THE FATE OF HEAVY METALS APPLIED IN SEWAGE SLUDGE TO GRASSLAND UNDER DIFFERENT MANAGEMENT PRACTICES

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SUMMARY

European policy regarding sewage sludge disposal lays down (i) that the disposal option chosen should entail the least possible cost to the community whilst at the same time safeguarding public health, and (ii) that it should limit environmental damage and recognise the value of sludge as a resource. Disposal of sludge on agricultural land appears to be the obvious choice. Not only is this method economical but at the same time makes full use of its fertiliser value. Given that proper guidelines are followed, hazards to health and environment can be significantly minimised.

Of all the problems associated with disposal of sludge on land, heavy metal build-up in the soil is of greatest concern. The application of potentially toxic elements in sewage sludge to grassland is now controlled in the EEC by legislation. The guidelines are based on metal levels in the receiving soil and in the sludge being supplied. However, the data upon which these guidelines have been formulated is far from complete. In particular, the influence of grassland management practices such as liming, fertiliser-N use and ploughing/reseeding programmes on the availability of heavy metals requires elucidation. In addition, there remains doubt as to whether three weeks no-grazing after sludge application is sufficient to reduce the risk of direct ingestion of sludge-borne metals by ruminants. Recently, concern has been expressed as to the potential reduction in soil microbial biomass in sludged soils.

Studies were conducted at grassland sites in west Scotland into factors

influencing herbage contamination by sludge adherence and the effects of liming, ploughing and fertiliser-N rate on heavy metal uptake. The effects of sludge application on the soil microbial biomass and nitrification potential were also investigated.

Experimental plots (involving all combinations of either ploughed and reseeded pasture or directly reseeded pasture, with no liming (pH 5.5) and liming (pH 6.3) and three rates of fertiliser-N (i.e. 0, 250 and 500 kg N/ha/year)) were established on an alluvial soil previously under permanent grass. The site had received regular additions of sewage sludge of industrial and domestic origins for around 50 years. Effects on the uptake by herbage (*Lolium perenne*) and on soil distribution of Cd, Cr, Cu, Ni, Pb and Zn were investigated. Metal enrichment was evident to a depth of 200mm, although metal accumulation was greatest over the 0-100mm depth. Although soil metal concentrations were above the lower limit set by the EEC (1986), their concentrations in herbage for the most part were neither phytotoxic nor zootoxic. However, herbage Cd, in all treatments approached a zootoxic concentration. Liming resulted in a significant decrease in herbage Cd, Cu, Ni and Zn whilst increasing the rate of fertiliser-N led to significant increases in herbage Cd, Cu and Zn concentrations which could not be explained by a change in bulk soil pH. Metal concentrations extracted by both 0.43M acetic acid and 0.05M EDTA did not reflect these changes in metal uptake. Chromium and Pb were unaffected by either liming or fertiliser-N rate. Ploughing and reseeding led to short term (i.e. over the first two cuts) increases in herbage Cu, Zn and Ni. However, the redistribution of metals within the soil profile following

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cultivation, resulting in lower metal concentrations in the top soil compared to the undisturbed profile, could be of considerable importance in lowering the potential dietary metal intake of grazing animals.

A pot trial was undertaken to study the relationship between soil pH and uptake of heavy metals by plants. A 50:50 soil:perlite mixture was used as a growing medium for perennial ryegrass (L. perenne L.). To this mixture the equivalent of 55m^3 of digested sludge was added. A series of pH treatments (pH 4.0-9.0 in increments of 0.5 units) was set up. With the exception of Pb, herbage metal uptake decreased as pH increased from 4.5-5.0. With a further rise in soil pH over the range of pH 5.0-9.0 a more gradual decrease in herbage metal uptake was evident. Herbage Pb concentrations were independent of soil pH.

At a second site in the west of Scotland 2% D.S. sludge was applied at a rate of 27.5m^3/ha and 67.5m^3/ha in late March to a L. perenne sward. At the same time, 12% D.S. sludge was also applied at the rate of 67.5m^3/ha. In addition, half the number of plots receiving 67.5m^3/ha of 2% D.S. sludge were cut one week before application. On the day of sludge application and at weekly intervals thereafter for 8 weeks, herbage from three replicate plots (0.5 x 0.5m) of each treatment was harvested and analysed. Where sludge containing 2% and 12% D.S. was applied at 67.5m^3/ha to an uncut sward, 35% and 55% respectively of harvested dry matter consisted of sludge solids on the day of application compared to 17% when 2% D.S. was applied to a previously cut sward. Applying 2% D.S. and 12% D.S. sludges at 67.5m^3/ha to uncut swards led to respective increases in herbage metal concentration on the day of application of as much as 8 and 28 fold compared to the control but the proportions of applied Cd, Cu, Mn, Ni, Pb and Zn adhering to herbage as defined by 'Adherence Index' were all between 12-23%. However, Cr and Fe appeared to behave differently on uncut herbage treated with 2% D.S. sludge on which a much higher proportion of around 60% of the metal applied remained.

On the day of application, total Fe, Cd, Pb (with the exception of the 27.5m^3/ha treatment) and Cu concentrations of sludge-treated herbage were above those suggested to be toxic to ruminants. However, within the 3-week no-grazing period, Cu, Cd and Pb concentrations fell to safe values where 2% D.S. sludge was applied but Fe required between 4-5 weeks. In contrast, application of 12% D.S. sludge led to herbage having potentially zootoxic concentrations of Cd, Pb, Cu and Fe for up to 8 weeks.

The dilution of adhering metals was brought about as a result of both plant growth and rainfall. However, their effects were not clearly distinguishable and could not entirely explain the observed dilution of metals. Hence, other factors may also have contributed to the removal of sludge metal from the leaf surfaces. The effect of rainfall in bringing about metal dilution especially that of Cr, Fe and Ni, derived from 2% D.S. sludge, was most evident when rain fell within one week of sludge application, but less so in grass given 12% D.S. sludge.

A pot experiment showed that drying the adhering sludge prior to the application of 'rain' resulted in no metal wash-off. The rate of dilution was not only influenced by the amount and timing of rainfall and the extent of plant growth but was also dependent on the dry solid content of the sludge. Adhering metals from the 12% D.S. sludge exhibited a higher dilution rate than the same metals from the 2% D.S. sludge. This may be related to factors such as the electrostatic bonding of metals to leaf surfaces.

Reductions in the total soil microbial biomass C, as measured by the chloroform-fumigation-incubation method, ascribed to elevated soil metal concentrations, were observed in a sludged soil. However, in certain situations, sludge application may stimulate microbial populations even though soil metal levels are increased. The environmental significance of any decrease in microbial numbers is uncertain in view of the fact that the sludged soil exhibited a similar base respiration rate to that of an unsludged control soil, i.e. in the sludged soil there was greater CO_2 production per unit weight of microbial biomass. This may possibly reflect some microbial adaptation to enhanced metal concentrations in soil.

The effects of extended sludge application on soil nitrification potential, measured in terms of NO_3-N production in a cyclic-continuous flow percolating system over a 20-32 day period, are not as distinct as the situation observed for microbial biomass C. Soil nitrification seems to be predominantly determined by soil pH. As pH increases, even over quite a narrow range, the delay period decreases whereas K_{max} increases. Sludge application to land may thus influence nitrification more by its resultant change in soil pH than by any resultant increase in concentrations of toxic compounds. In fact, provided sludged soil is limed, sludging may enhance the soil's nitrification potential.

The results of this work are interpreted and discussed in the light of current legislation and codes of practice governing sewage sludge applications to agricultural land in the United Kingdom.

CHAPTER 1

REVIEW OF LITERATURE - INTRODUCTION

Historically the primary objective of sewage treatment works in the United Kingdom has been to produce an effluent which meets the conditions set out by the Royal Commission on Sewage Disposal (1898 - 1915), (cited by Mason, 1981). These criteria allowed no more than 30 mg/1 of suspended solids and 20 mg/1 biological oxygen demand (B.O.D.), known as the 30:20 standard. By disposal into waterways, the Royal Commission envisaged that the effluent would be diluted with eight volumes of clean river water having a biological oxygen demand of 2 mg/1. In practice, such a dilution is not always possible and the receiving water may also fall short of the 2 mg/1 B.O.D. requirement, meaning that a more stringent standard than 30:20 may be required for the effluent. The 30:20 standard, coupled with the 1963 U.K. Water Resources Act (HMSO,1963), set out measures to control water pollution. This, in turn, has led to more efficient sewage treatment processes and the consequent production of larger quantities of sludge requiring disposal.

1.1 METALS IN SEWAGE SLUDGE

1.1.1 CONTENT AND ORIGIN

The heavy metal load of the wastewater entering a sewage treatment works, and ultimately the metal content of the sludge derived therefrom, varies widely depending on a number of factors, such as geographical location and the type and quantity of industrial input (Berrow and Webber, 1972). The metal contents of various sewage sludges have been measured by a number of workers (Berggren and Odens, 1972; Berrow and Webber, 1972; Blakeslee, 1973; Sterritt and Lester, 1981). Table 1.1 reproduces the results of surveys, carried out by Berrow and Webber (1972) and by Sterritt and Lester (1981), on the heavy metal content of sludges sampled from across England and Wales.

Metals in municipal wastewater originate from a variety of industrial, commercial and domestic activities (Klein et al., 1974; Gurnham et al., 1979 cited by Patterson and Kodukula, 1984), as well as storm runoff (Feiler, 1979). In some industrial towns as much as 50% of the total flow of raw sewage can be of industrial origin (Berrow and Webber, 1972). The presence of a particular industry can lead to large amounts of certain metals finding their way into sludge. For example, metals such as cobalt (Co), copper (Cu), nickel (Ni), and chromium (Cr) can be derived from electroplating, foundry processes and alloy production industries (Berrow and Webber, 1972). Even sludges from typically residential communities can contain Cu and zinc (Zn) concentrations in excess of 500 and 1000 mg/kg dry matter (D.M.) respectively (Page, Reference to 'Errata' page 9 has been inadvertently missed.

TABLE 1.1 TYPICAL CONCENTRATIONS OF HEAVY METALS (mg/kg DRY SOLIDS) IN U.K. SEWAGE SLUDGE AND SOILS.

METAL	SEWAGE SLUDGE METAL CONCENTRATION				SOIL METAL CONCENTRATION	
	Range(1)	Mean(1)	Range(2)	Mean(2)	Range value	Typical
Ag	5 - 150	32			<1	<1
B	15 - 1000	70			2 - 100	10
Ba	150 - 4000	1700			100 - 4000	1000
Be	1 - 30	5			<1 - 40	3
Bi	<12 - 100	34			<1	<1
Cd	<60 - 1500	<200	1 - 110	25	0.01 - 0.7	0.1
Co	2 - 260	24	11 - 2490	105	1 - 40	15
Cr	40 - 8800	980	57 - 5190	707	5 - 1000	100
Cu	200 - 8000	970	170 - 2080	721	2 - 100	20
Mn	150 - 2500	500	131 - 6120	667	100 - 3000	800
Mo	2 - 30	7	0.1 - 214	16	<1 - 5	1
Ni	20 - 530	510	16 - 2020	290	5 - 500	50
Pb	120 - 3000	820	27 - 45400	1550	2 - 200	30
Sn	40 - 700	160	3 - 329	57	<1 - 10	3
V	20 - 400	75			20 - 500	100
Zn	700 - 49000	4100	93 - 9210	1930	10 - 300	80

(1) Berrow and Webber (1972).

(2) Sterrit and Lester (1981).

1974). The sources of these high concentrates is uncertain but can be derived from foods, galvanized metal, pharmaceuticals, cosmetics and rubber (Williams, 1975).

Every year approximately 12,000 t of the most environmentaly significant heavy metals pass through U.K. sewage treatment works (Rossin et al., 1983), and much of this metal finds its way into sludge (Davis and Carlton - Smith, 1980). Sewage treatment essentially involves a sedimentation step whereby material capable of settling is deposited as sludge. At this stage much of the initial sewage metal load is removed as shown in Table 1.2.

Primary sedimentation, of sewage entering treatment works, removes only insoluble forms of heavy metals and has very little effect on the soluble metal concentration (Lester, 1983b), although some association of soluble metals with sewage particulates may occur prior to sewage entering the sedimentation tank.

Strong associations between sludge particulates and heavy metals, due to the formation of inorganic precipitates, organo - metallic interactions and by association with biomass, result during anaerobic digestion (Lester et al., 1983). The mechanisms involved in forming these associations have been reviewed by Brown and Lester (1979) and by Sterritt and Lester (1980). Several aspects of the activated sludge process have been shown to influence heavy metal removal efficiency such as nutrient supply and metabolic conditions of the biomass (Casey and Wu, 1977), growth rate and age of cells (Friedman and Dugan, 1968) and

TABLE 1.2 PERCENTAGE METAL REMOVAL OF INITIAL METAL LOAD IN SLUDGE DURING SEDIMENTATION.

METAL	% REMOVAL	REFERENCE
Cd	60 - 72	Lewin and Rowell,1973; Lester et al, 1979.
Cr	28 - 73	Stones, 1955; Stoveland et al, 1979.
Cu	45 - 70	Stones, 1958; Lester et al, 1979.
Ni	20 - 70	Stones, 1959a; Stoveland et al, 1979a.
Pb	54 - 73	Lewin and Rowell, 1973; Lester et al, 1979.
Zn	40 - 74	Stones, 1959b; Stoveland et al, 1979.

the association of metals with chelating and soluble complexing agents (Cheng et al., 1975; Stoveland et al., 1979b). Depending on the element concerned between 20 - 80 % of the metal content of settled sewage can be removed during the activated sludge process (Davis, 1980).

1.1.2 METALS OF CONCERN

The metal load of most sludges applied to agricultural land is generally much greater than that of the receiving soil (see Table 1.1) and includes metals such as Cu, Zn, tin (Sn), as well as silver (Ag), bismuth (Bi), and lead (Pb). High concentrations of Cr and Ni tend to be restricted to a small number of samples (Berrow and Webber, 1972). This, combined with the fact that the metals introduced into the soil via sludge application accumulate in the surface soil layers (see Section 1.3.4) is potentially a cause for concern especially when these substances are assimilated by living organisms (whether plant, animal or man) in sufficient concentrations, and for a sufficient period of time, for harm to occur (Lester, 1983b). Concern about these metals and safeguards to define their environmental impact is influenced by the metal's lability and hence its toxicity. Jaworski et al. (1984) have listed a number of characteristics which would lead to the labeling of an element as being either an existing or potential environmental problem: -

(i) The element or its form has been shown to have adverse effects on human or animal metabolism, cause mutations or is a known carcinogen (eg. Pb, Cd, As, Hg, methyl - mercury, Tl, Be, Cr, Cu, F, Mn, Mo, Ni, Sb).

(ii) The environmental concentration of the element is expected to increase with the use of existing technology (eg. Al, Cd, Ni, Cr).

(iii) New or developing technologies are expected to increase emissions, cause emissions in new geographic locations or emit new species of the element (eg. Be, Mn, Ga, As).

(iv) The rate of exposure or rate of accumulation is very high (eg . Cd, methyl - mercury, Pb).

(v) The dietary habits or geographical location of a certain subpopulation may make them more liable to higher rates of exposure or absorption possibly resulting in body levels near the critical level (eg. As, Pb, Cd, Hg, Ni, Ti).

(vi) Certain elements may $\overset{a}{e}$ffect the quality and quantity of the food supply available for human consumption resulting in food shortages (eg. Cu, Ni, Cr, Zn, Mo).

The number and complexity of forms of heavy metals, many of uncertain toxicity, which may enter the foodchain from sludge disposal on land, makes the compilation of a list of priority metal pollutants difficult and subject to continuous review as more information on a particular substance becomes available. At present, priority pollutants are those known to be toxic to bacteria involved in the sewage treatment process and those known to be phytotoxic or harmful to animals and man, especially carcinogens and those which accumulate in the foodchain (Davis, 1980). In this respect, field experiments carried out by Lunt (1959), cited by Berrow and Webber, 1972, showed that the amounts of Zn, Cu and boron (B) present in most sludges could, in acid soils, be toxic to plants. In addition, results from a long term field experiment at the Woburn Market Garden, reported by Le Riche (1968), showed that crop uptake of Cu, Ni, and Zn increased following repeated sludge applications, emphasizing the danger of these elements accumulating in the soil. The 1986 E.E.C. Directive on sewage sludge utilization on land (E.E.C., 1986) identifies Cd, Cr, Cu, Ni, Pb, Zn, and mercury (Hg) as the metals which need to be monitored in agricultural soils receiving sludge.

As far as Cu, Ni, Zn are concerned, plant yield depression starts at considerably lower tissue concentrations than those which could be harmful to animals or man consuming these plants, with the possible exception of Cu which, at relatively low levels in forage (below 20 mg/kg D.M.) may be toxic to sheep (Dalgarno and Mills, 1975). Consequently Cu, Ni,and Zn are recognized as being potentially phytotoxic elements (Rohde, 1962; Webber, 1972; Williams, 1975; U.S.E.P.A., 1976; Marks et al., 1980; Beckett and Davis, 1982).

On the other hand, Pb and Cd are present in sludge in amounts not normally high enough to have any adverse effect on plant growth, but they may accumulate in plant tissue to give concentrations which may be harmful to the health of animals and man consuming them (M.A.F.F., 1978; Williams, 1980; Kloke et al., 1984).

Concern for the potential toxicity arising from the introduction of Cr and Hg, via sludge into agricultural soils, is not unanimous. Although Cr is potentially phytotoxic (Davis, 1983), no limit has as yet been agreed upon for its inclusion into the E.E.C. Directive regulating sludge utilization on agricultural land (E.E.C., 1986). This is due to the fact that Cr is present in sludge in the cationic form, and as such is relatively non-toxic to plants (Christensen and Tjell, 1984; Webber, 1972; Williams, 1975). For its part, Hg is a well-known toxin and is of particular interest as it is a cumulative poison in animals and man (Davis, 1983; Dean and Suess, 1985). However, even when soils are greatly enriched with Hg, its uptake into the shoot is so small that levels are kept well below those that may be damaging to animals or man consuming these plants (Williams, 1975; Richardson, 1980; DoE, 1981;Davis, 1983). Of these seven priority metal pollutants in sludge i.e. Cd, Cr, Cu, Ni, Pb, Zn and Hg, it is widely accepted that Cd is the one of greatest concern, due to its rapid translocation into the edible parts of the plant and its acute and accumulative toxicity to man (Flick et al., 1971; Page and Bingham, 1973; Fleischer at al., 1974; Chaney,1975; Davis and Coker,1980; DoE, 1981; Naylor and Kresse, 1983; Cline and O'Connor, 1984; Kloke et al., 1984; Dean and Suess, 1985).

