for Zn has been reported at 500 ppm (26). Zinc toxicities are seldom experienced since soils are often deficient of zinc and must be added to the soil to support plant growth. However, high concentrations of soluble Zn such as those found in sewage sludge could cause soil levels to reach toxic levels.

Plant uptake of Zn is dependent upon soil pH and the amount of Zn applied to the soil. Greatest plant uptake occurs at low pH levels and increases as Zn concentrations are increased. Dowdy and Larson (8) found that barley seedlings increased their Zn uptake linearly with increased rates of Zn on acid soils. Using a sludge containing 820 ppm of total Zn, the uptake of this element was increased with the addition of 30 ton/ha sludge on acid soil. The barley grown on the alkaline soil contained 12 ug/100 g soil while the plants on the acid soil contained 62 ug/100 g soil (8).

Mitchell et al. (23) found that Zn was more toxic to lettuce than wheat when grown on acid soil. Using treatments of 10, 40, 320, and 640 ug Zn/g soil on a calcareous soil, the lettuce shoots contained 98, 106, 380, and 1265 ug Zn/g while the wheat leaves contained 67, 80, 189, and 412 ug/g of Zn respectively. Using the same treatments on an acid soil, the lettuce shoots contained 172, 297, 1585, and 1973 ug/g Zn while the wheat leaves contained 66, 81, 406, and 655 ug/g respectively (23).

Dowdy and Larson (7) using a sludge containing 1070 ppm zinc on an acid soil found that lettuce and radish plants accumulated higher amounts of Zn as the sludge treatments increased. Using sludge application rates of 112, 225, and 450 ton/ha, the radishes contained 40, 50, and 98 ppm while the lettuce contained 94, 155, and 225 ppm respectively (7).

Plant uptake of Zn has also been found to be dependent upon crop species. Boawm and Rasmussen (4) categorized plants into three groups: highly sensitive, medium sensitive, and tolerant, depending upon yield decreases due to increased applications of soil Zn. Highly sensitive crops included sweet corn, grain sorghum, barley, and wheat. Medium sensitive crops included alfalfa, English pea, lettuce, spinach, and tomatoes. Tolerant crops to high levels of Zn included clovers, potatoes, field beans, and snap beans.

It has also been reported that grass species accumulate higher concentrations of Zn and had greater reduction in growth than legumes. The maximum yield decrease for grasses ranged from 2% to 80% whereas the legume decreases ranged from 22% to 30%. These results were reported by Boawn and Rasmussen using Zn concentrations of 10 to 500 ppm in a calcareous soil. During the experiment, it was found that even the most sensitive crops tolerated Zn additions of up to 200 ppm without significant reduction in growth (4).

Cadmium

Cadmium (Cd) is a non-essential element for plant growth; however, plants typically are found to contain between 0.2 to 0.8 ppm Cd (26). Soils normally contain from 0.01 to 0.07 ppm. Cadmium can be found in high concentrations in sewage sludge which range from < 1.0 to 1500 ppm.

Cadmium has been one of the most studied heavy metals because of its association with certain health problems such as emphysema, hypertension, and chronic bronchitis (9).

Cadmium has also received great attention because of its ability to be absorbed in excessive amounts by many different food crops (22). Since crops take up Cd very readily, they provide a pathway for entry of the element into the food chain of man and animals.

Factors reported to affect the plant uptake and solubility of Cd in the soil include soil pH, concentration of Cd in the soil, and organic matter content. Increased soil pH decreases Cd uptake. Cadmium availability increases with increasing soil acidity which is due to increased solubility of Cd compounds such as hydroxides and carbonates (22). The lower the pH of the soil, the more Cd is released from the adsorption sites, entering into solution and consequently increasing availability to plants (1).

Mahler et al. (22) reported significant yield decreases for lettuce when grown on an acid soil with a sewage sludge contaminated with Cd. Using a sewage sludge containing Cd, it was discovered that a yield decrement of 50% for lettuce was associated with soil Cd concentrations varying from 98 to 285 ug Cd/g soil for plants grown on acid soil. At the highest Cd addition of 320 ug/g lettuce plants accumulated between 540 and 780 ug Cd/g on acid soils while on the calcareous soil, uptake was 300 ug Cd/g and less for the same treatments (22).

