SELECTED METALS IN EARTHWORMS, LETTUCE AND SOIL AMENDED WITH SEWAGE SLUDGE

by

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ABSTRACT

Earthworms (Lumbricus rubellus, Aporrectodea spp. and Octolasion cyaneum) were kept in soil treated with six application rates of milorganite. After ten days, the earthworms, their faeces and the soil were analyzed for cadmium, copper, lead and zinc. Cadmium and Zn were concentrated in the worm tissue of all three taxa, over soil levels, whereas Cu and Pb were not. Cadmium concentrations in the body tissue increased with increasing soil Cd, until soil concentrations reached 7 µg/gm, after which the body tissue concentrations levelled out. The body tissue Cd and Zn concentrations exceeded the concentration of these metals in the faeces. The faeces had higher Cu and Pb concentrations than the body tissue. The earthworms appear to be capable of accumulating Cd and regulating body tissue concentrations of Cu, Pb and Zn.

There were differences among earthworm taxa in the metal concentrations in both body tissues and faeces. Aporrectodea spp. had the lowest body tissue Cd concentrations, as well as, had lower faecal Cd concentrations than L. rubellus. Body tissue Cd concentrations for L. rubellus and O. cyaneum were not different. Copper and Pb concentrations in the body tissue of O. cyaneum were greater than those for the other two taxa, which did not differ significantly in their Cu and Pb concentrations. Lumbricus rubellus had higher Cu levels in its faeces than did Aporrectodea spp., whereas there were no differences in the faecal Pb concentrations for the three taxa. Zinc concentrations

were different in the body tissues of all three taxa and was highest in the _Aporrectodea_ spp. The latter taxon also had the lowest faecal Zn concentrations.

Earthworms might be useful biological monitors of Cd pollution, but not for Cu, Pb and Zn, in soils receiving sewage sludge.

In the experiment on the possible effects of milorganite and earthworms on growth and on mtal uptake by lettuce, the addition of the sewage sludge resulted in a significant increase in plant yield. The cadmium and zinc concentrations in leaf and root tissue were significantly increased by milorganite additions and nickel concentrations were decreased. Copper concentrations were unaffected by the sludge. Mean lead concentrations were higher in the milorganite treatments. However, the variability was great and there was not significant differences. The addition of milorganite to the soil resulted in increased concentrations of diethylene triamine penta acetic acid (DTPA) extractable metals. At an application rate of 20 gm milorganite/kg soil, DTPA extractable Cu, Ni and Zn were significantly correlated with lettuce leaf concentrations for these 3 metals. However, at the higher milorganite application rate of 50 gm/kg, only DTPA extractable Zn was significantly correlated with plant tissue concentrations.

The addition of earthworms to the soil did not affect lettuce yields or the concentrations of DTPA extractable metals. Lead concentrations in the roots were significantly lower when earthworms were present in the soil. Earthworms did not affect the concentrations of Cd, Cu, Ni and Zn in lettuce tissues.

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1. GENERAL INTRODUCTION

Sewage Sludge Application to Land - A Brief Overview

Sewage sludge is the by-product produced by municipal wastewater treatment. In Canada, approximately 10^5 tonnes of dry solids were produced in 1973 and the projected production by 1985 is 10^6 tonnes dry solids per annum (Webber, 1979). Disposal practices have included incineration, ocean dumping, fresh water dumping, landfilling and storage lagoons, as well as, application to land for disposal purposes, cropland improvement and for reclamation of disturbed lands such as mine spoils. In 1970, the Environmental Protection Service was established and is responsible for federal pollution control legislation. The major federal acts are the Environmental Contaminants Act, Clean Air Act, Ocean Dumping Control Act, Fisheries Act and Canada Water Act, as well as similar provincial legislation (Environment Canada, 1981; Sanderson et al. 1973). There is similar legislation in the United States (Parr et al., 1978). The main thrust of this legislation is to encourage municipalities to consider applying sewage sludge to land either for disposal or use in agriculture.

The addition of sewage sludge to agricultural lands is not new. For 300 years, beginning in 1559, sewage was spread on agricultural lands in Prussia. (Bates, 1972; Dowdy et al. 1976). Sewage farms were started in England's London area during the 1860's and agricultural land is still being used for sludge disposal today (Thompson and Dickens, 1979). About 25% of all sludge produced in the U.K. is applied to farmland as liquid digested sludge. (Coker, 1979). Since 1897, Melbourne, Australia has spread sewage sludge on land which is used primarily for livestock grazing (Seabrook, 1975). In the U.S. about 20% of municipal sludges are spread on land (Parr et al. 1978). Coote et al. (1981) reviewed the Canadian use of sewage on land. In British Columbia's Okanagan sewage sludge is spread on agricultural and parkland. Several major prairie cities also spread sludge on farmland. Ontario disposes of 34% of the sludge produced onto farmland. Both Quebec and the Atlantic Region do not practice land application of sewage sludge.

The addition of sewage sludge to agricultural land may have several beneficial effects, as well as, harmful effects on soil chemical properties. Sludges contain varying amounts of nitrogen, phosphorus and micronutrients and generally low levels of potassium and have been used to promote crop growth (Bates, 1972; Chawla et al. 1874; Coker, 1979; Dowdy et al., 1976, Parr et al., 1978; Stewart and Webber, 1976). Epstein et al. (1976) indicate that sewage sludges may contain from 2-8% nitrogen and 1-4°C phosphorus. Coker (1979) suggested that the nitrogen availability in sewage sludges could be estimated as the amount of ammonium - N present plus 1/6 of the organic - N present. Both Damprey (1979) and Coker (1979) suggested that the nitrogen and phosphorus content of sludges are suitable for grasses and cereal crops. The effects of sludge addition on soil pH is variable. Epstein et al. (1976) reported decreases whereas Coker (1979) indicated a rise in soil pH. This may reflect different processes utilized in sludge stabilization. The addition of lime is only one of several means of stabilizing sewage (Bates, 1972, Dowdy et al, 1976). Epstein et al. (1976) report an increase in soil cation exchange capacity when sludge was added to the soil. Various salts are used in wastewater treatment and when sludge is added to the soil, there is an increase in soil salinity and electrical conductivity. Excess salts can be harmful to plant growth and reduce germination while excess sodium can also be harmful to soil structure. (Dowdy et al., 1976; Epstein et al., 1976; Stewart and Webber, 1976).

The addition of sewage sludge to soil generally improves the physical properties. The organic matter in sewage sludge is relatively stable as the easily decomposed material is destroyed during processing (Coker, 1979). Webber (1978) and Gupta et al (1977) reported decreases in soil bulk density when sludge was added. Sludge additions can increase the percentage of water stable aggregates present in the soil. (Epstein, 1975; Webber, 1978). Water retention in soils treated with sludge ussually is increased. (Coker, 1979; Epstein, 1976; Gupta et al, 1977), although Webber (1978) and Epstein (1978) reported an increase in water holding capacity only at the $1.5 \times 10^6 N/m^2$ (15 bars) and no increase at the lower pressures.

Sewage sludges differ from other organic supplements added to soil in that they generally contain varying amounts of metals. The addition of large amounts of metals to soil is a potential hazard because many of the metals can be toxic to plants at certain concentrations, and once taken up by plants the metals enter the food chain and can threaten the health of livestock and humans. Long term application of sludge containing metals may result in a long term soil contamination and render the soil non productive for crop production (Bates, 1972; CAST, 1976; Dowdy et al., 1976; Parr et al., 1978). The movement of metals in the soil profile after sludge application has been reviewed by Page (1974) and Williams et al., (1980). In general, a large proportion of the metals present in sewage sludges occur in stable and nonmobile organic forms. Metals, including Cd, Cu, Ni, Pb and Zn, tend to remain in the surface and only a small percentage (< 7%) of the added metals in sewage sludges will be found below the plow layer. Williams et al. (1980) reported than Zn, Cu, Pb and Cd concentrations decreased with depth after sewage sludge was incorporated into the surface 20 cm and that for all 4 metals the concentrations from sludge treated soils and controls were equal at 25 to 30 cm, or 5 to 10 cm below the zone of sludge addition.

Most researchers refer to the metals as heavy metals. However, the term "heavy metal" has been used inconsistently in the literature. Heavy metals are those metals which have a density greater than 5,000 kg/m^3 yet in the literature concerning metal pollution, elements that are neither metals or heavy have been included in this group (Hughes et al., 1980). The metals of most concern are zinc, copper and nickel which can be phytotoxic and cadmium which can accumulate in plant tissue without the plant exhibiting toxic symptoms and become potential health hazards to livestock and humans. (Cast, 1976; Dowdy et al, 1976; Stewart and Webber, 1976).

Plants (and also animals) grown in soils amended with sewage sludge may either accumulate, concentrate or not retain metals in their tissues. When the concentration ratio (the ratio of metal concentration in the organism to the metal concentration in the soil) does not differ from zero, no retention of the metal by the organism has occurred. Accumulation occurs when the plant (or animal) tissue contains increasingly higher amounts of a metal as a result of being exposed to one level of the metal in the environment over a period of time, or at one given time the tissues contain increasing levels of metals in proportion to levels in the environment. Concentration of a given metal occurs when the concentration ratio is greater than unity (Hartenstein et al. 1980, Hughes et al., 1980; Van Hook, 1974).

The chemical composition of sludge varies widely from city to city and with the time of day, and depends on the type of industrial waste, treatment processes, storm drains, using the treatment plant. (Bates, 1972; Dowdy et al. 1976). Metal concentrations in sewage sludges from various centres have been published. In Canada, Chawla et al. (1974) review metal content in several Ontario sludges and Koch et al. (1977) present data on the composition of sludges from the Greater Vancouver Region. Page (1974) reviewed metal concentrations in sludges from a number of American cities, as did Sommers et al. (1976), Horvath and Koshut (1981) and Sommers (1977). Information for British and Welsh sludges has been summarized by Berrow and Webber (1972). All these reviews emphasize the variability in metal concentrations in different sludges.

Sommers (1977) indicates that prior to applying sludges to land, the composition including metal concentration should be known. Various countries have proposed guidelines to regulate the amount of metals which can be added to the soil in sewage sludge in an attempt to prevent metal concentrations from reaching toxic levels. (Freedman and Hutchinson, 1981).

Not only have municipalities spread sludge on land as a means of disposal but some cities have packaged sludge and marketed it as a fertilizer. In Britain, sewage was sold as manure during the 1800's. Since the turn of the century, Halifax, England has been packaging processed sludge and selling it as a fertilizer under the name "Organifax". Other processing plants in Britain also sell processed sludge under a variety of trade names (Wood, 1979). In 1927, Milorganite, a heat-dried activated sludge from Milwaukee, Wisconsin was the first sludge to be sold in the United States as a fertilizer (Anderson, 1959).

Earthworms have also been added to soil to increase crop growth. (Barley, 1961 and Ghabbour, 1966) and in New Zealand the addition of earthworms to pastures is a recommended procedure for increasing pasture production. (Stockdill, 1966).

Edwards and Lofty (1977) and Hughes et al. (1980) reviewed the uptake of metals by earthworms. In general, Cd and Zn are concentrated by earthworms and this poses a threat to organisms feeding on the earthworms. Also, when the earthworms die, the metals retained by the earthworms may become available for plant uptake. Ireland (1975) reported that decaying earthworms contained much more available Pb and Zn than did the soil or earthworm casts. Kirkman (1979) reported that earthworms increased the growth of wheat in soils both with and without sewage sludge as well as increased Mn uptake by wheat.

The present study consists of two parts. The objectives of the first part were:

1. To determine if earthworms belonging to three taxa would accumulate cadmium, copper, lead and zinc in response to increasing applications of milorganite to the soil in which the earthworms were kept.
2. To determine if there was a taxon difference in metal uptake by earthworms.
3. To determine if metal concentration in the earthworms was dependent upon body weight.
4. To determine if earthworms are potentially useful as biological monitors of metal levels in the soil.

This experiment was conducted in the controlled environment of a growth chamber. This study differs from other studies involving metal uptake by earthworms kept in sludge amended soils in that six application rates of sludge are used to test for accumulation by the worms in response to increasing metal loading of the soil.

The second part's objectives were:

1. To determine if the cadmium, copper, lead, nickel, and zinc concentrations in leaf lettuce would be increased when milorganite was added to the soil in the presence and absence

of earthworms.

2. To determine if milorganite and earthworms increased lettuce yields. This experiment was conducted in a greenhouse. Milorganite was used because it was readily available from gardening suppliers and was reported to have high concentrations of cadmium, copper, nickel, lead and zinc. (John and van Laerhoven, 1976). This experiment differs from the only other experiment testing the influence of earthworms on metal uptake by plants grown in sludge amended soil (Kirkman, 1979) in that several taxa of field earthworms were utilized rather than the manure worm (Eisenia foetida) which lives in compost heaps and in soils only with high organic matter contents (Reynolds, 1977).

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PART 1

Selected Metal Levels in Three Taxa of Earthworms (Lumbricidae) Kept in Soil Contaminated by Milorganite.

1 INTRODUCTION AND LITERATURE REVIEW

Earthworms are an important component of the soil fauna, particularly in temperate climatic regions. Their feeding and burrowing activities have been shown to increase soil infiltrability, soil porosity, soil aggregate stability and the mixing and translocation of soil constituents. Not all of the Lumbricidae affect the soil to the same degree, as some species are deep burrowers, while others are surface dwellers (Satchell, 1967; Edwards and Lofty, 1977).

Earthworms are a food source for many organisms especially a large number of bird species and moles, as well as, many invertebrates (Edwards and Lofty, 1977; Gish and Christensen, 1973). Ireland (1977) demonstrated that toads could accumulate lead from their diet, which consisted of earthworms with high tissue concentrations of lead.

The burrowing activities of earthworms have been shown to influence the redistribution of elements in the soil. In soil cores topped with litter spiked with ^{109}Cd, vertical migration of cadmium was greater in cores with earthworms, Lumbricus terrestris and Octolasion lacteum, than in cores with no earthworms present. The two species have different feeding habits and the former taxon also is a deeper burrower than the latter species. Lumbricus terrestris was more effective in the redistribution of cadmium (Oak Ridge National Laboratory, 1974). Reichle et al. (1971) and Crossley and co-workers (1971) demonstrated that Octolasion lacteum and Lumbricus terrestris were both important in organic matter turnover, and in redistribution of ^{137}Cs in forested soil systems in the southern U.S. Earthworms were shown to be important in the redistribution of ^{60}Co in the soil (Peredelsky et al 1957).

Various species of earthworms from both uncontaminated and contaminated soils have been found to accumulate, and in some cases, concentrate certain metals found in the soil into their tissues (see reviews by Edwards and Lofty, 1977; Hughes et al, 1980). The contaminated sites included: (1) urban and rural soils in the vicinity of extensively travelled highways; (2) soils to which sewage sludges with high levels of metals were added; (3) soils contaminated by mine tailings; (4) soils located downwind from lead - zinc smelters. The general findings were that Cd and Zn concentrations in earthworm tissue exceeded the concentrations of these metals in the soil. Cadmium concentrations in earthworms tended to increase with soil concentrations indicating Cd accumulation, whereas Zn concentrations tended to be regulated. Lead and copper tended not to be concentrated or accumulated by earthworms. Only earthworms sampled from soils contaminated by lead-zinc mine spoils were found to concentrate Pb above soil concentrations (Ireland, 1975, 1979; Ireland and Richards, 1977).

Van Rhee (1963, 1975, 1977) found decreased earthworm populations in orchard soils where copper fungicides such as copper sulphate had been used and in pasture soils where pig slurry containing high amounts of copper had been applied. High concentrations of copper were found to reduce earthworm reproductivity capacity as well as body growth. Copper levels in a mixed species sample of earthworms showed increasing levels of copper in the body tissue with increasing soil levels although the levels in the earthworms did not exceed that of the soil.

Ireland and his coworkers carried out a number of studies to determine the metal content in earthworms, primarily the acid tolerant species, Dendrobaena rubida, collected from soils with low pH's ranging from 3.6 to 5.1. Ireland and Wooton (1976) indicated that the lead concentrations in the tissues of D. rubida were seasonally variable and were significantly greater in the winter. They also found that Zn concentration in D. rubida were significantly lower in November for 2 of the 3 sites studied.

Accumulation of a metal by earthworms occurs when the earthworm tissue contains increasingly higher amounts of a metal as a result of being exposed to one level of the metal in the environment over a period of time, or at one given time the tissues contain increasing levels of metal in proportion to levels in the enviornment. Concentration of a given metal occurs when the ratio of metal concentration in the earthworms to the concentration in the soil is greater than unity (Hartenstein et al. 1980; Van Hook, 1974).

Ash and Lee (1980), Andersen (1979, 1980), Ireland and Richards (1977) and Ireland (1979) reported significant differences in metal uptake among different earthworm species.

Ireland and Richards (1977) suggested that species differences in body tissue concentrations may be a result of different metal tolerances between species, whereas Ireland and Richards (1981) indicated that differences in feeding habits may account for different metal concentrations among different earthworm species.

Carter et al. (1980) collected L. rubellus clitellate individuals and juvenile non-clitellate specimens from an agricultural field. The non-clitellate specimens had lower body tissue Cd concentrations than did the clitellate specimens.

Zinc regulation was reported by Bryan and Hummerstone (1973) for the marine polychaete, Nereis diversicolor. Zinc regulation in earthworms has been reported by Ireland and Wooton (1976), Ireland (1979), Ireland (1976) and Ireland and Richards (1977), as well as, Ash and Lee (1980). Earthworms also regulate copper (Ireland and Richards 1981).

Faeces are the major excreting sites for lead and zinc in the earthworm species Dendrobaena rubida (Ireland, 1976).

Van Hook (1974), Helmke et al. (1979), Czarnowska and Jopkiewicz (1978) and Carter et al. (1980) have suggested that earthworms are potentially useful biological monitors of metal levels in the soil. A biological monitor organism is a member of the biota indigenous to the study area and accumulates pollutants from the soil or ingested food in its tissue. The organism acts as an indicator in that the levels of a pollutant in its tissue are taken to be an index of the degree of pollutant contamination at the sampling site (Phillips, 1978).

The objectives of this study were as follows:

1. To determine if earthworms would accumulate the metals in proportion to the levels in the soils and, therefore, potentially be useful biological monitors of metal levels in the soil.
2. To determine if there were differences among three taxa of earthworms: Lumbricus rubellus, Aporrectodea spp. and

Octolasion cyaneum, in the metal concentrations of their body tissues.

3. To determine if the concentrations of metals in the body tissue was dependent upon the body weight of the earthworms.

2 MATERIALS AND METHODS

2.1 Sample Preparation and Chemical Analysis

Bulk soil samples were collected from the Ap horizon of a Crescent-Westham Island soil complex (Luttmerding, 1980) located on Mr. Hugh and Bob Reynolds farms on Westham Island in the Fraser Delta of British Columbia. (Soil profile descriptions are given in Luttmerding, 1981a,b). The soil was air-dried, crushed with a wooden roller and passed through a 6 mm screen. The soil (2760 grams) was throughly mixed with 0,5,10,25,50 or 100 grams of Milorganite per kilogram of soil on an air-dried basis. These application rates are equivalent to 0,11,25,22.50,56.25,112.50,225.0 metric tonnes of milorganite per hectare. The soil-milorganite mixture was packed (bulk density of 1200 kg/m^3) into plastic flower pots (15 cm × 17 cm). Milorganite is a heat dried activated sewage sludge sold by the Milwaukee Sewerage Commission. The sludge is highly contaminated and was used as the source of metals; cadmium, copper, lead and zinc. After filling the pots, they were watered with 1000 mls of distilled water (to approximate field capacity) weighed and the weights recorded. Four *Lumbricus rubellus* Hoffmeister, four *Aporrectodea* spp. (either *Aporrectodea trapezoides* Duges, *Aporrectodea tuberculata* Eisen, or a combination of these two species) and two *Octolasion cyaneum* Savigny were placed on top of the soil-milorganite mixture of each pot and allowed to burrow. Only clitellate adult earthworms were used. (See Plates 1-3 and Table 1 for descriptions of these earthworms). Earthworms were obtained by digging on Westham Island near the field where the soil was obtained.

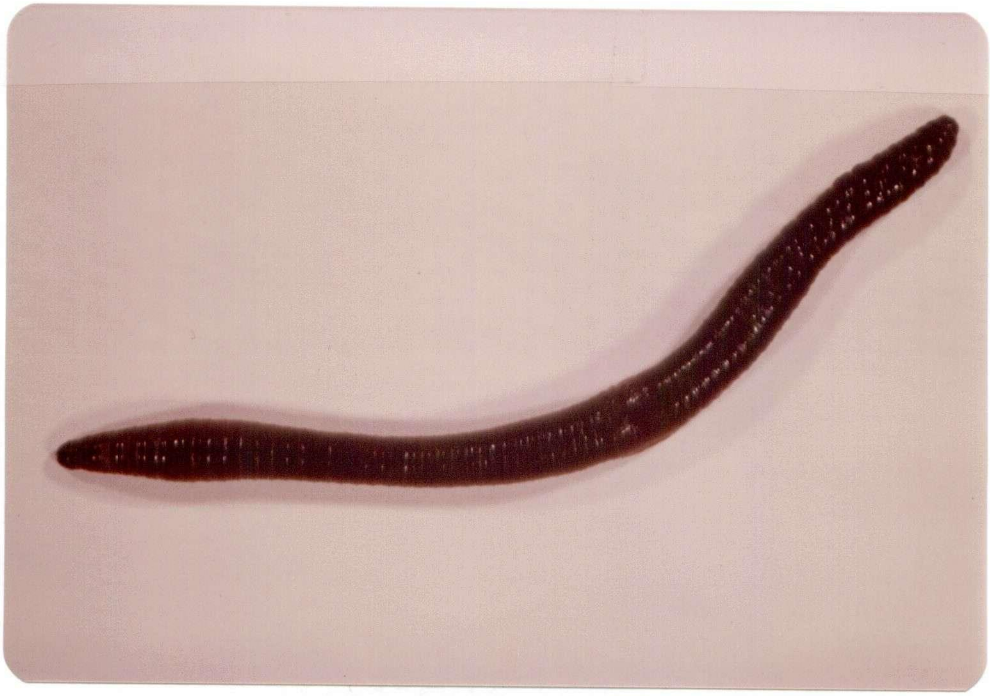


Plate 1. Clitellate Specimen of Lumbricus rubellus.