1.1.3 METAL SPECIES IN ANAEROBICALLY DIGESTED SEWAGE SLUDGE

Anaerobically digested sludge is a complex mixture of bacterial residues and particulate and colloidal mineral and organic matter (Fletcher and Beckett, 1987 a, b). On leaving the digestor, sewage sludge undergoes rapid oxidation and changes in microbial activity as a result of which chemical forms of heavy metals in sludge are in a complex equilibrium between several phases. Metal species in anaerobically digested sludges may be classified as follows (Gould and Genetelli, 1975; Elliott, 1984) :-

a) Soluble forms,
    (i) ionic forms
    (ii) organic complexes
    (iii) inorganic complexes
b) Co - precipitates in metal oxides
c) Precipitates
d) Adsorbed forms
    (i) weakly adsorbed
    (ii) chemisorption
    (iii) adsorbed to clay lattice
e) Organometallic complexes
    (i) simple complexes
    (ii) chelates
f) Biological residues

Physical and chemical properties associated with the treatment process, including such parametres as pH, oxidation-reduction potential and the presence of complexing agents will determine the relative distribution between the various forms of each metal (Gould and Genetelli, 1975; Adams and Sanders, 1984b).

Much of the metal in sludge is associated with the insoluble solid phase either through adsorption or precipitation (Davis and Carlton-Smith, 1980; Stover et al., 1976). Gould and Genetelli (1975) used elutriation and filtration to fractionate heavy metals in digested sludge on the basis of sludge particle size. All metals examined were seen to be associated with the solid phase, in excess of 90 % being found in the particulate fraction (>100 um) which contained 90 - 92 % of the total solids (Table 1.3). The supra-colloidal fraction (0.6 - 100 um) contained most of the balance with the remainder in the colloidal (0.002 - 0.6um) and dissolved fractions (< 0.002 um).

Mosey et al. (1971) reported that all heavy metals in sludge, with the exception of Cr, form extremely insoluble sulphide salts. Sulphide precipitation arises from bacterial reduction of sulphate under the reducing conditions of digestion (Mosey, 1976; Ahring and Westermann, 1983). Precipitated and organically-bound forms predominate for all the sludge metals (Lester et al., 1983). although a significant concentration of Ni may appear in an exchangeable form (Stover et al., 1976; Lake et al., 1984; Davis et al., 1985). Copper is generally present as a sulphide (Stover et al., 1976; Davis, R.D. 1981; Lake et al., 1984) although it has also been reported to occur in the organic matter fraction (Baldwin et al., 1983; Fletcher and Beckett, 1987). However, Stover et al. (1976) reported that only 10 % of the total Cu in anaerobically digested sludge is bound to organic matter. An important factor that may regulate the extent to which Cu is present in the soluble organic fractions is the pH of the sludge (Fletcher and Beckett, 1987b). At a pH of 5 - 8 there is a reversible reaction whereby Cu replaces protons on organic matter (Fletcher and Beckett, 1987b). Sludge pH is also important in determining the degree of Cd and Zn precipitation as carbonates in sludge (Mosey, 1976), Cd binding to carbonates occuring at pH $\geqslant$ 7.2 whereas Zn requires a pH $\geqslant$ 7.7 to form a carbonate (Mosey,1976).

TABLE 1.3 DISTRIBUTION OF METALS (% TOTAL RECOVERED) IN DIGESTED SLUDGE IN RELATION TO PARTICLE SIZE (FROM GOULD AND GENTELLI, 1975).

METAL	PARTICULATE	SUPRA-COLLOIDAL	COLLOIDIAL	DISSOLVED
Cd	90.4	8.2	1.4	-
Cr	92.9	7.1	-	-
Co	92.8	7.2	-	-
Cu	92.9	6.9	0.1	0.1
Fe	95.5	4.4	0.1	-
Mn	95.5	3.4	0.1	1.0
Ni	95.0	5.0	-	-
Pb	92.1	7.3	0.3	0.3
Zn	91.5	8.4	0.04	0.07

Cadmium and Pb are generally present in sludge as carbonates (Stover et al., 1976; Davis and Coker, 1980; Lake et al., 1984). However, Cd can also occur as a sulphide (Davis and Coker, 1980), or bound to organic matter (Davis and Coker, 1980; Davis et al., 1985) or as a phosphate (Davis and Coker, 1980). Chromium, as well as Zn, are generally predominant in the organic fraction of anaerobically digested sludge (Stover et al., 1976; Baldwin et al., 1983; Lake et al., 1984; Davis et al., 1985).

Although inorganic precipitates appear to predominate, there is mounting evidence to suggest that metal-organic interactions, adsorption onto microbial surfaces and assimilation by the biomass are important removal mechanisms (Lester et al., 1983). Most bacterial surfaces exhibit a negative charge over a fairly wide pH range (> pH 4.0) and the particles making up sewage sludge also exhibit charge in a similar fashion. On the basis of current knowledge, reactive groups such as carboxyl, hydroxyl, phenolic and sulphonate within the structure of the surface polymers may adsorb metals onto the cell wall (Foster, 1983). Structures with such a composition can be expected to have an affinity for cations and this sorptive capacity is a very important retention mechanism for potentially toxic metal ions in sludge. Several gram negative species of bacteria in activated sludge produce extracellular polymers as capsules (eg: *Klebsiella aerogenes*) or a gelatinous matrix (eg: *Zoogloea ramigera*) and these polymers have been shown to be involved in the adsorption of exogenous metal ions from solution (Brown and Lester, 1979). This adsorption may occur in order to facilitate percolation through the cell wall to reaction sites within the cytoplasm by metals necessary for growth and metabolism (Beveridge and Koval, 1981) or to enhance the polymer's protective function by increasing its resistance to decomposition (Martin, 1972; Martin et al., 1972). Of the total heavy metal content in anaerobically digested sludge 30 - 60 % of the Cd, Cr, Cu, Ni, Pb and Zn can be associated to the biomass (Hayes and Theis, 1976). Lester and Sterritt (1985) reported that microbial uptake of Cd, Cu, Mn and thallium (Th) are largely unaffected by a loss of viability, indicating that uptake of these metals is predominantly a passive process. In contrast, a loss of viability had a marked effect on the uptake of Ni (Lester and Sterritt, 1985). Intracellular uptake of heavy metals increases with metal load but levels off when the heavy metal concentration in the digestor approaches inhibitory or toxic levels (Hayes and Theis, 1976).

Sewage treatment results in a shift away from the more easily extractable metal forms which predominate in raw sludge, towards less soluble and precipitated forms in digested sludge (Lake et al., 1984). Nonetheless, metals in the 'exchangeable', 'sorbed' and 'organically-bound' fractions are likely to be comparatively mobile following sludge disposal to land and are, therefore, of particular interest (Davis et al., 1985). Williams (1975) states that Cu and Zn, in particular, are present in sludges in a highly mobile form compared with that in soils. As for Ni, although its mobility in sludges is generally high relative to that in soil, it is of no significance since, in practice, there are few sludges which contain very high Ni concentrations (see Table 1.1).

1.2 METALS IN SOIL AND THE SOIL PROCESSES AND CONSTITUENTS AFFECTING THEIR AVAILABILITY

1.2.1 HEAVY METAL FORMS IN SOIL

Sewage treatment causes chemical transformation of metals to take place in sludge and thereby alters their bioavailability to plants. However, once metals are applied to soil, regardless of the form in which they are, soil conditions determine their fate. These metals may (Page, 1974) :-

(i) pass through the soil unchanged,
(ii) form insoluble or sparingly soluble inorganic and organic compounds,
(iii) be sorbed by colloids as cations, anions or uncharged species,
(iv) be volatilized from the soil (eg. Hg, As, Se) and consequently lost from the soil system,
(v) be taken up by plants.

The ultimate form in which sludge metal is found in soil and its consequent fate is dependent upon rates of reaction and equilibrium between the incorporated sludge metal and soil constituents. Many soil materials can react to immobilize trace metals including clays, organic matter (i.e. organic acids, amino acids, humic acids and fulvic acid), hydrous iron and manganese oxides, carbonates, phosphates and living tissue and its residues (Camp, 1945; Lindsay, 1972, 1973; Chaney and Giordano, 1977; Keeney and Wildung, 1977; Adams and Sanders, 1985; Dean and Suess, 1985). In addition, genotype, age and growing conditions of the plant are important factors regulating bioavailability (Davis,1980). Since metal contamination persists in the top soil, any soil properties which reduce availability to plants will be important factors in determining metal toxicity, both to plants and to foraging animals.

Crop tolerance to toxic elements has been shown to be greater in soil than in hydroponic solutions, since soil can immobilize metals into unavailable forms (Melsted,1973). Immobilization has been clearly illustrated in experiments on the residual effects of trace element fertilizers. For example, following the application of Cu and Zn in fertilizers, less than 1 % of the Cu applied has been shown to become available to plants in the season of application (Boawn et al., 1960; Hodgson et al., 1966). Boawn (1974) showed that Zn applied to soil undergoes rapid immobilization in the first year and the remainder of available Zn is less rapidly tied up in the successive years. However, it is necessary to point out that metal application rates by fertilizer application are usually much lower than those originating from sludge disposal. Furthermore, extrapolation of results obtained from fertilizer application cannot be directly related to the situation where sewage sludge is applied as the metals forms within chemical fertilizers are different to those present in sludge.

A knowledge of the chemical forms of soluble heavy metals or of heavy metals associated with soil particles or colloids is essential for estimating bioavailability, mobility and chemical reactivity in soils. The distribution of metal cations in soils, can be subdivided into five arbitrary, but recognizable, chemical pools, (Viets,1962; Berrow and Burridge, 1980) :-

(i) Ionic, molecular or colloidal forms in soil solution,

(ii) Exchangeable forms,

(iii) Chelated or adsorbed forms existing as organic or organo-mineral complexes,

(iv) Incorporated forms in secondary clay minerals or sesquioxides,

(v) Fixed forms held within the crystal lattices of primary and secondary minerals.

In addition, Berrow and Burridge (1980) further subdivide pool (ii) into :-

(a) Readily exchangeable ionic forms on inorganic or organic 'exchange-active' material,

(b) More firmly bound ionic forms on exchange complexes.

The divisions are not as clear cut as this classification seems to suggest. The relative amount of each form depends on the element in question and on the prevailing soil conditions. Pools (i), (ii) and (iii) in Viets' (1962) classification, are believed to be in reversible equilibrium with one another, the equilibrium for each metal being affected by soil physico-chemical conditions and the metal's concentration in the respective pools. Pool (iii) is believed by Viets to be of most significance to plants because of its potentially greater size. The equilibrium between pool (iv) and pools (i), (ii) and (iii) is normally established very slowly. For this reason, pool (iv) is not considered to be an important source of plant available metals, except over long periods of time. Pool (v) is not in reversible equilibrium with any of the other pools and its metals are released by weathering. Viets speculated that pool (v) may release more metals in the available form than highly stable secondary minerals in pool (iv).

It is far from clear as to which forms of heavy metal are available for plant uptake and the technique which best measures plant available metal (see Section 2.1). For instance, Berrow and Cheshire (1985) observed that peat addition to Cu polluted soils decreased the exchangeable (i.e. acetic acid -extractable) more than the complexed (i.e. EDTA-extractable) Cu soil fraction and this resulted in a reduced uptake of Cu by plants. They concluded that exchangeable Cu is probably more closely related to plant availability compared to other forms of Cu in Cu polluted soils. In contrast, in unpolluted and Cu deficient soils, plant available Cu is more closely related to organically complexed forms of Cu (Berrow and Reaves 1985).The form of metal most likely to be immediately available for plant uptake is that contained in the soil solution (Alloway and Tills, 1983). This supply of metal to soil solution depends on a quantity/intensity relationship determined by soil buffering characteristics determined by soil pH and soil cation exchange capacity (Gupta and Stadelmann, 1983). It is thought that Cu complexes by virtue of their size or net charge can either facilitate or strongly reduce Cu transfer across biological membranes (Jaworski et al.,1984).

1.2.2 pH AND CATION EXCHANGE

Of the soil factors which determine that fraction of metal present in the soil solution and hence its availability for plant uptake, pH is widely recognized as being the most important (Leeper, 1972, Davis, 1983; Hani and Gupta, 1983). Acidification of the soil system results in increased plant uptake of Cd (Linnman et al., 1973; Haghiri, 1976; Webber and Beauchamp, 1975; MacLean, 1976; Miller et al., 1976; Davies, 1985), Ni (Patterson, 1971; Bolton, 1975; Bates et al., 1975; Adams and Sanders, 1983, 1985; Sanders et al., 1986b) and Zn (Wear, 1956; Peterson and Gschwind, 1972; Bolton, 1975; MacLean, 1976; Haq et al., 1980; Iyengar et al., 1981; Williams et al., 1984; Albasel and Cottenie, 1985) which can lead to toxicity. In contrast, Cu uptake and its toxicity have been shown to be pH dependent only below pH 5.5 and above this plant uptake is independent of soil pH (Rothamsted Experimental Station, 1985; Sanders et al., 1986). Such a clear-cut relationship between Pb uptake and soil pH has not been established. Plant uptake of Pb has been reported as being both dependent (Filipovic et al., 1961; Cox and Rains, 1972; Zimdahl and Foster, 1976) and independent (Bates et al., 1975; Andersson and Nilsson, 1976; Singh and Narwal, 1984; Davies, 1985) on soil pH.

The relative toxicities of metals may be linked to their comparative solubility at different pH levels (Filipovic et al., 1961; Davis and Carlton-Smith, 1984). In this way, acidification of a soil water suspension leads to an increased concentration of metal ions in solution, as a result of cation desorption with H^+, dissolution of solids and dissociation of organo-mineral complexes (Cottenie et al., 1984; Dean and Suess, 1985). Metal immobilization arising from increased soil pH may be partly attributed to the retention of metals, such as Cu and Zn, by H+ saturated montmorillonite (Kisk and Hassan, 1973) possibly as a result of hydroxide precipitation in the clay systems (Bingham et al., 1964). The retention of Cu, Pb, Zn and Cd by clay - humic acid mixtures under alkaline conditions is determined by competition between the ability of organic matter to form soluble metal humates and the tendency of clay to strongly retain the sparingly soluble metal hydroxyl species formed at pH >6 (Hatton and Pickering, 1980). Metal immobilization due to precipitation as hydroxides or carbonates, depending on the element, can also occur at a soil pH of 6 - 9 (Street et al., 1978; Dean and Suess, 1985). For instance, cadmium carbonate regulates Cd solubility above pH 7.25 (Street et al., 1978). Such pH dependent mobilization/immobilization processes are very complex and one mechanism could apply over only a narrow range of pH. Such is the case with Zn where at soil pH <5.5 its solubility is partly determined by nucleation or specific adsorption to hydrous oxide (McBride and Blasiak, 1979; Iyengar et al., 1981), and as soil pH increases to values >5.5 the adsorbing Zn is replaced by other cations. At soil pH >7.5 Zn mobility increases as a consequence of an increase in organic complexes in the soil solution (McBride and Blasaik, 1979).

Changes in metal solubility will also result in changes in metal diffusivity and leachability. In other words, as soil pH decreases metal diffusivity increases (Clarke and Graham, 1968; Melton et al., 1973). For instance, in one particular study the total concentration of Zn in the soil solution decreased about 100- fold as the pH of the soil increased from 4.4 to 7.5 (Jeffrey and Uren, 1983). Consequently, as metals are removed from the rhizosphere due to plant uptake, there is a more rapid replenishment of metals in the rhizosphere of acid soils than in more alkaline soils. In a similar fashion, the greater metal concentration in the soil solution in acid soils can result in greater leaching of metals such as Zn, Cu, Ni, Cu, Cr, Pb (Tyler, 1978) and consequently the residence time of these metals in the soil decreases with the risk of ground water contamination.

The metal fraction in a soil solution is not only governed by pH but also by the soil's cation exchange capacity. All soils contain negatively charged colloids whose charge is balancedby adsorbed cations. Calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) are the most common exchangeable cations in neutral soils, with hydrogen and aluminium (Al) ions dominating acid soils (Leeper,, 1972). Hence, metal cations added in soil amendments will enter into competition with those already present on the exchange complexes. The cation exchange capacity of soil is determined by the nature and content of organic matter and clay, and to a lesser extent hydrous iron and manganese oxides (Hani and Gupta, 1983; Gupta and Stadelmann, 1983). Humus becomes increasingly important as the seat for cation exchange as the pH of a soil rises, since for each unit increase in pH the change in cation exchange capacity of organic matter is several times greater than that of clays (Jenkinson, 1988).

Soils with a high cation exchange capacity can safely accept larger amounts of toxic metals relative to soils with low cation exchange capacity. In the U.S.A., metal uptake by crops has often been related to soil cation exchange capacity, i.e. for a given quantity of metal added, plant uptake is greatest from soils of low cation exchange capacity. Consequently it has been suggested that application of sewage sludge might be related to soil type, using cation exchange capacity as the criterion for regulating sludge disposal (Webber, 1980). It would appear that cation exchange capacity is particularly important for retention of Cd, in both polluted and unpolluted soils, than it is for either Zn and Ni (Soon and Bates, 1982). As cation exchange capacity increases Cd uptake decreases (Hagiri, 1974; Miller et al., 1976), this response being closely related to soil organic matter content. Lester et al. (1983) suggested that organic matter affects Cd adsorption more by its exchange capacity than by its chelating ability. The exchange capacity of humus is due mainly to carboxylic acid groups (R-COOH) which are dissociated to the carboxylate anion at the pH values found in all but the most acid soils (Jenkinson, 1988).