John (14) found that Cd uptake by radish plants was affected by both soil pH and increased concentrations of Cd. Cadmium was applied to limed and unlimed soils in the form of $CdCl_2$. He reported that the accumulations of Cd were greater in the acid soil and also that the radish tops accumulated more Cd than the roots. When Cd was applied at 0.5, 5, 25, 50, and 100 mg/kg soil, the radish tops grown on the acid soil contained 13, 36, 125, 227, and 402 mg Cd/kg tissue respectively while the radish tops on the limed soil contained 14, 30, 70, 105, and 183 mg Cd/kg tissue. Using the same treatments, the radish roots grown in the acid soil contained 8, 10, 34, 53, and 174 mg Cd/kg tissue while the radish tops grown on the limed soil contained 6.3, 8.3, 18, 25, and 28 mg Cd/kg tissue respectively.

Haghiri (10) showed that Cd uptake by plants is also influenced by crop species. Using a soil with a pH of 614 and Cd treatments in the form of $CdCl_2$ at 2.5 and 10 ppm, lettuce, celery, green peppers, and radishes were grown; and the tissue was analyzed for Cd content. It was found that the highest concentrations of Cd were found in lettuce while the least amounts were detected in green peppers and radish roots. At the 2.5 ppm Cd treatment, the lettuce, celery stalks, celery leaves, green peppers, radish tops, and radish roots contained 11, 6, 4, 4, 10.2, and 4.2 ppm Cd respectively. At the 10 ppm Cd treatment level, the same crops contained 27, 15, 11, 6, 16, and 6 ppm Cd respectively. It should be noted that the radish tops contained higher concentrations of Cd than the roots. It was also discovered that a depression in yields was experienced due to the application of Cd. The depression in yield was more prominent for lettuce than any of the other crops. In the study, it was reported that growth due to Cd toxicity can be depressed after plants have accumulated as little as 3 ppm of the metal.

Bingham et al. (3) showed that crops such as spinach, curlycress, and lettuce were very sensitive to Cd. These crops sustained a 25% reduction in yield when concentrations of 4 to 20 ug Cd/g soil was applied. Relatively tolerant crops such as rice, cabbage, and wheat sustained a 25% yield reduction when 20 to 50 ug Cd/g soil was applied. For radishes, it took a concentration of 96 ug Cd/g soil to result in a

25% yield reduction. In general, the lowest Cd concentrations were found in tubers, seed, and fruit tissue while the highest concentrations were found in the leafy tissues.

In a similar study by John (15), lettuce, spinach, broccoli, cauliflower, peas, oats, radishes, and carrots were grown on a soil with a pH of 5.1 with Cd treatments of 40 and 200 mg Cd/kg soil applied as $CdCl_2$. At the 40 mg treatment, the concentrations absorbed by the crops in descending order were carrot roots, pea roots, lettuce roots, radish tops, spinach leaves, cauliflower roots, broccoli roots, and oat roots. At the 200 mg level, the concentrations in descending order were carrot roots, broccoli roots, lettuce roots, pea roots, cauliflower roots, oat roots, spinach roots, and radish tops. At the lower Cd concentration treatment level Cd was accumulated in the roots except for the spinach and radish leaves. The same was true in the higher Cd treatment level except for the radish tops.

From these experiments, it can be seen that Cd is readily absorbed by plants when applied to the soil. Plant absorption was shown to occur when as little as 10 ppm of Cd was applied to the soil. Due to the plants' ability to accumulate high levels of Cd and its known effect to human health, this metal should be examined thoroughly when applying it to the soil. Efforts should be made to minimize the uptake of Cd by plants. Soil pH, Cd concentration, and crop species are factors which should be carefully considered.

Soil Analysis

Considerable research has been performed to determine the best extractant for predicting plant available Cd, Cu, Ni, Pb, and Zn. Varying concentrations of a wide range of acids, weak acids, neutral salts, and chelating agents have been tested as soil extractants with either method being capable of producing adequate correlation for these elements. The correlation depends upon the element being tested and the combination of variables such a pH, CEC, and organic matter.

Hag et al. (12) and Lindsay et al. (21) used nine different soil extractants to determine the correlation between plant and soil Cd, Cu, Ni, and Zn. Included in these extractants were DTPA, ammonia acetate, and acetic acid. The correlation of these extractants are shown in Table 2.

TABLE 2

CORRELATIONS OF PLANT AND SOIL Cd, Cu, Ni, AND Zn[a]

Extractant	Cd	Cu	Ni	Zn
	r			
DTPA	0.35	0.35	0.18	0.65
CH_3COONH_4	0.29	0.29	-	0.81
$(COOH)_2 + (COONH_4)_2$	-	0.31	0.08	0.01

[a]References 12 and 21.

In the preceeding discussion, it was reported that metal uptake by plants was greatly affected by sludge loading rates and soil pH. Also, many researchers have found plant species to vary widely in their ability to accumulate certain metals.

Municipalities today are faced with disposing large quantities of sewage sludge over rather limited areas of land. Consequently, loading rates could have the potential of being excessive, allowing metals to accumulate in the soil system. Therefore, an experiment was designed to evaluate sludge rates above those normally considered safe with respect to metal loading rates.