Plate 2. Clitellate specimen of Octolasion cyaneum.

Plate 3. Non-clitellate specimen of *Aporrectodae* spp.

Table 1 Diagnostic Characteristics of Four Earthworm Species (Adapted from Reynolds, 1977)

	Length (mm)	Diameter (mm)	Number of Segments	Prastomium	1st Dorsal Pore	Clitellum	Tuberculua Pubertatis	Setal Pairing	Genital Tumescenes	Pigmentation
Lumbricus rubellus (red marsh worm)	50-150 (usually 60 mm)	4-6	70-120	tanylobic	5/6-8/9	xxvi, xxvii-xxxi, xxxii	xxviii - xxxu	Closely	viii-xii (less frequently on x) xx-xxiii, xxvi-xxxvi	Ruddy brown or red violet irridescent dorsally, pale yellow ventrally
Octotasion cyaneum (woodland blue worm)	65-180	7-8	140-158	epilobic	11/12 or 12/13	xxix-xxxiv	xxx-xxxiii	Closely paired anteriorly widely paired posterioly	setae of x,xviii, xix,xx,xxi frequently on white genital tumescences	Blue-gray with (usually) lilac blue dorsal line, last 4-5 segments yellow, anterior segments pink, clitellum red-orange
Aporrectodea tuberculata	90-150	4-8	146-196	epilobic	11/12 or 12/13	xxvii-xxxiv	xxx,xxxi-xxxiii,xxxiv	closely	absent in xxxi and xxxiii, present in xxx, xxxii and xxxiv and frequently in xxvi	unpigmented almost white or greyish or sometimes with light pigmentation on the dorsum
Aporrectodea trapezoides	80-140	3-7	93-169 generally 130	epilobic	12/13	xxvii, xxviii-xxxiii, xxxiv	xxxi-xxxiii	closely	Genital tumescences including a and b setae only in ix-xi,xxxii-xxxiv, often in xxviii and occasionally in the region of xxvi-xxix	variable, often lighter behind the clitellum until near, the hind end, the deeper brownish-reddish

The tops and bottoms of the pots were covered with nylon mesh in order to prevent the earthworms escaping. The pots were placed in a Percival growth chamber set at a constant-temperature of 15°C and 12 hr photoperiod using a mix of incandescent and fluorescent light. Because of evaporation, distilled water was added daily to bring the soil-milorganite mixture back to the initial weight. The experiment was run for ten days. The six treatments were replicated five times. The pots were set up in a repeated measure design. In a repeated measures experiment, more than one measurement is made on the same experimental unit (Fisher and MacDonald, 1978; Lee, 1975; and Winer, 1971). In this experiment a flowerpot is an experimental unit, and there are three taxa of earthworms sampled from each unit. The earthworms are the repeated measure. The pots were set up as in a randomized block design.

After ten days, the surviving earthworms were recovered, rinsed with distilled water, placed on moist ashless filter paper (Whatman #40) in petri dishes and placed in an incubator at 15°C for four days to allow the earthworms to void their gut contents. During the four days the filter papers were changed daily so as to prevent possible coprophagy. Where possible, faeces from two individuals of each of *Lumbricus rubellus*, *Aporrectodea* spp. and *Octolasion cyaneum*, from each pot were collected and saved for analysis. All the surviving earthworms were killed and stored in a deep freezer until analysed.

The soil-milorganite mixtures were air-dried, crushed with a wooden roller and passed through a 2 mm stainless steel sieve. Three replicates for each soil milorganite mixture were prepared and analyzed for Cd, Cu, Pb and Zn. Samples weighing 0.5 gm were placed in acid-washed glass tallform 100 ml beakers and digested with 9 mls concentrated HNO_3 and 3 mls concentrated HCl on a Lindberg SB type H-2 hot plate on setting 4 (medium setting) for at least 1/2 hour. The beakers were covered with acid washed watch glasses. After digestion, the samples were filtered through Whatman #42 filter paper and brought to 25 ml volume with distilled water (van Loon and Lichwa, 1973). Analysis for Cd, Cu, Pb and Zn were done using a Perkin Elmer Model 306 atomic adsorption spectrophotometer (AAS) equipped with background correction and an air-acetylene flame. Wavelengths used were: 288.8 nm for Cd, 324.7 nm for Cu, 283.3 nm for Pb and 213.9 nm for Zn. The same procedure was also used to determine Cd, Cu, Pb and Zn concentrations in the bulk Ap sample and the milorganite.

The earthworms were placed in acid washed glass vials and partially freeze-dried. The worms and the faeces were then dried at 80°C for 3 days and then weighed on a Cahn 25 automatic electrobalance.

The sample preparation method for earthworm tissue and faeces depended on sample size. Earthworms less than 150 mg dry weight and faeces less than 100 mg dry weight were placed in acid washed Kimax glass vials (70 x 20 mm O.D.) and digested at 150°C and a block digester. One milliliter of concentrated reagent grade nitric acid was added to the vial and then evaporated to dryness. This was repeated twice. Then 1 ml of 30% reagent grade hydrogen peroxide was added to the vial and evaporated to dryness. This was repeated once. Next, 5 mls of 0.16 N HNO_3 were added to each vial. This procedure was adapted by T. Guthrie (personal communication) from Koirtyohann et al. (1976).

Earthworms heavier than 150 mg dry weight and faeces samples heavier than 100 mg dry weight were placed in acid washed 100 ml tallform beakers and digested on a hotplate at medium setting with 9 mls concentrated reagent grade HNO_3 and 3 mls concentrated reagent grade HCl. Beakers were covered with acid washed watch glasses. After digesting for at least 1/2 hour, or until the volume was reduced by one half, the samples were removed from the hotplate and allowed to cool. Five mls deionized water were then added and the samples filtered through Whatman #42 filter paper into acid washed 25 ml volumetric flasks. The samples were brought to volume using deionized water. The samples were then transferred to acid washed 50 ml plastic bottles and stored in a refrigerator until analyses. All glassware and plastic bottles used for preparing earthworm and faeces samples were acid washed with 8 N HNO_3 and rinsed with deionized water.

Cadmium, Cu, and Zn levels in the earthworm tissue and Cu and Zn levels in the faeces were determined using the atomic absorption spectrophotometer as described previously for the soil-milorganite mixtures.

Cadmium and lead levels in the faeces and lead levels in the earthworm tissues were determined using a Perkin Elmer model 306 AAS equipped with the Perkin Elmer Hollow Graphite Analyzer (HGA) model 2100 and a deuterium arc background corrector. Twenty microliter injections were made from each sample with an Eppendorf microliter pipet. These injections were repeated two or three times for each sample. Argon was used as the carrier gas. Furnace operating parameters, wavelengths and slit settings are given in Table 2. The results were recorded using a Perkin Elmer model 56 strip chart recorder set at 10 mv with a speed of 120 mm min. Peak heights were used for quantitative purposes.

Table 2 Instrument Parameters for HGA

element		slit number	Gas mode	Dry	Ash	Atomize
				HGA 2100 settings		
Cd	229.3 nm	3	normal	20 Sec at 90°C	10 Sec at 300°C	10 Sec at 2000°C
Ni	232.3 nm	3	normal	20 Sec at 90°C	10 Sec at 300°C	10 Sec at 2000°C
Pb	387.7 nm	3	normal	20 Sec at 90°C	10 Sec at 300°C	10 Sec at 2000°C

The same methods were used by Carter et al. (1980,1983) to determine metal concentrations in earthworm samples. Along with the earthworm samples, National Bureau of Standards orchard leaves, bovine liver and soil standards were run to test the accuracy of the methods. Some of the results are given in Tables 3 and 4. (Carter, written communication).

These data indicate that both methods are reliable for determining Cd, Cu and Zn concentrations and that Pb determinations are variable and inaccurate. Lead concentrations may have been near the detection limit of the instrument.

Selected chemical and physical properties were determined for the bulk Ap soil sample. The soil pH was determined by a pH meter for the

Table 3 Analyses of N.B.S. Orchard Leaf Standard

Method	sample dry wt. mg	μg/gm dry wt. Cd	Cu	Pb	Zn
Block Digestor	92.5	0.07	not done		22.2
	96.0	0.07	not done		23.4
	188	0.02	not done		19.4
	99.8	0.07	not done		24
	53.1	0.09	not done	19	25
	48.9	0.10	not done	20	23
Hot Plate	103.3	0	12		36
	211.5	0	10		26
	206.0	not done	8		24
	230.0	not done	not done		23
	200.0	0.05	11		33
Expected Value		0.1	12 ± 2	44	28

Table 4 Analyses of N.B.S. Liver Standard

Method	sample dry wt. mg	μg/gm dry wet Cd	Cu	Pb	Zn
Block Digestor	9.0	0.56	161	not done	117
	10.6	0.47	149	not done	132
	47.1	0.37	not done	0.27	130
	48.5	0.36	144	0.12	119
Expected Value		0.27	193	0.34	130

soil mixed 1:2 with 0.01 M $CaCl_2$. Total carbon (%C) was determined using the Leco Analyzer. Total nitrogen (%N) was determined by the auto analyzer after digestion in salicylic - sulfuric acid using the Technican Block Digestor, Model BD-20. The cation exchange capacity (CEC) was determined by NH_4 displacement and Semi-Microkjeldahl analysis. A portion of the extract from the CEC determination was used to determine the amount of exchangeable Ca, Mg, K and Na using an atomic absorption spectrophotometer. Particle size analysis using the hydrometer method was used to determine % sand, silt and clay. The methods for all these analytical procedures are described in detail in Methods Manual (Dept. Soil Science, 1977).

The data for metal concentrations were then analyzed statistically using the UBC Genlin programme for analysis of variance for repeated measures (Greig and Bjerring, 1977). Duncan's and Scheffe's Multiple Range tests were also used. The data were also analyzed using the Mann-Whitney test statistic. (Siegel, 1956). Relationships between earthworm body weights and metal content (μg) were determined for cadmium, copper, lead and zinc, using the stepwise regression anaylsis, UBC TRP programme. (Le and Tenisci, 1978).

2.2 STATISTICAL ANALYSIS

The experimental design utilized was the randomized block design having repeated measures. This design requires that more than one measurement is made on the same experimental unit. The experimental units in this study were the flowerpots filled with the various soil-milorganite applications. For each experimental unit, the metal concentrations for three taxa of earthworms (up to ten individual earthworms were analyzed) were determined. This is the repeated measures aspect of the design. This is a more useful and more powerful design than the regular randomized block design. If the repeated measures design was not used, then in order to test the concentrations of metals in the three taxa at six different milorganite applications with five replicates of each application would require ninity experimental units instead of thirty. The growth chamber used in the experiment held a maximum of thirty flowerpots. Also, each taxon within a given experimental unit is subjected to the same conditions which decreases the sources of variability. If the randomized block design, and, therefore, ninety experimental units were used the possibility of increased experimental variability is greater. The repeated measure design is, therefore, a more powerful experimental design as measurements from a single experimental unit should be more homogeneous than measurements from similar experimental units (Fisher and McDonald, 1978; Lee, 1975; Winer, 1971).

Little and Hills (1972) suggest that from four to eight replications are required to obtain reasonable precision for field and vegetable crop research. In this study each application of milorganite was repeated five time. The number of individual earthworms analyzed for each treatment varied with the taxon and the survival of the earthworms. The lowest number of Lumbricus rubellus individuals was fifteen for the 100 gm/kg treatment. The lowest number of Aporrectodea spp. individuals was eighteen for the 100 gm/kg treatment. For the 0 gm/kg treatment only seven speciments of Octolasion cyaneum were analyzed.

The suggested approach to determine differences between treatments is to use the analysis of variance F test. This test will determine if there were significant treatment differences, but if more than two treatments are involved, the experimenter will not know which treatments are different. In order to determine which treatments are significantly different, multiple comparison or multiple range tests are needed to compare each treatment mean with every other treatment mean. Duncan's multiple range test is most commonly used (Larkin, 1977; Little and Hills, 1972; Mendenhall, 1968; Steel and Torrie, 1960) although the Scheffe's test is more rigorous. Scheffe's test statistic is a more conservative method with regards to type I errors which reject H_0 when in fact it is true (Wilner, 1976). The UBC Genlin analysis of variance programme utilized the F test and if the F value is significant for the tested term, the multiple range tests are done (Greig and Bjerring, 1977).

The Mann Whitney U test was also used to detect differences among treatment means (milorganite application) for each taxon. The Mann

Whitney U test is a non-parametric test which is a ranking test. It is a powerful non-parametric test, having 95% of the power of the parametric t test, and it is often used as an alternative to the t test when the assumptions for the t test are not met. The non-parametric tests do not require that the samples come from normally distributed populations and also that the populations are not required to have the same variance. For the Mann Whitney U test, the null hypothesis is that the two populations from which the samples are drawn have the same distribution. This implies the null hypothesis that the two populations have the same means (Larkin, 1977; Siegel, 1956).

In the present study, treatment differences were determined with Duncan's multiple range test, Scheffe's multiple range test and the Mann Whitney U test. The significant differences detected were only slightly different for the body concentrations of cadmium for a given taxon. Although the F test value for the addition of milorganite was significant, for each taxon Ducan's and Scheffe's multiple range test detected no significant differences in the body tissue copper and zinc for each milogranite application. The Mann Whitney U test did find some significant differences in body concentrations of zinc and copper.

The significant differences detected by the 3 statistical tests were different for the metal concentrations in the faeces. The Mann Whitney U test tended to detect more significant differences in the faeces concentrations of metals among the milorganite applications.

The 3 statistical tests may not produce the same significant groupings of treatments for everal reasons. Both Duncan's and Scheffe's multiple range tests assume that the treatments being tested are uncorrelated and for unbalanced cases this assumption is violated to some extent for the predicted means (Greig and Bjerring, 1977). The Mann Whitney U test allows for unbalanced comparisons. Secondly, the assumptions that the observations being tested have the same variance and normal distribution may be invalid and, therefore, the non-parametric test would be the test to use.

The majority of studies involving the determination of significant differences in metal concentrations in earthworms from different treatments (soils with different metal concentrations) use parametric statistical tests. Student's t test has been used by Andersen (1979, 19800, Ash and Lee (1980), Ireland (1975, 1976), Ireland and Richards (1977, 1981). Duncan's multiple range test was used by Gish and Christensen (1973) and Ireland (1979). Ireland (1977) and Ireland and Wooton (1976) used Scheffe's Multiple range test, and two way analysis of variance. Roberts and Johnson (1978) used the Mann Whitney U test.

To determine the relationships between metal content (ug) and body weights a step wise linear regression analysis was used. Also calculated were the r^2 values for the equations. Boyden (1977) and Bryan and Hummerstone (1973) used regression analysis to determine relationships between metal content and body weights for a number of species. Regression analysis was also used by Hartenstein, et al. (1980) to determine relationships between metal concentrations and body weights for the earthworm; Eisena foetida. Parker, et al. (1980) used covariance analysis to determine the relationships between body concentrations of metals and body weights of the polychaete, *Arenicola marina*.

3 RESULTS AND DISCUSSION

3.1 Metal Concentrations in Milorganite, Soil, Earthworms and Faeces

Concentration of metals in milorganite are given in Table 5. These data are in agreement with those obtained by John and van Laerhoven, 1976; Carson, (written communication).

Table 5 Metal Concentration (μg/gm air dry weight) in Milorganite. (values are the mean of 30 determinations)

Metal	$\bar{x}$ (ISD)
Cd	99 (31)
Cu	316 (36)
Ni	67 (7)
Pb	573 (78)
Zn	792 (91)

Metal concentrations in the bulk Ap sample are given in Table 6. Selected chemical and physical data for the Ap Crescent Soil Series (Luttmerding, 1981) and the bulk Ap sample used in this experiment are given in Table 6. Metal concentrations in the bulk Ap sample are given in Table 7.

Table 6 Metal Concentrations (μg/gm air dry weight) in bulk Ap Sample. (Sample Size = 30)

Metal	$\bar{x}$ (ISD)
Cd	0.6 ± 0.4
Cu	32 ± 1.8
Ni	38 ± 4.5
Pb	12 ± 11
Zn	72 ± 5.2

Table 7 Selected Chemical and Physical Properties of Ap Samples
(Cresent Series adapted from Luttmerding, 1981b)

Sample	Depth (cm)	pH (0.01 $MCaCl_2$	%C (organic)	%N	Exchangeable Bases (NH_4OAC)				CEC neg/100g Soil	Particle Size		
					Ca	Mg	K	Na		% Sand	% Silt	% Clay
Ap Crescent Series	0–28	4.3	3.07	0.20	8.44	1.13	0.51	0.35	23.5	8.00	66.6	25.4
Ap bulk sample	0–20	4.1	2.30	0.27	5.55	0.97	0.16	0.12	22.8	4.50	66.05	29.45

Table 8 Cadmium Concentration (µg/gm dry weight) in Soil, Body Tissue and Faeces of Three Earthworm Taxa from Six Milorganite Application Rates. (Values ± 1 S.D., number of samples in brackets).

	Milorganite Application Rate (gm milorganite/kg Soil)					
	0	5	10	25	50	100
Soil Cd	0.8 ± 0.40 (5)	1.5 ± 0.76 (5)	2.5 ± 0.94 (5)	3.8 ± 0.94 (5)	7.3 ± 4.75 (5)	12 ± 3.49 (5)
Taxa			**Body tissue**			
Lumbricus rubellus	11.0 ± 5.14 (18)	18.0 ± 4.23 (19)	21.4 ± 6.07 (18)	32.4 ± 7.63 (16)	38.0 ± 7.97 (18)	38.8 ± 9.06 (15)
Aporrectodea spp.	8.4 ± 2.45 (20)	13.7 ± 2.79 (20)	13.4 ± 2.79 (20)	19.0 ± 5.48 (20)	27.1 ± 6.72 (19)	31.4 ± 6.28 (18)
Octolosion cyaneum	1.24 ± 4.39 (7)	18.5 ± 4.17 (8)	21.0 ± 5.42 (8)	27.2 ± 3.17 (8)	43.8 ± 9.68 (9)	41.0 ± 5.67 (8)
			Faeces			
Lumbricus rubellus	0.4 ± 0.20 (10)	1.4 ± 0.47 (10)	2.7 ± 2.21 (8)	6.3 ± 4.47 (10)	12.7 ± 3.67 (10)	9.0 ± 5.74 (9)
Aporrectodea spp.	0.5 ± 0.47 (11)	1.8 ± 1.26 (10)	2.0 ± 1.05 (8)	4.0 ± 1.81 (9)	5.4 ± 3.67 (9)	5.1 ± 2.34 (8)
Octolasion cyaneum	0.7 ± 0.64 (8)	1.1 ± 0.44 (8)	2.9 ± 1.17 (7)	3.7 ± 1.06 (9)	6.4 ± 2.29 (9)	13.6 ± 14.74 (8)

Table 9. Copper Concentration (μg/gm dry weight) in Soil, Body Tissue and Faeces of Three Earthworm Taxa from Six Milorganite Application Rates. (Values ± 1 S.D., number of samples in brackets).

	Milorganite Application Rate (gm milorganite/kg soil)					
	0	5	10	25	50	100
Soil Cu	37 ± 2.10 (5)	37 ± 2.51 (5)	40 ± 2.10 (5)	44 ± 2.11 (5)	52 ± 5.60 (5)	65 ± 3.11 (5)
Taxa			**Body tissue**			
<u>Lumbricus rubellus</u>	15.2 ± 6.11 (18)	16.2 ± 3.67 (19)	17.3 ± 4.53 (18)	19.4 ± 5.64 (16)	21.5 ± 5.34 (18)	20.2 ± 5.64 (15)
<u>Aporrectodea spp.</u>	10.5 ± 3.04 (20)	14.2 ± 3.31 (20)	18.4 ± 4.51 (20)	17.0 ± 11.07 (20)	16.3 ± 19.77 (19)	18.5 ± 19.19 (18)
<u>Octolosion cyaneum</u>	24.7 ± 3.90 (8)	24.9 ± 4.55 (8)	25.4 ± 4.39 (8)	26.6 ± 4.55 (8)	28.4 ± 2.90 (9)	29.6 ± 5.48 (8)
			Faeces			
<u>Lumbricus rubellus</u>	29.2 ± 6.75 (10)	38.1 ± 9.94 (10)	46.7 ± 14.3 (10)	58.0 ± 24.03 (8)	85.6 ± 21.11 (10)	61.9 ± 17.87 (9)
<u>Aporrectodea spp.</u>	29.6 ± 9.32 (11)	38.2 ± 4.57 (9)	39.3 ± 5.23 (10)	44.3 ± 7.57 (8)	52.2 ± 11.12 (9)	45.5 ± 9.14 (8)
<u>Octolasion cyaneum</u>	32.0 ± 5.62 (8)	37.1 ± 5.36 (8)	42.7 ± 8.70 (8)	45.4 ± 4.43 (7)	56.4 ± 8.51 (9)	83.7 ± 85.45 (8)

Table 10. Lead Concentration (µg/gm dry weight) in Soil, Body Tissue and Faeces of Three Earthworm Taxa from Six Milorganite Application Rates. (Values ± 1 S.D., number of samples in brackets).