The capacity of coarse textured soils to reduce heavy metal concentrations in solutions derived from applied sludge is generally less than that of fine textured soils (Page, 1974; Adams and Sanders, 1983, 1984). The concentrations of Pb (Doelman and Haanstra, 1979a), Zn (Shuman, 1975) and Cd (Cottenie et al., 1983; Kloke et al., 1984; Sauerbeck and Styperek, 1985) in the soil solution decreases as clay content increases. For instance, the threshold soil concentration at which Cd becomes toxic is < 2 mg/kg for light sandy soils but 4 mg/kg for loams (Sauerbeck and Styperek, 1985). Copper is also adsorbed by clays, adsorption decreasing in the order montmorillonite > illite > kaolinite (Kranskopf, 1970, as cited by Mortvedt et al., 1972).

Moreover, sandy soils (Archer, 1980) and topsoils derived from coarse textured parent material (Thornton, 1981; Reaves and Berrow, 1984), tend to be low in total Cu.

1.2.3 CHELATION BY ORGANIC MATTER

The nature and content of soil organic matter are important factors controlling the availability to plants of heavy metals present in soil. Metallic ions, particularly Fe and Al, are tenaciously held by humic preparations and can only be removed with difficulty (Jenkinson, 1988). The role of soil organic matter in immobilising heavy metals has been studied by several workers. High concentrations of soil organic matter lead to reduced plant uptake of Ni (Halstead et al., 1969), Cd (MacLean, 1976), Pb (Zimdahl and Foster, 1976) and Zn (Matsuda and Ito, 1970; Spotswood and Raymer, 1973; Claydon et al., 1974). However, MacLean (1976) found that soils high in organic matter increased Zn uptake by lettuce. The reduction in metal concentration in the soil solution is probably due to the formation of insoluble metal-organic complexes (Albrecht, 1941; Mortensen, 1963). However, complexing can also lead to increased metal solubility (Petruzelli et al., 1981). Soluble chelates generally carry a negative charge and consequently are not fixed to the negative surfaces of the soil. Moreover, it is thought that Cu complexes by virtue of their size or net charge can facilitate or strongly reduce Cu transfer across biological membranes (Jaworski et al., 1984). In this respect, Mercer and Richmond (1971) showed that organo-copper complexes with molecular weights of >1000 are available for plant uptake, whereas those with molecular weights >5000 are not.

The general reaction between a metal ion (M) and an organic ligand (A) can be described by the equation (Jenkinson, 1988) :-

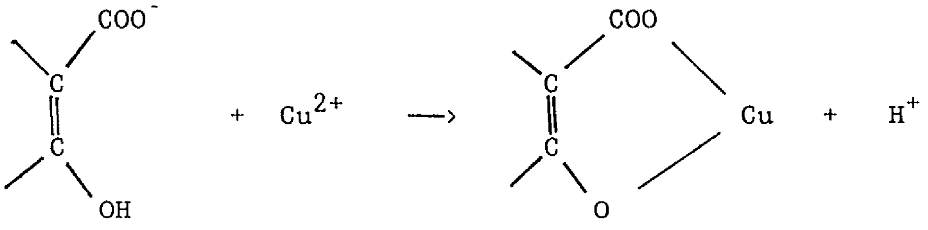
$$jM + iA \rightleftharpoons M_jA_i$$

However, little is known of the specific ligands which participate in individual complexing reactions in soils. Geering and Hodgson (1969) attempted to characterize the materials in soils responsible for metal-organic matter complexing. They separated soil organic matter into dialysable and non-dialysable fractions and found both fractions capable of complexing metals. The dialysable fraction was found to consist of acetic and other aliphatic acids, amino acids and peptides; the non-dialysable, largely of fulvic compounds with carboxyl functional groups. Soluble organo-metal complexes generally involve chelation with citric and oxalic acids (Leeper, 1972; Bondietti and Sweeton, 1973), although chelates with several leaf sap components, such as ellagic acid, are also present in soil solution (Gomah and Davies, 1974).

The stability constants for reaction between humates and metal, increases according to the stability of metal complexes (Jenkinson, 1988), with trivalent Fe and Al being the most strongly held, and followed by the divalent metals in the order :-

Cu > Ni > Co > Zn > Mn > Mg > Ca > Ba

This greater affinity of Cu for sorption onto humic acid has also been noted by Leeper (1972) and Kerndorf and Schnitzer (1980). In addition, Schnitzer and Skinner (1966) reported that of nine divalent ions Cu formed the most stable complex with fulvic acid. The exact nature of the chemical bonds between humic substances and metal ions is still uncertain (Stevenson, 1982). It is generally thought that they involve binding of metals by functional groups such as carboxyl, phenolic and imide (Randhawa and Broadbent, 1965) as well as carbonyl groups (Khanna and Stevenson, 1962). De Mumbrum and Jackson (1956) used infra-red adsorption spectra to study the exchange reaction mechanism of Cu and Zn and concluded that reaction of Cu and Zn with organic matter, in the form of peat, involves a chelating action associated with C=O, N=O and alcohol-OH groups. The fact that Cu retention by humic acids can be reduced by 50 % when the carboxyl and hydroxyl groups are blocked (Davies et al., 1969), further underlines their importance in the chelation mechanism. The chelation probably involves the metal linking to two adjacent functional groups (Van Dijk, 1971 - cited by Jenkinson 1988) :-



However, it is unlikely that a large proportion of cations are held by such a specific arrangement of functional groups (Jenkinson, 1988). Moreover, functional groups containing oxygen are not the only ones capable of reacting with metal ions, Cu in particular being able to react with the =N-H groups in the humic peptides (Stevenson, 1982) as well as with sulphhydryl groups (Dawson and Nair, 1950).

The reduction in metal availability due to complexing with organic matter, also results in reduced metal mobility within the soil system (Hodgson et al., 1965; Lund et al., 1976; Sposito et al., 1976; Holtzclaw et al., 1978). Nickel, Cd, Co and Zn being less strongly complexed than Cu are consequently more mobile than Cu. Copper is readily complexed by organic soil constituents, forming compounds of varying solubility. Copper deficiencies following the reclamation of peat bogs are common (Pizer et al., 1966). In fact, when organic soils of this type are treated with conventional soil extractants little or no Cu is removed, demonstrating that very stable organo-mineral complexes are present (Lucas, 1948). Incorporation of peat, when accompanied by liming to offset the acidification of peat, is effective in reducing Cu availability in contaminated soils (Berrow and Cheshire, 1985). The peat reduces the acetic-acid extractable (i.e. exchangeable) but not the EDTA extractable (i.e. complexed) Cu fraction in the soil (Berrow and Cheshire, 1985). This strong complexing to soil organic matter accounts for between 95 -99 % of total Cu present in the soil solution (Hodgson et al., 1966; Davies, 1975). In contrast, only 60 % of the Zn present in soil solution is in organic complexes (Knezek and Ellis, 1980) even though organic matter is more important than clays in retaining Zn (Himes and Barber, 1957) and up to 70 % of the total soil Zn content is bound to organic matter (Randhawa and Broadbent, 1965b). Moreover, Cu remains as an organic complex over a wide pH range (Williams et al., 1984). Humic acid and fulvic groups complex Cu at optimum pH of 2.5 - 3.5 and 6 respectively (Mitchell, 1964). This can account for the fact that unlike other metals such as Zn,whose solubility in the moderate pH range (5.5 - 7.0) is not controlled by soil organic matter (McBride and Blasiak, 1979), soil Cu availability is independent of pH above a threshold value of 5.5(Sanders et al., 1986).

1.2.4. HYDROUS METAL OXIDES

Hydrous Fe, Mn and Al oxides are considered to be important in controlling the concentration of heavy metals in soil solution and natural waters (Hodgson et al., 1966; Jenne, 1968;. Shuman, 1977; Kalbasi et al., 1978; Vuceta and Morgan, 1978). For instance, soils with high amorphous Fe oxide content generally show lower metal extractability than those low in amorphous Fe oxide (Kuo et al., 1985). Moreover, although pH is an important factor influencing metal availability for any particular soil, its influence can often be less important than that of amorphous Fe oxide (Kuo et al., 1985).

The mechanism by which these oxides remove metal ions from soil solution is still unclear. However, it is thought to involve initial adsorption and subsequent occlusion.

Hydrous Fe oxide is more important in regulating Cd availability than is hydrous Mn oxide (Sauerbeck and Styperek, 1985). On the other hand Cu is influenced more strongly by the soil's Mn oxide than by the Fe oxide (McLaren and Crawford, 1973). Manganese oxide probably determines initial Cu adsorption and Fe oxide, by virtue of its greater abundance in the soil, is responsible for long-term occlusion (McLaren and Crawford, 1973). The selectivity order of hydrous oxides for divalent metals is Cd > Ni > Zn > Cu (Quirk and Posner, 1975; Kinniburgh et al., 1976).

Jenne (1968) proposed that foreign metals are adsorbed on negatively charged sites, on hydrous oxides, for which Ca and Mg do not compete.

This mechanism would probably involve (Leeper, 1972):

$$\underset{\text{goethite}}{2FeO.OH} + Zn^{2+} \longrightarrow ZnFe_2O_4 + 2H^+$$

Such a mechanism is supported by the fact that raising the soil pH by one unit greatly strengthens the fixation of Zn (Leeper, 1972). However, goethite is not usually thought of as an acid but as a potential base which adsorbs phosphate and molybdate by exchanging hydroxyl groups (Leeper, 1972). The oxide's surface contains OH groups linked to one, two or three Fe ions (Parfitt et al., 1976), although some O^{2-} ions are also present in the surface layer. Moreover, it is only the singly linked Fe - OH group that is chemically active, (Parfitt et al., 1976, cited by Mott, 1988). Consequently, goethite probably reacts as a base and not as an acid and would preferentially bind with the anionic forms of metal such as Cu (Leeper, 1972).

While heavy metals may eventually be occluded in hydrous oxides, the rate of metal occlusion is likely to be very slow. Futhermore, in temperate soils these hydrous oxides may have a highly disordered structure and may be present in only small concentrations (Mott, 1988) unlike tropical soils which being more intensely weathered, the oxides are both crystalline and present in much larger proportions (Mott, 1988).

1.2.5 PHOSPHATES

Mantaining adequate reserves of available soil phosphate is an essential aspect of agricultural soil management. Application of sewage sludge can itself contribute significantly to maintaining soil phosphate levels. Liquid digested sludge contains about 1.6 % (on a dry solid basis) (DoE, 1981) and unlike N applied in sludge it is not prone to losses from the soil.

Soil phosphate is known to play some part in altering the availability of heavy metals. Over forty years ago it was realized that the availability of Zn for plant uptake depended, not only on soil pH, but also on its phosphate content (Camp, 1945), with high soil phosphate concentrations leading to Zn immobilization. Since then several workers have observed the decreased availability, not only of Zn (MacLean and Dekker, 1978), but also of other metals like Pb (Zimdahl and Foster, 1976), Cu (MacLean and Dekker, 1978; Racz and Haluschak, 1974) and Mn (Racz and Haluschak, 1974). In contrast, a high level of phosphate has been seen to increase the availability of Ni (Halstead et al., 1969; Crooke and Inkson, 1955). However, Pratt et al. (1964), working at high soil pH values, found that application of phosphate could alleviate Ni toxicity to plants. This pH dependent effect of phosphate on metal availability has also been reported for Cd (McLean, 1976) where phosphate application only reduces Cd uptake in acid soils, no such reduction occurring in neutral or alkaline soils. However, Zn uptake is reduced by phosphate addition at all soil pH levels (McLean, 1976).

It is generally assumed that antagonism between heavy metal and phosphate occurs within the plant. It is unlikely that reduced metal uptake in the presence of applied phosphorous is due to interaction between phosphates and metal in the soil. Leeper (1972) explains that some phosphates, such as $Zn_3(PO_4)_2$, are too soluble to explain poor Zn response in the presence of high concentrations of soil phosphate, although minerals of low solubility, such as plumbogummites or gorceixite, may play a role in immobilizing soil metals. It is also unlikely for the dilution of metal concentration in plant tissue arising as a result of the growth response due to greater phosphate availability (Wild and Jones, 1988). Phosphate application is seen to have a much greater effect on the Pb composition of shoots than on that of roots (Zimdahl and Foster, 1976), indicating that phosphate affect the translocation of Pb rather than its uptake. Similarly, phosphate is thought to interfere with the translocation or utilization of Zn within the plant (Olsen, 1972). However, no precise mechanism has been suggested for these effects.

1.3 EFFECTS OF SLUDGE APPLICATION ON SOIL CONDITIONS AND ITS METAL CONTENT

1.3.1 SOIL ORGANIC MATTER AND pH

The value of sewage sludge as a fertilizer has long been documented (Brenchley and Richards, 1920; Noer, 1926; Muller, 1929). It is a particularly valuable source of N and P, provided its application is correctly timed relative to sowing or planting of a crop. In addition, sewage sludge contains organic matter which provides humus and can, therefore, be a useful soil conditioner improving soil porosity (Pagliai et al., 1981; Guidi and Hall, 1983), aggregate stability (Borchert, 1983; Furrer and Stauffer, 1983), water holding capacity (Epstein, 1975; Guidi and Hall, 1983; Williams, 1975), and hydraulic conductivity (Epstein et al., 1976; Gupta et al., 1977).

At the same time, sludge application results in increased soil cation exchange capacity (Epstein et al., 1976; King and Morris, 1972). This is attributed to the introduction of organic matter (Gupta and Stadelmann, 1983). However, the Department of Environment (1981) points out that application of liquid sludges at normal rates will not usually have any noticeable effect on soil organic matter content, although regular applications of sludge cake can significantly increase soil organic matter content. In addition, while it is possible that the effect of added organic matter in reducing the phytotoxicity of added metals is due to the resultant increase in cation exchange capacity, it is more likely due to the formation of insoluble chelates unavailable to crops in the short term (DoE, 1981). However, the fraction of soil organic matter which are mostly markedly increased by sludge application are fats, waxes and oils (Hinsely et al., 1976) and these organic forms will not contribute significantly to immobilizing metals (Hinesly et al., 1976).

The application of sewage sludge to land can itself lead to changes in the pH of the receiving soil. Whereas sludge addition can increase the pH of acid soils (Bolton, 1975; Hemkes et al., 1980; Edgar, 1983) it generally leads to a decrease in soil pH (Cunningham et al., 1975; Kelling et al., 1977; Kuo et al., 1985). This soil acidification is probably associated with nitrification (Kirkham, 1974; Magdoff and Chromec, 1977; Robertson et al., 1982; Lester et al., 1983);

$$\text{i.e. } NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$$

Consequently, application of a wet sludge, high in ammonium, will result in a greater reduction of pH than would be the case where a dried sludge, low in ammonium, is applied (Chang and Broadbent, 1980; Williams et al., 1984). Soil acidification is also thought to arise from the oxidation of sludge organic matter, leading to the production of organic acids (Kirkham, 1974; Robertson et al., 1982).

The chemical treatment of sludge prior to disposal is also an important factor determining soil pH. In this respect, Soon et al. (1980) observed that soil pH is reduced by application of Fe- enriched sludge, slightly affected by Al-enriched sludge and increased by lime treated sludge. Hence lime treatment not only reduces odours and kills-off most of the pathogenic bacteria in sludge (DoE, 1981; SAC, 1986), but the resultant increase in soil pH can go some way in ensuring that polluting metals are kept in relatively unavailable forms. However, water-, acetic acid- and EDTA - extractable Cd concentrations in sludge may increase following addition of lime (Bloomfield et al., 1976) but acetic acid extractable concentrations of Cu, Ni and Zn do not (Bolton, 1975).

1.3.2 TOTAL METAL CONCENTRATIONS

A sound knowledge of soil/plant/animal relationships is essential in any appraisal of hazards associated with the utilization of sewage sludge on land. Plant uptake of the heavy metals applied is the first important step in determining the potential toxicity of these elements to the food chain. This is strongly influenced by the amount and availability of metals in the topsoil. The increase in total soil metal concentration arising from continued sludge application to land is well documented (Andersson and Nilsson, 1976; Sidle et al., 1976; Vitasalo, 1978; Keefer et al., 1979; Davis, 1980; Hemkes et al., 1980).

Aerial deposition and phosphate fertilizer application are far greater contributors to the metal load of agricultural soils than is sludge which is only of importance on a local basis (Davis, 1980). For example, in Denmark locally produced sludge was found to be a major factor in enhancing soil metal concentrations (Hansen and Tjell, 1978) but as sludge was only applied to a small proportion of the total agricultural land area, its contribution was only 4, 5 and 19 %, respectively, of the total national input of Pb, Cd, and Zn to soils. The remainder was accounted for by inorganic fertilisers and aerial deposition (Hansen and Tjell, 1978), eg. 90 - 99 % of the Pb content of ryegrass growing in a rural area of Denmark was as a direct result of aerial deposition (Tjell et al., 1979). In the U.K., land disposal of all the sludge produced would approximately require ⩽ 5 % of all the available agricultural land (Davis, 1980).

Increase in soil metal levels are a linear function of sludge metal loading (Andersson and Nilsson, 1972; Davis, 1981). Build-up of metals, such as Mn, Zn, Cu, Ni, Cr, Pb, Cd, Hg, and Co, in receiving soil can be brought about, not only by long term sludge disposal (Le Riche, 1968; Kirkham, 1975; Andersson and Nilsson, 1976; Webber,1980), but also by short term applications (Boswell, 1975; Hemkes et al., 1980).

1.3.3 AVAILABLE METAL CONCENTRATIONS

Following sludge application to land, there is an immediate increase in the concentration of heavy metals in the freely- available soluble and exchangeable fractions. In turn, this is followed by a more gradual release of metals by the solution of inorganic compounds and the decomposition of organic matter (Lewin and Beckett, 1980). Incorporation of sewage sludge into agricultural soils results in increased acetic acid, magnesium chloride, nitrilotriacetic acid (NTA)-, and diethylenetriaminpentaacetic acid (DTPA)-, extractable fractions of soil Cu, Ni and Zn (Bolton, 1975; Soon et al., 1980; Kuo et al., 1985) and ammonium acetate extractable soil Cd (Andersson and Nilsson, 1974). However, Singh and Norwal (1984) reported that the application of sludge did not result in an appreciable change in soil exchangeable, sorbed and carbonate fractions of metals except Zn where the DTPA- extractable fraction increased with sludge application rate.

