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THE INFLUENCE OF TWO SEWAGE SLUDGE SOURCES
UPON SOIL AND SOYBEAN GROWTH

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THE INFLUENCE OF TWO SEWAGE SLUDGE SOURCES UPON SOIL AND SOYBEAN GROWTH

By

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ABSTRACT

THE INFLUENCE OF TWO SEWAGE SLUDGE SOURCES
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Sewage sludge from two small municipalities were each incorporated at seven rates with the surface material of an Oshtemo sandy loam soil. The yield of soybeans grown in the greenhouse were decreased with each sludge material and each rate.

The sludge containing low levels of heavy metals adversely affected the physical condition of the soil and soybeans would not grow where rates exceeded 103 metric tons/ha. The sludge containing relatively high levels of Cu, Ni, Mn and Cr also contained considerable salt. Therefore leaching was required before soybeans would grow.

The use of this sludge increased the pH, organic matter and cation exchange capacity of the soil. Calcium and Mg levels were also increased while K and P levels decreased. Total Cu, Ni, Zn, Fe, Pb and Cr levels increased greatly while Mn and Cd levels were not greatly affected. The concentration of Ni in the soybean tops tended to increase with rate of sludge while the concentration of Cu, Zn, Mn and Fe tended to decrease. Sludge rates did not significantly affect concentrations of Cd, Pb or Cr.

Metal levels in the soil-sludge mixtures were also evaluated using HCl, DTPA and NH_4OAc as extractants.

To Ibe and Ediccio Jose

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INTRODUCTION

Sewage is a complex mixture of solid and liquid wastes derived from either domestic or industrial sources. Sewage sludge represents primarily the solid material that is precipitated in a sewage treatment plant, which is usually owned or operated by a municipality. Because so many sewage treatment plants are owned by local governments both industrial and doemstic wastes are mixed.

The mixing of waste from several sources results in wide ranges in chemical composition and physical properties. Several treatment processes increase the heterogeneity of sludge materials so that today no realistic average value can be reported to represent the chemical composition of sewage sludge derived from different municipilities.

Disposing of the sludge is a major problem for many municipalities, especially the larger ones. To illustrate the magnitude of the problem, Farrell (34) estimated that 4.7 million dry tons of sludge were produced in the United States in 1972 and that the number would increase to 6.6 million tons by 1985.

Land disposal represents one method of handling the evergrowing amount of sludge. Theoretically sludge should improve the productivity of soil because it contains considerable organic matter. In addition it contains varying quantities of many of the essential plant food elements.

Unfortunately many of the sludges produced today contain varying amounts of heavy metals, most of which at least theoretically can be toxic to plants and animals if present in sufficient quantities. The heavy metals originate primarily with industry. As a result industry is being asked to remove the toxic materials before the sewage is added to that of the city or town.

At the present time the sludge from some municipalities contain relatively large quantities of Cd, Zn, Ni, etc. The sewage from other smaller municipalities, those without industry, contain insignificant quantities of such materials.

The general purpose of this research was to determine the effects of two sludges with widely different chemical properties upon plant growth. More specifically the research was designed to investigate the movement of heavy metals from the sludges into the soil and finally into plants.

REVIEW OF LITERATURE

Copper in Soil

Copper is found in all soils, and its concentration usually ranges from 10 to 80 ppm. In soils, Cu occurs in association with hydrous oxides of Mg and Fe and as soluble and insoluble complexes with organic matter. Copper is found in soils principally as the Cu^{2++} ion. Keeney and Walsh (56) found that the extractable Cu content of sludge-treated soil decreased with time, which suggests that a reversion of the Cu to less soluble forms was occurring. Also, experiments have shown that Cu is adsorbed appreciably by quartz and much more strongly by clays (78, 2). Strong complexing of Cu by soil organic matter is believed to be an important factor explaining why Cu deficiencies are not as prevalent as Zn deficiencies on high pH soils (47, 32), even though both cations show similar decreases in solubility with increase in pH.

Copper compounds have been used for many years as fungicides and bactericides. Continued overuse of Cu containing fungicides and bactericides can result in soil accumulation of Cu toxic to economic crops (28, 45). Reuther and Smith (84) reviewed damage by added Cu to orchard soils in Florida. They emphasized the importance of pH and concluded that Cu adsorption was greatly increased with increased soil pH levels.

Copper in Plants

Copper is required by both plants and animals. The normal range for Cu concentration in plant tissue is between 5 and 20 ppm. When Cu concentration in plants is less than 4 ppm in the dry matter, deficiencies are likely to occur. When Cu levels exceed 20 ppm in mature leaves, toxicities may occur (53).

Many workers have reviewed different aspects of Cu toxicity in vineyards and orchards (27, 84). Since Cu accumulates in the topsoil, several investigators have pointed out that relatively shallow-rooted cereals are more sensitive to excess Cu than deep-rooted perennial plants (22, 38). Copper toxicities may be related to the effects of Cu on the uptake and utilization of other elements by plants. Reuther and Smith (85) showed that continued applications of Cu salts and Cu fungicides induced Fe deficiency in Florida citrus crops. Spencer (96) noted interactions between Cu and P in pot experiments with citrus seedlings.

Also, Cu toxicity may develop in plants from application of sewage sludge if the concentration of Cu in the sludge is relatively high. Copper toxicity to plants from sludge applications was reported by Cunningham et al. (25), who suggested that Cu is about twice as toxic as Zn. Corn and rye took up more Cu from soil treated with an inorganic salt of Cu than from soil treated with sewage sludge supplying an equal amount of Cu (26).

Nickel in Soil

According to Vanselow (106), soils normally contain from 5 to 500 ppm of Ni, with an average of 100 ppm. Soils derived from sandstone, limestone, or acid igneous rock generally contain less than 50 ppm Ni, while those soils derived from igneous rocks may contain from 5 to 500 ppm Ni. In a few areas, soils derived from ultrabasic igneous rocks contain as much as 2000 ppm of total Ni (47).

Nickel behaves similarly to Zn and Cu, although it forms stronger chelate links with organic groups (61). The ability of organic matter to retain Ni up to 2000 ppm has been related to plant growth (88). Nickel toxicity in nature has been observed chiefly on serpentine soils or Ni ore outcrops (22).

Nickel in Plant

Nickel has no known essential function in plants. It is toxic to plants at concentrations above 50 ppm in plant tissues. No significant plant toxicity due to Ni was observed by Cunningham et al. (24). However, Cropper (23) indicated that the addition of Cr, Cu, Zn and Ni as inorganic salts to a sandy soil decreased yields of corn. Patterson (83) found that land application of a sewage sludge high in Ni decreased yields, although liming the soil alleviated the problem. Webber (108) confirmed that decreased toxicity of inorganic Ni salts occurred as soil pH levels increased (39).

Roth et al. (89) used $NiSO_4$ in a pot experiment involving neutral peat, oats and soybeans. Both crops were unaffected at 1000 ppm and damaged at 2000 ppm, at which level the plant content exceeded 50 ppm. Hunter and Vergano (50) indicated that different crops vary considerably in their susceptibility of Ni toxicity. The crops tested and their resistance to Ni toxicity are listed in the following order: barley, wheat, ryegrass, beans, oats, clover, potatoes, turnips, swedes, cabbage, kale and beets.

Zinc in Soil

The total Zn content of soils varies from 10 to 300 ppm (1). In most soils the total Zn content exceeds crop requirements. Zinc availability is the important consideration. Some highly leached soils are very poor in Zn with total values ranging between 10 and 30 ppm. Soil solution concentrations and labile Zn levels in particular are often low, and Zn deficiency may result from the inherently low Zn content of the soil (33).

Zinc, under acid condition, occurs in solution as the Zn^{2+}. The most important mechanisms for Zn retention in soils are sorption on clay and hydrous Fe oxide surfaces and chelation by organic matter. Zinc deficiency in crops has been noted with increasing frequency and it has been suggested that some of this increase is due to field crop removal without the return of animal manure to the soil (107). Copper, Zn and Ni appear to be the metals most likely to become toxic to plants from sludge application (108). Lunt (69) and Rhode (86) attributed the poor growth of crops on sludge--amended soils to the toxicity of both Cu and Zn.

Zinc in Plants

Zinc was one of the first so-called trace elements (micronutrients) known to be essential for both plants and animals. Despite this, problems of Zn nutrition of plants, animals and people are still of pressing importance (27). High levels of Zn are more toxic to plants than animals or humans. King and Morris (57) recently described the phytotoxic effects of Zn contained in liquid sewage sludge. In this example, the yield of rye declined whenever rye foliage contained a concentration of 500 ppm or more of Zn. However, few indications of toxic effects of Zn are found in the literature.

Tiffin (103) and Ambler et al. (3) reported Zn concentrations in xylem exudates from decapitated tomato and soybean plants well above the ambient concentrations. Such an accumulation against a concentration gradient suggests an active absorption of Zn. Many workers have reported that Cu and Zn compete for the same absorption site (92, 32). It has been shown that high levels of Zn in the growth medium can materially lower the concentration of P and Fe in plant tissues. Melton, Ellis and Doll (71) conducted an experiment in which three levels of Zn were added to 20 Michigan soils and pea beans were grown. Pea bean growth was reduced by both Zn deficiency (below 20 ppm) and toxicity (above 50 ppm Zn in plant tissue).

Manganese in Soil

Manganese in soil exists primarily in two valence states as manganous (Mn^{2+}) and manganic (Mn^{4+}) ions. Above pH 8, Mn may be oxidized chemically to MnO_2 by the addition of citric and butyric acids (111) though autoxidation normally occurs at pH 10. Below pH 5.5, chemical equilibria tends to favor the Mn^{2+} ion.

The manganese contained in soils originated from the decomposition of ferromagnesian rocks (102). The quantities present vary from a trace to several thousand ppm (99). The most important fraction in plant nutrition is Mn^{2+}. In addition, easily-reducible Mn contributes to the plant supply. The levels of available Mn^{2+} in soils depend on oxidation-reduction reactions (36, 101). Manganese availability is also higher in acid soils due to the higher solubility of Mn compounds under low pH conditions (64, 82).