	Milorganite Application Rate (gm milorganite/kg soil)					
	0	5	10	25	50	100
Soil Pb	21 ± 2.91 (5)	21 ± 2.20 (5)	27 ± 5.02 (5)	33 ± 3.11 (5)	51 ± 11.31 (5)	76 ± 8.31 (5)
Taxa			**Body tissue**			
<u>Lumbricus</u> <u>rubellus</u>	1.2 ± 1.12 (18)	0.7 ± 0.48 (19)	1.3 ± 1.65 (18)	1.5 ± 1.38 (16)	4.0 ± 4.59 (18)	2.1 ± 1.53 (15)
<u>Aporrectodea</u> <u>spp</u>.	1.2 ± 1.09 (20)	2.2 ± 2.05 (20)	3.6 ± 4.93 (20)	1.6 ± 2.00 (20)	1.5 ± 1.54 (19)	2.5 ± 4.40 (18)
<u>Octolosion</u> <u>cyaneum</u>	5.0 ± 4.68 (7)	4.0 ± 5.44 (8)	5.7 ± 6.72 (8)	2.8 ± 3.20 (8)	3.0 ± 2.63 (8)	5.8 ± 7.80 (8)
			Faeces			
<u>Lumbricus</u> <u>rubellus</u>	12.8 ± 11.41 (10)	24.7 ± 13.32 (10)	28.5 ± 22.57 (10)	51.9 ± 43.50 (8)	113.3 ± 44.81 (10)	72.0 ± 46.84 (9)
<u>Aporrectodea</u> <u>spp</u>.	20.5 ± 20.66 (11)	17.4 ± 7.77 (9)	17.4 ± 9.74 (10)	52.7 ± 33.04 (8)	51.4 ± 22.91 (9)	54.3 ± 31.38 (8)
<u>Octolasion</u> <u>cyaneum</u>	7.0 ± 2.47 (8)	10.3 ± 5.65 (8)	18.4 ± 6.62 (8)	25.2 ± 12.56 (7)	44.2 ± 21.72 (9)	116.2 ± 138.02

Table 11. Zinc Concentration (μg/gm dry weight) in Soil, Body Tissue and Faeces of Three Earthworm Taxa from Six Milorganite Application Rates. (Values ± 1 S.D., number of samples in brackets).

	Milorganite Application Rate (gm milorganite/kg soil)					
	0	5	10	25	50	100
Soil Zn	77 ± 7.78 (5)	74 ± 7.38 (5)	80 ± 1.90 (5)	95 ± 6.89 (5)	116 ± 7.58 (5)	161 ± 21.87 (5)
Taxa	**Body tissue**					
Lumbricus rubellus	388.6 ± 122.51 (18)	317.2 ± 61.36 (19)	321.6 ± 89.34 (18)	293.5 ± 112.42 (16)	281.4 ± 63.78 (18)	307.2 ± 86.23 (15)
Aporrectodea spp.	378.2 ± 114.29 (20)	334.7 ± 120.52 (20)	332.9 ± 74.67 (20)	332.9 ± 87.30 (20)	343.1 ± 75.05 (19)	392.7 ± 77.75 (18)
Octolosion cyaneum	180.3 ± 46.94 (7)	184.8 ± 34.14 (8)	207.7 ± 46.55 (8)	217.0 ± 47.15 (8)	236.6 ± 24.34 (9)	225.7 ± 26.07 (8)
	Faeces					
Lumbricus rubellus	71.9 ± 28.47 (10)	65.7 ± 21.42 (10)	89.7 ± 35.73 (10)	106.4 ± 41.40 (8)	160.5 ± 34.57 (10)	135.8 ± 49.20 (9)
Aporrectodea spp.	67.5 ± 13.22 (11)	79.0 ± 15.40 (9)	89.1 ± 20.67 (10)	92.2 ± 23.09 (9)	101.8 ± 17.58 (9)	98.2 ± 15.94 (8)
Octolasion cyaneum	72.4 ± 7.52 (8)	75.5 ± 11.05 (8)	91.9 ± 23.80 (8)	97.4 ± 17.48 (9)	124.9 ± 17.51 (9)	136.0 ± 60.32 (8)

Figure 1. Effects of sewage sludge applications on cadmium levels in tissue and faeces of mature adults of <u>Lumbricus rubellus</u>.

Rates of sludge application (gm/kg air dried soil) in parentheses.

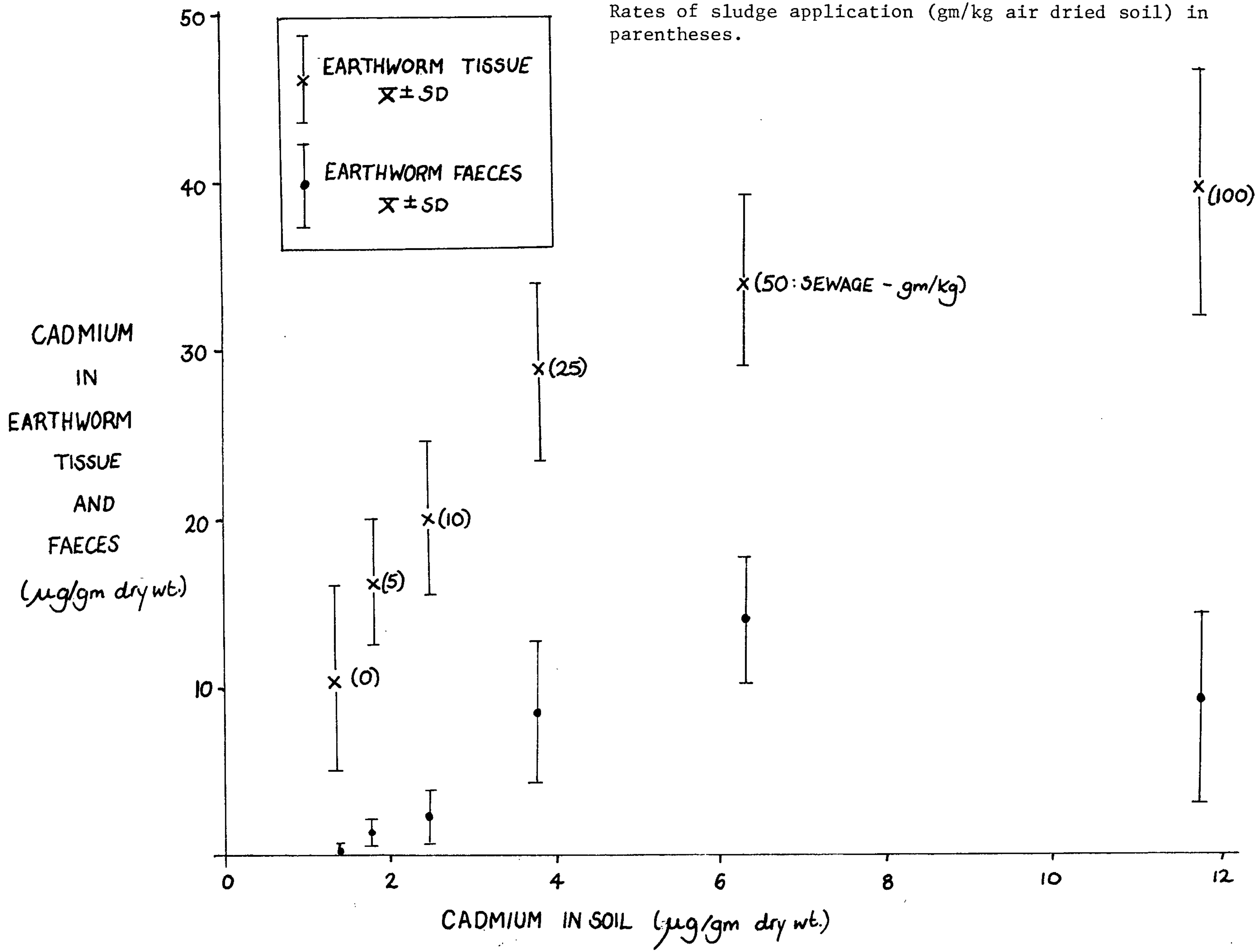
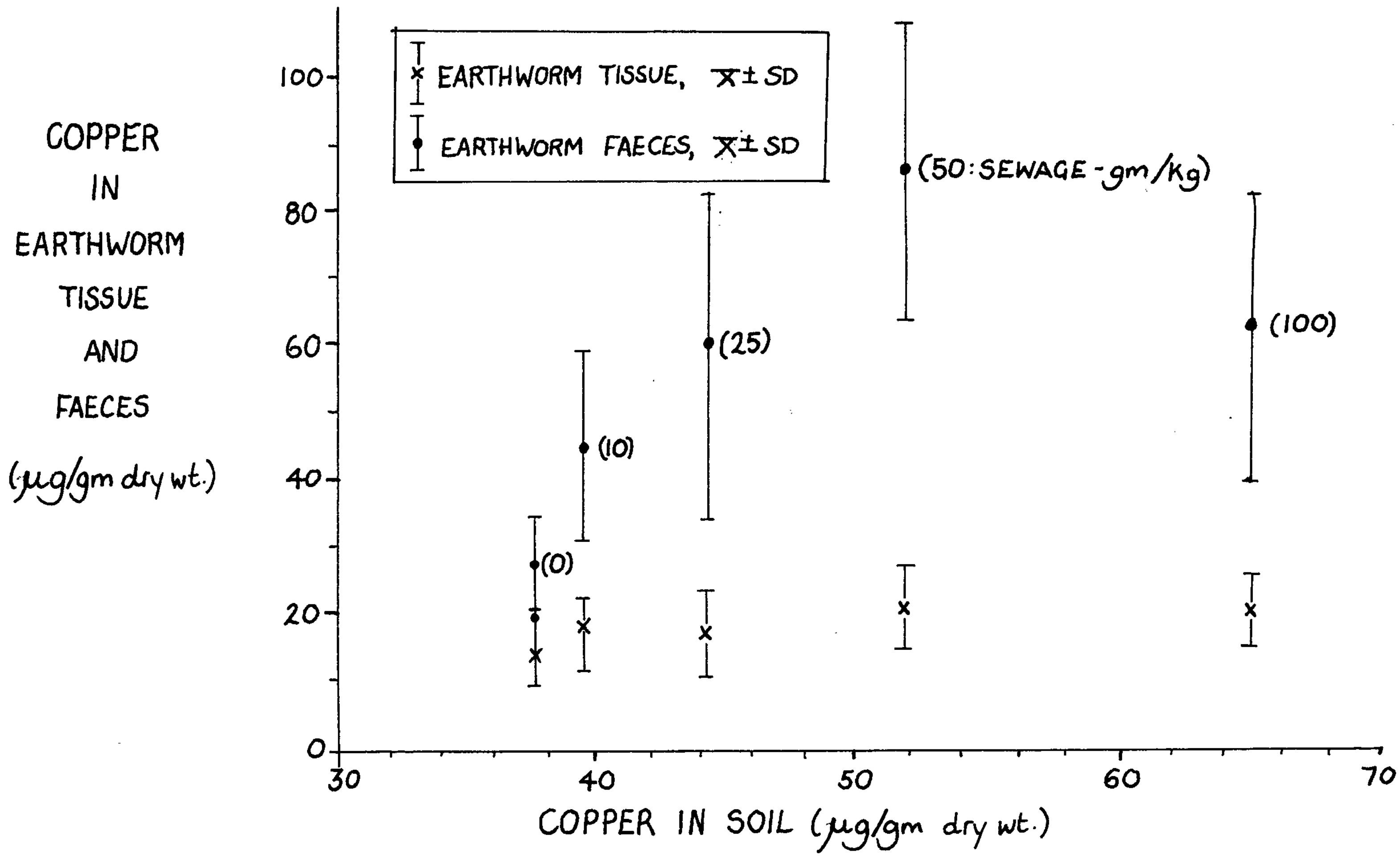


Figure 2. Effects of sewage sludge applications on copper levels in tissue and faeces of mature adults of Lumbricus rubellus.

Rates of sludge application (gm/kg air dried soil) in parentheses.



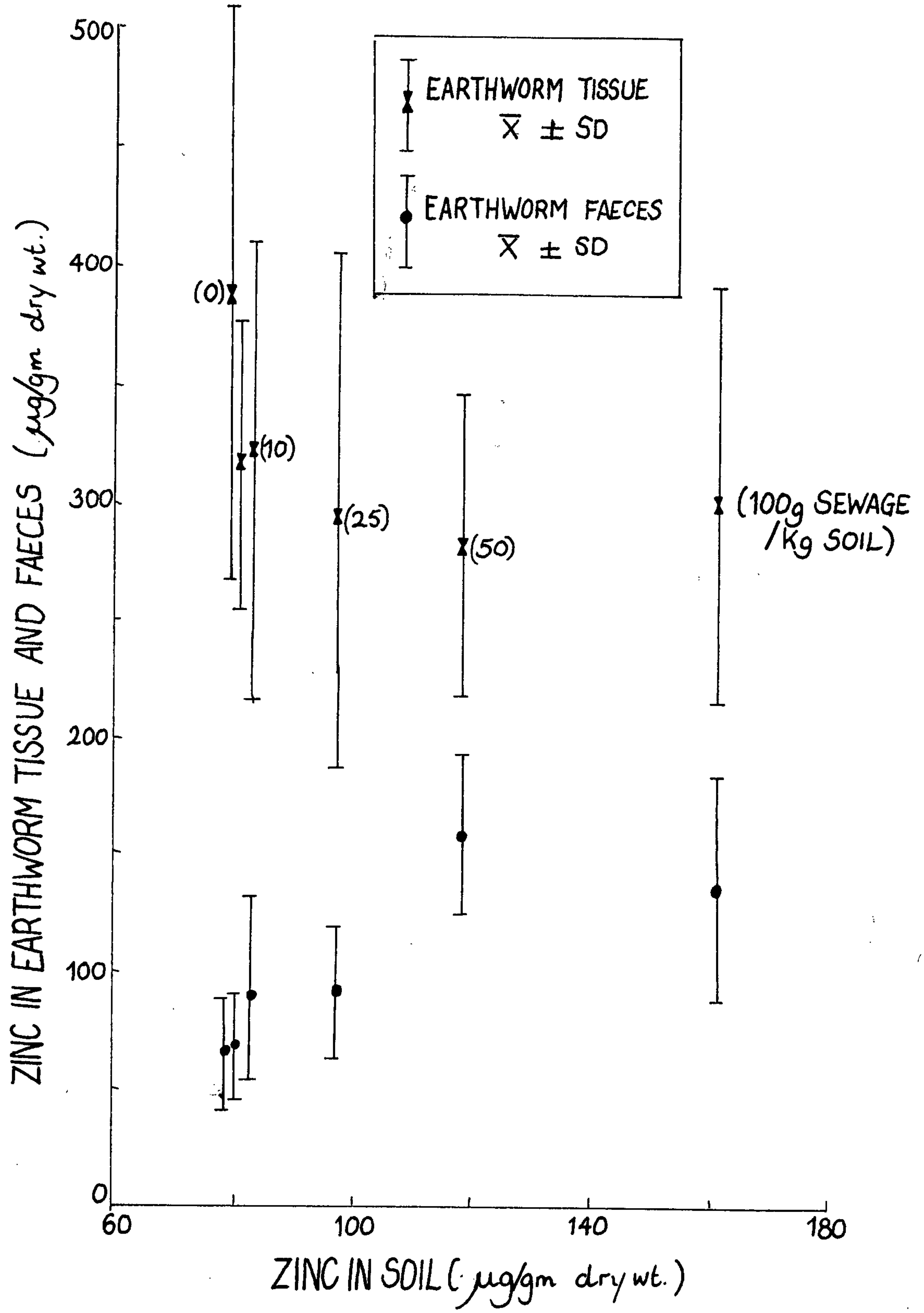


Figure 3. Effects of sewage sludge applications on zinc levels in tissue and faeces of mature adults of Lumbricus rubellus.

Rates of sludge application (gm/kg air dried soil) in parentheses.

Table 12 - ANOVA Table for Cadmium in Earthworm Body Tissue (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	4804.0	141.91	0.00000
Pots (Milorganite)	24	32.271	0.95327	0.52951
Taxa	2	1985.9	58.664	0.00000
Milorganite * Taxa	10	106.42	3.1435	0.00087
Residual	227	33.853		
Total	268			

Table 13 - ANOVA Table for Cadmium in Earthworm Faeces (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	348.46	141.91	0.00000
Pots (Milorganite)	23	22.742	1.4831	0.08940
Taxa	2	66.174	4.3155	0.01551
Milorganite * Taxa	10	45.264	2.9519	0.00239
Residual	119	15.334		
Total	159			

Table 14 - ANOVA Table for Copper in Earthworm Body Tissue (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	258.51	3.2063	0.00701
Pots (Milorganite)	24	80.626	1.0232	0.43741
Taxa	2	1900.6	24.120	0.00000
Milorganite * Taxa	10	34.607	0.43919	0.92608
Residual	227	78.798		
Total	268			

Table 15 - ANOVA Table for Copper in Earthworm Faeces (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	5369.2	12.347	0.0000
Pots (Milorganite)	23	783.62	1.8020	0.02210
Taxa	2	1703.3	3.9169	0.02252
Milorganite * Taxa	10	884.70	2.0345	0.03548
Residual	119	434.86		
Total	159			

Table 16 - ANOVA Table for Lead in Earthworm Body Tissue (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	16.49	1.6040	0.15996
Pots (Milorganite)	24	13.992	1.3669	0.12470
Taxa	2	115.51	11.284	0.00002
Milorganite * Taxa	10	18.140	1.7721	0.06678
Residual	226	10.236		
Total	267			

Table 17 - ANOVA Table for Copper in Earthworm Faeces (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	2200.5	18.739	0.00000
Pots (Milorganite)	23	3454.5	2.9417	0.00007
Taxa	2	3544.2	3.0181	0.05265
Milorganite * Taxa	10	3806.3	3.2413	0.00100
Residual	119	1174.6		
Total	159			

Table 18 - ANOVA Table for Zinc in Earthworm Body Tissue (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	19239.0	2.7889	0.01820
Pots (Milorganite)	24	12391.0	1.7889	0.01590
Taxa	2	0.33977×10^6	49.255	0.00000
Milorganite * Taxa	10	10170.0	1.4743	0.15003
Residual	227	6898.3		
Total	268			

Table 19 - ANOVA Table for Zinc in Earthworm Faeces (5% probability level)

Source	DF	Mean Square	F Ratio	Probability
Milorganite	5	16963.0	21.155	0.00000
Pots (Milorganite)	23	871.64	1.0870	0.36954
Taxa	2	4160.9	5.1891	0.00691
Milorganite * Taxa	10	2004.4	2.4997	0.00925
Residual	119	801.85		
Total	159			

Table 20 - Significant Groupings (5% probability level) of Milorganite Application Rates (gm/kg) for Cd Concentrations in 3 Earthworm Taxa Body Tissue and Faeces (Application Rates Within a Taxon Followed by a Common Letter are not Significantly Different at the 5% Level).

	Multiple Range Test		
Taxon (Body Tissue)	Duncan's	Scheffe's	Mann Whitney
Lumbricus rubellus	0a 5b, 10b 25c 50d 100d	0a 5ab 10b 25c 50c 100c	0a 5b 10b 25c 50c 100c
Aporrectodea spp.	0a 5b 10b 25c 50d 100e	0a 5ab 10ab25abc50bc100c	0a 5b 10b 25c 50d 100d
Octolasian cyaneum	0a 5ab 10bc25c 50d 100d	0a 5a 10a 25ad 50c 100cd	0a 5b 10b 25c 50d 100d
Taxon (Faeces)			
Lumbricus rubellus	0a 5a 10ab25bd50c 100d	0a 5a 10a 25ab50b 100ab	0a 5b 10c 25c 50d 100d
Aporrectodea spp.	0a 5ab 10ab25ab50b 100b	0a 5a 10a 25a 50a 100a	0a 5b 10b 25c 50c 100c
Octolasion cyaneum	0a 5a 10ab25ab50b 100c	0a 5a 10a 25ab50ab100b	0a 5a 10b 25b 50c 100c

Table 21 - Significant Groupings (5% probability level) of Milorganite Application Rates (gm/kg) for Cu Concentrations in 3 Earthworm Taxa Body Tissue and Faeces (Application Rates Within a Taxon Followed by a Common Letter are not Significantly Different at the 5% Level).

	Multiple Range Test		
Taxon (Body Tissue)	Duncan's	Scheffe's	Mann Whitney
Lumbricus rubellus	0a 5a, 10a 25a 50a 100a	0a 5a, 10a 25a 50a 100a	0a 5a 10a 25b 50b 100b
Aporrectodea spp.	0a 5a, 10a 25a 50a 100a	0a 5a, 10a 25a 50a 100a	0a 5b, 10c 25c 50c 100c
Octolasian cyaneum	0a 5a, 10a 25a 50a 100a	0a 5a, 10a 25a 50a 100a	0a 5a, 10a 25a 50a 100a
Taxon (Faeces)			
Lumbricus rubellus	0a 5ab 10abd25b 50c 100d	0a 5ab 10ab25ab50b 100ab	0a 5b 10bc 25ce50d 100e
Aporrectodea spp.	0a 5ab 10ab 25ab50b 100ab	0a 5a 10a 25a 50a 100a	0a 5ab 10b 25ab50b 100ab
Octolasion cyaneum	0a 5ab 10ab 25ab50b 100c	0a 5a 10a 25a 50a 100a	0a 5ab 10b 25b 50c 100c

Table 22 - Significant Groupings (5% probability level) of Milorganite Application Rates (gm/kg) for Pb Concentrations in 3 Earthworm Taxa Body Tissue and Faeces (Application Rates Within a Taxon Followed by a Common Letter are not Significantly Different at the 5% Level).

	Multiple Range Test		
Taxon (Body Tissue)	Duncan's	Scheffe's	Mann Whitney
Lumbricus rubellus	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a
Aporrectodea spp.	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a
Octolasian cyaneum	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a
Taxon (Faeces)			
Lumbricus rubellus	0a 5ab10ab25bd50c 100d	0a 5a 10ab25ab50ab100b	0a 5b 10bc25ce50de100e
Aporrectodea spp.	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10a 25b 50ab100b
Octolasion cyaneum	0a 5a 10a 25a 50a 100b	0a 5a 10a 25ab50ab100b	0a 5a 10b 25b 50b 100b

Table 23 - Significant Groupings (5% probability level) of Milorganite Application Rates (gm/kg) for Zn Concentrations in 3 Earthworm Taxa Body Tissue and Faeces (Application Rates Within a Taxon Followed by a Common Letter are not Significantly Different at the 5% Level).