Any such inconsistencies between the findings of workers in observed changes in metal extractability following sludge application may be explained by the ensuing temporary, but massive rise in soil biomass (Lewin and Beckett, 1980). The rapid growth in the microbial population may reduce, by assimilation, the increase in freely-available metal concentrations which follow sludge application. In addition, this may be coupled with a more gradual transfer of heavy metals into less labile organic complexes, adsorption sites and insoluble salts (Lewin and Beckett, 1980). Moreover, some sludge metal fractions are resistant to decomposition and release of metals from these fractions is very slow, if at all. Short term changes in metal extractability following sludge incorporation into soil were clearly demonstrated in a laboratory investigation carried out by Wollan and Beckett (1979). Over the first few days, subsequent to sludge incorporation, Zn extractability increased whereas extractable Cu decreased. However, this was followed by a more gradual equilibration period during which time the Zn extractable content fell and the Cu extractability increased in both cases to values higher than those present in the control soil. This greater extractability persisted for over 2 years (Wollan and Beckett, 1979). Peaks in the potassium nitrate and DTPA-extractable fractions of Zn, Cd and Pb have similarly been observed 30 days after sludge application to land (Petruzelli et al., 1981), further underlining the temporal effects of sludge application on soil metal availability.

Notwithstanding any observed metal fluctuations, the greater extractability of metals from sludged soils persists over that found in control soils for a number of years following application of sewage sludge to agricultural land (Berrow and Burridge, 1980; Petruzelli et al., 1981; Sanders et al., 1986a). There also appears to be a tendency for the ratio between acetic acid- and ethylenediaminetetraacetic acid-(EDTA) extractable Zn, Cu, Ni, Cr and Cd to reach a constant value in long term sludged soils irrespective of the metal forms or concentrations present in the sludge (Berrow and Burridge, 1980).

1.3.4 MOVEMENT OF SLUDGE DERIVED METALS

The environmental significance of an increase in soil heavy-metal levels, arising from sewage sludge application to land, becomes more apparent when it is realized that the applied metals, are normally retained within the top 20 cm of undisturbed soil, in which the highest concentration of plant root occurs. Most workers investigating this metal accumulation in sludged soils have, thus, tended to restrict their sampling to within this surface 20 cm depth. This general lack of metal (e.g. Cd, Cr, Cu, Ni, Pb, Mn, Co, Hg, As, Se, Zn) movement within the soil has been reported by many authors : Andersson and Nilsson (1972), Kirkham (1974), Giordano and Mortvedt (1976), Vitasalo (1978), Damgaard-Larson et al. (1979), Sommers et al. (1979), Williams et al. (1980), Chang and Broadbent (1980), Kuo (1981), Jones and Jarvis (1981), Emmerich et al. (1982), Elliot (1984), and Williams et al. (1984). Even as long as 20 years after prolonged disposal (20yrs), sludge metals are held within this surface layer of soil (Rothamsted Experimental Station, 1985). This accumulation of metals within the topsoil exhibits an asymmetrical pattern. Hemkes et al. (1980) observed that the accumulation of Cd, Cr, Cu, Ni and Pb was greater in the top 2 - 5 cm depth than in any other layer within the top 25 cm of soil.

One would expect to find the greatest degree of metal mobility in soils or sediments with a high sand content (see Section 1.2.2.). However, Ketchum and Vaccaro (1977) reported that, on sand filter beds which had been used for 33 years to filter secondary sludge effluent, metal accumulation was restricted to the upper 6 cm of these beds.

Under certain conditions metals may migrate to depths in excess of 20cm. Indeed, metal enrichment to depths as great as 3m has been observed under some sludge disposal ponds (Lund et al., 1976). Even where sludge is applied to land for relatively short periods of time (Hinesley et al., 1972) movement of Cr, Cd, Cu, Pb, Ni and Zn to depths below 15cm can occur.

Generally, in excess of 90 % of the total metal load applied to soil via sludge accumulates within the top 20cm depth (Sidle et al., 1976; Parker et al., 1978; Chang et al, 1984). However, individual elements have been shown to exhibit disparate retention. Page (1974) concluded that between 33 - 56 % of the total Pb, Ni and Zn applied to soil were retained in the surface 15 cm, that Cu retention was slightly lower at 27 - 31 % and that 17 - 26 % of the Cr and Cd was retained. This also implies that metal movements to depths greater than 15cm occurs, as the proportion of applied metals taken up by plants is very small (see Section 1.4.2.), and indicates that the degree of metal retention and, therefore, its movement down the profile, depends on the metal in question. In this context, Boswell (1975) noted that whereas Cd, Cr, Cu and Pb are all retained within the 15 cm layer, following the application of 16 t/ha of sewage sludge to an acid soil, Zn had moved down to depths as great as 30cm. This greater migration of Zn was also observed by Touchton et al. (1976). In this case surface applications of sludge within a 2-year period, had increased the concentrations of

Pb, Cu, Cd and Fe in the top 7.5 cm layer whereas Zn had moved as deep as 30 cm.

Metals differ in their mobility in soil and, in general, three groups can be discerned (Sidle et al., 1977; Sidle and Kardos, 1977; Holtzclaw et al., 1978; Tyler and McBride, 1982; Davis, 1983) :-

(i) mobile - Zn, Cd, Ni.

(ii) immobile - Pb, Cr, Ag.

(iii) intermediate - Cu.

In slight contrast, Hickey and Kittrick (1984) recorded the order of metal mobility in soils and sediments as being Cd > Zn > Cu = Ni. Leaching experiments have confirmed the general mobility of Zn and Cd and the relative immobility of Cu and Pb (Lagerwerff et al., 1976). It is widely accepted that of these contaminants Cd displays the greatest mobility (Tyler and McBride, 1982; Brown et al., 1983; Kiekens and Cottenie ,1983, 1985). Nonetheless Cd movement down the soil profile is very slow. In fact,analysis of soils which had received sewage effluent and sludge for 11 years (Sidle and Sopper,1976), demonstrated that no Cd movement occurred beyond the 0 - 5cm depth.

Limited metal movement down the soil profile is due to various soil constituents which react to immobilize trace metals (see Section 1.2). Apart from the reactivity of the element concerned,the physico-chemical properties of the soil itself affects the distributional pattern of applied metals. Immobility can be attributed to specific adsorption reactions with manganese oxides > organic matter > iron oxide > clay minerals (McLaren and Crawford, 1973).

Metals added to soil via sludge are therefore seen to persist in the uppermost layers of the soil for very long periods of time and are potentially available for uptake into plants and the foodchain. The forms of metals immediately available for the plant uptake are those in the liquid phase, i.e. in soil solution (Alloway and Tills, 1983). However, it is necessary to point out that many workers have used the terms 'available' and 'extractable' as though they were synonymous (see Section 2.1), as well as having correlated availability of an element or ion with its mobility. In this way, the mobility of an element or ion can be defined as its ability to transfer from the solid to liquid phase of the soil system (Cottenie et al., 1983), that is, its mass per unit weight or volume in the liquid phase plus the amount that can be transferred to the liquid phase by changing conditions in the soil system. It has been suggested (Lewin and Beckett, 1980) that there is, in fact, no distinct available fraction of any heavy metal in soil, though heavy metals are released, during crop growth, from one or more pools in which they are retained. In addition, it might be possible, to find extractants which release metals in proportion, but not equal to the quantities which are likely to become available to a crop (Lewin and Beckett, 1980) (see Section 2.1). However, the definition of relationships is dependent upon soil and crop types.

1.4 THE BIOAVAILABILITY OF METALS IN SEWAGE SLUDGE

1.4.1 INTRODUCTION.

The hazards associated with the introduction of heavy metal pollutants into soils from sewage sludge and their effects on the foodchain depend on potential metal toxicity and the degree of adsorption by plants, animals and man. As movement of sludge metals down through the soil profile is very limited (see Section 1.3.4.), the chance of metal contaminants reaching ground water supplies is very slim, with the possible exception of boron (B), molybdenum (Mo) and selenium (Se) (Davis and Carlton-Smith, 1980). However, the degree of metal movement down the soil profile will depend on the prevailing soil conditions, such as pH and cation exchange capacity. Hence the likelihood of contamination would be greatest where a sandy soil with a low organic matter content, overlies a shallow water table. Conversely, where the water table occurs at depth trace element contamination is very unlikely (Page, 1974; Higgins, 1984).

As only a very small proportion of the contaminating metal is lost from the topsoil by either leaching or plant uptake (where a crop is harvested) any capacity of the soil to convert, with time, the heavy metals into forms available to plants will be an important factor in governing their toxicity. When metal contaminated sludge is applied to land the metal fraction which is bound to organic matter will in theory become more available as the organic matter progressively decomposes. A situation could arise where, if organic matter supplementation of a metal polluted soil to cease, a point would be reached when, due to a gradual organic matter depletion, released metals attain levels toxic to plants - the so called 'Time-bomb' affect (Leeper, 1972; Chaney, 1973; Haghiri, 1974). This affect is of greatest relevance to those metals whose soil chemistry is significantly influenced by organic matter, (i.e. Cu, Zn and Cd). Sludge organic matter decomposes at a rate of 20 - 25% per annum (Spotswood and Raymer, 1973) and as such is relatively unstable. Simulated oxidation of organic matter by treatment with hydrogen peroxide resulted in increased Cd extractability (Lagerwerff et al., 1976) implying that a large proportion of the total Cd present in soil may be gradually solubilized and eventually become available for plant uptake. However, available evidence contradicts this theory and immobilization of sludge metals occurs with time (Giordano et al., 1975; Kelling et al., 1977; Healey, 1984; Kloke et al., 1984), indicating that the gradual solubilization of metals arising from the decomposition of sludge organic matter may be partially compensated by the adsorption capacity of the soil (Giordano et al., 1975). As heavy metals are released from their more labile forms, they accumulate in non-biodegradable organic complexes or in insoluble crystalline precipitates involving more common elements (Lewin and Beckett, 1980). Moreover, the organic matter groups that retain metals such as Cu represent a minuscule fraction of the total organic matter content of soils. Besides, soils have other sources of organic matter, such as roots, seeds, pollen, foliage and animal remains. The popular concept that a toxic flush of metals could be released when organic matter declines beyond a critical point now seems to be unfounded (Davis, 1980; Davis and Coker, 1980; Elliott, 1984).

Interpretation of experiments aimed at evaluating the phytotoxic effects of metals in sludge have not proved easy. Phytotoxicity is normally measured in terms of crop yield but, whereas some metals may reduce yield, the plant nutrients in sludge may increase it, confounding the interpretation of yield data. Much of the knowledge on the effects of metals on plant growth and composition has come from research using hydroponically grown plants or plants grown in soil to which metal salts have been added. Unfortunately, it is not possible to assess the effects on yield of metals in organic wastes directly from such investigations. Nevertheless, there have been a number of studies whereby direct comparisons of plant growth between the effects of metal salts and sewage sludges supplying similar amounts of metals have been made. Such investigations have shown that although metal salts provide a simplified system that allows the effects of metals to be identified without confounding effects arising from the presence of other sludge components, they invariably lead to higher metal uptake by crops (Dijkshoorn and Lampe, 1975; Mortvedt and Giordano, 1975). In fact, salt forms of Cd, Ni and Zn applied to soil are taken up by plants 5 - 7 times more readily than the same heavy metals applied in sewage sludge (Kloke et al., 1984).

Questions concerning the toxicity of metals in sludge could be answered much more easily if it were possible to hold constant the components of sludge, whilst altering the metal content to suit experimental requirements in such a way that the yield of the indicator crop depended entirely on the metal content of the sludge. Such a technique, widely used in the U.S.A., involves 'spiking' sewage sludge directly with soluble inorganic metal salts and allowing the mixture to equilibrate for a period of time, usually a few weeks, prior to its incorporation into the soil. Where such 'spiked' sludges have been compared to inorganic metal salts for their effects on crop yield, Ni, Cr, Cd, Cu and Zn concentrations in the test crops have invariable been lower for the 'spiked' sludge treatments (Cunningham et al., 1975; Giordano and Mays, 1977). Unfortunately, it would appear that metals added as inorganic salts to 'spike' the sludge remain in more soluble and readily available forms than do metals in unenriched sludge, which are generally present in a variety of insoluble forms (Stover et al., 1976) (see Section 1.1.3.). In this respect, the availabilities of Zn, Cu, Ni and Cr as determined by extraction with ammonium acetate, acetic acid and EDTA are considerably less in sludge formed by digestion in the presence of the metals than in sludge obtained by treatment with the metal salts added after the digestion process (Bloomfield and McGrath, 1982). Moreover, even after a yearly equilibration in the soil, Cd uptake from 'spiked' sludge remained greater than that from digested sludge containing a high Cd concentration (Webber, 1980). Extraction of this soil with acetic acid and EDTA confirmed that the added Cd in' spiked' sludge had remained in a more available form (Webber, 1980).

In view of these problems a new method for sludge enrichment has been developed in Britain by the Water Research Center. Sludges containing a controlled metal content are prepared by enriching the sewage, from which the sludge is produced, prior to treatment (Davis and Carlton-Smith, 1981). In plant uptake trials, the availabilitity to crops of metals in these experimental sludges correlated closely with the availability to crops of metals in real sludges collected from sewage treatment works (Davis, 1980).

1.4.2. PLANTS

Metal contamination of the surface soil arising from sludge application is known to be virtually permanent, removal by leaching and by the crops being negligible (Purves, 1972; Bates et al., 1975; Davis, 1981). However, although uptake of metals may be negligible, the effect of even short-term application of sewage on the metal composition of plants may be profound. Short-term sludge application to agricultural soils has resulted in increased plant tissue concentration of Zn, Cu, Ni and Cd (Boswell, 1975; De Vries and Tiller, 1978; Berrow and Burridge, 1980; Soon et al., 1980; Korcak and Fanning, 1985), but not of Pb and Cr (Bolton, 1975; Andersson and Nilsson, 1976; Kelling et al., 1977; Soon et al., 1980). These increases in metal concentrations are not only restricted to the crops harvested immediately following sludge application but persist for a number of years, even after a one-off sludging event (Chaney et al., 1977; Berrow and Burridge, 1980). However, although increased concentrations of potentially phytotoxic metals such as Cu, Ni and Zn can occur in plant tissue, these increases are generally not large enough to induce a yield reduction (King and Morris, 1972a; King et al., 1974;. Webber, 1980). Indeed as a result of nutrients added in sludge, crops often show an improved yield (Boswell, 1975). However, where metal rich sludge is applied, yield reductions do occur (Berrow and Burridge, 1980).

Unfortunately, there are relatively few examples of long term experiments designed to study the effects of sludge application on crop metal composition. Results from such investigations further illustrate the increase in plant Cu, Ni and Zn concentrations following sludge application to land (Rhode, 1962; Le Riche, 1968; Patterson, 1971) and the ensuing yield reductions (Rhode, 1962; Patterson, 1971), whereas Pb, Hg, Se, Co, Cr and Mo concentrations in plant tissue remain unchanged, even after prolonged sludge disposal (Andersson and Nilsson, 1972).

Crop species differ greatly not only in the case of metal uptake (Andersson and Nilsson, 1972; John, 1973; Davis, 1980; Davis, 1983) but also in their tolerance to heavy metals (Hunter and Vergnano, 1952; Marks et al., 1980). Such differences are also found between cultivars of the same crop (Bagchi, 1976; John, 1973). Generally the faster growing crops (or accumulator species) and in particular leafy vegetables like lettuce, take up metals more readily than slower growing crops like potatoes, carrots and runner beans (Dowdy and Larson, 1975; McIntyre et al., 1977; Richardson, 1980; Webber, 1980). Differences also exist between metals most readily taken up by the various accumulator species. For instance, lettuce can accumulate particularly high amounts of Pb, whereas red beet and leeks accumulate large quantities of Ni, even from uncontaminated sites (Richardson, 1980). Within individual plants there is also an asymmetrical distribution of metals, vegetative tissue containing higher metal concentrations than fruiting, root or tuber tissue (Dowdy and Larson, 1975).

In general, vegetables and potatoes are more sensitive to metal toxicity than are cereals which, in turn, are rather more sensitive than grasses (Chumbley, 1971). However, it has been suggested that the upper critical concentrations at which Ni, Cu and Zn cause yield depression does not vary between species (Beckett and Davis, 1982).

The effect on crops of potentially toxic metals will depend, to some extent, on interactions between the metals concerned, not only in the soil, but also within in the plant. These interactions can either enhance or alleviate toxic effects. Five ways in which two potentially toxic elements can interact have been identified (Beckett and Davis, 1982) :-

(i) Independent
(ii) Weakly additive
(iii) Additive
(iv) Synergistic
(v) Antagonistic

Independent behaviour is exhibited by metals such as Zn, Cu and Ni when their concentration in plant tissue are all below their toxic threshold (Davis, 1980). That is, at subcritical levels the element present in the highest concentration relative to its critical concentration determines the phytotoxic effect. However, when a plant is already suffering toxic reactions to a single element, its effects may be enhanced if a second element is also present in amounts sufficient, or nearly so, for it to be toxic by itself. In this case, the overall effect for metals such as Cu, Ni and Zn, may be additive and can cause greater toxicity than the sum of the two separate effects (Davis, 1980).