According to Geering et al. (37), the Mn^{2+} level of the soil solution of acid and neutral soils is in the range of 10^{-6} to 10^{-4}. These workers suggest that in soil solution Mn is present largely as organic complexes. On the other hand Lindsay (65) found that the affinity of Mn^{2+} for synthetic chelates is comparatively low and complexed Mn can easily be replaced by Zn and Ca.

Manganese in Plants

Manganese is absorbed by plants as the manganous ion, Mn^{2+} and molecular combinations with certain organic complexing agent such as EDTA. Manganese is required by plants in only small quantities, large amounts being toxic.

Like iron and others in the heavy metal group, Mn functions in the activation of numerous enzymes concerned with carbohydrate metabolism, phosphorylation reactions and with other metals in the activation of enzymes such as arginase, cysteine, etc. (62, 79). The rate of Mn uptake differs considerably between plant species. In its chemical behavior, Mn shows properties of both the alkali earth cations such as Mg and Ca and the heavy metals (Zn, Fe).

It is, therefore, not surprising that these ions species affect uptake and translocation of Mn in the plant or vice versa (42, 95).

Manganese excesses or toxicities commonly occur in strongly acid soils or in waterlogged soils. Loew and Sawa (66) found that barley showed toxicity symptoms when tops had 1000 ppm of Mn in the dry matter. Deatrick (29) showed that both the chloride and the sulfate of Mn in high concentrations exerted a toxic effect on wheat. Busler has described manganese toxicity symptoms in detail as mentioned by Mengel and Kirkby (72). Literature comparing toxicity symptoms in high pH levels due to overfertilization with Mn is lacking.

Iron in Soil

Iron makes up about 5% by weight of the earth's crust and is invariably present in all soils. The greatest part of soil Fe usually occurs in the crystal lattices of numerous minerals. This metal is trivalent in soils. The valence in soil applied Fe^{2+} is unstable in the ordinary pH range of soils and in the presence of oxygen (58). The solubility of Fe is largely controlled by the solubility of the hydrous Fe (III) oxides. These give rise to Fe^{3+} and its hydrolysis species (64): $Fe^{3+} + 3\ OH^{-} \rightarrow Fe\ (OH)_3$ (solid). The equilibrium is very much in favor of $Fe\ (OH)_3$ precipitation and is highly pH dependent, the activity of

Fe^{3+} falling with increasing pH. Therefore, acid soils are relatively higher in soluble inorganic Fe than calcareous soils where levels can be extremely low. This may well contribute to Fe deficiency in crops growing on these soils (51, 91).

Iron in Plants

Iron may be supplied to plants as Fe^{2+}, Fe^{3+} or as Fe chelates. However, absorption appears to be dependent on the ability of the root to reduce Fe^{3+} to Fe^{2+} (20). Many microelements cause an interveinal chlorosis, similar to that of simple Fe deficiency when they are present in phytotoxic concentrations (19).

Nicholas et al. (80) showed that Ni additions to soils induced low Fe chlorosis. It is generally recognized that Cu, Ni, Cr, Mn and Zn induce Fe deficiency when present in excess; Hewitt (41) has investigated the action of all these when supplied in nutrient solution and Millikan (75, 76) has dealt with all except Cr. Roth et al. (89) found that Ni and Cu caused chlorosis in oats and soybean in an organic soil at pH 6.5. Sewage sludge applications caused Fe-chlorosis of beets, spinach and bean in experiments by Lunt (68, 69).

Iron toxicity occurs when certain highly weathered Oxisols, Ultisols or Sulfaquepts are flooded. Iron toxicity has recently been identified as the primary cause of "manaranjamiento" disorder of Colombia (18).

Cadmium in Soil

Cadmium concentration in the soil ranges from 0.01 to 7 ppm with 0.06 ppm being common (1). The chemistry of Cd in soil is not well understood, but Cd appears to be influenced by certain soil chemistry properties. For instance, Haghiri (40) showed that the influence of soil organic matter in retarding the uptake of Cd by oat shoots was primarily due to its high cation exchange capacity rather than its chelating ability. Street et al. (98) and Hahne and Kroontje (44) suggested that as Cd formed relatively insoluble precipitates with carbonates, phosphates and hydroxides, it is quite probable that Cd will be less available to plants at higher soil pH values. Santillan-Medrano and Jurinak (96) noted that Cd solubility decreased as soil pH increased. They suggested, as did Street et al. (98), that solid phase $CdCO_3$ and $Cd_3(PHO_4)_2$ control the solubility of Cd in the soil solution.

More recently, Miller et al. (74) investigated Cd uptake and growth of soybeans in relation to soil pH, CEC and available P in nine soils exhibiting a range in these properties. They found that as the pH and CEC of soils increased, the absorption of Cd by soybeans decreased; available P had a positive effect on Cd uptake.

Cadmium in Plants

There is considerable current interest in Cd in plant nutrition. Cadmium and Zn are chemically very similar (35). Cadmium is thus able to mimic the behavior of the essential element Zn in its uptake and metabolic functions. Unlike Zn, however, CD is sometimes toxic to both plants and animals.

Cadmium is absorbed by roots and readily translocated to shoots, but most Cd is absorbed on the roots of important food crops (54). Cuttler and Rains (17) made a detailed study of the mechanism of Cd uptake by roots and concluded that diffusion coupled with sequestration might account for the accumulation of Cd in barley tissue.

Bingham et al. (8, 9) found that Cd concentration in the dry matter of edible tissues of various crops ranged from 1.7 to 80 ppm and that Cd additions to soils caused a 25% yield reduction. Haghiri and Shaeffer (40, 93) found that an increase in soil temperature led to an increase in uptake of Cd by crops whether the added Cd was inorganic or sludge-borne. Andersson and Nilsson (4) found no increase in Cd in rape fodder after 15 years of sludge applications.

Because many soil chemical properties are involved in the solubility of Cd and its subsequent uptake by plants, management needs to consider ways to keep the concentration of Cd in food and feed crops at a low level in sludge-treated soil.

Lead in Soil

Lead is a normal soil constituent with widely, varying concentrations generally in the 1 to 200 ppm range (52, 100). However, a few investigators have reported higher soil lead contents (16). In general, these higher levels occur near smelters or along heavily traveled highways and may range up to 1000 ppm or even beyond.

Plants take up Pb in the ionic form from soils. The amount of Pb take-up from soil decreases as soil pH levels increase, cation exchange capacity levels increase, and as available phosphorus levels of the soil increases (43). Soluble Pb added to soil reacts with clays, phosphates, carbonates, hydroxides and sesquioxides. Such reactions greatly reduce the solubility of Pb (55).

Baumhardt and Welch (10) added up to 1400 ppm Pb to corn and found no detrimental effects on growth or grain yield. Lagerwerff (59) found that a 10 fold increase in extractable Pb did not double Pb uptake by radish. Low Pb uptake by plants is probably due to the rapid fixation in soils (15). Baerut and Martinsen (6) found that increasing amounts of Pb in sludge did not result in higher concentration in the tubers.

Lead in Plants

Lead is a nonessential element that exhibts a low degree of potential toxicity to plants and a high degree of potential toxicity of animals (11). From the viewpoint of plant nutrition it is important to remember that Pb pollution mainly arises from airborne sources.

Toxic effects of Pb can result in a reduction in plant growth but this is not generally seen in the field and almost all detailed observations of Pb toxicity in plants are restricted to water culture experiments (12). The reason for Pb toxicity in plants is not clear at this time.

Today studies have centered on Pb uptake from soil sources, but the results of such investigations are inconclusive. Marten and Hammond (70) studied Pb uptake by bromegrass from sandy loam soils with a range in Pb content between 12 and 680 ppm. Results indicated that only plants grown in the soil with the 680 ppm level accumulated a significant amount of Pb. This accumulation was enhanced by the addition of a chelate, but the maximum accumulation was only 34 ppm. Rolfe (87) stated that most of the Pb taken up by plants seems to accumulate in the root system, and appreciable amounts are only translocated to leaves at relatively high soil Pb levels. This author has reported significant increases in levels of Pb in leaves. These investigations involved eight tree species grown in soils containing Pb in the range 75 to 600 ppm.

Chromium in Soil

Concentration of Cr found in soils range from 5 to 300 ug/g of soil (60). Chromium forms many pretty colors in solutions; hence, its name. Within the ranges of Eh and pH normally found in soils, it has the capability of existing in four states--two trivalent forms, the Cr^{3+} cation and the CrO_2^- anion, and two hexavalent anion forms, $Cr_2O_7^{2-}$ and CrO_4^{2-}. The trivalent cations tend to coordinate in octahedral configurations with oxygen--and nitrogen containing ligands (73). Bartlett and Kimble (13) found that Cr III formed a complex with soil organic matter at low pH levels and appeared to remain stable and soluble even when soil pH's were raised to levels where the Cr would be expected to precipitate. The hexavalent form of Cr in soils is of particular concern because this form is toxic in low concentrations to both plants and animals. There is evidence that it can be mobile in soils (104). Deutsch (30) reported ground water contamination by Cr (VI).

There have been few studies of the chemistry of Cr added in sludge to soils. The concentration of Cr in sludges ranges from very low values to well over 20,000 ppm. The decomposition of sludge in soils is likely to progress at a sufficiently slow rate so that the released Cr will change to insoluble compounds without a build-up of appreciable levels of soluble Cr in the soil (105).

Chromium in Plants

Chromium is one of the most recent additions to the list of elements essential in the nutrition of man and animals. Chromium in leafy plants has not been found to be nutritionally effective. Seed crops, especially wheat, contain very low levels of Cr (109). Although some instances of plant responses to soil applications of chromium have been reported, tests of the essentiality of Cr to plants growing in highly purified culture solutions have been negative (48).