	Multiple Range Test		
Taxon (Body Tissue)	Duncan's	Scheffe's	Mann Whitney
Lumbricus rubellus	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5b 10b 25b 50b 100b
Aporrectodea spp.	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50ab100b
Octolasian cyaneum	0a 5a 10a 25a 50a 100a	0a 5a 10a 25a 50a 100a	0a 5a 10ab25ab50b 100b
Taxon (Faeces)			
Lumbricus rubellus	0a 5a 10a 25ab50c 100d	0a 5a 10a 25ab50b 100b	0a 5a 10b 25bd50c 100cd
Aporrectodea spp.	0a 5ab10a 25ab50b 100b	0a 5a 10a 25a 50a 100a	0a 5a 10ab25ab50b 100b
Octolasion cyaneum	0a 5a 10a 25a 50bc100c	0a 5a 10a 25ab50b 100b	0a 5a 10bd25bd50c 100cd

The Cd, Cu, Pb and Zn concentrations in earthworm body tissue, casts and soil-milorganite media from the treatments are given in Tables 8 through 11. The body tissue metal concentrations for Octolasion cyaneum should be treated with caution, as this species did not effectively clear its guts, and the body tissue would be contaminated with faeces. As the concentrations of Cd and Zn in faeces are lower than body tissue levels, the contamination by casts may not be serious, whereas incomplete clearance of the gut would contribute to the body tissue Cu and Pb levels. Faecal concentrations of these two metals exceeded body tissue levels. Figures 1-3 present the Cd, Cu, and Zn concentrations in the body tissue and faeces of Lumbricus rubellus. ANOVA tables for the 4 metals and the treatments are presented in Tables 12 through 19. Tables 20 through 23 present the significant groupings of the milorganite applications with regards to the metal concentrations within the earthworm taxa.

The highest survival percentage was achieved by the Aporrectodea Spp. whereas Octolasion cyaneum had the lowest.

3.2 Cadmium

Cadmium was accumulated by the body tissues of all 3 earthworms taxa studied (Table 8). Tissue concentrations in all 3 taxa also exceeded those in the soil - milorganite mixture (the earthworm's food). There was a highly significant effect on earthworm tissue Cd concentrations resulting from the addition of milorganite (Table 12). Cadmium levels in tissue increased steadily as milorganite applications increased until soil concentrations exceeded 7 μg/gm Cd. Increasing the milorganite application rate from 50 gm/kg to 100 gm/kg did not result in significant increases in the Cd concentrations in the body tissues (Table 20). Figure 1 illustrates the effects of milorganite applications on Cd concentrations in the body tissue and faeces of L. rubellus.

The above results are in agreement with those from other studies. Helmke et al. (1979) determined that cadmium was accumulated by A. tuberculata. With increasing sewage sludge applications, the mean cadmium concentrations in the earthworm increased for 7 to 18 μg/gm for the controls to over 100 μg/gm for the highest application rate (60 tonnes/ha). Their lowest Cd value for the controls is similar to the control values for Aporrectodes spp. in this study, whereas the concentration for the highest milorganite application in the present study (equivalent to 225 tonnes/ha) was 31 μg/gm. There are several reasons why this difference may have occurred. In the present study, Aporrectodea spp., consists of A. tuberculata and A. trapezoides with possible differences between the species. The sewage sludge used by Helmke et al. may have had more available Cd, although the total Cd was similar to the total Cd in the milorganite. The study conducted by Helmke et al. was a field experiment conducted over a five year term, whereas the present study was a very short term laboratory study lasting for ten days. The earthworms in Helmke's study may have accumulated Cd in a step wise manner. Their activity may have decreased due to unfavourable temperatures and/or metal toxicity allowing the earthworms to reduce their body burden of cadmium. Feeding would again resume when conditions became favourable (Carter et al. 1981).

Hartenstein et al. (1980) found that E. foetida accumulated cadmium above levels in soil ammended with sewage sludge. They observed a maximum concentration of 50 μg/gm for their field studies. Higher concentrations, up to 170 μg/gm were found in earthworms raised in sewage sludge spiked with soluble $CdSO_4$. Andersen (1979) found that several species of earthworms strongly concentrated Cd over the levels in the soil. He found that the earthworms with the higher Cd concentrations were sampled from fields receiving sewage sludge with the higher concentrations of cadmium. Andersen also found that cadmium levels were highest in L. terrestris sampled from soils near busy roads where soil cadmium levels were greatest. In all cases, the earthworms had greater Cd concentrations than the soil. Ireland (1979) reported that L. rubellus concentrated cadmium over the levels in the soil. His values are similar to those for this study. Wright and Stinger (1980) determined the Cd concentrations in various species of earthworms sampled from soils downwind from a lead-zinc smelter. Their results again indicated that earthworms concentrated Cd above soil levels. The higher Cd body concentrations were in specimens sampled from sites with higher soil cadmium. The results quoted were similar to the results obtained in the present study. Gish and Christensen (1973), Czarnowska and Japkiewiez (1978) and Ash and Lee (1980) studying soils and earthworms near busy roads, all found that earthworms concentrated Cd above soil levels and accumulated the metal in response to increasing soil cadmium levels.

The ANOVA given in Table 13 indicates that the application of milorganite had a highly significant effect on the faecal Cd concentrations. The data in Table 8 show that the Cd concentrations in the casts increased with increasing milorganite application until the soil Cd levels exceeded 7 μg/gm and then dropped for L. rubellus and levelled out for Aporrectodea spp. In contrast, faecal Cd concentrations for O. cyaneum continued to increase with the 100 gm/kg application. However, the differences in Cd concentrations between casts from the 2 highest application rates are not significant (Table 20).

The results for Cd concentrations in earthworm casts obtained in this study are comparable to those reported in the literature. Czarnowska and Jopkiewicz (1978) found much lower Cd concentrations in the casts of L. terrestris than in the body tissues, as did Helmke et al. (1979) for A. tuberculata. In both studies, the Cd concentrations in the faeces were similar to soil Cd concentrations and in the latter study the Cd concentrations in the casts increased with increasing sewage sludge applciation. The same pattern was also noted by Ash and Lee (1980). Lumbricus terrestris and Aporrectodea chlorotica had higher amounts of Cd in their casts when sampled from soils with higher Cd levels. Cadmium in the faeces was much lower than the concentraiton in the body tissue of these two species. Similar results were obtained for Allolobophora longa in Denmark (Andersen, 1980). The casts had much lower Cd concentrations than did the earthworm body tissue and less than the soil when the soil Cd concentration was less than 0.65 μg/gm.

The data, obtained in this study suggest that at the two highest application rates of milorganite, feeding and excretory activities of the 3 taxa slowed down perhaps in a toxic response to levels of metals in the soil-milorganite medium. The Cd concentrations in the faeces were much lower than the concentration in the soil milorganite media. These results suggest that the ingested cadmium was effectively concentrated in the body tissue and the excretion of Cd is very slow. Friberg et al. 91979) indicates that for humans the excretion of cadmium is from 0.005 to 0.01% of the total body burden per day. Van Hook and Yates (1975) determined that crickets assimilated 17% of ingested ^{109}Cd and that tissue elimination of assimilated ^{109}Cd was 0.09-0.11% per day and that gut elimination was 0.8-0.9% per day. The elimination of ^{109}Cd by lycozid spiders was 0.007% per day.

There is a highly significant difference in the body tissue cadmium concentration of all three taxa (Table 12). Aporrectodea spp. contained significantly lower concentrations of Cd in their body tissue than did the other two taxa studied. (Duncan's and Scheffe's multiple range tests, $p<0.05$). There was no significant differences between the Cd concentrations in the body tissue of L. rubellus and O. cyaneum. There is a significant difference ($P<0.05$) between the Cd concentration in the casts of the different taxa (Table 13). The casts of Aporrectodea spp. had significantly lower Cd concentrations than the faeces of L. rubellus. The faeces of O. cyaneum had Cd concentrations that were not significantly different from the other two taxa. Differences between the taxa might have been due to differences in the absorption of cadmium from the diet or reflect differences in feeding and excretory activities. Lumbricus rubellus is a shallow burrowing species and a mixed feeder whereas the other two taxa are deep burrowers and may feed more on mineral soil.

Significant differences in cadmium concentration among different earthworm species have been reported in the literature. Ireland (1979) reported than Dendrobaena veneta contained significantly higher amounts of Cd than L. rubellus and Eisenielba tetraedra. The latter two species were not significantly different from each other in Cd content. L. rubellus and L. terrestris did not differ significantly in their Cd levels but were higher than Cd levels in A. chlorotica (Ash and Lee 1980). Wright and Stinger (1980) found that Allolobophora caliginosa had significantly higher Cd concentrations than did the five other species studied, which had no significant differences among them.

3.3 Copper

Copper concentrations in the body tissue of all three taxa showed a slight increase wth increasing application rates of milorganite, although at any application rate, copper concentrations in the earthworms did not exceed soil copper concentrations (Table 9). There was a significant effect due to the application of milorganite (Table 14). However, there were no significant miloganite x taxa interactions (ANOVA Table 14). The Mann Whitney U test did not find any significant differences ($p>0.05$) among the milorganite applications for Octolasion cyaneum. There were significant differences ($p<0.05$) among the applications of milorganite, using the Mann Whitney U test, for Lumbricus rubellus and Aporrectodea spp. (Table 21). The high mean body tissue copper concentrations for Aporrectodea spp. for the 50 gm/kg and 100 gm/kg are the result of several individual values which are much higher than the remaining individual determinations. These high values (92.9 μg/gm Cu for one individual from the 50 gm/kg treatment the next highest concentration for this application was 26.8 8 μg/gm Cu; 45.3 μg/gm Cu, 53.7 μg/gm Cu, 74.4 μg/gm Cu were the three highest concentrations obtained for individual Aporrectodea spp. specimens from the 100 gm/kg application, while the next highest concentration was 16.4 μg/gm Cu) may be outliers and a result of contamination. With these high values discarded, the mean body tissue copper concentrations for the 50 gm/kg and 100 gm/kg applications are 12.0 ± 7.28 μg/gm and 10.7 ± 4.30 μg/gm respectively.

The data in Table 9 compare favourably with the results from other studies. Helmke, et al. (1979) determined copper concentration in Aporrectodea tuberculata sampled from fields receiving increasing amounts of sewage sludge. There were slight increases in copper concentrations in the earthworms as the sewage sludge application increased, although no statistical evaluations of the data were done. The concentrations that Helmke, et al. reported were similar to the copper concentations obtained for the Aporrectodea spp. in the present study. Hartenstein, et al. (1979) found that copper concentrations in the earthworm, Eisenia foetida, feed grain or a manure-sewage sludge mixture were much higher in the earthworms feed the latter medium. Eisenia foetida, raised in sewage sludge, varied slightly in their copper concentrations, increasing with increased levels in the sludge, although the copper levels in the sewage sludge exceeded those in the earthworm. The copper levels reported by Hartenstein, et al. (1979) for *E. foetida* suggest there is only slight variation in tissue concentrations while there is a greater variation in the sludge copper levels.

In Ireland's study (1979), copper concentrations in *Lumbricus rubellus*, sampled from soils with differing copper concentrations, were not significantly different. The copper concentrations in the earthworms were lower than the soil copper concentrations. His data for copper concentration in *L. rubellus* were lower than those obtained in this study. Ireland and Richards (1977) compared the copper concentrations in *L. rubellus* and *Dendrobaena rubida* sampled from a sewage filter bed and soil influenced by a lead zinc mine spoil. although the soil concentrations were different at each site, there were no significant differences between the earthworm concentrations from each site for a given species.

Van Rhee (1977) reported that the copper content in a mixed sample of *Allolobophora* spp. and *L. rubellus*, from soils treated with copper fungicides, increased with increasing soil copper concentrations. There was a significant correlation between soil levels and earthworm levels.

The results reported by Ash and Lee (1980) suggest that copper was accumulated by *Lumbricus terrestris*, *L. rubellus*, and *Allobophora chlorotica* sampled close to roads with varying traffic densities. They found that *Lumbricus terrestris* sampled from the site with the lowest soil copper concentration had significantly lower body tissue levels of copper. The results obtained for L. terrestris for the other sites were not significantly different from each other. There were also significant differences in the copper body levels of A. chlorotica among the sites. Earthworms collected from soils, near Warsaw roads, with higher copper levels were found to have higher body tissue concentrations of copper. Copper was accumulated by L. terrestris but not concentrated as soil copper levels exceeded those in the earthworms (Czarnowska and Japkiewicz, 1978).

The present study also indicates that copper concentrations in the faeces of all three taxa increases with increasing miloganite application. Mean copper levels in the faeces of L. rubellus and Aporrectodea spp. declined once the soil sewage sludge mixture concentration of copper exceeded 52 μg/gm (Figure 4 and Table 9). Octolasion cyaneum's faeces continued to have higher mean copper concentrations at the highest milorganite application. This high mean value results from two exceedingly high values (276.4 and 137.9 μg/gm). When these two values are included in the mean for the 100 gm/kg application, the value is 78.5 ± 81.45 μg/gm Cu. Without these two values, the mean Cu concentration is 41.74 ± 12.36 μg/gm. These two high values are most likely the result of contamination and probably should be disregarded. When these values are disregarded, the copper concentration in the faeces of Octolasion cyaneum from the highest milorganite application also shows the same decline in copper concentrations demonstrated for the other two taxa studied. The decline in copper concentration in the faeces at the highest application of milorganite may indicate a drop in the feeding or excretion activities of these earthworms. At the highest application rate of milorganite, the metal levels in the soil milorganite mixture may have been toxic to the earthworms. Copper levels in the faeces of all three taxa exceeded the copper levels in the body tissue and suggests that copper is effectively excreted by the earthworms.

Copper levels in the faeces were significantly ($p<0.05$) affected as milorganite application rates increased (Table 15). The Cu concentations in the faeces of L. rubellus (Figure 2) and Aporrectodea spp. increased steadily and then declined once soil Cu levels exceeded 52 μg/gm Cu. The casts of O. cyaneum showed an increase in Cu concentration with the 100 gm/kg milorganite application rate, although this increase was not significant (Scheffe's and Mann Whitney tests, Table 21). The decrease in Cu concentration in the casts of L. rubellus from the 100 gm/kg treatment was significant by Duncan's and Mann Whitney range tests (Table 21).

Helmke, et al. (1979) indicated that the copper concentrations in the casts of Aporrectodea tuberculata were higher than the concentrations for the earthworm body tissues. The copper content in the casts also increased wth increasing sewage sludge applications. They did not observe a drop in the copper content in the casts from the highest application as was the case for the Aporrectodea spp. in the present study, although the decrease was not significant. However, the highest application of sewage sludge was 60 tonnes/hectare, whereas in the present study, the highest application of milorganite is equivalent to 225 tonnes/hectare. In contrast, to the present study and that of Helmke, et al. (1979), Ash and Lee (1980) reported that tissue samples of Lumbricus rubellus obtained from soils watered with a 1% copper salt solution had twice as much copper as did the faecal samples. The body tissue samples of L. terrestris, L. rubellus, and Allobophora chloratica obtained from their roadside study were also higher in copper than the faecal samples. These studies indicate that the chemical form of the element is important.

There was a significant difference among taxa in the copper concentrations of earthworm body tissue (Table 14). The overall mean for earthworm body tissue copper concentrations in O. cyaneum were significantly higher than the concentrations in L. rubellus and Aporrectodea spp., whereas the concentrations in the latter two taxa were not significantly different from each other (Duncan's and Scheffe's multiple range tests, $p<0.05$). Copper is an essential metal and O. cyaneum may have either a higher biological requirement for copper or may have a higher tolerance for copper than the other two taxa studied.

There was a taxa difference in the copper concentrations of the faeces (Table 15). The overall mean for copper concentrations in earthworm casts was significantly higher in L. rubellus than in Aporrectodea spp. Octolasian cyaneum did not significantly differ in the copper concentration of its casts from those of either Aporrectodea spp. and L. rubellus. This suggests that L. rubellus may be more efficient at excreting copper than the Aporectodea spp., as the body tissue copper concentrations of these two taxa were not significantly different (Duncan's and Scheffe's Multiple range tests, $p<0.05$).

The lack of signifiant increase in the body tissue copper concentrations of Octolasian cyaneum with increasing milorganite applications (Table 21) suggest that this species is regulating its body copper levels. The copper concentrations in the body tissue of Aporrectodea spp. from the 10 gm/kg, 25 gm/kg, 50 gm/kg and 100 gm/km were not significantly different (Mann Whitney test, Table 19) suggesting that this taxon is regulating copper concentrations once a certain threshold concentration is obtained. For Lumbrius rubellus, the 0 gm/kg, 5 gm/kg and 10 gm/kg applications were not significantly different and there were no significant differences among the 25 gm/kg, 50 gm/kg and 100 gm/kg applications. (Mann Whitney test. Table 21). The increasing copper levels in the casts of the earthworm taxa with increasing soil milorganite copper concentrations (Figure 2 illustrates the data for L. rubellus), indicating that the earthworms were ingesting the copper and excreting the excess copper, although as Table 21 indicates not all the increases in faecal copper were significant. The significant decline in the faecal copper concentrations of L. rubellus from the 100 gm/kg application compared with the 50 gm/kg and the lack of significance between the faecal copper concentrations of Aporrectodea spp. from the 25 gm/kg, 50 gm/kg and 100 gm/kg and also the casts of O. cyaneum from the 50 gm/kg and 100 gm/kg treatments (Table 21) suggests that the feeding and/or excretory activities of these earthworms decreased, perhaps due to toxic effects of the metals in the milorganite.

Ireland (1979) also observed no significant increase in the copper concentrations of L. rubellus sampled from soils with increasing concentrations of copper. The same results were obtained for L. rubellus and Dendrobaena rubida (Ireland and Richards, 1977). Both Ireland (19790 and Ireland and Richards (1977) suggest that copper concentrations are regulated by Lumbricus rubellus and Dendrobaena rubida. In vertebrates, the absorption of ingested copper is about 50 per cent and is normally regulated by homeo-static mechanisms (Piscator, 1979). In contrast to these studies, Van Rhee (1963, 1975, 1977) found decreased earthworm populations in orchard soils where copper fungicides such as copper sulphate had been used and in pasture soils where pig slurry containing high amounts of copper had been applied. High concentrations of copper were found to reduce earthworm reproductivity capacity as well as body growth. Copper levels in a mixed species sample of earthworms showed increasing levels of copper in the body tissue with increasing soil levels although the levels in the earthworms did not exceed that of the soil.

3.4 Lead

Lead concentrations were extremely variable in the body tissue of all three taxa (Table 10), and no pattern of increasing concentrations with increasing milorganite application could be established. Lead levels in the earthworms were lower than soil levels. Addition of milorganite had no significant effect on the lead levels of the body tisues of any of the three taxa studied (Table 16). As mentioned previously (Tables 3 and 4) the Pb analysis may not be accurate and the data suspect.

The published literature on lead concentrations in earthworms is variable. Van Hook (1974) found that a sample of mixed earthworm genera ranged in lead concentration from 4.0 to 5.5 μg/gm Pb which was lower relative to soil concentrations which ranged from 15 to 50 μg/gm Pb. Earthworms from soils with higher lead concentrations did not consistently have higher tissue lead levels. Andersen (1979) determined lead concentration in several species of Allolobophora sampled from fields treated with sewage sludges with differing lead concentrations. Lead levels in soil and earthworms were comparable to those obtained in the present study. In Andersen's study, lead concentrations in the earthworms did not exceed the soil lead concentrations. There was also no significant differences in lead content of the earthworms from the controls and from the two sewage sludges. These results are very consistent with the observations made in the present study.

Eisenia foetida could accumulate lead (up to 325 μg/gm) but tissue levels did not exceed the levels in the food source: sewage sludge spiked with lead acetate (Hartenstein, et al., 1980). Various studied report that there is a significant increase in lead content in earthworms sampled adjacent to busy roads (Gish and Christensen, 1973; Czarnowska and Japkiewicz, 1978; Andersen, 1979; Ash and Lee, 1980). In these four studies the soil lead concentrations were greater than those in the earthworms. Lead concentrations in the earthworms were greater than those recorded in the present study. Robert and Johnson (1978) found that there was a significant linear relationship between soil lead levels and levels of lead in Lumbricus terrestris, sampled from sites contaminated by a lead-zinc smelter, with higher lead levels in the organisms correlating with higher soil levels. Lumbricus terrestris and various species of Allolobophora had significantly higher lead concentrations in soils with higher lead levels although earthworm lead content was less than the soil levels (Wright and Stringer, 1980). The values reported for Allolobophora tuberculata are much greater than the levels for the Aporrectodea spp. used in this study (Allolobophora tuberculata = Aporrectodes tuberculata, Edwards and Lofty, 1977). However, the soil lead levels in Wright and Stringer's (1980) study were greater than those in the present study. Also their area was contaminated by fallout from a lead-zinc smelter and the soil lead may have been more available to the earthworm.

In soils contaminated by mine wastes, which had lead concentrations of 1314 μg/gm, Dendrobaena rubida and L. rubellus had lead concentrations as high as 7592 μg/gm and 3529 μg/gm respectively, whereas in soils where the soil lead concentration was 629 μg/gm, the lead concentration in L. rubellus was only 9 μg/gm (Ireland, 1979; Ireland and Richards, 1977). In another study, Ireland (1975) again demonstrated large concentrations of lead in D. rubida. From soils with lead concentrations of 127 μg/gm and 1713 μg/gm, the lead concentrations in this earthworm were 100 μg/gm and 4160 μg/gm respectively.

Addition of milorganite had a significant ($p<0.05$) effect on the concentration of Pb in the casts (Table 17). The faecal lead concentrations for O. cyaneum increased with increasing milorganite application (Table 10) suggesting that this species is effectively excreting ingested lead. The lead levels in the casts of L. rubellus increased with increasing milorganite application, until the soil lead concentration was 50 µg/gm. The lead content in the faeces of this species dropped with the 100 gm/kg milorganite application. This decline also suggests that the feeding and/or excretory activities of this species may be declining due to metal poisoning. When the lead concentration in the soil was between 20 and 30 µg/gm, the concentration in Aporrectodea spp. was fairly constant, between 17 and 20 µg/gm. As the soil lead concentrations increased from 30 µg/gm to over 70 µg/gm with increasing milorganite applications, the faecal lead concentrations for Aporrectodea remained fairly constant, around 52 µg/gm. This suggests that this taxon is not excreting lead efficiently, or has slowed down its feeding and/or excretory activities. However, the data is extremely variable and may not be accurate. There are not many significant differences in the Pb concentrations of the faeces of a given taxon from the different application rates of milorganite (Table 22).