Japanese scientists have shown that the addition of Zn to soil increases Cd adsorption by rice plants (as reported by Asami, 1984). They assumed that Zn dissociates the Cd fixed to binding sites in soil due to competition for these sites, consequently increasing Cd availability. A similar synergism between Zn and Cd has also been observed in rice plants grown in culture solution (Asami, 1984). In contrast, Lagerwerff and Biersdorf (1972) observed that increasing Zn levels in culture solutions suppressed Cd uptake by radish. They concluded that with the low Cd concentrations present in the nutrient solution, Zn competed with Cd for absorption at sites of uptake. It would appear that Zn interferes with both Cd absorption into the root and into the vacuole but once Cd is absorbed its rate of translocation into foliage is increased by Zn (Honma and Hirata, 1976). The ratio of Cd to Zn in solution is important in controlling the occurrence of synergism and antagonism between these two elements (Honma and Hirata, 1976). When the Zn/Cd ratio is low the presence of Zn facilitates Cd uptake (Honma and Hirata, 1976) whereas high Zn/Cd ratios lead to suppresed Cd uptake (Haghiri, 1974; Honma and Hirata, 1976). Similarly as the Cd/Zn ratio increases Zn solubility decreases (Bingham et al., 1976), possibly due to the precipitation of Zn with Cd as a mixed carbonate.

Antagonism has also been shown to occur between other metals besides Zn and Cd. For instance, Cu toxicity interfers with the uptake of other metals, notably Fe and also deranges the normal processes of nutrient accumulation by roots (Chapman, 1966). However, Cu uptake is itself depressed by both Ni and Zn (Dunne, 1956; Chaudhry et al., 1973; Davis and Carlton-Smith, 1984). Soil pH can influence the interactive behaviours of metals. Whereas at high pH levels Cu sorption is decreased by the presence of Zn and Cd, at pH levels < 5 their presence does not interfere with Cu uptake (Kuo and Baker, 1980). Similarly, Cu and Cd depress Zn sorption at low pH, while slightly enhancing Zn sorption at higher pH levels (Kuo and Baker, 1980).

1.4.3 ANIMALS AND MAN

In certain soils plants can grow at normal rates exhibiting no toxic effects and yet contain sufficient Se, Cd, Mo and Pb to cause either direct toxicity or metabolic imbalance in animals that consume these crops (Allaway, 1968). Other plants, however, may grow normally but contain insufficient concentrations of Co, Cr, Cu, Mn and Se to meet the dietary requirements of animals. Thus, with respect to certain trace elements added to soil via sewage sludge, the quality of feeds and foods could, under certain circumstances, be improved, while in others, detrimental effects may be encountered.

Few cases have been recorded of toxic effects in animals, such as reduced growth rates or mortality, arising from sludge application to agricultural land, although, in practice, cases are difficult to detect. One isolated case has been reported where stock was effected through the application to pasture of a high Mo-content sludge at heavy rates over many years, and another where sludge with an exceptionally high F content had been applied to land already contaminated with F (DoE,1981). Earthworms have been shown to concentrate elements such as Cd and Zn in their tissue (Helmke et al., 1979; Hartenstein et al., 1980; Kruse and Barrett, 1985). Such biological magnification may present a potential hazard for foodchain transfer of heavy metals - earthworms being an important food source for many amphibians, reptiles, birds and mammals. In recent years concern has also been expressed about possible soil microbial inhibition due to prolonged sludge disposal to agricultural land (Brookes et al., 1984; Long, 1985; Brookes and McGrath, 1986). Many have attributed this deleterious effect to the build-up of heavy metals resulting from disposal (see Chapter V).

Ultimately, it is the safeguarding of man's health that is of the utmost concern. Little is known about the metabolism of heavy metals from ingested plant material. A number of plant constituents such as pectate or phytate are expected to strongly reduce the bioavailability of metals to monogastric animals, whereas in the rumen of cattle insoluble sulphide may form, leading to decreased intestinal absorption.

Nevertheless, experiments in which animals have been fed with soluble metal salts show that after absorption most trace metals accumulate preferentially in a few organs or tissues, leaving the others relatively uncontaminated (Jaworski et al., 1984). For instance, Cd concentrations in animal tissues decrease in the order kidney > liver > lung > bone > brain > muscle, while in the case of Pb, the decreasing order is bone > kidney > lung > spleen > muscle. Thus, selective consumption of muscle rather than offal can act as a barrier to the transfer of heavy metals in humans even when pasture soils contain excessive amounts of metal. Processing of crops, prior to human consumption also lowers the metal contents of foodstuffs. Washing vegetables reduces Pb levels by 61 - 82 %, and Cd levels by 29 - 57 % (Kampe, 1983). In the case of potatoes, 94 % of the Pb and 50 % of the Cd is located in the skin, and peeling reduces the contents accordingly. Nevertheless, a survey of an old Pb and Zn mining area in north-east Wales revealed that an extensive area of agricultural land was contaminated by Cd, Pb and Zn and a subsequent study of women resident in the area demonstrated a clear association between blood Pb concentrations and the consumption of home grown vegetables (Gallacher et al., 1984).

1.5 REGULATIONS FOR SLUDGE DISPOSAL TO AGRICULTURAL LAND

1.5.1 THE 'ZINC-EQUIVALENT' CONCEPT

The first attempts in the U.K. to lay down standards to regulate sludge disposal to agricultural land was made by ADAS (Chumbley, 1971) based upon a series of pot trials and field experiments started in 1969. Until very recently these guidelines were still being used by sludge producers in the U.K.. These recommendations suggested that the relative phytotoxicities of Zn, Cu and Ni were in the ratio of 1:2:8 respectively. It was also assumed that the toxic effects of these three elements were related and additive and that the amount of toxic metals in sludge could be expressed as a single figure, i.e. the zinc-equivalent, by adding together the Zn content, twice the Cu and eight times the Ni content. The zinc-equivalent was related to a total loading (kg/ha) or to an available (EDTA-extractable) concentration in soil (mg/kg). Where there was no previous soil contamination, it was concluded that an addition to the soil (pH value close to 6.5) of 250 mg/kg of zinc-equivalent was permissible over a long period (about 30 years).

The zinc-equivalent was a convenient concept because all three elements (Zn, Cu and Ni) usually occur together in sludge in higher concentrations than they do in soil and are usually the only sludge-derived metals which adversely affect plant growth. Its introduction was thought to be opportune at a time of rapid expansion of sludge use on agricultural land in the U.K. and it represented the first real attempt to control metal contamination of soil and offset crop phytotoxicity. It undoubtedly served a useful purpose in ensuring that soil was protected.

Since that time, the zinc-equivalent concept has received much criticism. Work reported by Davis and Beckett (1978 a, b) and by Matthews (1978) suggest that the toxic effects of Zn, Cu and Ni only become additive when one or more approaches its individual toxic concentration. In addition the toxicities of Cu and Ni have been overestimated. For instance, Cu and Ni were found to be less than twice and less than three times, respectively, as toxic as Zn to red celery and lettuce, when based upon their total soil concentration (Doyle et al., 1978). Whereas, when based on their extractabilities Cu and Ni were only 0.4 and 5.9 times as toxic respectively as Zn (Doyle et al., 1978). A report from the U.S.A. Council for Agricultural Science and Technology (1976) stated that the toxicity of Zn, Cu and Ni were generally not additive and that the zinc-equivalent greatly underestimated the amounts of sludge-borne metals which could safely be applied to soils of near neutral or alkaline pH. It was also stressed that the zinc -equivalent does not uniformly apply to a broad range of crops. These conclusions have also been confirmed by numerous other field and pot trials with sludge treated soils (Bolton, 1975; Coker et al., 1982; Johnston et al., 1983). Crops vary greatly in their sensitivity to trace metals in soil, which makes the calculations of application limits very difficult except on the basis that they should allow even the most vulnerable crop to be grown safely (Webber, 1981). Although there are reservations (DoE, 1981), the zinc-equivalent, as originally proposed, has remained a guideline for sludge utilization on land in England and Wales. In contrast, this concept was never adopted by other countries including the Agricultural Advisory Services in Scotland (SAC, 1986) and in the recent E.E.C. Directive on the use of sludge on agricultural land (E.E.C., 1986), Zn, Cu and Ni are treated independently.

1.5.2 GUIDELINES

Since 1971, several countries have introduced guidelines regulating the use of sewage sludge on agricultural land, some of which had the force of law. However, it is important to note that since 1986, agreement between E.E.C. member countries has led to a common policy on sludge disposal (E.E.C., 1986). In a review of some of these guidelines, Webber et al., (1983) state that these are based on one or more of the following assumptions :-

a) that heavy metal concentrations in sludge applied to agricultural land may not exceed defined limits,

b) that heavy metal loadings to agricultural land may not exceed defined limits,

c) that heavy metals are less likely to cause problems if they are added to soil in several small increments during an extended period of time, rather than in one or few large increments, and

d) that heavy metal concentrations in soil may not exceed defined limits.

Guidelines which were in force in several European countries and in the U.S.A. and Canada are reproduced in Tables 1.4 - 1.7, as given by Webber

TABLE 1.4 MAXIMUM PERMISSIBLE HEAVY METAL CONCENTRATIONS (mg/kg DRY wt.) IN SLUDGES CONSIDERED TO BE ACCEPTABLE FOR USE AS AN AGRICULTURAL LAND (from Webber et al, 1983).

Element	Belgium	Canada*	Denmark	England & Wales	Finland	France	Germany	Holland	Norway	Sweden	Switzerland	Scotland**
As	10	75						10				150
Cd	10	20	8	20	30	20	20	10	10	15	30	20
Co	20	150			100							
Cr	500				1000	1000	1200	500	200	1000	1000	800
Cu	500				3000	1000	1200	600	1500	3000	1000	1000
F				3500								
Hg	10	5	6		25	10	25	10	7	8	10	7.5
Mn	500				3000				500			
Mo		20									20	25
Ni	100	180	30		500	200	200	100	100	500	200	250
Pb	300	500	400	2000	1200	800	1200	500	300	300	1000	800
Se	25	14				100						40
Zn	2000	1850			5000	3000	3000	2000	3000	10000	1000	2500

FOOTNOTES:

* Values apply to sludge and sludge-based products containing $\leqslant 5$ % nitrogen.

** SAC (1986)

TABLE 1.5 LIMIT VALUES FOR SLUDGE USE IN AGRICULTURE (FROM WEBBER et al., 1983).

Parameter	Canada	Denmark	England & Wales	Finland	France	Germany	Holland	Norway	Scotland	Sweden	U.S.A.
min. soil pH			6.5		6.0				5.5		
max. sludge loading to soil (t/ha)	200			20		167	200	20	10	5	
min. application period (yr)	45	20	30	5		100	100	10	5	5	
MAXIMUM HEAVY METAL LOADINGS TO SOIL (kg/ha)											
As	15						2		0.15		
Cd	4	0.2	5	0.1	5.4	8.4	2	0.2	0.2	0.075	5, 10, 20
Co	30							0.4		0.25	
Cr			1000		360	210	100	4	8	5	
Cu			280		210	210	120	30	10	15	125, 250, 500
F			600								
Hg	1		2		2.7	5.7	2	0.14	0.075	0.04	
Mn								10			
Mo	4		4						0.25		
Ni	36		70		60	60	20	2	2.5	2.5	50, 100, 200
Pb	100		1000		210	210	100	6		1.5	500,1000,2000
Se	2.8							5	0.4		
Zn	370		560		750	750	400	60	25	50	250, 500,1000
Zn-equivalent			560								

FOOTNOTES :

DENMARK - sludge with < 3mg/kg dry wt. may be applied as a P fertilizer (maximum solids loading 1-5 t/ha/yr) indefinitely.

ENGLAND & WALES - sludge may be applied to pasture land with pH >6.0. Zinc, Cu and Ni loadings are subject to the zinc-equivalent limitations. The Zn, Cu, Ni and zinc-equivalent loadings shown are those for non-calcareous soil and may be doubled for calcareous soil.

FRANCE - maximum heavy loading were estimated assuming the German normal background and French permissible concentrates in soil (Table 1.6) and that 1kg/ha metal results in 0.33 mg/kg increase in the soil concentration.

GERMANY - maximum heavy metal loadings were estimated assuming normal background and maximum permissible concentrations in soil (Table 1.6) and that 1 kg/ha metal results in 0.33 mg/kg increase in soil concetration.

HOLLAND - sludge solids and heavy metal loadings are for arable land. Loadings to pasture land are one-half the values.

SCOTLAND - no more than $55m^3$/ha of sludge in any one application and not more than once a month and maximum 2 t/ha (sludge D.M.) in any one year.

SWEDEN - five year loadings can be repeated.

U.S.A. - sludges with < 2 mg Cd/kg dry wt. may be applied to soil with pH < 6.5. Heavy metal loadings increase with increasing soil cation exchange capacity. The Pb loading to fruit and vegetable crops should not exceed 800kg/ha.

TABLE 1.6 NORMAL BACKGROUND (bkg) AND MAXIMUM PERMISSIBLE (perm) HEAVY METAL CONCENTRATIONS (mg/kg DRY wt.) IN AGRICULTURAL SOILS (FROM WEBBER ET AL., 1983).

Element	France	Germany		Scotland[1]		England & Wales		
	perm	bkg	perm	bkg[2]	perm	bkg		perm
						non-calc	calc[3]	
As				2-2.5	12	5	10	10
B						1	3.25	3.25
Cd	2	0.2	3	0.5-3	4	1	3.5	3.5
Cr	150	30	100	30-200	80	100	600	600
Cu	100	30	100	5-125	60	6	140	280
F						200	500	500
Hg	1	0.1	2	0.05-0.5	0.4	<0.1	1	1
Mo				0.5-4	2	2	4	4
Ni	50	30	50	25-100	40	1	35	70
Pb	100	30	100	50-150	80	50	550	550
Se	10			0.2-5	6	0.5	3	3
Zn	300	50	300	75-250	150	2.5	280	560
Zn-equiavlent						20.5	280	560

FOOTNOTES:

Values are total concentrations in soil except for England and Wales Zn, Cu and Ni extracted by EDTA and B extracted by hot water.

England and Wales - values shown are mg/l.

Maximum permissible Zn, Cu and Ni concentrations are subject to the zinc-equivalent limitation.

(1) SAC, 1986.
(2) Values are quoted as kg/ha (to 200mm sampling depth).
(3) calc = calcareous.

TABLE 1.7 E.E.C. 1986 DIRECTIVE RANGES FOR CONCENTRATIONS OF HEAVY METALS IN SOILS AND SLUDGES (mg/kg DRY-MATTER) (FROM COUNCIL OF EUROPEAN COMMUNITIES, 1986).

Element	Soil (1)	Sludge	Sludge (2) Annual addition (kg/hg) based on 10-year average
Cd	1 - 3	20 - 40	0.15
Cr (3)			
Cu	50 - 140	1000 - 1750	12
Ni	30 - 75	300 - 400	3
Pb	50 - 300	750 - 1200	15
Zn	150 - 300	2500 - 4000	30
Hg	1 - 1.5	16 - 25	0.1

FOOTNOTES:

(1) For soils with a pH value of 6 to 7. Higher limit values may be set by member states where the soil pH $\leqslant 7$.

(2) Member states may permit those levels to be exceeded in the case of the use of sludge on land which at the time of notifcation of this Directive is dedicated to the disposal of sludge but on which commercial food crops are being grown exclusively for animal consumption.

(3) No limit values for chromium have as yet been set.

et al. (1983) and SAC (1986). Examination of these tables indicates the wide variations that existed between guidelines adopted in different E.E.C. countries. For instance, although it is widely accepted that highly contaminated sludge should not be spread on agricultural land, there was disagreement as to what metals should be regulated and at what concentrations the limits for 'acceptable' sludge should be set. Of all the countries given in Table 1.4, only Denmark listed four metals whose sludge concentrations needed monitoring, the other countries specifing at least seven elements :- Cr, Cu, Hg, Ni, Pb, Cd and Zn. In addition, the guidelines for England and Wales (DoE, 1981) have specified control in three aspects of sludge use, these being (see Table 1.4) :-

(i) Pb applied to grasslands, garden or amenity areas

(ii) Fluorine (F) applied to grazing land (see Table 1.4)

It has also been suggested that more emphasis should be placed on 'safe' exposure standards based upon sludge metal ratios (Huisingh and Huisingh, 1974). Chaney (1974) recommended that the Cd content of sludge to be added to soil should not exceed 1 % of the Zn content and if possible less than 0.5 % and less than 15 mg/kg D.M.. At these concentrations of Zn and Cd it is believed that crop injury would occur from Zn before the Cd content of the crop constitutes a health hazard.

Most countries have set limits for the total loadings of heavy metals which may be added to soil via sludge application in a specified number of years (Table 1.5). Denmark and Finland are the only countries which regulate only Cd and no other metal. All other countries regulate at least five metals. In Denmark and Finland it is assumed that by restricting Cd loading the addition of other metals will remain within acceptable levels (Webber et al., 1983).

Maximum permissible heavy metal concentrations for agricultural soils (Table 1.6) were only defined by four countries : France, Germany, Scotland and England and Wales. In these countries sludge application must cease when one or more heavy metal concentrations in the soil equals the specified limits. The approach in England and Wales of placing limits solely on soil metal concentrations and not on the metal load of the sludge being applied, meant in effect that any sludge, even the most contaminated, could, in theory, be applied to agricultural land, a view not held by the other countries including Scotland.

Certain guidelines have taken into account plant factors when specifying the limits to be improved. In Germany, sludges had to be applied before the growing season to grassland, vegetables and fruit crops (Moller, 1982). In Holland, different quantities of sludge were specified depending on whether it was applied to arable land or pasture (Moller, 1982).

In the U.S.A. the quantities of metals which may be applied to land depend on the cation exchange capacity of the receiving soil. It is assumed that a soil with a cation exchange capacity of more than 15 meq/100g soil can tolerate a metal load four times greater than can a soil with a cation exchange capacity of less than 5meq/100g soil (U.S.E.P.A., 1977). The pH of the receiving soil is taken into account in the guidelines for England and Wales (DoE, 1981). These recommended a pH of 6.0 for grassland, 6.5 for arable crops and a pH above 7.0 for significantly contaminated soil.

1.5.3 THE 1986 E.E.C. DIRECTIVE

The very existence of hundred-fold differences in the limits of heavy metal loading that could be applied to agricultural land in different countries, despite the scientific evaluation which had been made on environmental impact remains a clear indication of the very large gaps in our knowledge from which 'safe' and 'unsafe' concentrations and loadings can be defined.