Mortvedt and Giordano (77) reported that in greenhouse experiments the Cr concentration in forage maize was unaffected by the Cr content of municipal waste. In contrast, equal levels of Cr added as sodium dichromate were highly toxic, presumably as a result of higher availability. Trivalent Cr, added as Cr sulfate was found by these authors to be considerably less toxic to maize than hexavalent Cr.

Toxicity effects have been observed by Hunter and Vergano (49) in oats supplied with excess of 5 ppm Cr as Cr sulphate. Plants suffering from severe Cr toxicity had small roots and narrow brownish red leaves, covered in small necrotic spots. Soan and Saunder (94) reported that the Cr content of tobacco roots were 20 times the content of the leaves of plants showing Cr toxicity. Cropper (14) reported Cr toxicity symptoms of corn similar to those reported by Soane and Saunder.

EXPERIMENTAL METHODS

Materials

Soil

An Oshtemo sandy loam (Soil Management Grout 3a) was selected for this study. Surface soil samples (0-20 cms) were collected and composited from the Warren Malkin farm in Clinton County (NW¼, SE¼, Sec 21, Victor Twp.). Soybeans were growing in the field at sample time. The soil was air-dried, screened through a sieve and stored in plastic containers. Samples for laboratory work were passed through a 2 mm stainless steel screen.

Plant nutrient analysis, pH, cation exchange capacity and the organic matter content were determined by Standard procedures in the M.S.U. Soil Testing Laboratory (112).

Sludge

The experimental plan included two types of digested sewage sludge, one poor and one rich in heavy metals. One sludge was collected from the Dimondale (Eaton County) sewage treatment plant, where the wastewater does not receive any chemical treatment. This sludge is derived primarily from domestic sources and is low in heavy metals. Diamondale has a population of approximately 1000 people. The other sludge was from the Saline (Washtenaw County) sewage treatment plant and was chemically treated with materials such as $FeCl_3$, Polymers, Zimite, Cl^- and Lime. This material represents a city/ industrial area and is high in heavy metals. The population of Saline is in excess of 6000.

Both sludges were transported in metal barrels lined with polyethylene bags to the M.S.U. soil barn. After air drying, they were ground to pass a sieve and then stored in plastic containers.

The sludges were analyzed for plant nutrients and total heavy metals by standard procedures (97).

Greenhouse Studies

The effect of sludge-borne heavy metals (in both low and high quantities) on growth and mineral composition of soybeans (Glicine max L. Amsoy) were evaluated in the greenhouse. A completely randomized treatment design was used in the low and high quantity sludge-borne heavy metal experiments. Six treatments in quadruplicate were involved. Sludge rates in both experiments were: 0, 26, 52, 103, 258, 515, 1030 tons (metric)/Ha. The rates used in these experiments far exceed the recommended rates and were intentionally high to induce maximum possible effects.

The sludges were hand mixed with the soil, but homogeneity was difficult to obtain. Also, it was discovered that the sludge adversely affected the soil water relations. As the sludge rates increased an extra quantity of water was required to reach field capacity. With the sludge from Diamondale, high rates caused poor drainage. Therefore, the higher treatments were not included in the research.

The soil amended with the sludge which contained the high heavy metal levels was placed in 2-liter plastic containers containing drainage holes to permit leaching of excess salts. The soil mixture was leached with sufficient water to bring the specific conductivity of the leachate to below 3 mmhos/cm. After drying, the soil/sludge mixture, as well as the soil amended with the sludge containing low heavy metal were placed in plastic lined pots and arranged on greenhouse benches to have a completely randomized design. The controls (without sludge) and sludge-amended soil treatments were not directly fertilized since fertilizer had been used in the field before the soil was collected.

All of the treatments received the proper amount of water to maintain the moisture level at field capacity. The soil was incubated aerobically two weeks prior to planting soybean. Ten soybean seeds were planted per pot. After emergency the seedlings were thinned to five plants.

Harvest was made after four weeks of growth. Plants were cut at the soil surface level, washed in 0.1 N HCl, and then twice in distilled water. The plant material was then put in paper bags and dried at 60°C. The plant samples were ground in a Wiley Stainless Steel Mill with a 20 mesh screen. Soil from the pots was air dried, screened (2 mm) and then placed in the oven for 24 hours at 60°C.

Plant Chemical Analysis

Chemical analysis of plant material involved a 0.5 g sample predigested with 15 ml of nitric acid and then digested with perchloric-nitric acid (2:1) in a 100 ml Kjeldahl flask. The total volume was brought up to 50 ml with deionized water and analyzed for Cu, Ni, Zn, Mn, Fe, Cd, Pb and Cr using a standard atomic absorption spectroscope.

Calcium and Mg levels were determined with a Perkin Elmer 303 atomic absorption spectrophotometer. K and P levels were evaluated with the use of a flame emission spectrophotometer and technicon autoanalyzer II (88 mm), respectively. Total nitrogen levels were determined by the semimicro-kjeldahl method (97).

Soil Chemical Analysis

Samples, 0.5 g from each pot, were predigested with 20 ml nitric acid, then digested with perchloric nitric acid mixture (5:1) in teflon beakers on a hot plate and evaporated to dryness. The final digestion was made using a $HF-HClO_4$ mixture (5:1), then dissolved in 6 N HCl (5 ml) and completed up to 50 ml for total heavy metals analysis by atomic absorption spectrophotometry. The M.S.U. soil testing laboratory analyzed the soil mixtures reporting pH, available P, exchangeable K, Ca and Mg, total carbon and cation exchange capacity.

Samples from soils amended with sludge high in heavy metal were analyzed with the atomic spectrophotometer after making three different extractions described as follows:

0.1 N HCl: Five grams of soil and 50 ml 0.1 N Hcl were shaken for 1 hour.

DTPA: Five grams of soil and 50 ml of a solution containing 0.05 M DTPA 0.1 M TEA and 0.01 M $CaCl_2$ (pH 7.3) were shaken for 1 hour.

NH_4OA_c: Five grams of soil and 50 ml of NH_4OAc (pH 4.8) were shaken for 1 hour.

RESULTS AND DISCUSSION

Chemical Characteristics of Sludge and Soil Material

From the time this research was initiated it was clearly evident that the two sludges had greatly different chemical and physical properties and that their effects upon soil and soybean growth were distinctly unique. For identification purposes and to facilitate discussion the sludge from Dimondale will be referred to as "D" since it originates primarily from domestic sources and contains little or no material from industry. The sludge from Saline will be referred to as "MI" since it represents a composite of municipal and industrial waste. This sludge is high in heavy metals.

Some of the chemical characteristics of the two sludges and the soil material used in this research are reported in Tables 1 through 3.

The information in Table 1 suggests salt and organic matter levels in the sludge are distinctly higher than in the soil material and that these affect the electrical conductivity and the cation exchange capacity. In Table 2, it is important to distinguish between "total" nutrients which are reported for the sludge materials and

TABLE 1.--Chemical Characteristics of Sludge and Soil Materials.

Chemical Characteristics	Sludge D	Sludge MI	Soil
pH	6.7	6.8	6.3
Electrical Conductivity mmhos/cm	5.4	4.5	.3
Organic Matter %	35.6	25.1	2.2
Cation Exchange Capacity meq/100 g			8.9
Total Nitrogen %	5.45	1.75	.01

TABLE 2.--Major Nutrient Levels in Sludge and Soil Materials.

	mg/kg Dry Matter		
Chemical	Sludge D	Sludge MI	Soil
Ca	13,085	21,580	600
Mg	2,505	3,570	40
K	10,550	10,150	135
P	23,400	30,000	245

TABLE 3.--Heavy Metal Levels in Sludge and Soil Materials.

	mg/kg Dry Matter		
Chemical	Sludge D	Sludge MI	Soil
Cu	365	5,217	41
Ni	25	10,785	13
Zn	1,501	1,076	38
Mn	195	346	264
Fe	11,252	14,456	9,139
Cd	8	8	1
Pb	201	285	30
Cr	245	5,496	89

"available" nutrients for the soil material. The "available" levels are those conventionally reported by the soil test laboratory. Available forms are those that are considered to be in such a state that they can be utilized by plants. They are either relatively soluble or in an exchangeable form.

In contrast with the information in Table 2, the data in Table 3 are all for total heavy metals. In addition to other differences between the two sludges the MI sludge is characterized by exceptionally high Cu, Ni and Cr levels. This was known before the research was initiated and in reality served as a basis for this thesis.

Chemical Characteristics of Soil: Sludge Mixtures

Some chemical characteristics of the soil-sludge mixtures are reported in Table 4. Soil reaction (pH) levels tended to increase with rates of sludge D, while the increase was definite for each rate of sludge MI. This is an important consideration because the solubility and availability of both plant nutrients and heavy metals is regulated by pH levels (81).

Organic matter levels decreased, as measured in percent, when small increments of sludge were used. However, where the higher rates were used, in excess of 100 t/ha, organic matter levels increased greatly. This is explained as a dillution effect. An explanation of the decrease was greater with sludge D which contained 10% more organic matter is not evident at this time. Since the samples for analysis were taken after an incubation period and after the soybean crop was grown, it could be related to the rate of decomposition of the organic matter in the sludge. Possibly there was greater microorganism activity in the sludge D soil mixture.

Theoretically the check pots of soil should have had the same cation exchange capacity (CEC). A slight difference, however, was obtained in that in the set with

TABLE 4.--Chemical Characteristics of Sludge-Soil Mixtures.