For all three taxa, the levels of lead in the faeces exceeded the concentrations in the body tissue and were in most cases lower than or similar to the soil levels. These results agree with those in the literature. Andersen (1979, 1981) found that the lead concentration in the faeces of Allolobophora longa was higher in the casts than in the earthworm body tissue. The levels in the casts were also higher than the soil levels. Czarnowska and Jopkiewicz (1978) found that the lead content in the casts of L. terrestris increased with increasing soil Pb levels. They also found, that the worm casts had lead levels similar to the soil lead content but far greater than the lead levels in the body tissue. Contrasting results were obtained by Ash and Lee (1980) for L. terrestris, A. chlorotica and L. rubellus. They found high lead levels in the earthworm body tissue. lead levels in the worm casts differed with differing soil lead levels.

There was a significant difference in the lead concentration of the three taxa body tissues (Table 16). Octolasion cyaneum contained significantly more lead than did the body tissue of L. rubellus and Aporectodea spp. (Duncan's and Scheffe's multiple range tests at $p<0.05$). The latter two taxa were not significantly different from each other. These findings suggest that O. cyaneum is either more efficient at absorbing lead from the diet or has a higher tolerance to lead body burdens or has a different feeding and excretory activity. There is no significant differences among the taxa in faecal lead content (Table 17, and this suggests that these taxa had similar excretion efficiences for lead.

Species differences in the body levels of Pb have been recorded in the literature. Ireland (1979) found that L. rubellus contained significantly lower levels of lead in its body tissue than did Dendrobaena venta and Eiseniella tetraedra. The latter two species were not significantly different from each other. Dendrobaena rubida had significantly higher amounts of lead than did L. rubellus (Ireland and Richards, 1977). Ash and Lee (1980) found higher lead levels in Allolobophora chloratica than in L. terrestris. L. terrestris was not significantly different from L. rubellus.

The lack of significant differences in the lead content of the body tissue with increasing milorganite application indicates that these three taxa of earthworms may be regulating the absorption of lead at the soil lead levels encountered in this study. This has not been reported for earthworms previously in the literature, although the data of Andersen (1979, 1980), and Van Hook (1974) suggest regulation of body tissue lead content. Lead levels in the worm casts exceeded the levels in the body tissues and also were similar to the soil levels. Lead regulation has been reported in marine annelids. Packer, et al. (1980) indicated that lead regulation by Arenicola marine occurred in sediments with high lead concentrations. Lead concentrations in the polychaete, Nereis diversicolor were extremely variable, especially where the sediments were highly contaminated by lead. (Bryan and Hummerstone, 1977).

3.5 Zinc

The data in Table 11 indicate that zinc concentrations in the body tissues of Lumbricus rubellus individuals treated with milorganite were fairly constant, even as the milorganite applications and soil zinc levels increased. The zinc concentrations were lower for the individuals kept in milorganite treated soil than were the concentrations of zinc in the body tissue of Lumbricus rubellus kept in the control soil without milorganite (Figure 3). The zinc concentrations in the body tissues of Aporrectodea spp. remained fairly constant, and the concentations of zinc in Octolasion cyaneum increased slightly with increasing milorganite applications and levelled off once the soil zinc concentration exceeded 116 ug/g (Table 11). The body tissue zinc concentrations are greater than the zinc concentrations of the soil-milorganite mixture. These results suggest that the three taxa studied concentrated zinc over and above the soil levels, but did not accumulate zinc in response to increasing environmental zinc levels.

There was a significant ($p<0.05$) effect on the zinc concentration of earthworms due to the application of milorganite (Table 18). Although there is no significant species x sludge interaction, the Mann Whitney test did detect a few significant differences among the treatments Zn concentrations in the body tissues of the 3 taxa (Table 23).

For *Lumbricus rubellus*, the highest concentrations of zinc occurred in the individuals from the control. There were no significant differences in the body zinc concentrations of this species kept in the milorganite treated soil. This result suggests that zinc regulation may be occurring in the individuals living in soils polluted with zinc. The decrease in the zinc concentration of *L. rubellus* from the control and the milorganite treated soil, may result from cadmium replacing some of the required zinc in the earthworm body tissue. Cadmium and zinc have similar chemical properties and are in the same periodic group, and the presence of cadmium can cause changes in zinc metabolism and distribution if cadmium is substituted for zinc in some enzymes or other essential biological systems (Friberg, et al., 1979; Nordberg, et al., 1979).

The data (Table 23) also suggest that *Aporrectodea* spp. and *O.*

cyaneum may be regulating their body Zn concentrations.

These data agree favourably with those found in the literature. Van Hook (1974) determined that a mixed sample of earthworm taxa concentrated zinc above soil levels by a factor ranging from 4 to 13. The concentration of zinc in the earthworms were similar to those determined in this study. Helmke, et al. (1979) observed that the zinc concentrations in A. tuberculata exceeded soil zinc concentrations and increased with increasing sewage applications. The zinc concentrations for the earthworms were within the range obtained in the present study. The compost worm, Eisenia foetida did not accumulate zinc from zinc acetate spiked sewage sludge (Hartstein, et al., 1980). When Zinc concentrations in the species raised on grain were compared with those fed on manure-sludge mixture, concentrations were considerably higher in earthworms fed on the latter medium. There were not significant differenes in the tissue zinc concentrations in L. rubellus and D. rubida sampled from two soils with different zinc concentrations (Ireland and Richards, 1977). Ireland and Wooton (1976) determined the zinc concentrations in D. rubida sampled from soils with three different zinc concentrations: 172, 286, and 880 μg/gm. The zinc concentrations in earthworms from the site with the lowest zinc level were significantly lower than those for the earthworms sampled from the two sites with higher soil zinc levels. Earthworms from the latter two sites did not differ significantly in their zinc concentrations. Lumbricus rubellus sampled from soils with Zn concentrations ranging from 100 μg/gm to 992 μg/gm did not differ significantly in their body tissue Zn concentration which ranged from 416 ug/gm (Ireland, 1979). Ireland (1975) found contrasting results for D. rubidia which has significantly higher zinc tissue concentrations when sampled from soils with low pH and exceedingly high zinc levels relative to the control site.

In roadside soils, Roberts and Johnson (1978) found that there was no significant linear relationship between soil zinc levels and L. terrestris body zinc levels. However, earthworms sampled from the highly contaminated sites had significantly more (twice as much) zinc than earthworms from the control sites. Wright and Stringer (1980) compared the conentrations of zinc in several species of earthworms sampled from soils contaminated by a lead-zinc smelter. The soils and earthworms sampled close to the smelter (4 km) had significantly higher concentrations than the soils and earthworms from the control site 9 km away from the smelter. A similar pattern was found in soil and earthworms sampled from increasing distance from several busy higheways (Gish and Christensen, 1973). Of the three highways studied, they found a significant ($p<0.05$) decrease in concentration in both soil and earthworms with increasing distance from two of the three highways studied. In one study site, the zinc concentrations in the earthwoms from all distances were not significntly different although soil zinc levels changed. In all cases the earthworm zinc concentrations were greater than those for the soil. The results from another roadside study (Czarnowska d Japkiewicz, 1978) indicates that earthworms had higher zinc concentrations than the soil. Zinc concentrations in the earthworms tended to be higher for those sampled from soils with higher soil zinc concentrations.

The data in Table 11 and Figure 3, indicate that there were slight increases in zinc concentrations in the faeces as soil zinc concentrations and rates of milorganite application increased. Addition of milorganite had a significant effect ($p<0.05$) on the Zn concentrations in the casts (Table 19). Within each taxon, there were significant differences in Zn levels in the faeces, as the milorganite application rates incrased (Table 23). The Zn levels in the casts of L. rubellus significantly declined at the highest application rate and the Zn concentrations in the faeces of the other 2 taxa levelled off. The lack of significance between the concentration of zinc in the casts of all three taxa from the 50 gm/kg and 100 gm/kg and 25 gm/kg and 100 gm/kg suggests that the excretion and/or feeding activities of the earthworms decreased at the highest application of milorganite perhaps due to metal poisoning.

Zinc levels in the faeces of all three taxa were lower than the zinc levels in the body tissue. The levels of zinc in the faeces were similar to the levels of zinc recorded for the soil milorganite mixture suggesting that the earthworms were excreting most of the ingested zinc.

Helmke, et al. (1979) observed similar results: zinc concentration in faeces increased with increasing sewage sludge application. The levels in faeces were also lower than the zinc concentrations in the body tissue. Czarnowska and Jopkiewicz (1978) also reported that the zinc concentration in earthworm faeces were lower than the zinc concentration in the body tissues. The faeces zinc concentration was variable but tended to increase with increasing soil zinc concentrations.

The apparent regulation of zinc observed in this study has been observed in other earthworm genera. Ireland (1979) observed zinc regulation by L. rubellus and in D. rubida (Ireland, 1975; Ireland and Richards, 1977). Bryan and Hummerstone (1973) determined that zinc was regulated by the marine annelid Nereis diersicolor. In vertebrates absorption of ingested zinc is highly variable, ranging from 10 to 90 per cent (Elinder and Piscator, 1979). Zinc is regulated by homeostatic mechanisms (Roberts and Johnson, 1978; Elinder and Piscator, 1979).

Zinc concentrations in the three taxa were significantly different (Table 18). Aporrectodea spp. had greater body concentrations of zinc than the other two taxa. Lumbricus rubellus had greater concentrations than Octolasion cyaneum (Duncan's and Scheffe's multiple range tests $p<0.05$). These taxa differences suggest a difference in the taxa's requirement for zinc and/or differences in body absorption efficiency of tolerances. Lumbricus rubellus is a shallow burrower and mixed feeder, whereas the other two taxa are deep burrowers and, therefore, some differences may be due to feeding activities or food intake.

Differences in body concentrations of zinc between earthworm species have been observed by other researchers. Wright and Stringer (1980) found significant differences in zinc content of various species of Allolobophora. Ireland (1979) found that L. rubellus had a significantly higher Zn concentrations than did Eiseniella tetraedra, which he attributed mainly to differences in feeding habits. The species differences in Zn tissue concentrations for L. rubellus and D. rubida was thought to be more related to differential absorption of zinc than differential feeding (Ireland and Richards, 1977).

There was also a significant difference in the faecal levels of the different taxa (Table 19). Duncan's multiple range test ($p<0.05$) indicates that Aporrectodea spp. had significantly lower concentrations of Zn in its faeces than did the other two taxa studied. Levels of Zn in the casts of L. rubellus and O. cyaneum were not significantly different from each other. As Aporrectodea spp. had significantly higher body zinc concentrations than did the other two taxa, the low concentration of zinc in the faeces reflects greater efficiency in absorbing zinc from the diet or that Aporrectodea spp. has a higher body requirement for zinc.

4 GENERAL RESULTS AND DISCUSSION

4.1 Soil Metal Concentrations

The metal concentrations of the soil increased with increasing application of milorganite (Tables 8-11). The soil cadmium concentrations increased appreciably with each increase in milorganite application. For copper the increases in soil copper concentrations were small and the control and lowest milorganite application had the same soil copper concentrations. Increases in the lead concentrations in the soil were slight until 50 gm/kg and 100 gm/kg milorganite were added to the soil. The 5 mg/kg milorganite application did not increase the soil lead levels over those of the control. The zinc concentrations in the soil increased appreciably as the milorganite application rates increased with the exception that the control and the lowest aplication rate of milorganite did not have different soil zinc concentrations.

In order to help prevent the build-up of metal concentrations to toxic levels, various government agencies in a number of industrial countries, including the Ontario Ministries of Agriculture and Food and, Environment have proposed guidelines for the recommended maximum metal concentrations in agricultural soils (Freedman and Hurchinson, 1981; Ontario Ministries Environment and Agriculture 1978). These guidelines are reproduced in Table 24.

The soil Cd levels for all Milorganite treatments exceeded the proposed Ontario guidelines for metal criteria for sewage sludge application. The soil Cu and Zn levels were within the proposed guidelines. The soil Pb levels at the 100 gm/kg application exceeded

Table 24 Proposed Ontario Guidelines for Maximum Metal Concentrations in Agricultural Soils and Maximum Metal Additions in Sewage Sludges (after Freedman and Hutchinson, 1981; Ontario Ministries Environment and Agriculture and Food, 1978).

Metal	Mean Concentration in Uncontaminated Ontario Soils (μg/gm dw)	Maximum Recommended Metal Concentration in Agricultural Soil (μg/gm dw)	Maximum Recommended Addition to Soil (kg/ha)
Arsenic	6.5	13.0	15.0
Cadmium	0.7	1.4	1.6
Cobolt	4.5	18.0	30.0
Chromium	14.0	112.0	220.0
Copper	25.0	100.0	168.0
Mercury	0.08	0.5	0.9
Moly bdenum	0.4	1.6	2.7
Nickel	16.0	32.0	36.0
Lead	14.0	56.0	94.0
Selenium	0.4	1.6	2.7
Zinc	54.0	216.0	363.0

the proposed guideline. These results indicate that the cadmium concentration in milorganite is sufficiently high enough to make this sewage sludge unsuitable for agricultural use.

4.2 Soil Metal Concentrations - Comparisons With Other Studies

The data from the present study and other studies using sewage sludge spiked with soluble metal salts and data from sites polluted by automobile exhaust or mining wastes may not be comparable. Beijer and Jernelou (1979) indicate that the type of chemical species present greatly influences the uptake and accumulation of metal by an organism. The total levels of metals in a given medium may not indicate the amount of metal in a form available to the organism. The majority of lead found in soils along roadsides will be in the form of the soluble halide, lead chlorobromide (PbCIBr) (de Haan & Zwerman 1976). These soluble forms of lead may be more available to earthworms as Hartenstein et al. (1980) found exceedingly high concentrations of lead in Eisenia foetida kept in sewage sludge spiked with lead acetate. The lead levels in earthworms sampled close to busy roadways were substantially higher than the lead levels observed in the earthworms used in this study. (Gish & Christensen, 1973; Czarnauska and Japkiewicz, 1978; Ash & Lee, 1980). The studies reported by Ireland (1975, 1979) indicated high levels of lead in earthworms and the soils sampled from sites contaminated by lead-zinc mine spoils. The lead in these soils may have been in an highly available form to the earthworms. In earthworms sampled from fields contaminated by pig manure earthworms were found to accumulate copper in response to increasing soil coper levels. (van Rhee 1977). The copper in pig manure is in the form of soluble salts (van Rhee 1975). Various studies (Ash & Lee, 1980; Hartenstein et al., 1980; and Ireland and Richards, 1981) demonstrated that earthworms kept in media treated with solutions of soluble metal salts had excessive concentrations of cadmium, copper, lead and zinc in the body tissues. The results of Ireland and Richards (1981) are quoted as an example. Lumbricus rubellus kept for 26 days on filter paper saturated with 5 μg/gm $CdCl_2$ had a body tissue concentration of 416 μg/gm Cd, compared to 6 μg/gm for the controls. In sewage sludge metals are likely to be complexed with organic matter and less available to organisms.

Differences in the results obtained by the various authors in the literature may also reflect differences in soil properties. The amount of extractable metals in the soil is influenced by the pH, organic matter and clay content (Machean and Langille, 1976). Cadmium (Chubin and Street, 1981), copper and lead (Haster, 1979), and zinc (Sehumas, 1975) adsorption is greater in soils with higher CEC, exchangeable bases, organic matter and clay content. The clay mineralogy and presence of iron and aluminum hydroxides also influences the adsorption of these metals. (Schuman, 1976; Haster, 1979; and Chubin & Street, 1981). Both Cavalloro & McBridge (1978) and Kuo & Baker (1980) indicate that pH influences Cd, Cu, and Zn adsorption by soils. The adsorption capacity of a soil are lower at lower pH.

Care must be exercised when comparing the results of the present study with field studies. In this study the milorganite was well mixed with the soil, a condition unlikely to be obtained in a field situation. In a field study preferential feeding by earthworms may lead to more variability in the metal content in the earthworms. Also sewage sludge under field conditions may undergo more extensive physical, chemical and biological changes under the influence of natural weather conditions over time (Hartenstein et al. 1980). Van Rhee (1965) indicates that generally earthworms do not like container conditions and after several months will either die or aestivate. In the present study in several of the pots, some of the individuals were aestivating and some had died; De Vries and Tiller (1978) compared levels of metals in vegetables grown in sewage sludge in greenhouse containers and in the field and found higher metal levels in the tissue from the greenhouse study. They warn that flowerpot studies may give totally different responses from field studies, as temperature, light, humidity and other environmental conditions will most likely between field and greenhouse situations.

4.3 Body Weights

The variability obtained in this experiment may, in part, be due to the natural biological variability within individuals. In order to minimize the variability due to reproductivity stage, all the earthworms used in this study were sexually mature, clitellate individuals. Carter et al. (1980) demonstrated that juvenille non-clietllate Lumbricus rubellus individuals had lower levels of Cd than mature clitellate specimens of the same species. Whole earthworms were analyzed to overcome variability due to sampling particular body parts. Ireland (1974) found that the highest concentration of lead and zinc in Dendribaeba rubida was in the posterior alimentary tract. Andersen (1980) reported that in the posterior most 10 segments of Allolobophora

Table 25 Regression Equations and r^2 Values Relating Body Dry Weights (mg) and Element Contents (μg) for *Lumbricus rubellus* for six Milorganite Treatments (5% probability level)

gm Milorganite/ kg soil	Body Weight (mg) $\bar{x}$ 1SD	μgCd	r^2	μgCu =	r^2	μgPb =	r^2	μgZn =	r^2
0	73.18 ± 18.48	.011 body wt	.310	0.015 body wt	.307	.084	-	27.59	-
5	90.30 ± 26.02	.018 body wt	.516	.009 body wt .00007 body wt	.804	.0008 body wt	.262	.327 body wt	.700
10	84.72 ± 28.41	.021 body wt	.590	.017 body wt	.657	.094	-	.309 body wt	.665
25	93.11 ± 40.48	3.59+160 body wt .0014 body wt^2 378x10^5 body wt	.818	.020 body wt	.760	.134	-	10.99 + .155 body wt	.487
50	97.20 ± 26.07	.039 body wt	.597	.021 body wt	.482	.378	-	.269 body wt	.384
100	91.51 ± 23.71	.037 body wt	.317	.020 body wt	.437	.172	-	.288 body wt	.242

Table 26 Regression Equations and r^2 Values Relating Body Dry Weights (mg) and Metal Content (µg) for <u>Aporrectodea</u> spp. for six Milorganite Treatments (5% probability level).

gm Milorganite/ kg soil	Body Weight mg $\bar{x}$ 1SD	r^2	µgCu =	r^2	µgPb =	r^2	µgZn =	r^2
0	135.91 ± 31.89	.229	.011 body wt	.571	.168	–	.369 body wt	.246
5	166.07 ± 47.81	.666	1.40+0.22 body wt	.785	.259	–	21.37+.214 body wt	.406
10	169.62 ± 33.67	.518	1.97+.030 body wt	.746	.662	–	.327 body wt	.342
25	171.21 ± 40.09	.404	.016 body wt	.109	.282	–	.311 body wt	.295
50	155.43 ± 34.27	.272	.016 body wt	.049	.0032 body wt+ .00003 body wt^2	.457	.322 body wt	.476
100	140.91 ± 37.52	.506	.239		.360	–	.402 body wt	.604

Table 27 Regression Equations and r^2 Values Relating Body Weights and Element Contents for *Octolosion cyaneum* from Six Milorganite Treatments (5% probability level).

gm Milorganite/ kg soil	Body Weight (mg) $\bar{X} \pm 1$ SD	Cd r^2	µgCu =	Cu r^2	µgPb=	Pb r^2	µgZn=	Zn r^2
0	236.57± 40.04	.325	.025 body wt	.807	1.057	0.0	.193 body wt.	.302
5	225.85± 41.82	.467	.025 body wt	.659	0.0	.010	.190 body wt.	.376
10	252.07± 124.82	.769	.027 body wt	.963	.0062 body wt	.450	.190 body wt.	.804
25	227.85± 62.86	.806	.027 body wt	.872	.0031 body wt	.270	.396 body wt. .00076 body wt^2	.026
50	187.27± 22.66	0.0	.028 body wt	.513	.543	.012	.239 body wt.	.299
100	148.50± 36.16	.849	.031 body wt	.854	.802	.038	.233 body wt.	.814

longa kept in sewage sludge amended soil, the coelomic sacs were filled with waste bodies containing higher levels of Pb and Cd than the gut wall.

In each of the treatments earthworms ranged in whole body weights. The relationship between body weight and metal content for Cd, Cu, Pb and Zn for the three taxa was determined using step-wise regression analysis. The results are given in Tables 25, 26 and 27. The cadmium content (μg) in all three taxa increased with increasing body size. The slopes of the equations increase with increaing milorganite application rate and, therefore, increasing soil Cd levels. This indicates that the earthworms at higher soil Cd levels had higher body burdens of cadmium. When the data is converted from absolute metal content (μg) to concentration (μg/gm or ppm) by dividing through by the individual body weights, the slope becomes zero. Concentration of Cd in all three taxa is, therefore, independent of body weight. The cadmium equations for Octolasion cyaneum 50 gm/kg treatment and for Lumbricus rubellus 25 gm/kg treatment do not conform to the trend established by the other treatments and may arise from random variation.

Copper content (μg) for all three taxa was also related to body weight. Unlike the trend exhibited by Cd, the slope for a given taxon for Cu content is relatively constant. For Lumbricus rubellus, the average slope is 0.019 and for Octolasium cyaneum the average slope is 0.027. The results for Aporectoedea spp. were more variable. Three of the treatments relate copper content to body weight and for these the average slope is 0.014. A constant slope for the different milorganite application indicates that the copper levels in body tissue are regulated. Converting the data to concentration, the slope relating body weight to copper concentration is zero indicating that copper concentration is independent of body weight.

Zinc content was also related directly to body weight for all three taxa. As for copper, the slope for a given taxa was relatively constant overall milorganite applications indicating regulation of zinc by the earthworms. Zinc concentration is independent of body size. The 25 gm/kg treatment for O. cyaneum, the 5 gm/kg treatment for Aporrectodea spp. and the 0 gm/kg and 25 gm/kg treatments for L. rubellus did not fit the trend established by the remaining treatments and may be the result of random variation.