In 1986 the Council of the European Community issued a Directive "on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture" (E.E.C., 1986). The purpose of this Directive (Table 1.7) was to ensure that man, animals, plants and the environment were fully safeguarded against possible harmful effects from the uncontrolled use of sewage sludge on agricultural land, and to promote its correct disposal. At the same time it was felt that "the disparity between the member states' provisions on the agricultural applications of sewage sludge might affect the functioning of the Common Market". The 1986 Directive stipulates that each member country must comply by producing guidelines not later than June 1989. Haigh (1987) explains that the Community had shown concern on the use of sewage sludge in agriculture even before the Community Environmental Programme had come into existence. In 1971 a research programme investigating certain aspects of sludge utilization was initiated as part of the work of European Cooperation in the field of Scientific and Technical Research and became known as COST Project 68. In 1983 conclusions drawn by the COST 68 Project and which were probably foreshadowed by the report of the Second Community Environment Programme, undoubtedly led to proposals for a Community Directive (Haigh, 1987). In 1982 a draft Directive was circulated among member states for comment and discussion.

The 1986 Directive requires that all sludge applied to agricultural land must have undergone pretreatment, but member states may authorize, under their own conditions, the use of untreated sludge, only if it is injected or directly worked into the soil. Maximum permissible soil metal levels are defined and sewage sludge application must be banned whenever the concentration of one or more metals in the soil already exceeds the limits laid down at the national level except on existing dedicated sites. The use of sludge is also regulated to ensure that heavy metal accumulation in the soil does not exceed these limits.

Regulation by member states may be either of the two methods :-

(i) upper limits can be set on the maximum quantity of sewage sludge which may be applied per unit area per year while observing the limits for metal concentrations in sludge selected from ranges laid down in the Directive;

(ii) the limits on metal loading per year as laid down in the Directive can be applied.

On grassland there is a mandatory period of not less than three weeks following sludge application after which grazing or harvesting is allowed. Sludge must not be used on land on which fruit and vegetable crops are growing (with the exception of fruit trees). Moreover, where fruits or vegetable crops are normally in contact with the soil and eaten raw, sludge application must occur at least ten months prior to harvesting.

Soil pH is also taken into account. Where the pH of the receiving soil is below 6.0, Member States are required to take into consideration the increased mobility and availability of metals and, if necessary, impose tighter limits. Where soil pH is above 7.0, Member States may permit the limit values they have fixed to be exceeded. However, the maximum authorized concentration of these metals must in no case exceed those values by more than 50 %. The official U.K. guidelines in response to this Directive are yet to be published.

CHAPTER 2

PREPARATION AND ANALYSIS OF SOILS AND HERBAGE

2.1 INTRODUCTION

The environmental significance of the accumulation of sludge-metals in agricultural soils depends largely on how much of the metals are availabe for plant uptake. As land application become more important as a disposal option for sewage sludge, an urgent need for a method of measuring 'available' metal fractions has been felt. The 'available' fraction should, strictly speaking, refer to that proportion of soil metal which can be absorbed by plants. However, as such, there is no distinct 'available' fraction of any metal, although heavy metals are released during crop growth from one or more of the pools in which they are retained (see Section 1.2.1.). Consequently, a full understanding of the effects of soil heavy metals on plants could only be achieved by a precise knowledge of metal speciation and plant response to each species (Lester et al., 1983). The complexity of mechanisms and reactions in which soil-heavy metals are involved, namely dissolution-precipitation, adsorption-desorption and complexation-decomplexation reactions (Cottenie et al., 1983), make the accurate determination of soil metal status very difficult. Determination of the 'available' fraction in terms of actual plant uptake is a very slow process and so soil scientists have developed more rapid analytical methods using chemical extractants. In theory, these extractants, model plant roots in their ability to remove trace elements from the soil; that is the extractants dissolve metals from the various soil pools in quantities that are proportional, but not equal, to the quantities that are likely to be released to a crop. The choice of extracting solution has often been based on empiricism, rather than on purely theorectial considerations (Kiekens and Cottenie, 1985), although some theoretical aspects may have inspired their adoption. Extracting solutions have been used for either selective determination of one single element or for simultaneous determination of a number of elements. Their value is normally judged as a function of more or less satisfying correlations obtained between the extracted amounts and the metal content of plant tissue. In such instances it would, therefore, be more appropriate to use the term 'extractable' rather than 'available' metal.

Soil constituents, especially trace elements, can be brought into solution stepwise, using extractants of increasing strength, starting with pure water and in the final attack, strong acids, to obtain the total amount (Viets, 1962). Such a series of extractants, which mobilise the various forms of soil metal species (see Section 1.2.1.) in a stepwise succession, has been proposed by Berrow and Burridge (1980) :-

(i) water;
(ii) ion exchange extraction using neutral salts (eg. ammonium acetate);
(iii) dilute acid extraction (eg. acetic acid), which also attack some insoluble salts;
(iv) extraction by chelating agents (eg. EDTA);
(v) total extraction by strong acids.

Each extractant will displace some, if not all, of the fraction displaced by the less vigorous reagents preceeding it (Berrow and

Burridge, 1980). The relative amount extracted will depend on the element in question and on the prevailing soil conditions.

Sequential extraction has been employed to portion heavy metals between various soil fractions. Such procedures utilize a series of selective extractants to remove metals from the various soil fractions. For instance, McLaren and Crawford (1973) developed a sequential extracton procedure using 0.05M calcium chloride ($CaCl_2$), 2.5 % acetic acid, 1M potassium pyrophosphate, acid diammonium oxalate (pH 3.25) and hydrofluoric acid, to separate soil Cu into soluble-exchangeable, weakly-adsorbed, organically bound, occluded and residual fractions respectively. Most sequential extraction procedures have been used to study native or background levels of trace metals (Le Riche and Weir, 1963; Shuman, 1979; Tessler et al., 1980). Recently, attempts have also been made to fractionate the heavy metals in contaminated systems, such as harbour sediments (Gupta and Chen, 1975) and soils and road dust (Stover et al., 1976; Elsokkary and Lag, 1978; Harrison et al., 1981; Miller and McFee, 1983; Kuo et al., 1983) and has proved very useful in assessing the relative importance of metal forms in soils and sediments. However, the method is very slow, tedious and labour intensive and is unsuited for routine use.

Measurement of total trace metal concentrations in soil, by extraction with strong acids, is not a good indicator of that metal fraction available for plant uptake (Cottenie et al., 1983; Hani and Gupta, 1983; Kuo et al., 1985; McGrath et al., 1985). Determination of total soil metal concentration indicates the extent of contamination but not its biological significance, since a significant fraction may be present in the non-available form. Nonetheless, Purves and Ragg (1962) found that where total Cu concentration was $\leqslant$ 2 mg/kg, in soils of the Eckford series, (developed from old sandstone in south-east Scotland), this provided a useful indication of soils likely to give rise to Cu deficiency in cereals. Similarly, total soil Cu content provides a good indication of Cu availability in chalk soils (Davies et al., 1971).

In Britain, 0.5M acetic acid is used on a routine basis to measure plant availabilty of a wide variety of metals, except for Cu which is measured by 0.05M EDTA (Mitchell, 1964). Both these reagents have been extensively used to identify metal contamination of soil (Le Riche, 1968; Purves and McKenzie, 1969; Beavington, 1973; Webber, 1974). In a Scottish context, acetic acid and EDTA have been used, by the Macauly Institute for Soil Research, in the mapping of extractable trace metal contents in soil (Berrow and Reaves, 1984). Acetic acid is used to measure extractable Cr, Co, Pb, Mo, Ni, Ti, V and Zn, whereas EDTA is used for Cu, Mn and Zn. Moreover, Chumbley (1971) proposed that in regulating sludge disposal to land, Ni and Zn in soil should be monitored in terms of their extractability in 0.5M acetic acid and EDTA used for monitoring Cu.

EDTA extracts the exchangeable, readily soluble and organically complexed fractions of soil metals, as well as part of that fraction sorbed by sesquioxides (Davies, 1975). Extraction with EDTA has been widely used as an indication of plant available Cu under deficiency conditions (Borggaard, 1976; Robson and Reuter, 1981) and good correlations between EDTA-extractable Cu and crop uptake have been obtained (Oien, 1966; Lakanen and Ervio, 1971; Beyers and Hammond, 1971;

Osiname et al., 1973; Tills and Alloway, 1983).

EDTA extraction is also a good predictor of plant available Pb (Alloway and Tills,1983; Davies, 1975, 1985), Zn (Viro, 1955; Trierweiler and Lindsay, 1969; Berrow and Burridge, 1983) and Cd (Davies, 1985). In this respect, the incoporation of soil pH and organic matter content into the predictive equation provides better results for estimating plant uptake of Zn (Haq and Miller, 1972) and Cd (Haq et al., 1980).

For its part acetic acid is the most widely used extractant (Mitchell, 1970). By virtue of being a weak acid and at the same time showing some complexing ability via its associated anion (ie. acetate), makes acetic acid a useful reagent for estimating the mobile reserves of certain elements in soil (Leeper, 1972). It has proved particularly valuable in determining plant available Cd, Zn and Ni (Hunter and Vergnano, 1953; Ng and Bloomfield, 1962; John et al., 1972; Haq et al., 1980;. Berrow and Burridge, 1983).

In recent years, there has been a shift away from using acetic acid to that of weak salts which are more sensitive to soil conditions especially soil pH, which as previously discussed (see Section 1.2.2.) plays a major part in determining soil metal speciation and hence availability. Ammonium acetate (Symeonides and McRae, 1977; John et al., 1972 ; Andersson and Nilsson, 1974), sodium nitrate (Gupta and Stadelmann, 1983; Hani and Gupta, 1983), calcium cholride (MacLean and Dekker, 1978; Sanders et al., 1986a) as well as water (Adams and Sanders, 1985) have all received considerable attention.

The soil solution is the interface between the plant root and the soil. Consequently, the concentration of metal in soil solution is likely to be closely related to the bioavailable fraction. The main advantages of using water extraction are (Adams and Sanders, 1985):-

(i) it involves no chemical reagents,
(ii) it does not lead to destructive sampling of the soil, and
(iii) it extracts that fraction which is immediately available to plants or which is in rapid equilibrium with labile forms.

Good correlations have been obtained between the concentrations of Cu, Ni and Zn displaced by water extraction and their concentrations in plant tissue (Bingham et al., 1976; Davis, 1979; Valdares et al., 1983). However, on practical grounds, soil solution measurements are not suitable for routine soil metal analysis since as well as being time comsuming, they require sensitive analytical techniques due to the very low concentrations extracted, and water extraction is prone to contamination problems (McGrath et al., 1985).

Neutral salts extract larger concentrations of most metals than are present in the soil solution, but not as much as acetic acid or EDTA (McGrath et al., 1985). Besides displacing those metals present in the soil solution, their cations can exchange for weakly held cations on soil exchange sites (Viets, 1962). One such widely used extractant is ammonium acetate. It is seen to yield good correlations between the levels of Ni, Cd and Zn extracted and that taken up by plants (Soane and Saunders, 1954; Misra and Pande, 1974; Haq et al., 1980). However, Andersson and Nilsson (1974) point out that ammonium acetate extraction is only sensitive to pH when it is prepared at pH 7 and not when prepared at pH 4.8, at which it masks any influence of soil pH on metal availability.

Sodium nitrate (0.1M) is especially useful in determining plant available Cd (Sauerbeck and Styperek, 1985; Hani and Gupta, 1985). Its two main advantages according to Hani and Gupta (1985) are :-

(i) the amounts extracted from different soil types correlate well with the amounts taken up by plants, and

(ii) regardless of the physico-chemical properties of the soil an almost uniform soil metal content, results in defined biological effects in plants, where the values for Zn, Cd and Cu are 1.0, 0.06 and 1.5 mg/kg respectively.

However, the amounts of metal, and Cd in particular, dissolved by sodium nitrate from soils are often very small (Hani and Gupta, 1983; Sauerbeck and Styperek, 1985). Consequently, even with flameless techniques the soluble metal fraction in most soils cannot be ascertained. For instance, whereas the maximum permissible Cd concentration in soil receiving sludge is 3 mg/kg (E.E.C., 1986), sodium nitrate-extractable Cd in soil containing 2 - 4mg Cd/kg (total) can be too low for detection (Sauerbeck and Styperek, 1985).

The use of calcium chloride (0.1M) extraction is very promising. It is a slightly stronger extractant than sodium nitrate, removing more metal and consequently poses less of an analytical problem (Juste and Solda, 1985). The most important advantage of calcium chloride extraction over sodium nitrate is the fact that it dissolves a sufficiently large proportion of available Cd, even in situations of low Cd contamination

(1 - 2 mg/kg) (Sauerbeck and Styperek, 1985). At the same time it is still sufficiently weak to reflect the modifying effects of individual soil properties (Sauerbeck and Styperek, 1983). Furthermore, calcium chloride extractable Cd is seen to reflect changes in Cd uptake which occur over the years following sludge application and will also reflect the difference in availability between artificially enriched sludge and sludges with naturally high levels of metals (Sauerbeck and Styperek, 1985). Good correlations have also been obtained between calcium chloride extractable Zn and Ni fractions and their relative uptake by plants (Sanders et al., 1986 a, b).

Many other extractants have also been used with varying degrees of success, eg. nitrilotriacetic acid (NTA) (Soon et al., 1980), hydrochloric acid (Wear and Sommer, 1947), DTPA (Keeney and Walsh, 1975; MacLean and Dekker, 1978; Adams and Sanders, 1985; Hani and Gupta, 1985), ammonium nitrate (Davies, 1985). However, many of these extractants are so condition-specific that a blank adoption of them without proper assessment prior to their being used to determine the 'available' fraction is not advisable (Singh and Narwal, 1984). Knowledge gained from previous correlation studies for a given extractant may or may not be valid under different soil conditions and for different crops or for sludges derived from different sources (Keeney and Walsh, 1975). Measurements made with these non-conventional and conventional extractants can, therefore, only be of value in predicting plant uptake, and hence toxicity, if appropriate calibration curves plotting extractable soil metal against plant uptake are at hand for the particular soil and crop under consideration (Davis, 1979), otherwise the results obtained with extractants may be misleading. As the extracted amounts represent the equilibrium concentration obtained by the contact of the solid soil phase with the extracting solution, the analytical results will be influenced by the operating conditions such as soil/solution ratio, extraction time and ambient temperature (McLaren and Crawford, 1973; Kiekens and Cottenie, 1985).

In spite of numerous attempts, no universal extractant has yet been found which can predict metal availability to a wide variety of plants under a wide variety of conditions. It would, therefore, appear that the only reliable test for the affects of sludge application on crop metal content is the actual analysis of the crops growing on the receiving soil. Plant tissue analysis offers a direct method of measuring the physiological status of plants in connection with either deficiency or toxicity, the concentration of trace elements in plants having a direct bearing on the health of animals consuming them. Crop tissue analysis can provide a useful complement to soil analysis for monitoring purposes, i.e. soil analysis indicates the extent of contamination, while plant analysis indicates its significance. Plant analysis offering a direct method of measuring available metals in soil, since the concentrations of metals in plant tissue are the integral result of all the factors which have affected uptake up to the time of sampling (Davis and Carlton-Smith, 1980), no soil analysis being able to predict or replicate this.

Unlike chemical extraction, bio-assay techniques are relatively slow. Such methods have been criticised in that in the field situation they will only indicate the toxicity problem after it has already arisen

(Page, 1974). whereas what is needed is a procedure that will identify the problems before they occur. However, plant analysis need not be retrospective since small scale tests with a sludge and the soil to which it will be added can be completed before sludging of the field has occurred and the results used to predict the hazard. Moreover, regular sampling of crops growing on sludged fields affords an essential monitor for assessing future sludge disposal practice.

Sampling plants grown under controlled conditions has the advantage that problems of leaf contamination are minimized and growing conditions can be well-defined and controlled. In addition, glasshouse trials offer ease of husbandry, large replication, are cheap to mantain and subject to less experimental error than field trials (Kuntze et al., 1983). Plants can also be grown at any time of the year and the uptake observed after one application of sludge providing a basis for assessing the probable hazard of subsequent applications.

There is evidence that the tissue metal concentrations are greater in plants grown under glass than those grown in the field (Davis, 1981; DeVries and Tiller, 1978). This is probably due to the more favourable growing conditions prevailing in the greenhouse. Watering of pots is much more rapid and regular than in the field, glasshouse temperatures exceed those in the field by several degrees both during the day and night. This results in increased evo-transpiration which in turn can result in increased amounts of metals reaching the leaves (Kuntze et al., 1983). The higher soil temperature in the pot can also result in increased solubility of some metal complexes (Stephenson and Collis-George, 1974).

Thanks to the higher metal concentrations found in plants grown under greenhouse conditions as opposed to those found in plants grown in the field situation, the results obtained from pot experiments can be considered as the 'worst-case' situation, thus affording a margin of safety for assessing sludge disposal practices to agricultural land (Davis and Carlton-Smith, 1980). However,it would be unwise to base soil metal limits for land application of sewage sludge on results obtained from glasshouse trials (Kuntze et al., 1983), disposal guidlines should ultimately rely on date obtained from actual field experiments.

Whichever method is ultimately adopted, i.e. chemical extraction or plant analysis, there is a need to 'standardise' the plant species used. Ryegrass and barley are well suited for such a purpose as they are common crops in Britain and easily grown and harvested. In addition, their upper critical concentrations for many metals are well known (MacNicol and Beckett, 1985). The more information obtained for a particular plant species or chemical extractant, the more useful it becomes as its adoption will be based upon a wider spectrum of soil/sludge/plant combinations.

In the following sections the general methods employed throughout this work for the analysis of soil, herbage and sludge are described.

The results pertaining to the extraction of soil using acetic acid, EDTA and aqua regia are described in the following chapter (Chapter 3).

2.2 MATERIALS AND METHODS

All analyses were carried out in duplicate. Where appropriate, blanks were used to quantify background contamination. All equipment used for trace element analysis was acid washed in 0.5M HNO_3.

2.2.1 SOILS

2.2.1.1 SAMPLE PREPARATION

Fresh soil, was rubbed through a 5mm stainless steel wire sieve to remove stones and plant material. The sieved soil was then transfered to aluminium trays, spread out into a thin layer, placed into an aerated oven at 30°C and left to dry for 24hrs (minimum) (ADAS, 1986). The air-dried sample was then gently ground to pass through a 2mm round-hole sieve (see Appendix A).