	Sludge D tons (metric)/ha				Sludge MI tons (metric)/ha						
Chemical Characteristics	0	26	52	103	0	26	52	103	258	515	1030
pH	6.0	6.0	6.4	6.3	6.0	6.5	6.8	7.2	7.3	7.4	7.5
Organic matter (%)	2.8	1.7	1.9	2.9	2.7	2.4	2.4	3.2	4.7	6.7	9.5
Cation exchange capacity (meq/100 g)	7.4	8	7	8.7	6.6	5.9	6.6	7.3	11	16	24
---------- Kg/ha ----------											
Ca	1672	1912	2181	2359	1403	1763	1942	2480	4092	6093	11350
Mg	57	131	179	242	30	66	63	66	116	156	205
K	157	193	273	428	123	105	90	74	76	72	54
P	581	747	791	1104	525	503	413	402	271	162	37

sludge D treatments, the CEC averaged 7.4 meq/100 g, and with the other set averaged 6.6. This possibly could reflect the effect of the leaching process used with sludge MI that was necessary before soybeans would grow.

CEC levels with sludge D varied slightly, but with sludge MI there was an increase with each of the extra amounts of sludge used. It is theorized that this indicates that much of the organic matter in sludge MI was in a relatively stable colloidal form. It seemingly was more resistant to microbial decomposition than sludge D.

In evaluating CEC levels, it is also important to recall the exceptionally high heavy metal levels in this sludge (sludge MI). It is possible that the levels of heavy metals reduced the activity of the organic matter decomposing microorganisms, thus influencing the CEC levels.

The relatively high CEC levels represent a desirable situation especially when heavy metals are added to the soil. The higher the CEC, the greater the ability of the soil mixture to bind heavy metal and other toxic materials. They then become less available to plants (40).

As previously shown (Table 2) both sludge materials contained large amounts of both Ca and Mg which undoubtedly explains the high pH levels as related to rates of sludges. Exchangeable Ca and Mg levels in the soil-sludge mixtures increased proportionally to the amount of sludge used. The sludge also contained relatively large amounts of total K. When sludge D was mixed with soil material, exchangeable levels of K increased greatly. In other words, this sludge is a good source of K.

The opposite occurred with sludge MI. Exchangeable levels of K decreased with increased rates of this sludge. An explanation of this is not available at this time, but obviously a tremendous amount of K was fixed into nonexchangeable or unavailable forms.

While both sludge materials contained relatively large amounts of P, the effects upon soil test levels were distinctly different. With sludge D, available P levels increased with sludge rates. The opposite occurred with sludge MI. One explanation to this situation is related to the metal composition of the two sludges. With low metal levels in sludge D, there was little opportunity for the P to react with the metals to form relatively insoluble compounds. Since the metal content of sludge MI was relatively high, the more soluble forms of P in the soil could react with the metals to produce forms of phosphates which are relatively insoluble and unavailable to plants.

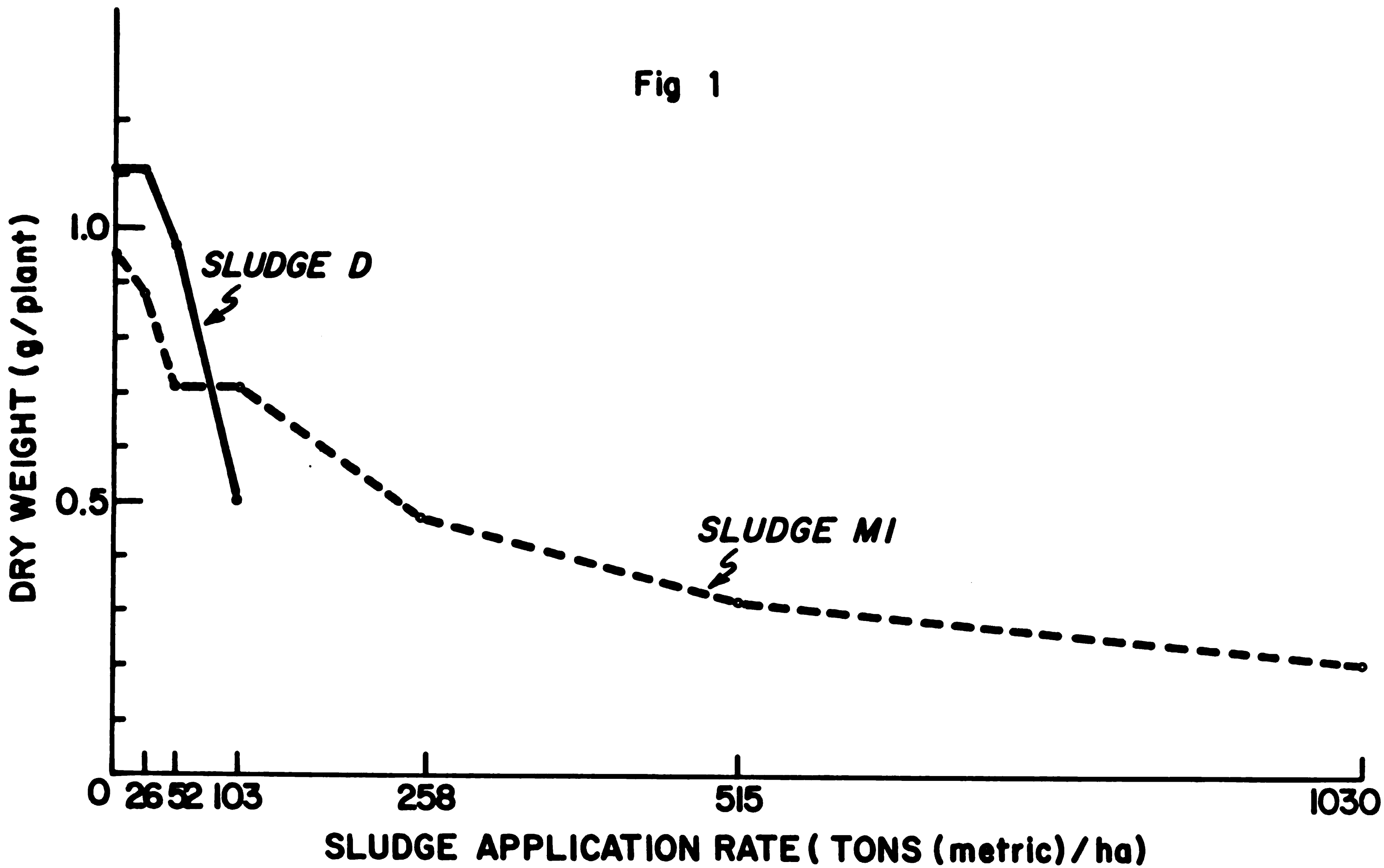
Soybean Yields

The dry matter yields of soybean tops as affected by two sources of sludge are shown in Figure 1. No yields are reported for the three highest rates of sludge D because this material adversely affected the physical condition of the soil and no way could be discovered to eliminate this situation. Sludge D is an exceptionally hydrophilic material and when used at rates in excess of 103 metric tons/ha water would not enter the soil-sludge mixtures. This situation was not anticipated since there is no reference to it in the literature. The experience, however, is significant and should be evaluated by others who anticipate using this sludge or similar materials in field crop production or in other research projects.

Dry matter production decreased significantly with increasing levels of both sludges. The plants grown in the soil enriched with MI sludge showed growth and color differences during early stages of plant development (two weeks after emergence). Seedling emergence was uniform in both soil-sludge mixtures. However, the soybean plants exposed to MI sludge were stunted and more chlorotic in comparison to the control treatment. In addition, leaf size and number of trifoliate leaves were reduced by the higher rates of MI sludge. In contrast, the soybeans grown in the D sludge pots appeared to be normal.

Figure 1.--Yields of dry matter as influenced by soil-applied sludge.

Fig 1



DRY WEIGHT (g/plant)
1.0
0.5
SLUDGE D
SLUDGE MI
0
26
52
103
258
515
1030
SLUDGE APPLICATION RATE (TONS (metric) / ha)

While root development was not part of this research, certain observations should be reported. Root size and distribution were similar in all of the pots that contained sludge D. This was not the situation with sludge MI. Dense and healthy roots filled the soil volume where rates up to and including 103 metric tons/ha were used. Root volumes and size were greatly reduced in those pots where rates in excess of this level were used. As will be shown, levels of heavy metals in the soybean tops were not sufficient to cause a phytotoxic condition. Since roots were not analyzed, it is assumed that there may have been some heavy metal accumulation in or on the roots which contributed to a reduction in top growth. Such an evaluation is based on the research of Wells and Whitton (110), who observed that in tomato plants, the heavy metal concentration in roots were more closely related to soil concentrations than the concentrations in leaves or fruit.

Macronutrient Levels in Soybean Tops

Levels of the macronutrients Ca, Mg, K, P and N in soybean tops as well as yields are reported in Table 5. Calcium levels in the tops of soybeans grown on sludge D decreased with yield ($r = 0.99$). The relationship was not as strong with sludge, but the trend was significant ($r = 0.94$). See Table 6.

TABLE 5.--Yield and Macronutrient Content of Soybean Plants.

Treatment	Yield	Concentration (in percent)				
		Ca	Mg	K	P	N
Tons metric/ha	g/plant					
<u>Sludge D</u>						
0	1.11	1.41	0.44	2.02	0.23	3.43
26	1.12	1.57	0.47	2.69	0.65	4.97
52	0.97	1.22	0.47	2.82	0.80	5.43
103	0.51	0.54	0.47	2.31	0.68	6.76
<u>Sludge MI</u>						
0	0.95	1.13	0.34	1.70	0.40	1.82
26	0.88	1.10	0.42	1.46	0.46	2.10
52	0.71	0.90	0.43	1.62	0.40	2.53
103	0.71	0.65	0.40	1.50	0.20	3.30
258	0.47	0.58	0.37	1.23	0.23	5.70
515	0.32	0.43	0.36	1.00	0.30	7.80
1030	0.21	0.46	0.27	1.12	0.36	8.30

TABLE 6.--Simple Correlation Coefficients Between Soybean Yield and Rates of Sludge, Macronutrient Levels in Plants and Rates of Sludge and Between Macronutrient Levels in Plants and Yield.