The objective of this experiment was to determine the effects of various rates of a municipal sewage sludge on the uptake of Cd, Cu, Ni, Pb, and Zn in lettuce, radish, and tall fescue.

CHAPTER II

MATERIALS AND METHODS

A greenhouse experiment was conducted using a Decatur silt loam, a Rhodic Paleudult (clayey, kaolinitic, and thermic). It was obtained from The University of Tennessee (Plant Science Field Laboratory on Government Farm Road) south of Knoxville, Tennessee.

The sewage sludge was obtained from the Third Creek sewage treatment facility at Knoxville. This material was found to contain substantial concentrations of heavy metals which probably originate from industries utilizing the Third Creek facilities. The heavy metal analysis of this material was found to be Cd, 25.5 ppm; Cu, 692.0 ppm; Ni, 108.7 ppm; Pb, 336.5 ppm; and Zn, 1187.0 ppm. The sludge was applied at 10%, 20%, and 30% rates which corresponds to 224, 448, and 672 mt/ha respectively.

The pH level of the soil used in the greenhouse experiment was 5.5 and 6.5 for that which was treated with lime. Sludge treatments consisted of a control — 10%, 20%, and 30% sludge on a dry weight basis. This resulted in two pH levels and four sludge level rates. The experiment was conducted as a 2 by 4 factorial arrangement of treatments in a randomized complete block design with three replications.

The crops grown for the experiment were tall fescue (*Festuca arundinacea*), lettuce (*Lactuca sativa*), and radishes (*Raphanus sativus*). Tall fescue samples were collected three times at 60-day intervals to determine the rate of uptake over an extended period of time. Lettuce tops were harvested 90 days from planting. Radish tops and roots were collected to determine where the plant accumulated heavy metals. Radish samples were collected 60 days after planting. After the vegetation samples were collected, soil samples were collected and prepared for analysis.

Vegetation samples were extracted by the dry ashing method; and the soil samples were extracted by three methods — diethylenetriaminepenta-acetic acid (DTPA), acetic acid, and ammonia acetate. All samples were analyzed for Cd, Cu, Ni, Pb, and Zn.

The plant material was prepared for analysis by drying in a forced-air oven at 70°C, grinding to pass a 2.0 mm sieve, and storing in plastic bags. The ground plant material was analyzed by weighing 0.500 g material in a porcelain crucible and ashing at 500°C for four hours. The ash was allowed to cool, placed in a 50 ml beaker with 10 ml of 1 N HCl, filtered through a Whatman No. 40 filter paper, collected in a 50 ml volumetric flask, and brought to volume with deionized water. The solution was analyzed by flameless atomic absorption spectrophotometry.

The soil was extracted by three selected chemicals as outlined below. With the DTPA method 10.00 g of finely ground soil was placed in a 125 ml Erlenmeyer flask with a 20 ml mixture of 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 MTEA; adjusted to pH 7.3; and shaken for two hours on a rotary shaker. The extract was then filtered into a 50 ml volumetric flask and held for analysis.

The ammonium acetate method was carried out by weighing a 5.00 g sample and placing it in a 125 ml Erlenmeyer flask. To this, 50 ml of 1.0 N ammonium acetate adjusted to pH 7.0 was added; and the material was shaken for 30 minutes on a rotary shaker. The mixture was then filtered into a 50 ml volumetric flask and held for analysis.

Similarly, for the 0.5 N acetic acid procedure, 5.00 g of soil was weighed and placed in a 125 ml Erlenmeyer flask. To this, 50 ml of 0.5 N acetic acid was added; and the mixture was shaken for 30 minutes on a rotary shaker. The mixture was then filtered into a 50 ml volumetric flask and held for analysis.

All analyses were performed on an atomic absorption spectrophotometer using a flameless graphite furnace.

Duncan's Multiple Range Test for mean comparisons and Pearson's test for coefficients of correlations were performed with significant differences being reported at the 0.05 level of significance. Data were analyzed at the Union Carbide Computing Center on an IBM 360/195 utilizing the Statical Analyses System outlined by Barr et al. (2).

The data were analyzed individually for each experiment. Before computing analysis of variance, all variables were tested for normality by computing the Shapiro-Wilk W-statistic. Those variables which were not normally distributed were transformed by use of a natural logarithmic transformation. In all cases the transformed variables were either normally distributed or more closely approached a normal distribution than the non-transformed data.