2.2.1.2 pH DETERMINATION

The pH of 10cm^3 <2mm air dried soil, was measured using a 2.5 : 1 (V/V) water to soil mixture (ADAS, 1986).

A 2 : 2 (V/V) water to soil ratio was used where the pH of moist soil was required.

2.2.1.3 LIME REQUIREMENT

Lime requirement was measured using a soil-lime incubation technique. $10cm^3$ soil samples were mixed with increasing amounts of calcium hydroxide ($Ca(OH)_2$), stored moist with 25ml distilled water for 10 days and shaken daily for 2hrs. Soil pH was measured at the end of the incubation period. The amount of $Ca(OH)_2$ necessary to increase soil pH to desired levels was determined from a calibration curve of pH vs weight of $Ca(OH)_2$ added.

A similar procedure was followed to determine the weight of aluminium sulphate ($Al_2(SO_4)_3$) required to lower soil pH to desired value.

2.2.1.4 AIR-DRY MOISTURE CONTENT

Samples of 10g (± 0.01g) <2mm air dried soil were weighed into silica crucibles and placed in an oven (105^oC) for 24hrs. The crucibles were then cooled in a dessicator and weighed. Loss of weight was expressed as % air-dried moisture (Avery and Bascomb, 1974).

2.2.1.5 TOTAL OXIDISABLE ORGANIC MATTER

The procedure followed is that described by ADAS (1986), involving the oxidation of soil organic matter by gently boiling with a solution of potassium dichromate, sulphuric acid and orthophosphoric acid. Excess dichromate was determined by titration with ferrous sulphate solution.

2.2.1.6 LOSS ON IGNITION

Air-dried (<2mm) soil was ignited at 680°C in a muffle for 18hrs (Avery and Bascomb, 1974). The loss on ignition was expressed as a percentage loss of weight of oven dry soil.

2.2.1.7 SOIL PARTICLE DISTRIBUTION

Following the destruction of organic matter by hydrogen peroxide of <2mm air-dried soil the clay and silt fractions were determined by the hydrometer method (Bouyoucos, 1951). The sand fraction was then collected and separated using nested sieves.

2.2.1.8 TOTAL NITROGEN CONTENT

The method used involved the conversion of soil organic nitrogen to ammonium-nitrogen by digestion with sulphuric acid and sodium sulphate, using a copper-selenium catalyst (ADAS, 1986). The ammonium is then converted to ammonia by means of sodium hydroxide and removed by steam distillation, and collected in boric acid. The ammonia is then determined by titration with dilute sulphuric acid.

2.2.1.9 EXTRACTABLE PHOSPHOROUS AND POTASSIUM

Soil P and K are extracted by 0.43M acetic acid at 20 ± 1°C (MISR/SAC, 1985). A 1:40 soil/solution ratio was used where 5g of <2mm air dried soil were shaken with 200ml 0.43M acetic acid on an end-over-end shaker for 2hrs and filtered immediately. The extract was measured spectrophotometrically.

2.2.1.10 EXTRACTABLE NITROGEN

2M potassium cholride is used to extract NH_4^+- and NO_3^-- nitrogen from moist soil. The ammonia is released by adding magnesium hydroxide and removed by distillation to be determined by titrating with sulphuric acid. Nitrate nitrogen is similarly determined following its reduction to ammonia by Devarda's alloy (ADAS, 1986).

2.2.1.11 EXTRACTABLE Cd, Cu, Ni, Pb AND Zn BY 0.43M ACETIC ACID

Five grams (± 0.01g) <2mm air-dried soil was extracted in 200ml 0.43M acetic acid (DoE/Nat. Water Council, 1982), in a 500ml PTFE bottle, shaken overnight (16 hrs) on an end-over-end rotator at 20rpm at 18°C. The solution was filtered through Whatman No. 540 filter paper and the first few mls rejected. The remaining filtrate was used for determination by atomic absorptiometry.

2.2.1.12 EXTRACTABLE Cu AND Zn BY 0.05M EDTA

A 1:5 soil:extractant ratio (W/V) was used in which 15g <2mm air-dried soil, was extracted in 75ml 0.05M EDTA at pH 7.0 (DoE/Nat. Water Council, 1982) in 250ml PTFE bottles. The solutions were shaken for 1hr on a 20 rpm end-over-end rotator, at 18 ^{o}C, and immediately filtered through Whatman No. 540 filter paper, rejecting the first few mls. The remaining filtrate was used for metal determination by atomic absorption.

2.2.1.13 TOTAL (AQUA REGIA EXTRACTED) Cd, Cr, Cu, Ni, Pb AND Zn

The method employed is one based on the procedure described by Berrow and Stein (1983), and used for routine analysis at the West of Scotland College.

Three grams (± 0.01g) of <2mm air-dried soil was placed into a 100ml graduated boiling tube. To this 22.5ml of 6M redistilled HCl and 7.5ml redistilled 15.7M HNO_3 were added and allowed to stand overnight at room temperature (20 ± 1 ^{o}C). The tubes were then placed in a Tecator digestion block and left for 30mins at 80 ^{o}C or until the frothing subsided (whichever was the longest). The temperature was then increased to 140 ^{o}C and left for 2 hrs for the digestion to be completed.

After cooling, distilled water was added to bring the final volume to 100ml. The digests were then shaken and filtered through Whatman No. 542 filter paper. The filtrate was then used for heavy metal determination by atomic absorption.

2.2.2. HERBAGE

2.2.2.1 SAMPLE PREPARATION

Immediately on receipt, approximately 200g of fresh herbage was subsampled and placed in an aluminum tray, lined with greaseproof paper, and dried overnight at 95°C in a forced draught Unitherm oven.

After cooling, the samples were weighed to determine dry matter content. The dried material was then ground in a 20cm steel hammer mill (Scott et al., 1971). The ground samples were stored in paper bags.

A 5g subsample of dried herbage was placed in a glass vial, oven-dried at 100°C and then cooled and stored in a $CaCl_2$ filled dessicator.

2.2.2.2 TOTAL HERBAGE Cd, Cr, Cu, Fe, Mn, Ni, Pb AND Zn BY DRY COMBUSTION

The underlying principle behind this method is that the organic matter of the plant material is destroyed by dry combustion. The soluble mineral constituents in the resulting ash are then dissolved in hydrochloric acid. Any silica present is dehydrated and thereby made insoluble.

The method followed is based on that described by Scott et al., (1971). Two grams (± 0.01g) of dried milled material, taken from a 5g subsample, was ashed overnight at 470°C in a Lenton Thermal Design Ltd. muffle furnace, allowed to cool, moistened and taken to dryness. This was followed by a second overnight ashing. The residual ash was then refluxed with 5ml of 6M HCl for 20mins., taken to dryness, baked for 20mins. and refluxed with 2.5ml 6M HCl for a further 15mins. The solution was then taken up to 25ml with distilled water, filtered through Whatman No. 541 filter paper, ready for trace metal determination by atomic absorption.

2.2.3 SEWAGE SLUDGE

2.2.3.1 PREPARATION OF SAMPLE

A bulk sample of sludge of approximately 1l was homogenised by vigorous hand shaking.Approximately 150mls of known weight of fresh homogenised sludge was poured into a clean aluminium tray and left overnight to dry in a forced-drought oven at 95°C. The dried sludge was then milled using a Tecator Cyclone Sample Mill and stored in plastic bags for subsequent analysis.

2.2.3.2 TOTAL Cd, Cr, Cu, Fe, Mn, Ni, Pb AND Zn CONCENTRATION IN SEWAGE SLUDGE

The digestion procedure employed is that previously described for the determination of total soil metal concentrations (see Section 2.2.1.13).

2.2.4 DETERMINATION OF HEAVY METALS

2.2.4.1 PREPARATION OF STANDARD SOLUTIONS

Multi-element standards solutions were prepared in the relevant extracting solution. In so doing differences in physical and chemical interferences during atomic spectrophotometry between the standards and the extracts were minimized (Kirkbright and Sargent, 1974).

2.2.4.2 ATOMIC ABSORPTION SPECTROPHOTOMETRY

Cadmium, Cr, Cu, Ni, Pb and Zn concentrations in soil, herbage and sludge extracts were read on an Instrumentation Laboratory AA/AE spectrophotometer, model 757, equipped with a single-slot burner and an air-acetylene flame, except for Cr which was determined in a N_2O-C_2H_2 flame (see Table 2.1)..For the determination of all the elements, except Cu, a Smith-Hieftje background correction was applied.

Each individual sample was read over three consecutive 3sec. integration periods and the mean reading recorded. To minimise sample evaporation, all the extractants were read within 48 hrs of preparation. Moreover, EDTA extracts were read within 6hrs of extraction to prevent precipitation of EDTA complexes.

To reduce errors and interferences, flame conditions were optimised each time the instrument was used. Standard solutions were read at regular intervals and calibrations adjusted as necessary.

TABLE 2.1. ATOMIC ABSORPTION SPECTROPHOTOMETER, OPERATION PARAMETERS AND SENSITIVITIES.

ELEMENT	Cd	Cr	Cu	Ni	Pb	Zn
Wavelength nm	228.8	357.9	324.7	232.0	217.0	213.0
Lampcurrent mA	2.5	4.0	3.0	8.0	3.0	3.0
Slit width um	320	160	320	40	320	320
Sensitivity ng/ml/18ABS	0.01	0.06	0.03	0.06	0.10	0.01
Upper limit to linear range ng/ml	2	5	4	5	15	1
Detection limit* ug/ml	0.001	0.003**	0.002	0.01	-	0.00

FOOTNOTES:

* Kirkbright and Sargent (1974).

** The detection limit for a N_2O-C_2H_2 flame is expected to be lower than this value quoted for an air-CPV$2_{H}2$ flame.

Copper, Fe, Mn and Zn concentrations in sludge contaminated samples were determined using a Thermo Electron Plasma 100 emission spectrometer (see Table 2.2). Each individual sample was read over five consecutive 1sec integration periods and the mean reading recorded.

TABLE 2.2. PLASMA EMISSION SPECTROMETRY; OPERATING CONDITIONS AND DETECTION LIMIT.

ELEMENT	WAVELENGTH nm	TORCH HEIGHT mm	DETECTION LIMIT ug/ml
Cu	324.75	14	0.0054
Fe	238.20	20	0.0046
Mn	257.61	20	0.0014
Zn	213.856	14	0.0018

CHAPTER 3

EFFECTS OF CULTIVATION, LIMING AND NITROGEN FERTILIZER RATE ON THE DISTRIBUTION AND UPTAKE OF HEAVY METALS BY GRASS

3.1 INTRODUCTION

The most common soil management practices in agriculture generally involve the enhancement of soil nutrient availabilities by fertilizers and lime application and the amelioration of soil physical conditions by cultivation and drainage.

Lime application in Britain has a long history dating back to Roman times. Spreading chalk on some loam and clay soils was considered, among other things, to make these soils easier to cultivate. Nowadays, the need for liming is seen primarily as a means of maintaining a satisfactory soil pH for crop growth (ADAS, 1981) since plant species differ in their tolerance to soil acidity (Table 3.1). At low pH values the growth of many agricultural and horticultural crops is reduced and at high soil pH levels some plants are susceptible to trace element deficiencies. Soil pH has a very strong influence on the mineral composition of plants (see Section 1.2.2). Consequently liming, with its associated increase in soil pH, results in reduced uptake and hence lessens the risk of toxicity of Ni, Zn, Pb, Cd and Cu (John and Van Laerhoven, 1972, 1976; King and Morris, 1972a; MacLean and Dekker, 1978; Naylor and Kresse, 1983; Asami, 1984; Davies, 1985). However, increased soil pH leads to increased Mo uptake (Davies and Jones, 1988) and can result in Mo-induced Cu deficiency in ruminants (Thornton and Webb,1975).

TABLE 3.1 SOIL pH VALUES BELOW WHICH CROP GROWTH IS ADVERSELY AFFECTED ON MINERAL SOILS (FROM ADAS, 1981).

	pH
Barley	5.9
Clover (white)	5.6
Lettuce	6.1
Lucerne	6.2
Oats	5.3
Potato	4.9
Rape	5.6
Ryegrass	4.7
Tomato	5.1
Wheat	5.5

Large quantities of fertilizers are regularly applied to soils in intensive cropping systems to provide adequate N, P and K for crop growth. In Britain, the use of chemical fertilizers has increased dramatically over the past century (Table 3.2). The application of N is recognised as being important in increasing the productivity of grasslands in temperate regions. On average, in 1986, 162kg N/ha was applied to grazing land on Scottish dairy farms (SAC, 1986), with about 220 and 107kg N /ha applied to grassland cut for silage and hay respectively (SAC, 1986). Fertilizer application affects the mineral compostion of crops. A well known example of this is induced Mg deficiency in crops following the application of K^+, NH^{4+} or Ca^{2+} containing fertilizers (Mengel and Kirkby, 1987; Marschner, 1986). In anion uptake, antagonistic effects are less common although Cl^-, SO_4^{2-} and $H_2PO_4^-$ uptake can be stimulated when NO_3^- uptake is strongly depressed (Kirkby and Knight, 1977). The most common anion interaction is that between NO_3^- and Cl^-. High Cl^- levels depress NO_3^- uptake and vice versa (Mengel and Kirkby, 1987). Fertilizer may also affect concentrations of minerals in plant tissue not present in the fertilizer such as micronutrients (eg. Cu, Mo and Co) (Hemingway, 1961, 1962; Klessa et al., 1989). Whether these concentrations increase or decrease depends largely on the soil status with respect to the mineral in question (Fleming, 1973). The more rapid growth stimulated by the application of one element may produce a dilution effect on a second element under conditions of marginal soil availability, or luxury uptake if sufficient is available. These effects occur with both nitrogenous and phosphatic fertilizers and are generally more marked with the former (Little, 1981). The application of fertilizers can

TABLE 3.2 FERTILIZER USE IN THE U.K. IN SPECIFIED YEARS FROM 1913 TO 1984 (THOUSANDS OF TONNES) (FROM WILD, 1988).

	N	P_2O_5	K_2O
1913	29	183	23
1939	61	173	76
1950	229	468	238
1969	803	484	465
1982	1416	446	483
1984	1588	488	559

also result in changes in sward composition. As plant species differ widely in their ability to take up metals (see Section 1.4.2) a shift in sward composition can result in a change in the net mineral composition of the sward. For instance, in areas of high soil Mo, non-nitrogenous fertilizer application has resulted in molybdenosis in stock. This has been attributed to the increase in clover, which contains higher Mo content than grass (Fleming, 1973).

Soil cultivation has a number of objectives (Davies and Payne, 1988) :-

(i) to obtain a seedbed,
(ii) to kill weeds,
(iii) to undo damage done by previous traffic over land,
(iv) to incoporate crop residues,
(v) to increase permeability of the surface soil or subsoil.

However, the effect of cultivation on metal uptake has not been elucidated. In theory, the resultant improvement in soil permeability would be the most likely factor to have an effect on metal availabilty through improved root growth and drainage. Moreover, following cultivation there is an increase in organic matter decomposition (Jenkinson, 1988), whose rate declines with time (Foth, 1978). This increased decomposition rate and the lower soil organic matter content can result in increased heavy metal mobility and, hence, plant availability (see Section 1.2.3).

AIMS AND OBJECTIVES

As outlined above the adoption of a particular agricultural technique (eg. liming, cultivation and fertilizer use) can have a significant influence on the mineral composition of crops. The aim of the work described in the following sections was to investigate the influence of farming practices (i.e. fertilizer N rate, cultivation and liming) on sludge metals in grassland soils in the west of Scotland with respect to their;

i) uptake by grass,
ii) accumulation and distribution in soil profiles,
iii) extractability in acetic acid and EDTA as related to (i).

For this purpose a field experiment was set up on permanent grassland with a long history of sludge disposal on site. Concurrently, a pot experiment investigating the effects of pH on metal uptake by ryegrass was also carried out.

3.2 EXPERIMENT 1 - FIELDWORK

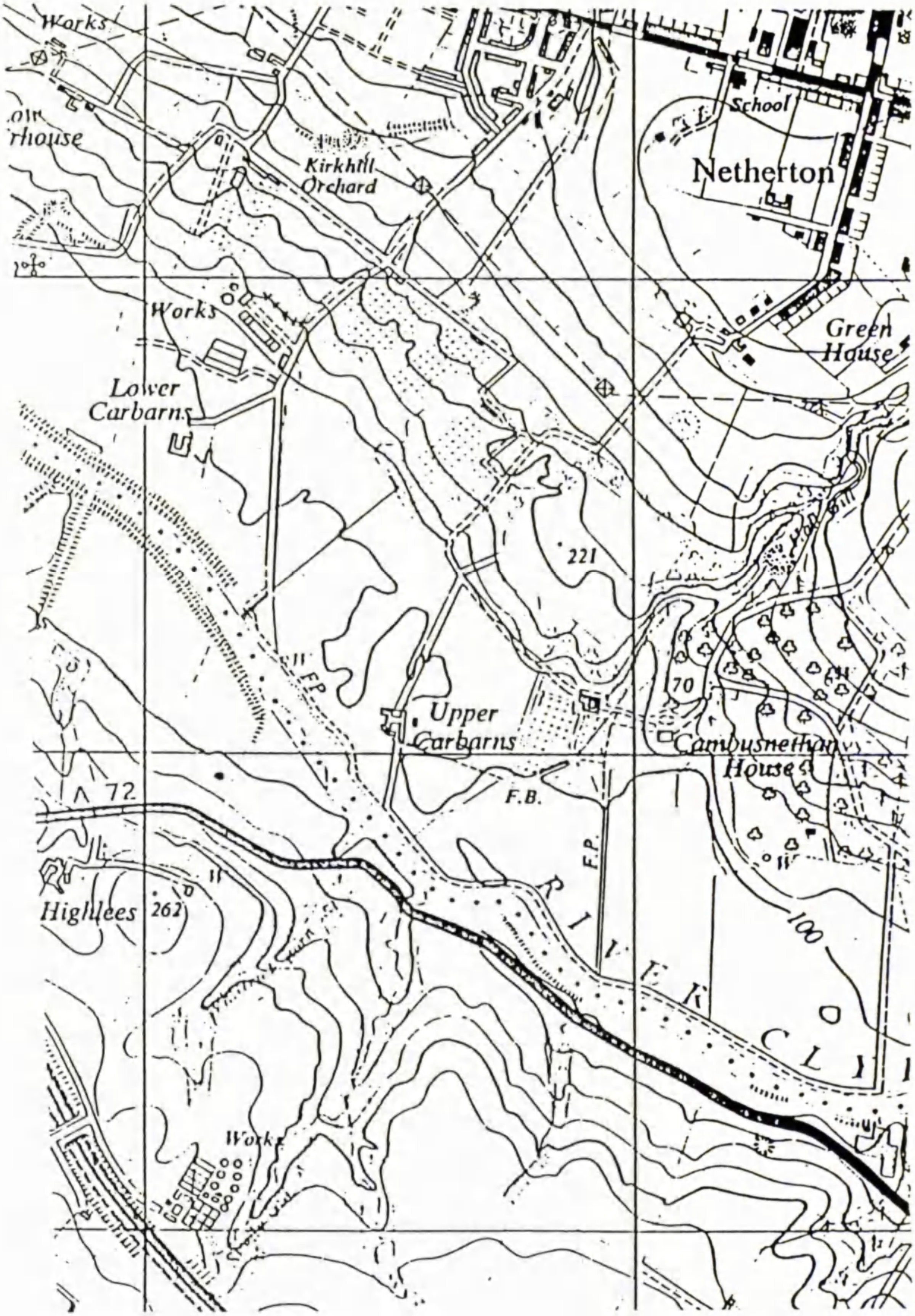
3.2.1 SITE AND SOIL DESCRIPTION.