	Sludge D		Sludge MI	
Yield	Rates	Yield	Rates	Yield
Yield	-0.95**	--	-0.90**	--
Element				
Ca	-0.92**	0.99**	-9.76*	0.94**
Mg	NS	NS	-0.85*	NS
K	NS	NS	-0.79*	0.91**
P	NS	NS	NS	NS
N	-0.97**	-0.86*	-0.91**	0.98**

*, **Significant at the 0.05 and 0.01 levels, respectively.

In contrast with Ca, Mg levels of soybeans grown in sludge D were not affected. This was not the situation with the sludge MI treatments where Mg levels varied between 0.43 and 0.27%. The concentration of Mg in the tops tended to decrease with both yields and increased rates of sludge.

Yields and rates of sludge D, as well as K levels in soybean tops, were not closely related. K levels in the tops of soybeans grown on sludge Mi were almost one-half those grown on sludge D. Yields were also less. In contrast with what occurred with sludge D, there was a significant correlation between K level and yield ($r = 0.91$) with sludge MI.

Phosphorus levels in soybean tops were increased with the use of sludge D, but were not closely associated with yield. The levels in tops that were grown in sludge MI were somewhat lower than those grown on sludge D and also were not closely related to either yield or rates of sludge.

In contrast, N levels in soybean tops increased with rates of application of both sludges. Nitrogen levels increased as yields decreased. The correlations of N levels with yields were both negative and significant to the 0.05 and 0.01 levels, respectively ($r = -0.86$ for sludge D and $r = -0.98$ for sludge MI). An explanation to the differences in macronutrient composition of soybean tops as affected by two sources of sludges is not clearly evident, but is thought to be closely related to soil-plant relationships as affected by the rates of sludge used. Since root size was reduced by increasing increments of sludge MI, the levels could be partially explained on the basis of nutrient mobility, N having the most and P the least.

Heavy Metals in Soybean Tops

Considering the wide variations in heavy metal levels within the two sludges, wide levels within the soybean plants were expected. Heavy metal levels in soybean tops are reported in Table 7. Copper levels were relatively stable and not greatly affected by the relatively high levels in either sludge. Mean Cu levels in plants treated with both sludges were identical, equaling 31.1 ppm.

These observations are in agreement with those of Dowdy and Larson (32), who worked with barley. They suggested that the Cu may be immobilized by the organic matter in the sludge or that they are not translocated to the top. As was the situation with most of the metals, the use of low rates of sludge, especially sludge MI, had little effect upon Cu uptake (Table 8). Increasing rates of both sludges tended to reduce the uptake of Cu.

The Ni levels in the tops of plants grown with sludge D treatments, were not determined because Ni levels in the sludge were low. This was also the situation with Cd, Pb and Cr.

The use of sludge MI always greatly increased the uptake of Ni, but the level was not closely related to either sludge rate or yield (Table 8). Some researchers (5) report that Ni toxicity is a possibility when soil is treated with high Ni sludges. No visual symptoms of Ni

TABLE 7.--Yield of Dry Matter and Metal Concentration of Soybeans Grown on Sludge-Amended Oshtemos Sandy Loam.

		Plant Metal Concentration							
Treatment	Yield	Cu	Ni	Zn	Mn	Fe	Cd	Pb	Cr
Tons metric/ha	g/plant	ppm							
Sludge D									
0	1.11	32		31	74	83			
26	1.11	32		76	141	109			
52	0.97	30		78	290	118			
103	0.51	32		83	308	92			
Sludge MI									
0	0.95	29	8	39	145	100	< 1.4	15	< 3
26	0.88	26	30	18	224	83	1.4	16	< 3
52	0.71	32	46	27	187	92	1.4	18	< 3
103	0.71	35	39	25	104	75	1.4	21	< 3
258	0.47	32	37	21	77	66	1.4	22	< 3
515	0.32	30	38	21	64	75	1.4	18	< 3
1030	0.21	35	56	22	44	75	1.4	18	< 3

TABLE 8.--Yield of Dry Matter and Metal Uptake of Soybeans Grown on Sludge-Amended Oshtemo Sandy Loam.

		Plant Metal Concentration							
Treatment	Yield	Cu	Ni	Zn	Mn	Fe	Cd	Pb	Cr
Tons/acre	g/plant	----------	----------	----------	mg/plant	----------	----------	----------	----------
Sludge D									
0	1.11	35		35	82	92			
11.5	1.11	35		84	157	121			
23.0	0.97	29		74	281	114			
46.0	0.51	16		72	157	47			
Sludge MI									
0	0.95	27	7	37	138	96	1.3	14	3
11.5	0.88	23	27	16	198	73	1.2	14	3
23.0	0.71	23	33	19	132	65	1.0	13.0	3
46.0	0.71	25	28	18	74	53	1.0	15	3
115.0	0.47	15	17	10	36	31	0.7	10	3
230.0	0.32	10	12	7	21	25	0.5	6	3
460.0	0.21	7	12	5	9	15	0.3	4	3

toxicity, as previously described, occurred on the soybeans as they were grown in the greenhouse. Reduced yields at the higher rates of sludge MI are attributed to a combination of several factors and not just high Ni levels.

The Zn concentration in soybean tops increased with the rates of sludge D and tended to decrease slightly with sludge MI (Table 7). The higher levels associated with sludge D is explained by the fact that sludge D contains higher concentrations of this metal. As frequently occurs, uptake of Zn tended to decrease as the growth of plants decreased. This was the situation for both sources.

Degree of oxidation or reduction regulate the solubility of such metals as Fe and Mn which could partially explain the greatly increased concentration of Mn with increased rates of sludge D. In contrast, Mn levels decreased with increased rates of sludge MI. In explaining these data it is also important to note that MN levels in sludge D are significantly lower than in sludge MI (Table 3). Also pH levels tend to be higher in soil-sludge MI mixtures (Table 4). As with all of the other metals, the uptake of MN decreased with increased rates of sludge MI (Table 8).

Levels of Fe within the soybean plants associated with both sludges varied so greatly, they are difficult to interpret. In contrast with this, the uptake of Fe decreased as the rate of sludge MI increased. This was associated with a decrease in yield of soybean tops.

Cadmium levels in soybean plants as affected by sludge MI were very low, less than 1.4 ppm. The uptake of this metal was proportional to the yield. Levels of Pb in plant tissue were increased slightly with increased rates of sludge MI. Uptake, however, tended to decrease with increasing amounts of the sludge. Such observations have been previously made (6, 63).

Levels of Cr in plants grown with sludge D, for reasons previously mentioned, were not determined. Levels in soybean tops were less than 3 ppm with the MI sludge. This is in agreement with the data of Dowdy and Ham (31) who found that Cr in sludge is not sorbed by soybean plants.

Total Metal Levels in Soil-Sludge Mixes and Soybean Tops

Frequently there is a poor relationship between total metal content of a soil and utilization of the metal by plants. This explains why total analyses are not used as a basis for fertilizer recommendations and why modern research on waste materials sometimes reports metal contents of soil sludge mixtures on an "extractable" basis.

The total metal analysis for soil-sludge MI mixes is reported in Table 9. Sampling time occurred after the soybean harvest.

As anticipated, the metal content of the mixes tended to increase with an increase in rate of sludge. Levels of Cu, Ni, Zn, Fe and Cr increased dramatically because they were present in the sludge in large amounts. Levels of Mn, Cd and Pb increased only where relatively high rates of sludge were used because the sludge contained very low levels of these metals.

The effect of rates of sludge upon metal content of soybean tops are reported in Table 10. Levels of Cd and Cr were low and were not affected by the use of the MI sludge. Copper levels were variable with maximum levels being less than 6 ppm higher than that in plants that received no sludge. Levels of Ni tended to increase with rates of sludge, but there was variability as related to rates. All levels of Zn in soybean tops grown in soil-sludge mixtures were lower than the soybeans grown without sludge. Low rates of sludge tended to increase Mn levels, but the levels in the plants grown with the higher rates of sludge decreased rapidly to levels below those obtained in the check treatments. Levels of Fe were variable and were highest where no sludge was used. Lead levels were variable, but were slightly higher in the soybean tops representing sludge treatments.

TABLE 9.--Total Heavy Metal Levels of MI Sludge-Soil Mixtures.

	Control	Sludge Application Rates, Tons metric/ha					
Metal	0	26	52	103	258	515	1030
	mg/Kg dry matter						
Cu	34	79	122	224	625	2046	2449
Ni	13	151	259	500	1407	2980	5125
Zn	45	51	59	80	155	312	514
Mn	257	232	208	220	257	308	333
Fe	8617	9080	10493	12600	20444	41268	69871
Cd	<1	1	1	1	1	2	4
Pb	61	61	61	61	102	116	144
Cr	52	118	164	203	1198	2085	2685

TABLE 10.--Metal Concentration in Soybean Tops as Influenced by Rates of Sludge.

	Control	MI Sludge Application Rates, Metric tons/ha					
Metal	0	26	52	103	258	515	1030
	ppm						
Cu	29	26	32	35	32	30	35
Ni	8	30	46	39	37	38	56
Zn	39	18	27	25	21	21	22
Mn	145	224	187	104	77	64	44
Fe	100	83	92	75	66	75	75
Cd	<1	1	1	1	1	1	1
Pb	15	16	18	21	22	18	18
Cr	<3	<3	<3	<3	<3	<3	<3

The information in Tables 9 and 10 illustrate well why agronomists do not use total metal analysis as a basis for fertilizer recommendations and why researchers on soil sludge mixtures are finding it difficult to relate total metal content of treated soil to toxic levels in a crop.

Extractable Levels of Metals from Soil-Sludge Mixes

At the moment, several researchers are reporting extractable levels of metals from sludge-soil mixtures by two or more methods. Seemingly, the researchers use one method for interpretation of results as related to previous investigations at their experiment station, and another so that the data can be easily interpreted by others who have not had experience with such procedures. DTPA appears to be the standard now used in many laboratories, while historically some laboratories have used HCl or NH_4OAc. Because of this situation, the soil-sludge mixtures were extracted with these three extractants.