CHAPTER III

RESULTS AND DISCUSSION

Lettuce Analysis

Heavy metal concentrations of lettuce leaves are shown in Table 3. It should be noted that there were only 18 observations made in this experiment. Missing values occurred in the 10% sludge without lime, 20% sludge without lime, 30% sludge without lime, and 30% sludge with lime treatments. Repeated attempts to grow lettuce in these pots failed to produce plants.

TABLE 3

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn IN LETTUCE LEAVES AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level	Lime rate	Cd	Cu	Ni	Zn	Pb
% by wt.	g/pot	ppm				ln (ppm+1)
0	0	22.1a[a]	51.2a	4.9ab	94.8c	1.1c
10	0	16.4a	53.1a	2.5ab	250.4bc	0.7c
20	0	14.5a	76.5a	11.6a	595.2a	3.4a
30	0	15.3a	66.3a	14.5a	268.6a	2.8ab
0	16.5	18.7a	47.0a	2.0b	71.1c	1.9bc
10	16.5	22.5a	52.8a	5.8ab	288.8bc	1.0c
20	16.5	13.6a	54.3a	6.5ab	703.4a	2.4a
30	16.5	11.6a	47.7a	10.8ab	526.4ab	1.1c

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

It was found that only the Pb and Zn concentrations were increased as sludge rates increased. Cadmium, Cu, and Ni concentrations were found not to increase as a result of increased levels of sludge. However, the Cd concentration had a tendency to decrease as sludge rates increased. The plants grown on the unlimed soil ranged from 22 ppm for the control to 15 ppm for the 30% sludge level while plants grown on the limed soil ranged from 18 ppm for the control to 12 ppm for the 30% sludge level. The relationship of decreasing Cd levels with increasing sludge levels might be attributed to the length of time the plants were allowed to grow in the soils. John et al. (18) found that leaf lettuce concentration of Cd decreased as exposure increased from three to five weeks. Following five weeks of exposure, it was found that Cd concentrations had declined as the Cd levels increased from 10 to 50 ppm. Presumably, the additional growing period diluted plant concentrations, suggesting that the plants grew faster than the Cd was translocated into the plant. In this experiment, the lettuce plants were harvested after a six-week growing period allowing the plants to dilute Cd concentrations. Cadmium uptake was also found not to be affected by liming treatments.

Like Cd, the uptake of Cu by lettuce leaves was not affected by either sludge or liming treatments. These results were similar to those reported by John et al. (17), who also reported similar results with Cu uptake in lettuce.

Nickel uptake was unaffected by increased sludge rates or liming treatments.

Uptake of Pb increased as sludge rates increased. Plants grown on the unlimed soils with sludge levels of 0% and 10% had lower Pb concentrations than the plants grown on the 20% and 30% sludge levels. For the limed pots, the 20% sludge level was the only treatment level showing an increase. The only rate of uptake affected by pH was the 30% sludge level. The ln of the concentrations for the 30% level for the unlimed and limed treatments were 2.8 and 1.1 respectively.

Zinc uptake by lettuce plants was found to increase as sludge rates increased. The 0% and 10% sludge levels had lower Zn concentrations than the plants grown on the 20% and 30% sludge levels. The limed soils showed higher leaf concentrations at the 20% sludge level than the 0% and 10% sludge level. No differences were found between liming treatments. John et al. (18) also reported differences of Zn uptake by lettuce as Zn levels increased in the soil but was not affected by pH.

Radish Analysis

Both radish tops and roots were analyzed to determine if the heavy metals accumulated in the edible portion or were translocated to the leaves or top portion of the plant. Tables 4 and 5 show the uptake concentrations for the radish tops and roots respectively.

Zinc was the only element to show increased uptake with increased levels of sludge application in the leaves. Nickel, Cu, and Zn showed a response to added sludge in the roots. Zinc was found to be translocated into the plant at higher rates than the other metals. Cadmium uptake was not affected by sludge rates or lime treatments for either tops or roots. John et al. (14) also showed that Cd uptake by radish plants was not affected until a treatment of 50 mg Cd/kg soil was applied. This concentration is greater than the Cd concentration in the present experiment which indicates that at only very high Cd levels does the radish plant have a tendency to accumulate the metal. The uptake of Cd by radish plants was also found not to be affected by liming treatments.