The lead data were variable, but for all three taxa the lead content was constant for a given application of milorganite with the following exceptions: the 50 gm/kg treatment for Aporectodea spp., the 5 gm/kg treatment for Lumbricus rubellus and the 10 gm/kg and 25 gm/kg treatments for Octolasion cyaneum. These four results may be a result of random variation. Lead concentration is, therefore, dependent upon body size with the higher concentrations in smaller individuals. Body size could significantly affect the Pb results but the lead content in the body tissue is extremely variable making interpretations difficult (Table 10). Also the lead data may be suspect and inaccurate.

Boyden (1977) reviewed metal content and size relationship for several species of shellfish. For the oyster, Ostrea edulis, zinc concentration (ppm) was independent of body size for oysters from metal

(1980). They indicated that the concentration of Mg, Fe, Al, Nn, and Zn were greatest in the smaller individuals. In contrast the larger individuals had higher concentrations (μg/gm) of copper.

The relationship between body weight and metal concentrations varies from emtal to metal and from species to species. The relationships determined in this experiment were not inconsistent with relationship determined for other species.

Boyden (1977) also reported that the slope of the regression line relating cadmium content (ug) to body weight of the limpet, Potella, increased with increasing Cd concentration in the environment. The same trend was observed for all the earthworm taxa studied in this experiment.

Earthworms as Biological Indicators of Metal Contamination

The second objective of this study was to determine if the earthworm taxa studied could be used as biological monitors of metal contamination of soil. There are several criteria that a soil organism should meet before it can be considered useful as an indicator of soil etal concentration: 1) contain the metal in proportion to its concentation in the soil, i.e., the concentration of metal in the organism should change predictably with the soil concentrations, 2) the organism should accumulate the metal but not regulate the metal, 3) be of reasonable size to provide sufficient tissue for analysis, and 4) be easy to sample and identify and be able to survive in the laboratory, to allow for defecation before analyses, and for laboratory studies of metal uptake (Bryan and Hummerstone, 1977; Philips, 1978; Wieser, et al., 1976). Helmke, et al. (1979), Carter, et al. (1980), Czarnowska and Jopkiewicz (1978) and Van Hook (1974) mention that earthworms may be useful indicators of metal pollution int he soil. Feeding and burrowing activities of earthworms expose them to the soil. Individual earthworms contain enough tissue to allow for analyses. They are ubiquitous and are relatively easy to collect. Adult clitellate earthworms can be identified using morphological characteristics without dissection, for example using the key produced by Reynolds (1977).

The concentration ratios of the four metals for the three taxa are given in Table 28. The concentration ratio is defined as the ratio of μg/gm element in the consumer's organism tissue (in this case, earthworm) to the μg/gm element in its food items (soil) (Van Hook, 1974). My results are in general agreement with those reported by Van Hook (1974), Martin and Coughtrey (1976), and Wright and Stringer (1980) and those calculated from the data of Gish and Christensen (1973), Ireland (1975, 1979) and Andersen (1979, 1980). The results in Table 28 verify that lead and copper are not concentrated by the 3 earthworm taxa, above soil levels for these two elements. As copper soil concentrations increased from 37 to 52 μg/gm, the concentration ratio for copper in Lumbricus rubellus remained fairly constant indicating that there was a near linear relationship between increasing soil copper and earthworm body tissue concentrations. However, the increases in soil copper were very slight and there were very few significant differences in the earthworm body tissue copper concentrations. When copper concentrations increased to 65 μg/gm the concentration factor for

Table 28 Soil Metal Concentrations and Concentration Factors for Cd, Cu, Pb, Zn, for Three Taxa of Earthworms

		Lumbricus rubellus	*Aporrectodea* spp.	*Octolasion cyanium*
Cadmium:	**Soil Cd µg/gm**			
	0.8	13.8	10.5	15.5
	1.5	12.0	9.1	12.3
	2.5	8.6	5.4	8.4
	3.8	8.5	5.0	7.2
	7.3	5.2	3.7	6.0
	12	3.2	2.6	3.4
		$\bar{x}$ 8.5 ± 4.0	$\bar{x}$ 6.0 ± 3.1	$\bar{x}$ 8.8 ± 4.4
Copper:	**Soil Cu µg/gm**			
	37	0.41	0.28	0.67
	37	0.44	0.38	0.67
	40	0.43	0.46	0.64
	44	0.44	0.39	0.60
	52	0.41	0.31	0.55
	65	0.31	0.28	0.46
		$\bar{x}$ 0.41 ± 0.05	$\bar{x}$ 0.35 ± 0.07	$\bar{x}$ 0.60 ± 0.08
Lead:	**Soil Pb µg/gm**			
	21	0.06	0.06	0.24
	21	0.03	0.10	0.19
	27	0.05	0.13	0.21
	33	0.05	0.05	0.08
	51	0.08	0.03	0.06
	76	0.03	0.03	0.08
		$\bar{x}$ 0.05 ± 0.02	$\bar{x}$ 0.07 ± 0.04	$\bar{x}$ 0.14 ± 0.08
Zinc:	**Soil Zn µg/gm**			
	77	5.0	4.9	2.3
	74	4.3	4.5	2.5
	80	4.0	4.2	2.6
	95	3.1	3.5	2.3
	116	2.4	3.0	2.0
	161	1.9	2.4	1.4
		$\bar{x}$ 6.0 ± 3.1	$\bar{x}$ 3.8 ± 0.95	$\bar{x}$ 2.2 ± 0.44

concentration factor for Lumbricus rubellus decreased the copper concentration factor for Aporrectodea spp. was variable but tended to decrease as soil concentrations increased suggesting copper regulation. The copper concentration factors for Octolasion cyaneum decreased as soil concentrations increased. This is consistent with the hypothesis that this species is regulating copper concentrations in its body. The zinc concentration factors for all three taxa decreased as soil concentrations increased, again suggesting that zinc is regulated. The concentration factors for zinc are greater than unity, an indication that body tissue zinc concentrations are greater than the concentrations in the soil. The lead concentration factors are variable but tend to decrease with increasing soil lead concentrations supporting the hypothesis that lead is regulated by the three taxa. Cadmium was concentrated by all three taxa to a much greater extent than zinc. Even though the cadmium concentrations in all three taxa continued to increase as soil cadmium concentrations increased from 0.8 μg/gm to 7.3 μg/gm, the concentration factors decreased. This suggests that the soil cadmium concentrations are increasing to a greater degree relative to the earthworm body tissue concentrations, as the rates of milorganite application increase. The relationship between soil cadmium concentrations and earthworm tissue concentrations is not linear, even though the soil-milorganite mixture is the source of cadmium. The soil cadmium concentration values used in the concentration factor calculation were determined using a total soil cadmium concentration and may not be indicative of the actual amount of cadmium available to the earthworm. At the highest soil cadmium concentration it was suggested that earthworm feeding and or excretory activities had slowed down perhaps in response to metal poisoning. The data suggest that if soil cadmium concentrations are to be predicted using the earthworm body tissue concentrations and the average concentration factor the soil cadmium concentration could sometimes be underestimated or over estimated. For example, using the average concentration factor of 8.5 for L. rubellus would tend to underestimate soil Cd levels in soils with cadmium levels greater than 4 μg/gm and over estimate the soil Cd concentration where soil concentration is less than 2 μg/gm. This requires further work to determine the Cd levels in one of the given taxa and use the quoted concentration factors to predict soil Cd values. Comparing predicted soil Cd values with actual values determined by reliable analysis would indicate the usefulness of earthworms as biological monitors of soil contamination by Cd. The data in Table 28 also suggest that there are taxa differences among the concentration ratios which reflects the taxa differences in metal concentrations discussed previously. The results indicate that thse three taxa of earthworms would not be an appropriate biological monitor for Cu, Pb and Zn as these three elements appear to be regulated by the organism. These taxa, therefore, fail to meet criterion #1, for these three metals. As the lead and copper concentrations are much lower in the earthworms relative to soil levels it would be easier to detect soil Pb and Cu contamination by analyzing the soil. The earthworm taxa studied may be used to indicate Cd contamination in the soil but there was not a linear relationship. If earthworms are to be used to indicate soil Cd levels, then different taxa should not be pooled.

5 CONCLUSIONS

For all three taxa of earthworms the tissue concentration μg/gm of Cd, Cu, and Zn was independent of body weight. Lead concentration data were extremely variable. However, the data indicated lead concentrations were higher in smaller individuals.

The body tissue concentrations of copper, zinc and lead appear to be regulated in all three taxa studied. In contrast, cadmium concentrations in the body tissue increased with increasing soil Cd concentrations. The concentrations of Cd, Cu, Pb and Zn increased in the casts with increasing rates of milorganite application. In *L. rubellus* for all four metals studied, at the highest milorganite application rate, there was a decrease in the faecal metal concentrations suggesting a slow down in feeding and excreting activities perhaps the result of metal poisoning. For *Aporrectodea* spp. the concentrations of metals in the casts tended to level out at the highest application rate of milorganite, suggesting that activities were starting to slow down in response to metal loading of the soil. In contrast, the Cd and Zn concentrations in the faeces of *O. cyaneum* continued to increase at the highest milorganite application rate while the Cu and Pb concentrations levelled out supporting differences in metal tolerances.

There were significant differences ($p<0.05$) in the metal concentrations in the faeces and body tissue among the taxa. There was no consistent pattern for the uptake either for a given metal or given taxon. As the pattern of metal uptake is not consistent, these differences may reflect different excretory activities and different tolerances and requirements for metals rather than selective feeding.

The results suggest that the earthworm taxa studied would not be useful biological indicator organisms for soil Cu, Pb and Zn concentrations. Earthworms might be useful to predict cadmium soil concentrations but further work would be required to validate this conclusion. This study indicates that results from different taxa should not be pooled as species differences in metal concentrations did occur.

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PART 2

The Uptake of Cadmium, Copper, Lead, Nickel and Zinc by Lettuce (*Lactuca sativa* L.) Grown in Soil Amended with Milorganite and in the Presence of Earthworms (Lumbricidae)

1. INTRODUCTION AND LITERATURE REVIEW

Sewage sludges have been applied to agricultural lands as a means of waste disposal and utilizing the nutrients in the sludges to promote plant growth. Many sludges contain metals such as cadmium, copper, nickel and zinc at concentrations which may be toxic to plants and their consumers. The uptake of metals by plants from sewage sludges has been reviewed by Page (1974) and more recently by Webber (1979, 1980). The uptake of metals by lettuce grown in sludge amended soils has been studied by a number of researchers, in paticularly MacLean (1974, 1976), MacLean and Dekker (1978), John (1975), John and van Larhoven (1972, 1976, 1976) and Singh (1981). In general, sewage sludge promotes plant growth and results in higher concentration of cadmium, copper, nickel and zinc in plant tissues. Lead concentrations in plant tissue tend to be unaffected by sewage sludge additions. Plant species respond differently to metal concentrations in the soil. In general, leafy vegetables such as lettuce, tend to have higher metal concentrations in their tissues than do other vegetables. The amount of metals taken up by plants is influenced by a number of factors including soil properties, properties of the metals and plant species.

Soon and Yates (1982) indicated that there are five chemical pools for metal cations in the soil: (1) water soluble, (2) easily exchangeable, (3) complexed or adsorbed and exchangeable only by other cations with higher affinities for the sorption/complexation sites, (4) occluded by or coprecipitated with metal oxides, carbonates of phosphates, and other secondary minerals (5) metals held in primary minerals. The first three pools are generally thought to be available to plants, while the last two pools are considered to be unavailable to plants. Total metal concentrations in soils are often determined by digestion with concentrated acids. Although the relationship between total metal concentrations and plant uptake have been questioned (MacLean and Langille, Symeonides and MacRae, 1977, 1976) guidelines for sewage sludge disposal on to land are based on total metal concentrations in the sludges. (Freedman and Hutchinson, 1981). Total metal concentration in soil indicates the degree of pollution of soil by metals but does not necessarily indicate the amount that plants can utilize.

Many extracting solutions which have been utlized to determine the amount of available metals in soil. In many British laboratories, the concentration of metals extracted by 0.5 N HOAC (acetic acid) is considered to indicate the amount of metals in soil or sludges that is available for plant uptake (Page, 1974). In the United States and Canada, 0.1 N HCl has been used as an extracting agent. (Brown et al., 1971; MacLean and Langille, 1976). More recently, the amount of metals extracted by DTPA (0.005 M diethylenetriaminepentaacetic acid, pH 7.3) has been used to determine plant available metals with good correlation between extracted metal concentrations and plant tissue concentrations. (Lindsay and Norvell, 1978; Karim et al., 1976; Korcak and Fanning, 1978. Karim et al. (1976) indicated that acid extracting solutions may overestimate the available metals and that amount of metals extracted by the chelating agent, DTPA was a more reliable estimate of plant available metals in the soil. The amount of extractable metals in the soil or sewage sludge is influenced by a number of properties such as pH, organic matter content, metal species etc. (Webber and Corneau, 1975; Silviera and Sommers, 1977; Korcak and Fanning, 1978; MacLean and Dekker, 1978; MacLean, 1974; John, 1972; Haq et al., 1980).

Many researchers have indicated that earthworms can increase plant yields although this conclusion is often reached on the basis of faecal analysis (see reviews by Barley, 1961; Ghabbour, 1966; and Edwards and Lofty, 1977). Few researchers have experimentally studied metal uptake by plants grown in the presence of earthworms, or the effect of earthworm activity (ingestion and excretion) on the available pool of metals.

Lunt and Jacobson (1944) found that earthworm faeces contained higher amounts of total N and nitrate N, organic matter, total and exchangeable Ca, exchangeable K and Mg and available P, than did the surrounding soil. Total Mg in the earthworm faeces was similar to soil levels. Ireland (1975) determined that acetic acid extractable Zn and Ca had higher concentrations in earthworm casts than the surrounding soils, whereas extractable Pb was greater in the soil than earthworm casts. Putrified earthworm tissue had higher concentrations of extractable Pb, Zn and Ca than did the surrounding soil. Total concentrations of Pb, Zn and Ca were higher in the decomposing earthworm tissue but equal in the faeces and soil. Edwards and Lofty (1977) indicated that earthworms increased the amount of plant available Mo in pasture soils.

In laboratory studies, concentrations of N, Mg, Cu and Zn were similar in sludge amended soils with and without earthworms but there were higher concentrations of dilute acid extractable P, K, Ca, Fe and Mn in the sludge treated soils with earthworms than without earthworms (Kirkman, 1979). She also found higher Mn concentrations in the roots of wheat grown in the presence of earthworms than in their absence. The concentrations of Fe, Cu and Zn in wheat were unaffected by earthworms. Previously, Peredel'skii et al. (1957) reported that Co^{60} uptake by a number of plant species was greatly increased when grown in the presence of earthworms.

In the present study, lettuce (Lactuca sativa L. C.V. early curled Simpson (leaf).) was grown in the presence and absence of earthworms (Lumbricus rubellus Hoffmeister, Aporrectodea spp. either Aporrectodea trapezoides Duges or Aporrectodea tuberculata Eisen, or a combination of these 2 species, and Octolasion cyaneum Savigny) and in the presence and absence of the sewage sludge, milorganite which is high in metal concentrations. The objectives of this study were to determine: 1) if Cd, Cu, Ni, Pb and Zn, uptake by lettuce would be affected when grown in sludge amended soil and in the presence of earthworms. 2) if yields of lettuce would be increased by the addition of the milorganite and earthworms to the soil; 3) if DTPA extractable Cd, Cu, Ni, Pb and Zn would be influenced by the presence of earthworms.

2. MATERIALS AND METHODS

2.1 Experimental Design and Preparation

Bulk soil samples were collected from the Ap horizon of a Cresent-Westham Island soil complex located on Mr. Hugh Reynold's farm on Westham Island in the Fraser Delta of British Columbia. Soil profile descriptions and soil maps are given in Luttmerding (1980, 1981 a, b).

The soil was air-dried, crushed with a wooden roller and passed through a 6 mm screen. For the sludge treatments, the soil (6313 gm) was thoroughly mixed with 158 gm of milorganite (50 gm/kg which is equivalent to 56 T/ha). Twelve flower pots (21 cm d x 21 cm h) were filled with the soil-milorganite mixture and 12 were filled with soil (6313 gm) and all pots were packed to a bulk density of 1200 kg/m^3. After filling, the pots were watered with a fertilizer solution containing 100 μg/gm each of nitrogen, phosphorus and potassium to ensure adequate nutrient supply. Each pot was watered with 2300 mls of distilled water, to approximate field capaacity. The pots were weighed and the initial weights recorded.

There were four treatments: 1) soil with no earthworms or milorganite added; 2) soil with only earthworms added; 3) soil with only milorganite added; 4) soil with both earthworms and milorganite added. Each treatment was replicated six times utilizing a randomized block design.

For treatments 2 and 4, ten mature, clitellate earthworms were placed on top of the soil medium and allowed to burrow. Four <u>Lumbricus</u>

rubellus Hoffmeister, four Aporrectodea spp. (a combination of A. trapezoides Duges and A. tuberculata Esien) and two Octolasion cyaneum Savigny were used in the earthworm treatments (approximate density in 1 m^2 is 263 earthworms). The earthworms were obtained by digging on Westham Island near the field where the soil was obtained. The bottom of the pots were covered with nylon mesh in order to prevent the earthworms escaping. Five seeds of leaf lettuce (Lactuca sativa L. cv. early curled Simpson) were planted in each pot. There was a sixteen hour photoperiod provided by a mix of incandescent and fluorescent lighting. This lighting regime is recommended for lettuce (Bickford and Dunn, 1972). Temperature and relative humidity were monitered by a hydrothermograph (Weather Measure Corporation, Model H311). The day temperature during most of the experimental period (December 11, 1978 to February 9, 1979) was 21°C ± 2°C and the night temperature was 19°C ± 2°C. During the last week of December and first week of January the night temperatures were mainly 10°C and the day temperatures were mainly 15°C. Relative humidity fluctuated between 40% and 60%. Distilled water was added daily to bring the flower pots back to the initial weight.

2.2 Sample Preparation and Chemical Analysis

After two months, the plants were harvested. The leaf material from each pot was pooled and fresh weights were obtained. The lettuce leaves were then oven dried at 80°C for three days. After weighing, the samples were ground in a stainless steel Wiley mill. The main roots from each pot were collected, washed with deionized water, oven dried at

80°C as above and weighed on an electronic microbalance.

One gram samples of oven dried (80°C) leaf material were dry ashed overnight at 480°C in a muffle furnace. The cooled ash was dissolved in 5 mL of 2N HCl and evaporated to dryness on a hotplate. The residue was then dissolved in 5 ml of 2N HCl, filtered through Whatman #42 filter paper and brought to 25 ml volume (Dept. Soil Sc., U.B.C., 1977).

Cadmium, copper, lead, nickel and zinc in the leaf extract were determined by atomic absorption spectrophotometry using a Perkin Elmer Model 306 atomic absorption spectrophotometer with an air acetylene flame and the following wavelengths: 288.8 nm for Cd; 324.7 mn for Cu, 283.3 nm for Pb, 232.0 nm for Ni and 213.9 nm for Zn. The instrument was equipped with background correction.

The roots were weighed, placed in acid washed Kimax glass vials (70 x 20 mm O.D.) and digested in nitric acid using a block digestor set at 150°C as described previously in part 1 for earthworms under 100 mg dry weight. This method was used as insufficient material was obtained to use the same method as the leaves. These 2 methods give comparable results (Carter, personal communication). Lead and nickel concentrations in the roots were determined by flameless atomic absorption spectroscopy, using the Perkin Elmer Hollow Graphite Analyzer (HGA) Model 2100 equipped with deuterium arc background correction. The furnace operating parameter, wavelengths, and slit settings for the HGA are given in Table 2 of Part 1.

The soil and soil-milorganite mixtures were air dried, crushed with a wooden roller and passed through a 2 mm stainless steel sieve. Three replicates of the medium from each flower pot were prepared and analyzed for cadmium, copper, lead, nickel and zinc. Samples weighing 0.5 gm were placed in an acid washed glass tallform 100 ml beakers and digested with 9 mls of concentrated HCl and 3 mls of concentrated HNO_3 (aqua regia) on a hot plate set at medium for at least half hour. The beakers were covered with acid washed watch glasses. The samples were filtered Whatman #42 filter paper and brought to 25 ml volume (Van Loon and Lichwa, 1973). Cadmium, copper, lead, nickel and zinc were determined using flame atomic absorption spectroscopy as described previously, in Part 1.

Extractable metals in the soil and soil-milorganite mixture were determined using the diethylenetriaminepentaacetic acid (DTPA) extractant. Ten grams of air dried soil were weighed into 125 ml wide mouth plastic bottles with 20 mls of DTPA extracting solution and shaken on an Eberback two-speed horizontal shaker with an 8.0 cm stroke and a low speed of 12- cycles/min. for 2 hours at 25°C. The samples were then filtered through Whatman #42 filter paper and the filtrate analyzed for Cd, Cu, Ni, Pb and Zn by flame atomic absorption spectroscopy as described previously. (Department Soil Science, 1977).

The DTPA extractant was chosen over the 0.5 N HOAc (acetic acid) extractant as described by Webber and Corneau (1975) and the 0.1 NHCl extractant as described by Department of Soil Science, U.B.C. (1977). In a separate study lettuce (<u>Lactuca sativa</u> L. cv early curled Simpson) was grown in the soil-milorganite mixture (20 gm milorganite/kg soil) as described previously. The extractable Cd, Cu, Ni, Pb and Zn in the soil-sludge medium were determined using DTPA, 0.5 N HOAc and 0.1 N HCl. Total metal levels in the soil-milorganite mixture and lettuce leaf tissue were also determined by the methods described previously. The DTPA extract of soil had the best correlation of the three extractants with copper, nickel and zinc concentrations determined for the leaf tissue.