Extractable Cu levels increased with rate of sludge with all three extractants, with HCl removing the greatest amount (Table 11). This was also the situation with Ni and Zn, and with two exceptions with Fe. Levels of Cd, Pb and Cr tended to increase with rates of sludge, but the increases were not as consistent and were proportionally less than with other metals.

TABLE 11.--Extractable Metal Levels in Soil-Sludge Mixtures as Influenced by Rates of Sludge.

Extractant	Metal	Rate of Sludge-Metric T/ha						
		0	26	52	103	258	515	1030
		mg/Kg dry material						
0.1N HCl	Cu	13	46	80	193	413	837	802
	Ni	2	86	130	264	872	1114	1245
	Zn	5	12	21	54	96	156	268
	Mn	148	147	148	144	140	119	117
	Fe	462	733	868	1396	3223	3668	3207
	Cd	<0.4	0.8	0.7	0.8	0.8	1.1	1.4
	Pb	4	4	12	4	16	15	< 4
	Cr	< 2	27	44	92	318	285	38
0.05 M DTPA	Cu	4	23	45	93	144	280	639
	Ni	2	22	36	55	95	136	337
	Zn	3	4	6	10	22	31	65
	Mn	26	21	23	18	19	37	25
	Fe	64	61	68	77	114	196	357
	Cd	0.1	0.1	0.1	0.1	0.1	0.2	0.4
	Pb	<3.0	5	5	5	5	10	13
	Cr	<0.1	0.2	0.2	0.5	0.2	4	1.1
1.0 M NH_4OAc	Cu	2	12	33	78	201	8	782
	Ni	1	19	39	75	185	280	679
	Zn	3	5	8	15	34	62	147
	Mn	20	25	33	38	59	61	64
	Fe	13	17	22	47	192	662	952
	Cd	0.2	0.2	0.2	0.3	0.2	0.4	0.6
	Pb	< 3.2	3.2	3.2	3.2	6.4	6.4	9.6
	Cr	2.2	2.2	3.0	2.2	7.2	17.8	32.1

Extractable Mn levels are usually difficult to interpret because the degree of oxidation affects its extractability as well as availability to plants. The degree of oxidation of this heavy metal apparently changes very rapidly and can change even in the process of sampling and preparing the sample for analysis. With the HCl extractant, levels tended to decrease slightly with increased rates of sludge. With DTPA, extractable levels varied and there was no significant trend associated with rates of sludge. Levels of NH_4OAc extractable Mn increased with rates of sludge.

Correlations Between Soybean Yields, Metal Levels in Soybeans and Methods of Testing for Metals in Soil-Sludge Mixes

Simple correlations coefficients between the heavy metal concentrations in soybean plants and the yield as affected by increasing rates by both sludges are reported in Table 12. Only the coefficients for Mn were statistically significant. When interpreting these data, it should be remembered that the soil used in the research represented a highly productive soil despite the fact that K and Mg tests were relatively low. The second point to recall when interpreting these data is that the rates of sludge used represents extremely high levels of some metals, especially Cu, Ni, Zn, Fe and Cr.

TABLE 12.--Simple Correlation Coefficients of Heavy Metal Concentrations in Soybean Plants as Related to Yields.

Element	Sludge D	Sludge MI
Cu	-0.11	-0.58
Ni	--	-0.73
Zn	-0.54	0.50
Mn	-0.77*	0.84*
Fe	0.20	0.70
Cd	--	-0.50
Pb	--	-0.50
Cr	--	0.10

*Significant at the 0.05 levels.

Correlation coefficients between the total metal content of soil-sludge mixtures and the metal content of soybean plants are reported in Table 13. Little is known about the forms of metals that occur in specific sludges. In the MI sludge, the metals are present as relatively insoluble materials or else they reacted with soil components to form compounds that are insoluble or are very slowly available to plants. One other explanation is possible. Plants may not have the ability to translocate metals in proportion to that in the soil to the tops of

TABLE 13.--Correlation Coefficients Showing Relation Between Total Metal Content of Soil-Sludge Mixtures ** and Metal Concentrations in Soybean Plants.

Metal	
Cu	0.40
Ni	0.64
Zn	0.34
Mn	0.75
Fe	-0.45
Cd	0.40
Pb	0.30
Cr	0.20

*MI Sludge

plants. Correlation coefficients illustrating the relationship between total metal content of the soil-sludge mixture and the concentrations within the soybean tops were low. The range extended from 0.75 down to 0.20 with most of the coefficients being less than 0.50. When interpreting these data, it is well to recall the exceptionally wide range in the metals of the soil-sludge mixtures.

The correlation coefficients in Table 14 illustrate well one of the major problems faced by researchers

TABLE 14.--Correlation Coefficients Showing Relation Between Total Metal and Extractable Metal Levels of Soil-Sludge Mixtures.

Metal	Extractant		
	HCl	DTPA	NH_4OA_c
	--------Correlation Coefficient --------		
Cu	0.97**	0.93**	0.95**
Ni	0.92**	0.98**	0.98**
Zn	0.94**	0.99**	0.99**
Mn	-0.92**	0.60	0.72
Fe	0.76	0.99**	0.99**
Cd	0.85*	0.98**	0.90**
Pb	0.25	0.90**	0.99**
Cr	0.42	0.72	0.96

who are attempting to relate plant nutrient or heavy metal levels in soil-sludge mixes to plant growth. Already it has been shown that total metal analysis of soil-sludge mixtures and plant growth or metal contents of plants are not closely related. The correlation coefficients in this table suggest a close relationship between total metals in soil-sludge mixes and extractable levels. If this is the situation, then high correlations between metal levels in plants or crop yields and extractable levels of metals could not be expected. This thought suggests the need for an improved way to relate heavy metal levels in soil materials to plant growth.

Interestingly, there was a statistically significant negative correlation coefficient with HCl extractable Mn. Also there was a high coefficient for more metals extracted with NH_4OAc than DTPA in that the coefficient involving Cr was significantly higher with the NH_4OAc extractant.

As anticipated, there was no close relationship between metal levels in soybean tops and the three metal extractants used on the soil-sludge mixtures (Table 15). The only significant correlation coefficients obtained were with HCl and NH_4OAc extractable Mn. The coefficients were low enough to be significant to only the 5% level. Interestingly, with the HCl extractant, the correlation was positive and with the NH_4OAc, the coefficient was negative.

Correlation coefficients between metal levels as extracted from soil-sludge mixtures are shown in Table 16. The highest and most frequently significant correlations were between metals extracted with DTPA and NH_4OAc. The only instance where correlations were not exceptionally high involved Mn. Curiously, the coefficient was relatively high and statistically significant at the 5% level when the HCl and NH_4OAc extractants were compared. Again

TABLE 15.--Correlation Coefficients Showing Relation Between Extractable Metal Content of Soil-Sludge Mixtures and Metals in Soybean Plants.

Metal	Extractant		
	HCl	DTPA	NH_4OAc
	--------Correlation Coefficient -------		
Cu	0.43	0.55	0.52
Ni	0.59	0.70	0.66
Zn	-0.38	0.35	-0.32
Mn	0.78*	-0.10	-0.86*
Fe	0.53	-0.42	-0.45
Cd	0.52	0.21	0.31
Pb	0.52	0.52	0.34
Cr	0.20	0.10	0.20

TABLE 16.--Correlation Coefficients Between Three Extracting Agents for Heavy Metals.

Metal	DTPA and HCl	DTPA and NH_4OAc	HCl and NH_4OAc
	---------Correlation Coefficient------		
Cu	0.86*	0.99**	0.90
Ni	0.86*	1.00**	0.87*
Zn	0.99*	1.00**	0.98**
Mn	-0.50	0.06	-0.86*
Fe	0.73	0.98**	0.80*
Cd	0.83*	0.88**	0.86*
Pb	-0.03	0.89**	0.21
Cr	-0.15	0.84*	0.14

these data suggest that there is a similarity of results obtained with the three extractants and that there is a need for an improved method for relating heavy metal levels in soil materials and in soil-sludge mixes to plant growth and metal utilization.

SUMMARY AND CONCLUSIONS

The low heavy metal content sludge (D) affected the physical condition of the soil so that soybeans would not grow when rates in excess of 103 tons/ha were used. Soybean yields decreased with an increase in rate of this sludge. This was also the situation with all rates the heavy metal sludge (MI) which did not adversely affect physical condition of the soil.

To grow soybeans in the MI sludge treated soil it was necessary to leach the excessive salts from the soil-sludge mixes.

Organic matter, pH, and cation exchange capacity were increased with the use of both sludges. Exchangeable Ca and Mg levels were also increased. Soybean yields were higher with D sludge than MI sludge. Plants grown with the MI sludge were stunted, and chlorotic. Treatments with this material also reduced leaf size and number of trifoliate leaves. There was also a great reduction in root volume associated with increased rates of MI sludge.

Calcium concentrations in soybean tops decreased with increased rates of both sludges. Magnesium levels were not greatly affected. Potassium concentrations tended to increase with D sludge and decrease with sludge

MI. Phosphorus concentrations in soybean tops were not closely related to rates of sludge applications. In contrast, N levels increased greatly with increased rates of both sludge materials.

The concentration of heavy metals in soybean tops varied greatly. Copper and Fe levels were not affected by sludge D while Zn and Mn concentrations increased greatly. In contrast, with sludge MI, Zn and Mn levels decreased significantly while Cu, Cd, Pb and Cr levels were not greatly affected. Nickel level increased greatly with rate of sludge.

The uptake of heavy metals by soybean plants was greatly affected by the yield. With sludge D, Cu uptake decreased with increased rates of sludge. Manganese levels tended to increase while Zn and Fe levels were variable. The uptake of most heavy metals by soybean plants decreased with increased rates of sludge MI with Ni and Cr being exceptions. With this sludge Ni levels tended to increase while Cr levels were very low.