TABLE 4

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn IN RADISH TOPS AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level % by wt.	Lime rate g/pot	Cd	Cu	Ni	Pb	Zn
		log (ppm+1)				ppm
0	0	2.4ab[a]	3.8a	1.4bc	2.7ab	78.6d
10	0	2.3ab	3.9a	1.5bc	2.9ab	287.6cd
20	0	2.5ab	4.2a	2.1ab	2.8ab	598.3ab
30	0	2.5ab	4.5a	2.3ab	2.6ab	574.3ab
0	16.5	1.9b	3.7a	0.9c	2.4b	85.0d
10	16.5	3.1a	4.2a	1.9abc	2.6ab	441.3bc
20	16.5	2.7ab	4.5a	2.1ab	3.4a	589.0ab
30	16.5	2.5ab	4.4a	2.5a	3.3ab	731.3a

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

TABLE 5

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn IN RADISH ROOTS AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level % by wt.	Lime rate g/pot	Cd	Cu	Ni	Pb	Zn
		ppm				
0	0	7.0a[a]	36.8cd	10.7b	28.8a	162.6d
10	0	9.1a	60.4bc	10.1b	29.2a	539.7c
20	0	6.6a	71.0ab	12.0ab	25.3a	589.3c
30	0	8.7a	93.2a	14.2ab	33.3a	648.2bc
0	16.5	4.7a	22.6d	3.3c	13.4a	115.5d
10	16.5	7.0a	40.0cd	7.7bc	12.5a	518.7c
20	16.5	9.1a	62.3bc	12.2ab	17.6a	797.6b
30	16.5	8.5a	86.6ab	19.3a	23.8a	994.4a

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

Only the radish roots were found to be higher in Cu concentrations as affected by increased sludge rates. The 20% and 30% sludge levels differed from the 0 level at both lime treatments. The range of Cu uptake for the unlimed and limed soils were from 37 to 93 ppm and 23 to 87 ppm respectively. These results are in contrast to those reported by Dowdy and Larson (19) who reported that radishes were very effective in excluding Cu from their edible tissues. They reported Cu uptake of > 0.3 ppm for all sludge treatment rates.

The uptake of Cu by the tops or roots was not affected by liming treatments.

Nickel concentrations were found to be higher in radish roots than tops. The concentration of Ni in the tops ranged from 2.5 to 12.2 ppm while the roots ranged from 3.3 to 19.3 ppm. Increases in this element were found for the radish roots at the 20% and 30% sludge rate on the limed soils. No differences were found as a result of adding lime to the soils.

Lead accumulated at much higher concentrations in the radish roots than in the tops. No differences were noted as a result of liming this soil-sludge mixture. The root uptake for the unlimed and limed soil ranged from 29 to 33 ppm and 12 to 24 ppm respectively while the tops ranged from 13.5 to 18.2 ppm and 11.0 to 30.0 ppm respectively.

Zinc was the only element to exhibit increased uptake with increased levels of sludge application in the leaves. Zinc was also found to be accumulated at higher concentrations in the roots than in the tops. Uptake rates increased for both roots and tops as sludge rates increased with increases being observed at the higher sludge rates for each plant part.

Tall Fescue Analysis

Tall fescue was harvested for three successive cuttings to determine what effect time and plant maturity have on the uptake of heavy metals. The concentrations for each harvest are shown in Tables 6, 7, and 8.

Data from the first harvest showed that Zn concentrations in tall fescue were increased over the checks at both the 20% and 30% sludge levels with both lime treatments. These increases were again noted in the second harvest with lime. In the third harvest all levels of added sludge increased the Zn concentrations in both the limed and unlimed treatments.

Only in the third cutting did Cd show a consistent increase at the two highest levels of sludge application. A similar trend was shown by the Cu data. In the first and second cuttings sludge levels had little effect on Cu uptake. But in the third cutting Cu uptake was enhanced by each increment of sludge at both lime levels.

With the exception of the second harvest, the highest two levels of the sludge applications caused the Ni uptake to increase. This was true at both lime levels.

Lead uptake was not affected to a large degree by either sludge or lime applications. This is consistent with that reported in the literature.

Comparisons of metal uptake for all plant samples are shown in Table 9. Only the 30% sludge rate was considered in order to determine which plant species accumulated the highest level of metals. It was found that tall fescue was a less efficient accumulator of Cd, Cu, and Zn than the other crops. In this study both Cu and Zn levels in all plants appeared to be rather high. Of the 20 lime no-lime comparisons shown in

TABLE 6

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn FOR THE FIRST HARVEST OF TALL FESCUE AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level % by wt.	Lime rate g/pot	Cd (ppm)	Ni (ppm)	Zn (ppm)	Cu ln (ppm+1)	Pb ln (ppm+1)
0	0	1.7c[a]	7.6c	52.6c	2.8b	2.5b
10	0	4.0ab	9.6bc	117.3bc	3.8a	2.7ab
20	0	5.0a	14.6ab	225.0ab	3.7a	3.0ab
30	0	5.3a	15.6a	277.0a	3.7a	2.8ab
0	16.5	3.3b	10.0bc	58.6c	3.2ab	3.2ab
10	16.5	3.3b	8.6c	117.3bc	3.5a	2.5b
20	16.5	5.0ab	17.3a	224.3ab	3.7a	3.4ab
30	16.5	4.3ab	15.3a	197.0ab	3.6a	4.1a