2.3 Statistical Analysis

The data were statistically analyzed using the UBC-MFAV programme for analysis of variance. The data for zinc concentrations in lettuce leaves and roots were statistically analyzed using the UBC-BMD programme for the analysis of variance, as this programme can handle missing data. The tissue from one replicate was contaminated with zinc during the analytical procedures. Paired-difference tests were used to determine if metal concentrations in the root tissue. The paired-difference test was used instead of the t-test to determine differences between two means, as the t test requires the two samples be independent and random. The lettuce leaf and root tissue from the same plant would not be independent. The paired difference test eliminates the effect of the flowerpot to flowerpot variability and yields more information on the mean difference in the metal concentrations of the different tissue types. (Mendenhall, 1971).

3. RESULTS

3.1 Survival and Activity of the Earthworms

There was limited activity of the earthworms in the flower pots. The majority of earthworms (82%) survived the experiment but all the *Aporrectodea* spp. and *Octolasion cyaneum* individuals which were recovered were aestivating (see plate 1), and were located near the bottom or centre of the pots. The surviving *Lumbricus rubellus* individuals were all recovered near the surface and most were active. *Lumbricus rubellus* had the poorest survival record (58%) of the three taxa used while 92% of the *Octolasion syaneum* and 96% of the *Aporrectodea* spp. survived. In some of the earthworm treated flower pots, there was no evidence of earthworm activity (no burrows or casts present). The soil in which earthworm activity was evident also tended to be more crumbly than soil with no earthworm activity.

3.2 Yield of Lettuce Tissue

The fresh and dry weights of harvested lettuce leaf tissue are given in Table 1. Analysis of variance indicated that the addition of milorganite was highly significant ($p<0.05$). The addition of milorganite resulted in an increase in the lettuce leaf tissue. Earthworms had no effect on the production of tissue.

3.3 Metal Concentrations in Milorganite

Metal concentrations in miloganite are presented in Table 2. These data are similar to the results presented by John and van Laerhoven (1976) and Carson (written communication).

Plate 1 Aestivating earthworms. The taxon, on the left is Aporrectodea spp. and Octolasion cyaneum is on the right. There is some evidence of burrowing activity to the left and above the Octolasion cyaneum individual.

Plate 2 An example of the soil from treatment with earthworms, to illustrate the burrowing activity.

Plate 3 An example of the soil from treatment with no earthworms.

Table 1 Fresh and Dry Weight (80°C) and Standard Deviations for Lettuce Leaf Tissue Harvested After Two Months Exposure to Treatment.

Treatment	Grams Fresh Weight	Grams Dry Weight
No earthworms, no milorganite	10.902 ± 5.079	0.703 ± 0.383
Earthworms, no milorganite	11.082 ± 1.371	0.710 ± 0.136
No earthworms, 50 gm milorganite/kg soil	64.059 ± 11.781	5.039 ± 1.063
Earthworm, 50 gm milorganite/ kg soil	59.851 ± 11.548	4.734 ± 0.949

Table 2 Total Cadmium, Copper, Lead, Nickel and Zinc Concentrations (μg/gm) in Lettuce Leaf Tissue Grown in Soil Amended with 20 gm Milorganite/kg soil, the *Milorganite and Soil.

	Cd	Cu	Pb	Ni	Zn
Lettuce	21.7± 3.35	9.6± 2.33	7± 2.7	8 ±2.1	67.2±12.50
Milorganite	99 ±31	316.0±36	573±78	67±7	792 ±91
Soil	0.6± 0.4	32.0± 1.8	12±11	38±4.5	72 ± 5.2

*The mean and standard deviation for milorganite and soil based on 30 determinations.

Table 3 Total and Extractable Soil Cadmium, Copper, Lead, Nickel and Zinc Concentrations ($\mu g/gm \pm 1$ Std. dev.) Extractable Metals Also Expressed as Percentage of Total Soil Metal

	Cadmium		Copper		Lead		Nickel		Zinc	
Extractant	µg/gm	% Total	µg/gm	% Total	µg/gm	% Total	µg/gm	% Total	µg/gm	% Total
Conc. HCl & HNO_3 (Total Soil Metal)	3.0 ± 0.45	100	25.7 ± 3.24	100	2.8 ± 5.2	100	5.0 ± 8.3	100	123.2 ± 27.51	100
DTPA	2.3 ± 0.16	77	10.0 ± 0.93	39	3.0 ± 0.51	11	3.2 ± 0.31	6	11.8 ± 1.21	10
0.1 HCl	2.6 ± 0.15	88	9.9 ± 0.49	39	3.8 ± 0.42	13	4.4 ± 0.26	9	18.7 ± 1.29	15
0.5 HoAc	1.6 ± 0.26	60	2.0 ± 0.45	8	nonedetectable		nonedetectable		11.7 ± 5.5	9

Table 4 Correlation Coefficients[1] Between Different Soil Extractants and Metal Levels in Lettuce Leaves, Grown in Soil Amended with 20 gm Milorganite/kg soil.

Extractant	Cadmium	Copper	Lead	Nickel	Zinc
Conc. HCl & HNO_3 (Total Soil Metal)	-0.08	0.57	-0.77*	0.79**	0.68*
DTPA	0.13	0.82**	-0.18	0.95**	-0.91**
0.1 N HCl	-0.21	0.99**	0.64*	0.43	-0.93**
0.5 N HoAc	-0.62*	-1.00**	-	-	-0.95**

[1]Coefficients not significant unless otherwise stated

* = sig. $p<0.05$
** = highly sig. $p<0.01$

3.4 Metal Extractability and Correlations with Lettuce Metal Contents

The total metal concentrations determined by concentrated nitric and hydrochloric acid and extractable metal concentrations determined by DTPA, 0.1 NHCl, and 0.5 HOAc in the soil mixed with 20 gm milorganite/kg soil are given in Table 3. The metal concentrations in the lettuce leaf tissue are given in Table 2. In Table 4, the correlation coefficients (r values) obtained between the extractable metals and metal concentrations in the lettuce leaf tissue are presented. The results indicate that at the 20 gm/kg application rate of miloganite, the copper, lead, nickel and zinc concentrations in the lettuce were correlated with the total concentrations of copper, lead, nickel and zinc in the soil. The correlation coefficients between the extractable metals and lettuce concentrations varied. The DTPA extractable copper, nickel and zinc was highly significantly correlated with lettuce leaf concentrations for these three metals, which are the metals often considered to be of most concern in soil metal pollution. The three extractants had highly significant correlation with leaf copper and zinc concentrations. Extractable cadmium determined by 0.5 NHOAc was significantly correlated with lettuce cadmium concentrations. The acetic acid, however, failed to extract any nickel or lead from the soil-miloganite mixture. As DTPA extractable copper, nickel and zinc correlated significantly with lettuce concentrations for these metals, it was chosen as the extractant to be used in the second experiment.

DTPA extracted slightly less cadmium, lead, nickel and zinc and equal amounts of copper than did 0.1 N HCl, and more metals than 0.5 N

Table 5 DTPA Extractable Metals (μg/gm dry weight ± I.S.D.) in Soil After Two Months Exposure to Treatment.

Treatment	Cd	Cu	Ni	Pb	Zn
No earthworms, no milorganite	0.12 ± 0.04	8.3 ± 0.14	4 ± 0.4	1 ± 0.45	1.9 ± 0.26
Earthworms, no milorganite	0.12 ± 0.04	8.4 ± 0.16	4 ± 0.0	2 ± 1.6	1.8 ± 0.72
No earthworms 50 gm milorganite/kg soil	2.3 ± 0.12	10.8 ± 0.27	5 ± 0.5	2 ± 0.41	10.3 ± 0.20
Earthworms, 50 gm milorganite/kg soil	2.3 ± 0.25	10.7 ± 0.52	5 ± 0.5	2 ± 0.63	9.9 ± 1.98

Table 6 Correlation Coefficients[1] (r values) for Metal Concentration in Lettuce Leaves, Total Soil Metal Concentration and DTPA Extractable Metals, With and Without Milorganite Added to the Soil.

	Cd		Cu		Ni		Pb		Zn	
Treatment	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
Treatments without milorganite	-0.52	0.29	-0.33	-0.21	-0.25	0.36	0.03	-0.33	-0.22	0.50
Treatments with 50 gm milorganite/kg soil	-0.43	-0.26	-0.09	-0.15	-0.26	-0.24	0.48	-0.65*	0.83**	0.79**

[1]For explanation of significance levels, see footnote Table 4.

HOAc, with the exception that DTPA and 0.5 N HOAc extracted equal amounts of zinc. The acetic acid extractant did not extract any lead or nickel. Much of the total cadmium present in the milorganite amended soil was extracted by the three extractants (76%). Based on the percent of the total metals extracted, the overall effectiveness of DTPA and 0.1 N HCl, was in the order cadmium > copper > zinc = lead > nickel; whereas for 0.5 N HOAc the order of effectiveness was cadmium > copper = zinc.

Table 5 gives the concentrations of DTPA extractable metals in soil without and amended with 50 gm milorganite/kg soil. The addition of milorganite significantly ($p < 0.05$) increased the amount of DTPA extractable metals present in the soil. At least 40% of the zinc and cadmium present in the milorganite was DTPA extractable. The presence of earthworms did not significantly effect the amount of DTPA extractable metals.

Table 6 presents the correlation coefficients for metal concentrations in lettuce leaves and total soil metal concentrations and DTPA extractable metals. As earthworms had no significant effect on the DTPA extractable metals, the correlation coefficients were calculated pooling the treatments without milorganite and with milorganite. There is much variation in the correlation coefficients. In the milorganite treatments the DTPA extractable zinc had correlation coefficients which were significant. The correlation coefficients presented in Table 6 and Table 4 also are inconsistent, and this may reflect the different application rates of milorganite, and subsequent

Table 7 Metal Concentrations (μg/gm dry weight ± S.D.) in Lettuce Leaves After Two Months Exposure to Treatment.

Treatment	Cd	Cu	Ni	Pb	Zn
No earthworms, no milorganite	5.9 ± 2.02	12.4 ± 2.95	19.0 ± 4.3	8.5 ± 5.6	58.0 ± 15.79
Earthworms, no milorganite	5.9 ± 1.40	12.2 ± 1.70	19.8 ± 1.8	11.3 ± 2.8	65.4 ± 12.52
No earthworms 50 gm milorganite/kg soil	50.7 ± 6.41	10.2 ± 1.25	9.0 ± 2.1	24.5 ± 14.0	89.9 ± 24.29
Earthworms, 50 gm milorganite/kg soil	55.2 ± 4.01	10.9 ± 0.39	11.3 ± 1.5	24.7 ± 14.3	93.1 ± 9.69

Table 8 Metal Concentrations (μg/gm dry weight ± I.S.D.) in Lettuce Roots After Two Months Exposure to Treatment.

Treatment	Cd	Cu	Ni	Pb	Zn
No earthworms, no milorganite	4.5 ± 2.65	49.6 ± 8.28	95.2 ± 39.3	18.8 ± 8.5	87.4 ± 25.76
Earthworms, no milorganite	4.3 ± 1.85	39.5 ± 5.72	67.3 ± 24.0	9.3 ± 3.4	75.4 ± 29.16
No earthworms 50 gm milorganite/kg soil	48.4 ± 6.21	54.7 ± 16.43	41.5 ± 15.7	12.7 ± 1.9	148.1 ± 14.74
Earthworms, 50 gm milorganite/kg soil	56.0 ± 12.13	45.2 ± 12.52	39.7 ± 10.5	10.2 ± 1.2	129.5 ± 39.51

differences in total and DTPA extractable metals in the soil and differences in metal concentrations in the tissue between the two milorganite applications.

Comparison of Tables 3 and 5 indicates that the 20 gm/kg and 50 gm/kg applications of milorganite did not result in differences in the amounts of DTPA extractable cadmium copper, lead and zinc. Extractable nickel was slightly higher in the 50 gm/kg application.

3.5 Metal Concentrations in Lettuce Tissues

The concentrations of cadmium, copper, nickel, lead and zinc in the lettuce leaf and root tissues are given in tables 7 and 8 respectively. The addition of milorganite significantly ($p < 0.05$) increased cadmium and zinc concentrations in the leaf and root tissues. milorganite had not significant effect on the copper or lead concentrations in lettuce leaves and roots. The average lead concentration in the leaf tissue from the milorganite treated soil was greater than that from the non-treated soil. There was a large standard deviation associated with the milorganite treated plants and this may explain the lack of significance due to the addition of the sludge. Nickel concentrations in the leaves and roots were significantly lower ($p < 0.05$) when milorganite was added to the soil.

There was only one significant effect due to the presence of earthworms. When earthworms were present, the lead concentration in the lettuce roots was significantly lower ($p < 0.05$) than the lead concentration in the roots grown without earthworms present.

Paired difference tests between the cadmium concentrations in the

Table 9 Total Metal Concentrations (μg/gm dry weight ± S.D.) in Soil After Two Months Exposure to Treatment.

Treatment	Cd	Cu	Ni	Pb	Zn
No earthworms, no milorganite	1.6 ± 0.16	34.8 ± 0.82	37 ± 3.4	19 ± 1.8	69.0 ± 1.32
Earthworms, no milorganite	1.7 ± 0.15	34.8 ± 0.84	37 ± 6.2	22 ± 3.0	65.8 ± 2.20
No earthworms 50 gm milorganite/kg soil	4.7 ± 0.47	41.1 ± 1.30	36 ± 2.9	38 ± 2.6	88.4 ± 2.46
Earthworms, 50 gm milorganite/kg soil	4.4 ± 0.36	40.9 ± 1.47	35 ± 3.1	35 ± 5.7	86.5 ± 3.43

leaves and roots indicate that there was no significant difference between the two tissue types. Copper and nickel concentrations in the roots were significantly higher than the copper and nickel concentrations in the lettuce leaf tissue. The lead concentrations in the tissues were variable and the paired difference test ($p < 0.05$) detected significant differences between the lead concentrations in the leaf and root tissues in 2 of the 4 treatments Zinc concentrations in the root tisues from three of the four treatments were significantly higher than the leaf zinc concentrations. The zinc concentrations in the root tissue from the treatment with earthworms and no milorganite on average were greater than the leaf tissue but the difference were not significant.

3.6 Metal Concentrations in the Soil

Table 9 presents the metal concentrations in the soil after two months exposure to treatment. The addition of milorganite significantly increased the total concentrations of cadmium, copper, lead and zinc and did not significantly increase the nickel concentration ($p < 0.05$). The concentration of nickel in milorganite is low relative to the concentrations of the other four metals studied (Table 2). The addition of earthworms to the soil did not affect the total metal concentrations in the soil.

Comparison of the total metal concentrations in the soil with 50 gm/kg milorganite application (Table 9) with the soil amended with 20 gm/kg milorganite (Table 2) indicates that the increased application of milorganite resulted in slightly higher cadmium, copper, and lead concentrations. Nickel and zinc concentrations were higher in the soil from the 20 gm/kg milorganite application. Also, the copper concentrations from the control (no added milorganite, Table 9) were higher than the copper concentrations than the soil treated with 20 gm/kg milorganite. This suggests that the two application rates may not have resulted in different total metal loadings of the soil and may reflect the variability in metal levels in the miloganite and in the soil (Table 2).

3.7 Soil Properties

Selected physical and chemical properties of the soil used in the present study are presented in Table 10. For complete descriptions of the Westham and Crescent soils the reader is referred to Luttmerding, 1981.

Table 10 - Selected Soil Properties of the Buk Ap Soil Samples and of an Ap Horizon from the Crescent Soil Series (Luttmerding, 1981b).

Treatment	Depth (cm)	pH ($CaCl_2$)	% C	% N	Exchangeable Bases (NH_4 OAc) meq/100 gm soil Ca	Mg	K	Na	CEC meg/100 m Soil	Particle Size % Sand	% Silt	% Clay
Crescent - Ap Soil Series	0.28	4.3	3.07	0.20	8.44	1.13	0.51	0.35	23.5	8.00	66.60	25.40
Bulk Sample - Ap	0.20	3.9	2.36	0.18	4.71	0.82	0.17	0.15	20.3	3.12	68.38	28.50

4. DISCUSSION

4.1 Yield

The results (Table 1) suggest that the addition of the fertilizer solution containing nitrogen, phosphorus and potassium was inadequate and that the addition of milorganite increased the supply of nutrients available to the lettuce. Milorganite contains 1.3% total P, 6.6% total N (360 mg/kg NH_3 -N and 3.0 mg/kg NO_3 -N) and 0.63% total K (Carson, written communication). Anderson (1959) reported similar nutrient analysis: 5.96% total N, 3.96% total P_2O_5; 0.41% acid soluable K_2O.

Increases in crop yield as a result of sewage sludge additions have been reported by various workers. From a review of the literature, Webber (1979) reported that in nearly all the studies reviewed, sewage sludge increased the yields of cereal crops. John and Laerhoven (1976) reported an increase in lettuce yield grown in soil amended with 5 gm/kg and 10 gm/kg applications of milorganite (a greenhouse experiment). An application rate of 100 gm/kg resulted in yields significantly lower than the control. The yield obtained for the control in the present greenhouse study was substantially lower than the yields obtained by John and Laerhoven (1976).

The earthworms were inactive in this experiment and consequently there was no significant effect on yield due to their presence. The greenhouse conditions may not have been suitable for earthworm activity.

Other researchers have reported beneficial effects of earthworms on plant growth. Kirkham (1978) reported that the dry weight yields of wheat grown in soils amended with sewage sludge and without sludge were increased when the earthworm, Eisenia foetida, was added to the soil. Root growth of wheat was unaffected by the presence of earthworms. the increase in wheat yield was attributed to the earthworms increasing the water use efficiency of the wheat. Atlavinyté and Vanagas (1973) reported the growth of barley was increased when earthworms (Allolobophora caliginosa) were present. They found that the earthworm activity increased the amounts of plant available phosphorus and potassium in the soil and that this accounted for the increased barley yield when earthworms were present. Atlavinyte, et al. (1968) reported increased barley yields when grown in flower pots, in the presence of earthworms. Yields were greater where there were greater numbers of active earthworms. They also report that Lumbricus rubellus had a poor survival record (18-30% surviving). the earthworm, Allolobophora caliginosa did not result in increased yields of spring wheat and peas grown in clay soils packed into flower pots. Van Rhee (1965) also reported that the earthworms did not like flower pot conditions, and after seven months most of the earthworms were missing or dead. Those that survived were aestivating. Van Rhee (1965) also determined the effects of four earthworm species on the yields of grass, spring wheat, peas, berseem and Egyptian clover, utilizing artificial cultures. Earthworms resulted in substantial dry matter yield increases over the controls for grass (287%), wheat (111%) and clover (877%) and a 39% decrease in the yield of peas. Barley (1961) reviewed a number of pot-culture experiments that tested the effects of earthworms on plant growth, and concludes that earthworms can significantly increase yields of plants grown in pot-cultures, and that the increases are not merely the result of nutrients released from dead earthworms.

Earthworm densities similar to the density used in this present study resulted in increased yields of barley grown in flower pots (Atlavinyté et al., 1969) as well as increased availability of phosphorus and potassium (Atlavinyté and Vanagas, 1973). These workers indicated that increasing the density of earthworms in the flower pots increased the yields of barley and available phosphorus and potassium. In the present study, had the earthworms been active, the density should not have been limiting.

4.2 Extractable Metals

As expected, the amounts of metals extracted by aqua regia were greater than the amounts of metals extracted by the 3 extracting agents. The concentrations determined by extraction with the concentrated acids are considered to be the total metal concentrations in the soil (Ellis et al., 1976).

As noted in Table 3, DTPA extracted slightly less cadmium, lead, nickel and zinc and equal amounts of copper than did 0.1 N HCl, and more metals than 0.5 N HOAc, with the exception that DTPA and 0.5 N HOAc extracted equal amounts of zinc. Acetic acid did not extract any lead or nickel. Much of the total cadmium present in the milorganite amended soil was extracted by the three extractants (76%). Based on the per cent of the total metals extracted, the overall effectiveness of DTPA and 0.1 N HCl, was in the order cadmium > copper > zinc = lead > nickel; whereas for 0.5 N HOAc the order of effectiveness was cadmium > copper = zinc.

Acetic acid is a weak acid and is only partially dissociated whereas hydrochloric acid is a strong acid and dissociates completely in water. The hyrdochloric acid extractant, therefore, has more hydrogen ions which can replace metal cations on the soil exchange complex, than does acetic acid. This accounts for the higher concentrations of metals extracted by the 0.1 N HCl. Symeonides and MacRae (1977) and Karim, et al. (1976) indicated that hydrochloric acid and other acid extracting solutions may also dissolve metals from the labile solid phases. DTPA is a chelating agent which combines with the free metal ions in the soil solution to form soluble complexes which reduces the activity of the free metals ions in solution. To replenish the free metal ions in solution, metal ions desorb from the soil surfaces or dissolve from the labile solid phases (Lindsay and Norvell, 1978). The concentrated acids not only provide hydrogen ions to replace metal cations on the exchange site, dissolve labile solid phases, but also dissolve metal precipitates such as the oxides, carbonates and phosphates (Soon and Yates, 1982). The metal precipitates are not considered important for plant uptake.

The results from the present study are comparable to the results reported in the literature. Symeonides and MacRae (1977) determined that 1 N HCl extracted 100% of the total cadmium in soil, whereas 5% N HOAc extracted, on average, 50% of the total soil cadmium. Webber and Corneau (1975) reported that 0.5 N HOAc was not effective in extracting nickel from soils mixed with sewage sludge. They also reported that DTPA extracted less zinc from soils than did the acetic acids, whereas in the present study, DTPA and 0.5 N HOAc extracted equal amounts of zinc. Korcak and Fanning (1978) found for sludge treated soils, that the average percentages of extractable metals was related to the total metal concentrations in the sludges. The increase in DTPA extractable metals from soils amended with sludges was also observed by Silviera and Sommers (1977). This was also true for the present study.

Earthworms did not significantly (at the 5% probability level) affect the level of DTPA extractable metals in the soil (Table 5). As determined previously, in Part I the total concentrations of cadmium, copper, lead and zinc in the earthworm faeces were similar to the total concentrations of metals in the soil. In the present study, the DTPA extractable metals for the soil with earthworms was determined for soil plus any faeces present. The extractable metals were not determined for the faeces. Insufficient faecal material could be collected for analysis of extractable metals, due to the inactivity of the earthworms. If there was indeed any increase in extractable metals in the casts, the increase would likely be diluted by the metals from the soil. As the earthworm body tissue concentrated cadmium and zinc above soil levels, there may be possible affects on available cadmium and zinc when these earthworms die and decompose.