Heavy metal concentrations in soybean plants were not closely related to yield with one exception. With both sludges the correlation coefficients involving Mn were statistically significant. All correlation coefficients relating total metal content of soil sludge mixtures and metal concentrations in soybean plants were not statistically significant.

In evaluating HCl, DTPA and NH_4OAc for extracting heavy metals from the sludge-soil mistures, NH_4OAc extractable metals were closely related to total metal levels with one exception, Mn. The fewest statistically significant correlation coefficients were obtained with HCl. Such data suggests that there is need to improve the methods currently used to relate heavy metal levels in soil materials and in soil-sludge mixes to plant growth and metal utilization.

LITERATURE CITED

LITERATURE CITED

1. Allaway, W. H., 1968. Agronomic controls over environmental cycling of trace elements. Advanced Agronomy. 20:235-274.

2. Allaway, W. H., 1975. The effect of soils and fertilizer on human and animal nutrition. Agriculture information bulletin No. 378. Agriculture Research Service and Soil Conservation Service. United States Department of Agriculture.

3. Ambler, J. E., J. C. Brown, and H. G. Gauch, 1970. Effect of zinc translocation of iron in soybean plants. Plant Physiol. 46:320-323.

4. Anderson, A., and K. O. Nilsson, 1972. Enrichment of trace elements from sewage sludge fertilizer in soils and plants. Ambio 1:176-179.

5. Bingham, F. T., A. L. Page, G. A. Mitchell, and J. D. Strong, 1979. Effects of liming an acid soil amended with sewage sludge enriched with Cd, Cu, Ni and Zn on yield and Cd content of wheat grain. J. Environ. Qual. 8: No. 2, 202-207.

6. Baerug, R., and J. H. Martinsen, 1977. The influence of sewage sludge on the content of heavy metals in potatoes and on tuber yield. Plant and soil 47:407-418.

7. Bowen, J. E., 1969. Absorption of copper, zinc and manganese by sugar cane tissue. Plant Physiol. 44:255-261.

8. Bingham, F. T., A. L. Page, R. J. Mahler, and T. J. Ganje, 1975. Growth and Cd accumulation of plants grown on a soil treated with a Cd enriched sewage sludge. J. Environment. Qual., 4: 207-211.

9. Bingham, F. T., A. L. Page, R. J. Mahler, and T. J. Ganje, 1976. Yield, and Cd accumulation of forage species in relation to Cd content of sludge amended soil. J. Environ. Qual., 4: 267-272.

10. Baumhardt, G. R. and L. F. Welch, 1972. Lead uptake and corn growth with soil-applied Pb. J. Enviorn. Qual., 1:92-94.

11. Bryce-Smith, D., 1975. Heavy metals as contaminants of the human environment. The educational techniques subject group. Chemistry Cassette. The Chem. Soc. London.

12. Brewer, R. F., 1966. Lead, pp. 213-217. In H. D. Chapman: Diagnostic Criteria for Plants and Soils. Univ. of California, Div. of Agric. Sciences.

13. Bartlett, R. J. and J. M. Kimble, 1976. Behavior of chromium in soils: II. Trivalent forms. J. Environ. Qual., 5(4):379-383.

14. Cropper, J. B., 1967. Greenhouse studies on nutrients uptake and growth of corn on sludge-treated soils. M.S. Thesis, University of Illinois, Urbana, Illinois.

15. Chapman, H. D., 1966. Diagnostic Criteria for Plants and Soils. Univ. of California, Div. Agric. Sci., pp. 203-212.

16. Cannon, H. L. and J. M. Bowles, 1962. Contamination of vegetation by tetraethyl lead. Science 137: 765-766.

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

18. CIAT, 1971-1972. Annual Reports. Centro Internacional de Agricultura, Cali., Colombia.

19. Chapman, H. D., G. F. Liebig and A. P. Vanselow, 1939. Some nutritional relationship as revealed by a study of mineral deficiency and excess symptoms on citrus. Soil Sci. Soc. Am. Proc. 4:196-200.

20. Chaney, R. L., J. C. Brown and L. O. Tiffin, 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiol. 50:208-213.

21. Cervato, A., 1958. Agronomical aspects of the accumulation of Cu in soil. Ann. Fac. Agr. S. Cuore, 66:88-117. I.F.E.

22. Crooke, W. M., 1956. Effect of soil reaction on uptake of Ni from a serpentine soil. Soil Science 81:269-276.

23. Cropper, J. B., 1969. Greenhouse studies on nutrient uptake and growth of corn on sludge-treated soils. M.S. Thesis, Univ. of Illinois, Urbana, Illinois.

24. Cunningham, J. D., J. A. Ryan and D. R. Kenney, 1975b. Phytotoxicity in metal uptake from soil treated with metal amended sewage sludge. J. Environ. Qual., 4:455-460.

25. Cunningham, J. D., D. R. Kenney and J. A. Ryan, 1975c. Phytotoxicity and uptake of heavy metals added to soils as inorganic salts or in sewage sludge. J. Environ. Qual., 4(4):460-462.

26. Cunningham, J. D., D. R. Kenney, and J. A. Ryan, 1975a. Yield and metal composition of corn and rye grown on sewage sludge amended soil. J. Environ. Qual., 4:448-454.

27. Delas, J., 1963. The toxicity of Cu accumulating in soils. Agrochimica 7:258-288.

28. Delas, J., J. Delmas and C. Dimas, 1960. Isotopic dilution of Cu incorporated in soils over a 10 year period. Ann. Agron. 13:31-53.

29. Deatrick, E. P., 1919. The effect of manganese compounds on soils and plants. New York (Cornell) Agric. Expt. Memoir. 19:365-402.

30. Deutsch, M., 1972. Incidents of Cr contamination of ground waters in Michigan, pp. 149-159. In W. A. Pettyjohn (ed.), Water quality in a stressed environment, Burgess Publishing Co., Minneapolis, Minn.

31. Dowdy, R. H. and G. E. Hamn, 1977. Soybean growth and elemental content as influenced by soil amendments of sewage sludge and heavy metals: Seedling studies. Agronomy J., 69:300-303.

32. Dowdy, R. H. and W. E. Larson, 1975. Metal uptake by barley seedlings grown on soils amended with sewage sludge. J. Environ. Qual., 4(2):229-235.

33. Elgawhary, S. M., W. L. Lindsay and W. D. Kemper, 1970. Effect of complexing agent and acids on the diffusion of zinc to a simulated root. Soil Sci. Soc. Amer. Proc. 34:211-214.

34. Farrell, J. B., 1974. Overview of sludge handling and disposal. In Municipal Sludge Management, Proceedings of the National Conference, Pittsburgh, Pa., pp. 5-10.

35. Fulkerson, W. and H. E. Goeller (eds.), 1973. Cadmium, the dissipated element. Oak Ridge Natl. Lab Rep. NSF-EP21.

36. Graven, E. H., O. J. Attoe and D. Smith, 1965. Effect of liming and flooding on manganese toxicity in alfalfa. Soil Sci. Soc. Amer. Proc. 29:702-706.

37. Geering, H. R., J. F. Hodgson and C. Sdano, 1969. Mictonutrients cation complexes in soil solution: IV. The chemical state of manganese in soil solution. Soil Sci. Soc. Amer. Proc. 33: 81-85.

38. Gilbert, F. A., 1957. Mineral nutrition and the balance of life. Univ. Oklahoma Press, Norman.

39. Halstead, R. L., B. J. Finn and A. J. McLean, 1969. Extractability of Ni added to soils and its concentration in plants. Can. J. Soil Sci. 49:335-342.

40. Haghiri, F., 1974. Plant uptake of Cd as influenced by cation exchange capacity, organic matter, zinc and soil temperature. J. Environ. Qual. 3:180-183.

41. Hewitt, E. J., 1948b. Experiments on Fe metabolism in plants. I. Some effects of metal-induced iron deficiency. Rep. Agric. Hort. Res. Sta. Bristol.

42. Hewitt, E. J., 1948. Relation of manganese and other metal toxicities to the iron status of plants. Nature 161:489-490.

43. Hassett, J. J., 1974. Capacity of selected Illinois soils to remove from aqueous solution. Comm. Soil Sci. Plant Anal. 5:499-505.

44. Hahne, H. C. and W. Kroontje, 1973. Significance of pH and Chloride concentration on behavior of heavy metals pollutants: Hg (II), Cd (II), Zn (II), and Pb (II0. J. Environ. Qual., 2: 444-450.

45. Hill, J. F. and O. C. Bryon, 1937. Nutritive relation of Cu on different soil types in Florida. J. Amer. Soc. Agron. 29:809-814.

46. Heydeman, A., 1959. Geochim. Cosmochim. Acta, 15:305-329.

47. Hodgson, J. F., W. L. Lindsay and J. F. Trierweiler, 1966. Micronutrient cation complexing in soil solution. II. Complexing of zinc and copper in displaced solution from calcerous soils. Soil Sci. Soc. Amer. Proc. 30:723-726.

48. Huffman, E. W. D. and W. Allaway, 1973. Growth of plants in solution culture containing low levels of Cr. Plant Physiol. 52:72-75.

49. Hunter, T. G. and O. Vergano, 1953. Trace elements toxicities in oats. Ann. App. Biol. 40:761-777.

50. Hunter, J. G. and O. Vergano, 1952. Nickel toxicity in plants. Ann. Biol. 39:279.

51. IRRI, 1963-1973. Annual Reports. International Rick Research Institute, Los Baun, Philippines.

52. John, M. K., 1971. Lead contamination of some agricultural soils in western Canada. Environ. Sci. Technol. 5:1199-1203.

53. Jones, J. B., 1972. Plant tissue analysis for micronutrients, pp. 319-341. In: Micronutrients in Agriculture, Soil Sci. Soc. Amer., Inc., Madison, U.S.A.