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

TABLE 7

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn FOR THE SECOND HARVEST OF TALL FESCUE AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level % by wt.	Lime rate g/pot	Cd ln (ppm+1)	Zn ln (ppm+1)	Ni (ppm)	Pb (ppm)	Cu (ppm)
0	0	0.8a[a]	4.5abc	6.3bcd	13.0ab	20.3abc
10	0	0.1b	4.8ab	4.0d	1.0d	25.0abc
20	0	0.9a	4.9a	10.3abcd	8.0bc	27.3ab
30	0	1.2a	5.7a	14.0abc	5.0cd	30.3a
0	16.5	0.9a	3.4c	5.3cd	8.6bc	15.6bc
10	16.5	0.7ab	3.6bc	2.8d	1.5d	12.6c
20	16.5	0.9a	5.4a	15.3a	14.6a	27.6ab
30	16.5	0.9a	5.5a	14.6ab	5.3cd	33.3a

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

TABLE 8

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn FOR THE THIRD HARVEST OF TALL FESCUE AS AFFECTED BY SLUDGE AND LIME TREATMENTS

Sludge level % by wt.	Lime rate g/pot	Cd	Cu	Ni	Pb	Zn
		ln (ppm +1)				
0	0	0.6c[a]	2.1e	0.6e	1.2ab	2.6e
10	0	0.8c	3.6d	1.7cd	0.7b	4.9d
20	0	1.4a	4.8bc	2.0bc	0.9ab	5.9c
30	0	1.5a	5.3a	2.6ab	1.7a	6.4ab
0	16.5	0.6c	2.2e	1.3d	0.7b	2.5e
10	16.5	1.1b	3.6d	1.6cd	0.8ab	5.2d
20	16.5	1.5a	4.5c	2.1bc	1.2ab	6.1bc
30	16.5	1.4a	5.0ab	2.8a	1.1ab	6.5a

[a]Values followed by the same notation within each column do not differ at the 0.05 level of significance, according to Duncan's Multiple Range Test.

TABLE 9

MEAN CONCENTRATIONS OF Cd, Cu, Ni, Pb, AND Zn BY LETTUCE, RADISH TOPS, RADISH ROOTS AND TALL FESCUE AT 30% SLUDGE RATE

Crop	Lime (g/pot)	Cd	Cu	Ni	Pb	Zn
		ppm				
Lettuce	0	15.4	66.3	14.5	15.4	768.6
Lettuce	16.5	11.5	44.7	10.8	1.4	526.4
Radish top	0	11.0	97.6	11.0	13.6	574.3
Radish top	16.5	11.6	91.0	12.8	34.0	731.3
Radish root	0	8.7	93.2	14.1	33.4	648.2
Radish root	16.5	8.5	86.6	19.3	23.8	994.4
Tall fescue	0	4.0	33.3	14.9	13.1	308.2
Tall fescue	16.5	2.8	31.4	15.2	6.2	217.1

Table 9, liming was found to have a depressing effect on the metal uptake of 14 of these comparisons.

Nickel uptake only ranged from 10.8 ppm to 19.3 ppm for all species, which indicates that these species of plants were similar in their ability to accumulate Ni. Radish roots and tops accumulated more Pb than the lettuce or tall fescue.

Coefficients of correlation (r) for tall fescue harvests with radish roots and tops are shown in Tables 10 and 11. Significant r values were observed for Zn at all harvests of tall fescue and for the limed and unlimed soils. The analysis also shows that the second and third harvests correlated for all metals at each liming treatment except for Cd and the third harvest Ni. The only other significant correlations observed were between the first and second harvest for Ni at the unlimed treatment, and between the first and third harvest for Pb at the limed treatment. Therefore, one would expect concentration values to be well related throughout the growing season for Zn, but not for the other metals.

The correlation analysis for radish tissues indicate that significant r values existed between radish roots and tops for Cu, Ni, and Zn at the unlimed treatment, and for Ni and Zn at the limed treatment. Correlation for lettuce tissues were not performed due to the number of missing values which would invalidate the determinations.