4.3 Correlations Between Extractable Metals and Lettuce Metal Concentrations

Table 6 gives the correlation coefficients obtained between metal concentrations in lettuce leaf tissue grown in soil amended with 50 gm/kg milorganite application rate, and total and DTPA extractable metals in the soil-sludge mixture. The correlation coefficients between total metals and plant concentrations indicate poor correlation. This is in agreement with what is expected, as it is generally accepted that total metals overestimate the amounts that plants can actually utilize (Karim, et al., 1976). Total cadmium for the control was not significantly correlated ($p>0.05$) with plant cadmium. This accounts for only 27% of the variability in the lettuce cadmium, however. There was also poor correlation between DTPA extractable metals and the concentrations in the plant. Only DTPA extractable zinc from the sludge treated soil was significantly correlated ($p<0.01$) with lettuce zinc. These results were surprising as DTPA extractable copper, nickel and zinc from the soil treated with 20 gm milorganite/kg soil were significantly correlated with lettuce tissue metal concentrations. However, comparing the concentrations of metals in the lettuce tissue from the 20 gm/kg milorganite application with the lettuce concentrations from the second experiment, indicates that the higher application of milorganite resulted in higher concentration of cadmium, lead, nickel and zinc and only slightly higher copper concentrations in the lettuce. As the concentrations of DTPA extractable metals in the two milorganite-soil mixtures were approximately equal and the concentrations of metals in the lettuce tissue were higher in the plants from the 50 gm/kg milorganite application, it is no longer surprising that the corelation coefficients are not equal. The results suggest that at the higher application rate of milorganite the plants are taking up metals from other pools not measured by DTPA.

The correlation coefficients between DTPA extractable metals and plant tissue from the 50 gm/kg milorganite application were low and accounted for only a small amount of the variability in metal concentrations in the lettuce tissue. Although DTPA and other extractants have been widely used and discussed in the literature (see review Lindsay and Norvell, 1978) with varying degree of correlation with metal uptake by plants, DTPA was initially developed by Lindsay and Norvell as a test to access copper and zinc deficiencies in calcareous soils. In acidic soils with large concentrations of metals due to the addition of sewage sludges, the use of DTPA may be inappropriate and this would be reflected by poor correlation with plant uptake. Silviera and Sommers (1977) warn that although DTPA extractable metals may correlate with plant uptake, the addition of sewage sludge may result in a large increase in DTPA extractable metals and consequently the concentration of DTPA used in the extraction may not be appropriate for soil treated with sewage sludge.

4.4 Lettuce Cadmium Concentrations

Lettuce leaf tissue concentrated cadmium above soil levels and also accumulated Cd with increasing soil Cd concentrations. Cadmium concentrations in the leaf tissue from the controls exceeded the soil concentrations by a factor of 3.6 and for the 50 gm/kg application of milorganite the concentration factor was 11.7. Lisk (1972) reported that the ratio of cadmium concentration in plant tissue to soil concentration is generally around 10 to 1.

The increase in cadmium concentration in the lettuce tissue with the application of milorganite and hence increased soil cadmium concentrations is consistent with the literature. From his review of the literature, Webber (1979) indicates that the concentrations of cadmium in vegetable crops grown in acid soils amended with sludges with high cadmium concentrations can be quite large. The concentrations of cadmium for the control plants are higher than those reported by Page et al. (1981) for lettuce grown in uncontaminated soil. However, the soil cadmium concentration of the control (1.6 µg/gm) is above the mean of 0.88 µg/gm Cd for noncontaminated soils in the Lower Fraser Valley (John, 1978), and may indicate contamination, perhaps by superphosphate fertilizers. John (1973) reported lettuce had cadmium concentrations of 4.8 µg/gm in lettuce leaf tissue and 2.8 µg/gm in root tisue when grown in non-contaminated soil collected from the Ap horizon of a Hazelwood, silt loam. He also reported lettuce leaf cadmium concentrations of 7.1 µg/gm and root concentrations of 4.8 µg/gm for plants grown in soil from the Ap of a Hjorth silty clay loam with a pH of 4.04 and a cadmium concentration of 0.67 µg/gm determined by IN HNO_3 (John, 1972). The values for his controls are similar to the values for the controls determined in the present study. John (1972) also reported a concentration of 50.8 µg/gm cadmium in the lettuce leaf tissue of plants grown in the Hjorth soil amended with 5 µg/gm cadmium chloride. However the magnitude of the cadmium concentrations in the plant tissues determined in my study are similar only to the concentrations of lettuce tissue in studies using cadmium salts or sewage sludges spiked with cadmium salts. John and van Laerhoven (1976) grew lettuce in soil from the Ap of a Monroe silt loam amended with various application rates of milorganite. They report plant concentrations much less than those obtained in the present study and also reported that increases in milorganite application resulted in decreased cadmium concentrations which was associated with decreased yields at applications exceeding 10 gm/kg. The increase in organic matter associated with increased applications of sludge was credited with reducing the amount of cadmium available for plant uptake. Lettuce cadmium concentrations increased threefold when sewage sludge applications were increased from the control to 220 t/ha (Zwarich and Mills, 1982). However the sludge used in their study had a cadmium concentration of only .22 μg/gm and plant concentrations increased from 1.4 μg/gm when no sludge was utilized to 4.3 μg/gm when 220 t/ha sludge was applied. Singh (1982) determined cadmium concentrations in lettuce which was grown in sludge amended soil (silt-loam, pH 6.7 and sludge from Sarnia with 99 μg Cd/gm and soil amended with various inorganic cadmium salts). The concentrations in lettuce grown in the cadmium salt amended soils were similar to the concentrations in lettuce grown in milorganite, whereas the cadmium concentrations in the lettuce grown in the soil amended with the Sarnia sludge were much lower than those obtained in the present study. Mitchell, et al. (1978) reported a cadmium concentration of 43 μg/gm for lettuce harvested from a calcaeous, silt loam amended with a cadmium salt enriched sewage sludge to produce a cadmium concentration of 5 μg/gm. These researchers reported increased cadmium concentrations in lettuce tissue (up to 413 μg/gm) as soil cadmium concentrations increased up to 320 μg/gm.

In the current study, the paired difference test at the 5% probability level did not detect a significant difference between the cadmium concentrations in the lettuce leaf and root tissue. Webber (1980) reports that cadmium concentrations in leafy vegetables tend to be higher in the edible parts of the plants rather than the roots. John (1972) reported significantly greater cadmium concentrations in the above ground parts of lettuce and radish plants than the roots from both controls and cadmium amended soil. However, John (1973) found that although the control plants had higher cadmium concentrations in the leaf tissue relative to the roots, the reverse was the case for lettuce grown in cadmium amended soil. These results suggest that the lettuce plants vary in their ability to immobilize cadmium in the roots.

4.5 Lettuce Copper Concentrations

The results suggest that copper concentrations may be regulated by the plant or that copper uptake is reduced when the concentration of other metals is increased, or that additional copper in the milorganite is not utilized by the lettuce plants. Copper in the sewage sludge may be immobilized by forming stable organic copper complexes.

Several other authors also report that coper concentrations in lettuce tissues did not increase with increasing soil copper concentrations. Mitchell, et al. (1978) reported that copper concentrations in lettuce remained unchanged at an average concentration of 7.1 μg/gm copper even though the soil copper concentrations increased from 0 to 320 μg/gm. When the soil copper increased to 640 μg/gm, there was a significant increase of lettuce concentrations to 9.3 μg Cu/gm.

Milorganite additions to a Monroe silt loam, resulted in increasing concentrations of 1.0 N HNO_3 extractable copper, but did not result in increasing copper concentrations in lettuce leaf tissue. The results reported by John and van Laerhoven (1976) report a higher copper concentration for lettuce (19.9 μg/gm for plants grown in soil amended with 25 gm/kg milorganite) than the copper concentration in the plants of the present study. MacLean and Dekker (1978) determined copper concentrations in lettuce grown in soil amended with sewage sludge spiked with varying concentrations in lettuce remained fairly constant as the soil concentration increased. Zwarich and Mills (1982) also applied sewage sludge to soil in increasing application rates and determined copper concentrations in lettuce. They report an increase in lettuce copper with increased sludge application. However, the concentration of copper from two sludge treatments, 55 t/ha and 110 t/ha were not significantly different either but were from the control and the 220 t/ha application.

In the current study, the concentration of copper in the root tissues was significantly higher ($p<0.05$) than the copper concentrations of the leaf tissue. This agrees with Webber (1980) who concluded that leafy vegetables tended to have greater concentrations of copper in their roots than in their leaves. Mitchell, et al. (1978) found higher concentrations of copper in the roots of wheat than in wheat grain and wheat leaves. They suggest that copper once taken-up by the roots remains bound up by amino acids and forms stable organic complexes which are not translocated to the leaf tissue. Czuba and Hutchinson (1980) also found higher copper concentrations in lettuce roots than in the lettuce leaves.

4.6 Lettuce Nickel Concentrations

Addition of milorganite resulted in significant decreases in the nickel concentrations in the lettuce leaf tissue (Table 7) and lettuce root tissue (Table 8), even though the total concentration of nickel in the soil from the control treatments and milorganite treatments were unaffected. These results suggest that the organic matter added in milorganite may be binding with the nickel although there was a slight but significant increase in DTPA extractable nickel when milorganite was added to the soil. Halstead et al. (1969) determined that Niconcentrations in alfalfa and oats grown in Nicontaminated soils were reduced when the soil organic matter content was increased. This was attributed to the formation of stable Ni-organic matter complexes which were unavailable to plants. Hutchinson (1981) indicates that the addition of organic matter decreased ammonium acetate extractable nickel in soils, while Hag, et al. (1980) indicated that DTPA extractable nickel concentration in soil was positively correlated with organic matter and that nickel concentration extracted by acetic acid was decreased by increased organic matter content.

The increased concentrations of the other metals present in the soil milorganite mixture may result in the interference with nickel uptake by the plant. Hughes, et al. (1980) indicated that nickel uptake by soybean seedlings was reduced when the concentrations of copper and zinc were increased.

The addition of sewage sludge to soil generally results in increased nickel concentrations in leaf and root tissues of a wide range of crop plants (Page, 1974; Webber, 1979; Hutchinson, 1981). Nickel concentrations in plant tissues do not exceed soil concentrations and usually are less than 10 µg/gm for plants grown in soil uncontaminated by nickel (Hutchinson, 1981). MacLean and Dekker (1978) determined nickel concentrations in corn and lettuce which had been grown in soils amended with increasing amounts of nickel sulphate salts and to which sewage sludge had also been added. They found that nickel concentrations in the plant tissues increased with corresponding increases in soil concentrations. However, the plants grown in soil to which the sludge had also been added had lower nickel concentrations than plants grown in soils amended only with the inorganic salt. Mitchell, et al. (1978) grew lettuce and wheat in soil amended with metal spiked sewage sludge and determined that nickel concentrations in the plants increased as soil nickel concentrations increased. Nickel concentrations in lettuce and onions grown in soil amended with sewage sludge increased for plants grown in pot cultures in a greenhouse but did not increase in plants grown in the field, amended with equivalent application rates of sludge (de Vries and Tiller, 1978). John and van Laerhoven (1976) reported that the addition of milorganite to unlimed soils did not result in increases in the nickel concentration of lettuce tissue or beet tubers but did result in increased concentrations of nickel in the beet tops. The addition of Iona sludge to unlimed soil lead to significant increases in the nickel concentrations of lettuce, did not affect the concentration in beet tops, and reduced the nickel concentrations in the beet tubers (John and van Laerhoven, 1976).

Paired difference tests at the 5% probability level indicated that lettuce roots had significantly higher concentrations of nickel than did the leaf tissues, in this study. Mitchell, et al. (1978) found higher nickel concentrations in the root tissues of wheat than in the leaf and grain tissues. They attribute the higher root concentrations to effective binding of nickel by organic molecules within the root, and that the root retains these nickel-organic complexes against transolocation to the leaf tissue (same as copper).

4.7 Lettuce Lead Concentrations

Lead concentrations in the leaf and root tissues of lettuce were not significantly affected by the addition of milorganite to the soil.

The average lead value for the leaf tissue from the milorganite treatment was much greater than the value for the control. However, there were large standard deviations associated with both values and, therefore, the differences were not significant.

Page (1974), de Vries and Tiller (1978), Zwarich and Mills (1982) indicated that the addition of sewage sludge to soil did not lead to significant increases in lead in a variety of crop plants including lettuce. John and van Laerhoven (1976) indicate that although the addition of milorganite significantly increased soil lead levels, the concentrations of lead in lettuce tissues remained unchanged. Adding 1000 μg/gm of lead as inorganic lead salts to soil did result in significant increases in lettuce concentrations (John and van Laerhoven, 1972), therefore, maybe the form of the lead is important.

There was significantly higher concentrations of lead in the roots than in the leaf tissue for two of the four treatments. (the treatments were treatment 1) no earthworms and no milorganite, and treatment 4) earthworms and milorganite, paired difference tests at the 5% probability level). Webber (1980) reported that leafy vegetables tend to have higher lead levels in roots relative to leaf tissue. Czuba and Hutchinson (1980) found that in their study the lead concentrations in lettuce root tissue were not consistently higher than leaf tissue. The literature on lead uptake by plants was reviewed recently by Koeppe (1981). He reports that the roots of plants grown in lead contaminated soils always had more lead than the above ground plant parts. Koeppe (1981) indicated that lead was adsorbed by the cell walls of roots of some plants and in others lead was precipitated by cell organelles.

Lettuce Zinc Concentrations

The addition of milorganite to the soil resulted in significant ($p < 0.05$) increases in the zinc concentrations of both lettuce tissue types. Soil total zinc concentrations were similar to or slightly less than the leaf tissue zinc concentrations whereas the concentrations in the roots exceeded the concentrations in the soil.

Other workers also report increased zinc concentrations in plants, including lettuce, when sewage sludge is added to the soil (Page, 1974; Mitchell *et al.*, 1978; Webber, 1979; Schauer *et al.*, 1980). Zwarich and Mills (1982) reported that there was a linear relationship between lettuce zinc concentrations and the soil zinc concentrations. John and van Laerhoven (1976) reported that although additions of milorganite increased soil zinc concentrations, lettuce grown in unlimed soil had a significant decrease in tissue zinc concentrations. They attribute this to zinc being bound by the increasing additions of organic matter.

For this study, paired difference tests at the 5% probability level indicated that the roots had significantly higher zinc concentrations than the leaf tissue did in three of the four treatments. The variability in zinc concentrations in the roots of the lettuce grown without milorganite in the presence of earthworms was too large to detect any differences between the leaf and root zinc concentrations. Webber (1980) indicated that leafy vegetables tended to have higher concentrations of zinc in above ground tissues rather than in the roots. Zinc in wheat roots was higher than in wheat leaves and grain (Mitchell et al., 1978). Lindsay (1972) reported that when plants were grown in soils with high available zinc concentrations in plant tops tended to be less than the levels in the roots, and that if the supply of soil zinc is adequate luxury consumption of zinc by roots often occurs.

4.9 Effects of Earthworms on Lettuce Metal Concentrations

The decrease in lead concentration in roots in the presence of earthworms ~~if~~ is not due to a reduction in the lead availability as earthworms had no effect on the concentration of DTPA extractable lead. Earthworms are not accumulating the lead in their own tissue. (refer to previous experiment Part 1) and thereby reducing the amount available to the plants. However, DTPA extractable lead was only weakly correlated (r = -0.33) with plant lead when no milorganite was present and when milorganite was present the correlation was increased (r = -0.65). DTPA extractable lead only accounts for 11% of the variability of lead tissue concentrations for plants without milorganite treatment and for only 42% of the variability in plant tissue lead concentration for plants treated with milorganite. It is possible that earthworms have tied up lead that is not measured by DTPA but is in a form that would be used by the plants. Ireland (1975) indicated that earthworms did not increase acetic acid extractrable lead.

Although not very active in the soil, it is possible that there was a slight increase in the aeration of the soil due to burrowing activity, resulting in a decrease in the availability of lead. Metals generally are less available when the supply of oxygen is increased.

Only two reports in the literature exist concerning metal uptake by plants in the presence of earthworms. Peredelsky et al. (1957) reported that when mustard was grown in the presence of earthworms there was a fivefold increase in the cobalt concentration in plant tissues. Earthworms also resulted in an increased cobalt concentration in several cereals, legumes, hemp and buckwheat. When wheat was grown in sludge amended soils in the presence of the earthworm, _Eisenia foetida_ there was a significant increase in the concentration of manganese in the roots. Earthworms had no effect on the copper and zinc concentrations in the wheat roots or shoots (Kirkham, 1979).

5. GENERAL DISCUSSION

The results obtained in this experiment are in general agreement with those reported in the literature, although the metal concentrations in the lettuce tissue tended to be higher than those determined by other researchers. There are several reasons for this variation between studies and involve differences due to sludge variation, differences in soil properties, and differences in metal uptake by plant varieties.

Sewage sludges from different treatment plants vary not only in total metal concentrations but also in amounts of available metals, metal species, pH and organic matter content. (Page, 1974). As sludges contain different amounts of available metals it is reasonable that plants grown in different sludges would have variations in tissue metal contrations. Also, many of the studies reported in the literature involve growing plants in soils amended with sewage sludge spiked with inorganic metal salts or growing plants in soils amended with inorganic metal salt solutions. Webber (1980) concluded from his literature review that studies using inorganic salts at high concentrations to simulate sludges with high metal content should be viewed with caution. High concentrations of metal salts tend to result in decreased yields and high plant metal concentrations. (Mitchell et al., 1978; Korcak and Fanning, 1978; Zwarich and Mills, 1982). Singh (1981) reported that the Cd concentration was 5 times higher in lettuce grown in soil treated with Cd salts than in lettuce grown in the same soil treated with sewage sludges with comparable Cd concentrations. These studies demonstrate that metals in sewage sludges are less available than metals in inorganic salts.

Soil properties such as pH, organic matter content, cation exchange capacity and clay content influence the amount of metal taken up by plants (Webber, 1980). Liming soils amended with sewage sludges or inorganic metal salts reduced the Cd, Cu, Ni, Pb and Zn concentrations in lettuce (John and van Laerhoven, 1972, 1976; MacLean, 1974; 1976; Mahler et al., 1982). MacLean (1989) reported that the addition of organic matter to soils decreased Pb uptake by plants. Organic matter addition decreased Cd and increased Zn uptake by lettuce (MacLean, 1974, 1976). Gaynor and Halstead (1976) indicate that the concentrations of Cd, Cu, Pb and Zn in letuce were less in plants grown in finer textured soils. Generally plants grown in soils with low cation exchange capacities tend to have higher metal concentrations than do plants grown in soils with high cation exchange capacities (Webber, 1980).

Care must be exercised when comparing the results of the present study with field studies. In this study the milorganite was well mixed with the soil, a condition unlikely to be obtained in a field situation. Also, sewage sludge underfield conditions may undergo more extensive physical, chemical and biological changes under the influence of natural weather conditions over time (Hartenstein et al., 1980). De Vries and Tiller (1978) and Page et al., (1981) compared levels of metals in vegetables grown in soil amended with sewage sludge in greenhouse containers and in the field and found higher metal concentrations in the tissues from the greenhouse study. Page et al.,

(1981) indicated that container-grown plants root entirely within a contaminated soil whereas plants grown in the field may extend roots below the contaminated layer. Also, the variations in light, temperature, humidity, moisture and other environmental conditions may be more pronounced in a field experiment compared to one in a greenhouse which may affect metal uptake by plants (de Vries and Tiller, 1978).

Not only do different plant species differ in their ability to take up metals but there are also differences among plant varieties (Page, 1974; Webber, 1980; Page et al., 1981). John and van Laerhoven (1976) determined the Cd concentration in 9 varieties of lettuce (6 head, 2 leaf, iromaine) grown in solution culture with $CdCl_2$. There was significant differences among the varieties in their tolerances to Cd phytotoxicity and Cd concentration. The highest and lowest Cd concentrations were recorded for head lettuce varieties. The data of Giordano et al., (1979) also suggest differences in Cd and Zn concentrations among different lettuce cultivars.

In the present study, earthworms did not influence plant growth which is contrary to most results reported in the literature. (Barley, 1961; Atlavinyté et al., 1968). Van Rhee (1965) indicated that generally earthworms do not like container conditions and after several months will either die or aestivate. The surviving earthworms in the present study were aestivating and some had died. Also, the taxa used in this study were different from the species and commonly used in the literature, although the density of earthworms used was similar to those used in the literature.

6. CONCLUSIONS

The concentrations of total soil Cd, Cu, Pb and Zn but not Ni and the concentrations of DTPA extractable Cd, Cu, Ni, Pb and Zn were increased when milorganite was added to the soil. The concentrations of both total and DTPA extractable metals were unaffected by the presence of earthworms.

The concentrations of Cd and Zn in both leaves and roots were increased when milorganite was added to the soil. Nickel concentrations decreased in both tissue types and Cu and Pb concentrations were unaffected by milorganite amendments to the soil. Only the Pb concentrations in lettuce roots were affected by the presence of earthworms. With earthworms present in soil both with and without milorganite the Pb concentrations in the roots were lower than when the earthworms were absent.

The concentrations of Cu, Ni and Zn in lettuce leaf tissue were significantly correlated with the concentrations of DTPA extractable metals when milorganite was added to the soil at the rate of 20 gm/kg. Only Zn concentrations in leaf tissues were significantly but negatively correlated with DTPA extractable Zn when milorganite was added at the rate of 50 gm/kg. These results demonstrated that at increased levels of sludge application the plants are utilizing metals from soil metal pools not measured by DTPA.

Yields of lettuce were increased when milorganite was added to the soil. The additional N, P, and K added in the sludge resulted in increased yields. However, the Cd concentrations in the soil after the addition of milorganite exceed the proposed Ontario guidelines for sludge additions to land. (OME, OMAF, 1978). Milorganite is not recommended as a fertilizer.

Earthworms were not very active in the flower pots and most were recovered in resting positions. The presence of earthworms did not affect plant yields.

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