54. John, M. K., H. H. Chua and C. J. Van Jaerhoven, 1972. Cadmium contamination of soil and its uptake by oats. Environ. Sci. Technol. 6(7): 555-557.

55. Jurinak, J. J. and J. Santillan-Madrano, 1974. The chemistry and transport of lead and cadmium in soils. Research Report 18, Utah Agr. Exp. Sta., Utah State University, Logan, Utah.

56. Keeney, D. R., W. Lee and L. M. Walsh, 1975. Guidelines for the application of wastewater sludge to agricultural land in Wisconsin, Wisconsin Dept. Nat. Resources. Tech. Bulletin 88, p. 36.

57. King, L. D. and H. D. Morris, 1972. Land disposal of liquid sewage sludge. II. The effect of soil pH, Mn, Zn and growth and chemical composition of rye (Seeale Cereale L.). J. Environ. Qual., 1:425-429.

58. Krauskopf, K. B., 1972. Geochemistry of micronutrients, pp. 17-23. In: Micronutrients in Agriculture, Soil Sci. Soc. America, Inc., Madison, U.S.A.

59. Lagerwerff, J. V., 1971. Uptake of cadmium, lead and zinc by radish from soil and air. Soil Sci. 111:129-133.

60. Leep, H. R., 1974. Effect of soil heavy metal contamination upon growth and nutrient composition of corn. Ph.D. dissertation, Michigan State University, East Lansing, Michigan.

61. Leeper, G. W., 1978. Managing the heavy metals on the land. Pollution Engineering and Technology. R. Young and P. N. Chereuisinoff, editors.

62. Lehninger, A. L., 1975. Biochemistry, the molecular basis of cell structure and function. Worth Publisher, Inc., New York.

63. LeRiche, H. H., 1968. Metal contamination of the soil in the Woburn Market-Garden experiment resulting from the application of sewage sludge. J. Agric. Sci. Caurb. 71:205-208.

64. Lindsay, W. L., 1972. Inorganic phase equilibria of micronutrients in soils, pp. 41-57. In: Micronutrients in Agriculture, Soil Sci. Soc. America, Inc., Madison, U.S.A.

65. Lindsay, W. L., 1974. Role of chelation in micronutrient availability. In: E. W. Carson: The Plant Root and its Environment, pp. 507-524. University Press of Virginia.

66. Loew, O. and S. Sawa, 1902-1903. The action of manganese compounds on plants. Imp. Unit. (Tokyo). Coll. Agric. Bull. S:161-172.

67. Lunt, H. A., 1953. The case for sludge as a soil improver with emphasis on value of pH control and toxicity of minor elements. Water and Sewage Works. 100:295-301.

68. Lunt, H. A., 1959. Digested sewage sludge for soil improvement. Conn. Agric. Exp. Stn. Bull. 622:1-30.

69. Lunt, H. A., 1959. Digested sewage sludge for soil improvement. Conn. (New Haven), Agric. Exp. Sta. Bull. No. 622.

70. Marten, G. C. and P. B. Hammond, 1966. Lead uptake by bromegrass from contaminated soils. Agron. J. 58:553-554.

71. Melton, J. R., B. G. Ellis, and E. C. Doll, 1970. Zinc, phosphorus, and lime interaction with yield and zinc uptake by phaseolus vulgaris. Soil Sci. Soc. Amer. Proc. 34:91-93.

72. Mengel, K. and E. A. Kirkby, 1978. Principles of plant nutrition. International Potash Institute, Switzerland.

73. Mertz, W., 1969. Chromium occurrence and functions in biological systems. Physiol. Rev. 49:165-239.

74. Miller, J. E., J. J. Hassett and D. E. Koeppe, 1976. Uptake of cadmium by soybeans as influenced by soil cation exchange capacity, pH. and

75. Millikan, C. R., 1947. Effect of Mo on the severity of toxicity symptoms in flax induced by air excess of either manganese, zinc, copper, nickel or cobalt in the nutrient solution. F. Aust. Inst. Agric. Sci., 13:180.

76. Millikan, C. R., 1948. Antagonism between molybdenum and certain heavy metals in plant nutrition.

77. Mortvedt, J. J. and P. M. Giordano, 1975. Response of corn to zinc and chromium in municipal wastes applied to soil. J. Environ. Qual., 4(2):170-174.

78. Muller, J., 1960. Ann. Agron. 11:75-91.

79. Mumford, F. E., H. M. Stark and D. H. Smith, 1962. A naturally-occurring cofactor for indoleactive acid oxidase. Plant Physiol. 37:xiv.

80. Nicholas, D. J. D., C. P. Lloyd-Jones, and D. J. Fisher, 1957. Some problems associated with determining iron in plants. Plant Soil 8:367-377.

81. Page, A. L., 1974. Fate and effects of trace elements in sewage sludge when applied to agricultural lands. Environ. Technol. Ser. EPA-670/2-74-005. Cincinnati, Ohio, p. 98.

82. Page, E. R., 1962. Studies in soil and plant manganese. II. The relationship of soil pH to manganese availability. Plant and Soil 16:247-257.

83. Patterson, J. B. E., 1971. Metal toxicities arising from industry. Techn. Bull., Min. of Agric. Fish, Food, Agric. Develop and Adv. Serv., Cambridge, England, 21:193-207.

84. Reuther, W., and P. W. Smith, 1954. Proc. Sci. Soc. Florida 14:17-23.

85. Reuther, W. and P. F. Smith, 1953. Effects of high copper content of sandy soil on the growth of citrus seedlings. Soil Sci. 75:219-224.

86. Rhode, G., 1962. The effects of trace elements on the exhaustion of sewage-irrigated lang. J. Inst. Sewage Purif. Part 6:681-585.

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

88. Roth, J. A., E. F. Wallihan, and R. G. Sharpless, 1971. Soil Sci., 112:338-342.

89. Roth, J. A., E. F. Wallihan, and R. G. Sharpless, 1971. Uptake by oats and soybeans of copper and nickel added to a peat soil. Soil Sci. 112:338-342.

90. Santillan-Medrano, J., and J. J. Jurinak, 1975. The chemistry of lead and cadmium in soil: solid phase formation. Soil Sci. Soc. Amer. Proc. 39:851-856.

91. Sanchelli, V., 1969. Trace elements in agriculture. Van Nostrand Reinhold Co., New York, pp. 39-57, 151-171.

92. Schmid, W. E., H. P. Haag, and E. Epstein, 1965. Absorption of zinc by excised barley roots. Plant Physiol. 18:860-869.

93. Shaeffer, C. C., A. M. Decker, and R. L. Chaney, 1975. Effects of soil temperature and sludge applications on the heavy metal content of corn. Agron. Abstr.

94. Soane, B. D., and D. H. Saunder, 1959. Nickel and chromium toxicity of serpentine soils in Southern Rhodesia. Soil Sci. 88:322-330.

95. Somers, J. J. and Shive, J. W., 1942. The iron-manganese relation in plant metabolism. Plant Physiol. 17:582-602.

96. Spencer, W. F., 1966. Effect of copper on yield and uptake of phosphorus and iron by citrus seedlings grown at various phosphorus levels. Soil Sci. 102:296-299.

97. Stewart, E. A., H. M. Grimshaw, J. A. Parkinson and C. Quarmby, 1974. Chemical Analysis of Ecological Material, p. 388. Blackwell Scientific Publications, Oxford, London.

98. Street, J. J., W. L. Lindsay and B. R. Savey, 1977. Solubility and plant uptake in soils amended with cadmium and sewage sludge. J. Environ. Qual. 6:72-77.

99. Swaine, D. J., 1955. The trace element content of soils. Soil Sci. Techn. Comm. No. 48. Herald Printing Works, Coney St., York, England.

100. Swaine, D. J., 1955. The trace element content of soils. Commonw. Bur. Soil Sci. Tech. Commun., No. 48. Herald Printing Works, York, England, p. 157.

101. Tanaka, A. and S. Yoshida, 1970. Nutritional disorders of the rice plant in Asia. Intern. Rice Res. Inst., Technical Bulletin 10.

102. Tisdale, S. L. and W. L. Nelson, 1975. Soil Fertility and fertilizer. Third edition. Macmillan Publishing Co., Inc., London, p. 317.

103. Tiffin, L. O., 1967. Translocation of manganese, iron, cobalt and zinc in tomato. Plant Physiol. 42:1427-1432.

104. Turner, M. A., and R. H. Rust, 1971. Effect of chromium on growth and mineral nutrition of soybean. Soil Sci. Soc. Am. Proc. 35:755-758.

105. U.S. Environmental Protection Agency, 1976. Application of sewage sludge to cropland: appraisal of potential hazards of the heavy metal to plants and animals, p. 25. EPA-43019-76-013. November 15, 1976.

106. Vanselow, A. P., 1966. Nickel. In: Diagnostic Criteria for plants and soils. H. D. Chapman, ed. Div. Agri. Sci., U. Calif., Berkley, 2:118-119.

107. Viets, F. G., Jr., 1966. Zinc deficiency in the soil-plant system, pp. 90-128. In A. S. Prased (ed.), Zinc metabolism. C. C. Thomas Publisher, Springfield, Illinois.

108. Webber, J., 1972. Effects of toxic metal in sewage on crops. Water Pollution Contr. Fl: 404-413.

109. Welch, R. M. and E. E. Cary, 1975. Concentration of chromium, nickel and vanadium in plant materials. J. Agric. Food Chem. 23:479-482.

110. Wells, N. and J. S. Whitton, 1977. Element composition of tomatoes grown on four soils mixed with sewage sludge. N. Z. Journal of Experimental Agriculture 5:353-359.

111. Zajic, J. E., 1969. Microbiology, Biogeochemistry. Academic Press, New York, p. 345.

112. Recommended Chemical Soil Test, Procedures for the North Central Region, 1975. North Central Regional Publication No. 221, Bulletin No. 499, North Dakota State University.