Soil Analysis

Soil samples were taken at the end of the experiment and analyzed for Cd, Cu, Ni, Pb, and Zn. Three extractants were used — DPTA, acetic acid, and ammonium acetate. Different extractants were used in order to

TABLE 10

CORRELATION COEFFICIENTS OF Cd, Cu, Ni, Pb, AND Zn CONCENTRATIONS AS RELATED TO HARVESTS OF TALL FESCUE AT TWO LIME RATES
n = 24

Variable	Lime rate (g/pot)	Cd	Cu	Ni	Pb	Zn
				r		
First and second harvest	0	0.002	0.53	0.76[a]	-0.26	0.83[a]
First and third harvest	0	0.69[a]	0.31	0.54	-0.25	0.85[a]
First and second harvest	16.5	0.08	-0.06	0.55	-0.16	0.79[a]
First and third harvest	16.5	-0.03	0.14	0.46	-0.67[a]	0.84[a]
Second and third harvest	0	0.40	0.58[a]	0.66[a]	0.63[a]	0.80[a]
Second and third harvest	16.5	0.11	-0.61[a]	0.62[a]	0.13	0.66[a]

[a]Indicates r was significantly different from zero at $p \leq 0.05$.

TABLE 11

CORRELATION COEFFICIENTS OF Cd, Cu, Ni, Pb, AND Zn CONCENTRATIONS AS RELATED TO RADISH ROOTS, TOPS, AND LIME RATES
n = 24

Variable	Lime rate (g/pot)	Cd	Cu	Ni	Pb	Zn
				r		
Radish root and radish top	0	-0.38	0.72[a]	0.68[a]	0.28	0.69[a]
Radish root and radish top	16.5	0.22	0.41	0.71[a]	0.11	0.92[a]

[a]Indicates r was significantly different from zero at $p \leq 0.05$.

determine the best method for evaluating the plant availability of the various metals. Correlation values between plant uptake and soil concentrations are shown in Table 12.

The r values for linear correlations between Cd uptake in plant tissues and soil concentrations were positive only with DPTA and acetic acid for the tall fescue-unlimed treatment, and with DPTA for the radish root-limed treatment. The Cd correlation coefficients showed that extractable Cd with the methods tested would be a poor predictor of plant Cd.

Copper correlations were found to be significant when compared with extractable Cu for all three extractants for radish root-unlimed and radish root-limed treatments, but in only one instance with the radish tops. As the roots are of more interest with this crop, it is fortunate that the extractants performed well here. This data indicates that all three extractants could be used to predict root uptake of Cu from soil extractable Cu when it is used as the only variable.

DTPA and acetic acid were the only extractants found to give any correlation between plant uptake and soil extractable Ni. Acetic acid correlated with the tall fescue-limed and -unlimed treatments, lettuce-unlimed treatment, radish tops at the limed and unlimed treatments, and radish roots-limed treatment. DTPA was observed to have significant r values for tall fescue-limed treatment, lettuce-limed and -unlimed treatment, radish tops-limed treatment, and radish root-limed treatment. Of the three extractants, acetic acid was the only one which consistently correlated between plant Ni and soil extractable Ni. Of the eight crop-lime treatments, significant correlations were observed for seven of the observations.

TABLE 12

CORRELATION COEFFICIENT OF DTPA, ACETIC ACID, AMMONIUM ACETATE EXTRACTABLE Cd, Cu, Ni, Pb, AND Zn WITH LETTUCE, RADISH TOPS, RADISH ROOTS, AND TALL FESCUE CONCENTRATIONS AT TWO LIME RATES

n = 24

Crop	Lime rate (g/pot)	Soil extractant	Cd	Cu	Ni	Pb	Zn
			r				
Tall fescue	0	DTPA	0.70[a]	0.28	0.49	0.52	0.69[a]
Tall fescue	0	Acetic Acid	0.71[a]	0.31	0.84[a]	-0.05	0.80[a]
Tall fescue	0	Ammonia Acetate	0.31	0.35	0.00	-0.33	0.63[a]
Tall fescue	16.5	DTPA	0.43	0.25	0.31	0.17	-0.78[a]
Tall fescue	16.5	Acetic Acid	0.48	0.29	0.62[a]	0.18	0.79[a]
Tall fescue	16.5	Ammonia Acetate	0.24	0.31	0.00	0.01	0.76[a]
Lettuce	0	DTPA	-0.52	0.45	0.63[a]	-0.59	0.95[a]
Lettuce	0	Acetic Acid	0.00	0.47	0.76[a]	0.37	0.99[a]
Lettuce	0	Ammonia Acetate	-0.45	0.47	0.34	0.56	0.99[a]
Lettuce	16.5	DTPA	-0.29	0.08	0.63[a]	0.59	0.72[a]
Lettuce	16.5	Acetic Acid	-0.30	0.15	0.46	-0.22	0.66[a]
Lettuce	16.5	Ammonia Acetate	-0.49	0.14	0.00	-0.47	0.65[a]
Radish top	0	DTPA	0.26	0.37	0.49	0.34	0.84[a]
Radish top	0	Acetic Acid	0.28	0.60[a]	0.78[a]	0.12	0.95[a]
Radish top	0	Ammonia Acetate	0.24	0.58	0.00	-0.48	0.92[a]
Radish top	16.5	DTPA	-0.04	0.25	0.72[a]	0.01	0.90[a]
Radish top	16.5	Acetic Acid	-0.17	0.24	0.67[a]	0.60[a]	0.94[a]
Radish top	16.5	Ammonia Acetate	-0.13	0.25	0.00	0.37	0.93[a]

TABLE 12 (Continued)

Crop	Lime rate (g/pot)	Soil extractant	Cd	Cu	Ni	Pb	Zn
			r				
Radish root	0	DTPA	-0.13	0.64[a]	0.36	-0.04	0.59[a]
Radish root	0	Acetic Acid	-0.08	0.80[a]	0.43	0.31	0.75[a]
Radish root	0	Ammonia Acetate	-0.06	0.86[a]	0.00	-0.47	0.72[a]
Radish root	16.5	DTPA	0.74[a]	0.83[a]	0.95[a]	-0.23	0.95[a]
Radish root	16.5	Acetic Acid	0.49	0.85[a]	0.91[a]	0.30	0.95[a]
Radish root	16.5	Ammonia Acetate	0.49	0.84[a]	0.00	0.10	0.93[a]

[a]Indicates r was significantly different from zero at $p \leq 0.05$.

Zero r values were found to exist between plant uptake and ammonium acetate extractable Ni. All Ni concentrations performed with ammonium acetate were 1.0 ppm, indicating that this extractant is not favorable to use for Ni extractions.

Neither of the extractants were found to be successful in predicting Pb uptake. The only correlation for Pb was observed with acetic acid at the radish top-unlimed treatment.

All extractants were observed to have significant correlations for all treatments between plant uptake and soil extractable Zn. This indicates that any of the three extractants could be used successfully as a soil test in order to predict plant uptake of Zn. The average r values for DTPA, acetic acid, and ammonium acetate are 0.80, 0.85, and 0.81 respectively.

Finally, soil pH was determined periodically during the course of the experiment. Although each pot was not sampled each time, it was found that soil pH values tended to decrease as the experiment progressed.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Research was conducted on a Decatur silt loam soil amended with three levels of sewage sludge in a greenhouse study with three crops — lettuce, radishes, and tall fescue. The three levels of sludge were 10%, 20%, and 30% by weight mixed with the soil plus a check treatment. Two pH levels were used — the native of 5.5 and a second level of 6.5 was obtained by the addition of lime.

Tissue samples were analyzed for Cd, Cu, Ni, Pb, and Zn; and the soil was extracted for these metals by DTPA, acetic acid, and ammonium acetate.

Most crops were found to be affected primarily by the rate of sludge applied to the soil. Copper and Zn concentrations were increased more by sludge rates than were the other metals. The plant concentration was also dependent upon the plant species. Lettuce was found to accumulate Cd while radish roots and tops accumulated Cu, Pb, and Zn. Tall fescue accumulated slightly higher rates of Ni than the other species.

As stated earlier, metal uptake was primarily affected by sludge rate rather than soil pH. This could be attributed to the pH levels used during the experiment. It was discovered that at the end of the experiment, a portion of the pH of the limed treatments had decreased as low as 5.8. Therefore, the differences in uptake between the two pH levels would not be expected. This decrease in the pH might be attributed to the organic acids released during the biodegradation of the sludge and the nitrification occurring during this same period. This observation should be considered if sludge is applied to the soil for crop production. The soil pH should be adjusted in order to compensate for the acids released during decomposition of the sludge.

The soil analysis indicated that Zn was the only metal examined that could be effectively extracted by all three extractants used. DTPA and acetic acid were found to correlate well with the plant uptake of Ni. The lettuce, radish tops, and radish roots all had significant correlation with DTPA; while the acetic acid was correlated with tall fescue, lettuce, radish top, and radish root concentrations of Ni. Lead did not correlate with either of the extractants used in the experiment. The only significant r values for Cd were between tall fescue, DTPA, acetic acid, and also for the DTPA and lettuce relationship. From the data given, it would be difficult to recommend either of these as a universal extractant for heavy metals.

Considering the metals studied in this experiment, DTPA and acetic acid were the best extractants for predicting plant uptake of Cu, Ni, and Zn. None of the three methods could be recommended for the prediction of plant uptake of Cd and Pb.

Considering the effectiveness of the extractants according to the pH of the soil, it was found that the significant correlations were equal in number for the limed and unlimed soils. Therefore, soil pH would not be a factor in determining the soil extractant.

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