Trial work was carried out at Lower Carbarns Farm (Nat. Grid Ref. NS 773 537), one of the few long-term sewage sludge disposal sites on agricultural land in the West of Scotland.

The field borders the southern perimeter fence of the Carbarns Sewage Treatment Works (see Plate 3.1). Digested sludge has been applied to the farm since 1934. For many years the sludge was actually used to irrigate the fields and the remnants of disused irrigation ditches lay witness to this.

The sewage draining into the Carbarns Sewage Treatment Works is mainly of domestic origin, although some industrial wastes are also present, particularly abattoir, light engineering and film processing wastes. However, before the decline in Scottish steel industry, the works received effluent from metal plating, steel pickling and store polishing industries, which undoubtedly contributed to the metal load of the disposed sludge. Currently, the Carbarns Works serves a population of 44100 and produces 20000 gallons of digested sludge per day, all of which is disposed on to agricultural land (McCluskey, personal communication).

PLATE 3.1 PART OF SURVEY MAP (SHEET NS 75) SHOWING LOWER CARBARNS



SITE DESCRIPTION

Relief - Gently sloping alluvial terrace in river valley of the Clyde.

Elevation - 100m

Slope - 3°

Aspect - SSW

Slope form - Straight.

Climate - Warm moist lowland.

Rainfall - 900mm

Vegetation - Permanent grass.

Land use class - 3.2

Soil parent material - Till derived from shales and sandstone from carboniferous sediments.

Soil Association - Rowanhill.

Soil series - Caprington series.

Major soil Group - Cambic Stagnogley.

Drainage - Imperfectly drained.

PROFLIE DESCRIPTION.

Horizon	Depth	Description
Ap	0-19cm	Dark reddish brown (5YR 5/2) clay loam. Moderately developed, fine to medium subangular blocky structure breaking to fine or medium crumb. Low packing density; moderately porous with fine fissures and some coarse fissures randomly orientated in horizontal and vertical planes. Medium size macropores; brittle; moderately firm; moderately sticky and very plastic; slightly moist. Common very fine distinct and clear reddish brown (5YR 4/4) mottles; moderate organic matter; fine abundant fibrous grass roots; few very small rounded stones; gradual irregular boundary into B horizon.
B	19-62cm	Dark reddish brown (5YR 3/4) clay loam. Moderately developed medium prismatic peds; medium packing density; moderately porous, with medium fissures. Semi-deformable, moderately weak; very sticky and very plastic. Few, fine, prominent and sharp black (5YR 2/1) mottles. Common, very fine, faint and clear reddish-brown (5YR 4/3) mottles. Low organic matter as common, medium, distinct and diffuse brownish black (5YR 3/1) mottles. Fine and many fibrous roots changing to common with depth. Few very small rounded stones.

An area 24m x 23m immediately adjacent to the perimeter fence of the Carbarns Works was enclosed by a fence in Spring 1985 to exclude cattle from the neighbouring field.

This area was divided into four blocks, each block consisting of 12 plots of 2m x 1.5m, on which 12 treatments were randomized (see Section 3.2.2). Each plot was separated from its neighbour by a 1m discard strip. A 5m discard was allowed between the experimental area and the enclosure fence.

To enable the long-term effects of sludge application on the concentration of soil heavy metals to be quantified, an 'unsludged' soil was sampled on the same farm at a distance of about 135m from the trial site (Nat. Grid Ref. NS 772 536), immediately adjoining the east side of the farm steading. This area was previously used as an orchard and now, following uprooting of the trees, serves as a small paddock where a mixed sward exists.

3.2.2 TREATMENTS

Twelve treatments, each replicated four times were set up. The treatments involved :-

a) Cultivation - not ploughed (0),
ploughed (1).

b) Liming - not limed (0),
limed to pH 6.3 (1).

c) Fertilizer N rate - 0 kg/ha (0),

250 kg/ha (1),

500 kg/ha (2).

These were combined in all possible ways, i.e. :-

Cultivation	Liming	Fertilizer N rate
0	0	0
0	0	1
0	0	2
0	1	0
0	1	1
0	1	2
1	0	0
1	0	1
1	0	2
1	1	0
1	1	1
1	1	2

3.2.2.1 CULTIVATION

During the second week in March, 1986 the 24 plots that had been designated for cultivation were dug up. As a tractor could not be used for the job, due to insufficient plot width, the plots were dug manually. To best mimic the action of a motorised plough, it was decided to invert the top 20 cm of soil by spade. The inverted grass layer was then vigorously broken up using a spade.

In order to obtain a comparable sward on both cultivated and uncultivated plots, the existing sward on the latter was killed off using glycophosphate i.e. 'Roundup'. To maximise kill-off a first dose was applied during the last week of March, followed by a second application 20 days later. On 27th May, 1986, following a base dressing of fertilizer, (see Section 3.2.2.3), the entire experimental area was sown to perennial ryegrass (*Lolium perenne* L. cv Springfield). Sowing was carried out by means of a controvertor. A seeding rate of 45 kg/ha was used.

3.2.2.2 LIMING

Soil samples from each of the 24 plots that were to be limed to pH 6.3 were collected before sowing. Using a hand auger, 15 individual cores, to a depth of 10 cm, from each of these plots were taken. The samples were then bulked and the pH determined on <2mm air-dried soil. The lime requirement of six samples (3 each from the ploughed and unploughed), chosen at random from the group of 24 samples, was measured. As the buffering capacity of each of the six samples analysed was very similar, the mean lime requirement was used to calculate the amount of $Ca(OH)_2$ required to increase soil pH to 6.3. In calculating the weight of $Ca(OH)_2$ needed for each individual plot, it was assumed that there is 2500t soil/ha to a depth of 20cm. Calcium hydroxide was used in preference to calcium carbonate as the latter, unlike $Ca(OH)_2$ is slow acting and contains a number of impurities making the attainment of the target pH that much more difficult, especially in trials of short duration.

The lime was surface applied by hand to the plots at the beginning of May, that is 3 weeks prior to sowing. Due to the time scale of this experiment (2 yrs) and the well-known difficulties in obtaining and mantaining target pH in fields trials, liming was carried out only on this one occasion.

The pH of all plots, for each depth sampled (see Section 3.2.2.4), up to and including 75 - 100mm, were measured at the end of the second season, using the soil samples collected for heavy metal analysis. Moreover, the pH of the eight control plots, i.e. cultivated and uncultivated with no lime and no nitrogen, was measured for all the depths sampled.

3.2.2.3 FERTILIZER N TREATMENTS

The first dressing of fertilizer N, as NH_4NO_3 (34.5% N), was applied one week prior to sowing (20th May 1986). Thereafter, N was applied one week after the spring and summer grass cuts.

The rates of N applied were 0 kg/ha, 250 kg/ha and 500 kg/ha. These rates represented the total N applied during one growing season and were split into three applications :-

	kg N /ha	
	Low N rate	High N rate
Before spring cut	105	210
Before summer cut	85	170
Before autumn cut	60	120
Total application	250	500

At the same time as fertilizer N application, a base dressing of 110kg P_2O_5/ha and 190 kg K_2O/ha as triple superphosphate (43% P_2O_5) and granular potash (60% K_2O) was given to all the 48 plots. In common with N, the total dressings were split over three applications as follows :-

	kg P_2O_5/ha	kg K_2O/ha
Before spring cut	50	80
Before summer cut	30	70
Before autumn cut	30	40

Prior to sowing, each plot received an NPK dressing equivalent to that applied before the summer cut. In other words, in the first years only 145kg N/ha and 290kg N/ha were applied for the 250kg/ha and 500kg/ha treatments respectively.

The first fertilizer dressing of each season, that is the one prior to the spring cut, was spread about one month before the first forcasted harvesting date.

3.2.3 SOIL SAMPLING

Prior to sowing but following cultivation and sward kill-off, soil samples were collected from each of the 48 plots on the 24th April 1986, and five soil cores collected per plot. These cores (54mm i.d.) were taken randomly from outside the herbage cutting area of each plot and each core subdivided into seven depths :-

0 - 25 mm
25 - 50 mm
50 - 75 mm
75 - 100 mm
100 - 150 mm
150 - 200 mm
200 - 300 mm

The corer used consists of a tall stainless steel pipe with a wide rim attached at the 20cm mark, serving both as a 'depth gauge' and as an aid to pushing the corer into the substrate. The boring end is tapered to facilitate removing the soil core. The 200 - 300mm samples were collected using a dutch auger. The 0 - 200mm core was divided on a graduated wooden board using a stainless steel knife. The five replicate cores, at each individual depth, were then bulked and kept in polythene - lined paper bags for subsequent analysis.

Soil sampling was repeated at the end of the second year, that is at the end of the second growing season on the 15th October 1987. On this occasion, the five soil cores were randomly collected from the whole area of each plot.

The 'uncontaminated' site was similarly sampled on the 24th April 1986 and the 15th October 1987. Five samples were collected, each composed of five cores taken in a zig-zag fashion from throughout the paddock.

3.2.4 HERBAGE SAMPLING

Five grass cuts were taken; two during the first growing season and three during the second, i.e. :-

13th August	1986
6th October	1986
27th May	1987
13th August	1987
7th October	1987

The grass was harvested with a motorized 'Agria' grass cutter which provides a 1.2m wide cut. As a result of this cutting width a 0.15m wide 'buffer zone' was present at each side of the cutting edge from which the first year soil samples had been taken (see Section 3.2.2.4). The fresh weight yield of each plot was recorded on site and herbage samples returned to the laboratory for drying and heavy metal analysis (see Section 2.2).

In view of the fact that some grass from each plot would be required for heavy metal analysis, samples were taken using stainless steel hand shears from a central strip running the length of the plot prior to the grass being harvested with the mechanised grass cutter. This procedure was followed to avoid soil comtamination which would have been inevitable had the samples been taken from the swath of the 'Agria'. These samples were included in the fresh-yield measurements. At the end of each harvesting session, the entire trial site was cut and the grass removed to ensure a uniform sward. For the first cut (August 1986) much of the sward consisted of chickweed (*Stellaria media* L.). Although this was included for fresh weight determination, it was excluded from the samples destined for trace metal analysis. After this first cut the chickweed was out-competed by the faster growing ryegrass.

3.2.5 EXPERIMENT II - POT WORK

Top soil (0 - 10cm) from Temple Field at the West of Scotland College (Nat. Grid Ref. 381 237) was sampled on the 3rd December 1986 and air-dried and milled (as described in Section 2.2.1.1). This soil is an imperfectly drained loam on reddish brown clay till of the Bargour series. A 50 : 50 (v/v) soil-perlite mixture in plastic potting bags (5l) was used as a growing medium for perennial ryegrass (*Lolium perenne* L. cv Springfield). Perlite was added to <2mm air-dried soil to facilitate drainage.

A series of pH treatments (pH 4.0 - 9.0; in increments of 0.5 units) were set up by adding appropriate amounts of $Ca(OH)_2$ and $Al_2(SO_4)_3$ to soil. Digested sewage sludge, from the Maudslie Sewage Treatment Works near Lanark, equivalent to an application rate of $55m^3$/ha was then incorporated into each of the soil-perlite mixtures. The composition of the sewage sludge is given in Table 3.3. After a two month

TABLE 3.3 COMPOSITION OF MAUDSLIE SEWAGE SLUDGE APPLIED

pH	6.8
% dry soilds	1.4
METAL	mg/kg dry solids
Cd	3.6
Cr	65
Cu	297
Ni	22
Pb	326
Zn	616

'equilibration' period during which time the pots were kept moist, the pots were sown with ryegrass at a rate of 50 kg/ha. To ensure an adequate water supply, each pot was kept on a perlite bed regularly watered with deionised water. In the greenhouse, the pots were placed into four seperate blocks (representing the 4 replicates per treatment). Within each block the 11 pH treatments were placed in a random fashion. So as to maintain good and healthy growth throughout the experiment a standard liquid feed (see Appendix B) was applied to the soil surface on three occasions. Two herbage cuts were obtained, the grass being cut when 15 - 20cm long. The soil pH of each of the 44 pots was recorded at the start, i.e. after the addition of $Ca(OH)_2$ or $Al_2(SO_4)_3$, and after the second cut. Fresh and dry weight herbage yields were recorded and herbage and soil analysed for total Cd, Cr, Cu, Ni, Pb and Zn.

3.3 RESULTS

3.3.1 CARBARNS FIELD TRIAL

Soil concentrations of total (aqua regia-extractable) Cd, Cr, Cu, Ni, Pb and Zn concentrations within the depths 0-25, 25-50, 50-75, 75-100, 100-150, 150-200 and 200-300mm of samples collected in March 1986 and October 1987 were measured. With the exception of Cr and Cu, total soil metal concentrations recorded in both years were similar (Appendix C). Chromium concentrations of the first year samples, when considered on a depth by depth basis, were, however, seen to be significantly ($P < 0.001$) greater than those recorded in the second year. In contrast, when considering depth as the sole source of variation, or in conjunction with cultivation, total Cu concentrations in the 1987 samples were significantly ($P < 0.05$) higher than those measured in 1986.

In the uncultivated soil, metal concentrations were seen to change significantly ($P < 0.001$) with depth (Appendix C). With the exception of Ni which showed a significant ($P < 0.05$) increase between the 50 - 75mm compared to the 0 - 25mm depth, all other metals showed no significant change in concentration within 0 - 100mm (Table 3.4). However, at depths below 100mm metal concentrations decreased significantly ($P < 0.05$) as depth increased (Figures 3.1 - 3.6).

Significant ($P < 0.001$) differences existed between the total metal concentrations, in the soil profile of the sludged uncultivated plots and the unsludged soil (Appendix C). In contrast to the previously

TABLE 3.4 CHANGES IN TOTAL Cd, Cr, Cu, Ni, Pb AND Zn CONCENTRATIONS (mg/kg AIR-DRY SOIL) WITH DEPTH IN THE UNSLUDGED (UNSL.), UNCULTIVATED (UNCULT.) AND CULTIVATED (CULT.) SOIL PROFILES (CARBARNS FIELD EXPERIMENT).

		Cd			Cr	
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	1.54	1.24	0.87	113.7	100.8	97.1
25-50	1.45	1.30	0.95	118.1	106.0	99.3
50-75	1.46	1.36	0.93	120.1	110.8	103.0
75-100	1.47	1.43	0.94	116.9	112.8	103.9
100-150	1.25	1.32	0.87	108.4	109.8	107.0
150-200	1.05	1.11	0.89	101.1	103.0	107.5
200-300	0.79	0.78	0.90	103.2	103.7	114.1
S.E.D.(1)		0.13			4.87	
S.E.D.(2)		0.07			2.86	
S.E.D.(3)		0.16			6.27	

		Cu			Ni	
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	78.3	64.1	39.5	27.2	24.3	28.1
25-50	80.8	69.6	41.4	28.7	26.3	29.3
50-75	82.1	75.0	41.1	30.6	28.5	30.6
75-100	81.5	77.5	41.5	30.8	29.1	30.5
100-150	71.7	74.9	42.9	26.7	27.4	32.2
150-200	61.6	65.5	40.8	23.2	24.3	32.8
200-300	50.3	49.4	44.0	20.5	21.4	32.8
S.E.D.(1)		6.74			1.78	
S.E.D.(2)		3.96			1.05	
S.E.D.(3)		8.68			2.29	

		Pb			Zn	
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	169.4	127.4	108.8	255.8	209.3	164.5
25-50	179.3	146.7	111.6	252.2	228.5	167.2
50-75	185.4	158.7	119.3	266.1	251.2	167.0
75-100	183.5	166.0	116.8	275.3	256.0	158.2
100-150	163.0	156.2	114.6	245.7	242.9	155.2
150-200	120.2	123.6	112.9	193.6	205.6	153.3
200-300	80.0	86.3	132.7	139.0	142.8	143.8
S.E.D.(1)		29.43			30.03	
S.E.D.(2)		17.28			17.64	
S.E.D.(3)		37.87			38.64	

FOOTNOTE: S.E.D.(1) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTHS BETWEEN EITHER UNSLUDGED VS. UNCULTIVATED OR UNSLUDGED VS. CULTIVATED.

S.E.D.(2) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTH BETWEEN CULTIVATED VS. UNCULTIVATED.

S.E.D.(3) = VERTICAL COMPARISON OF DEPTHS WITHIN UNSLUDGED.

FIGURE 3.1 CHANGES IN TOTAL CADMIUM CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES

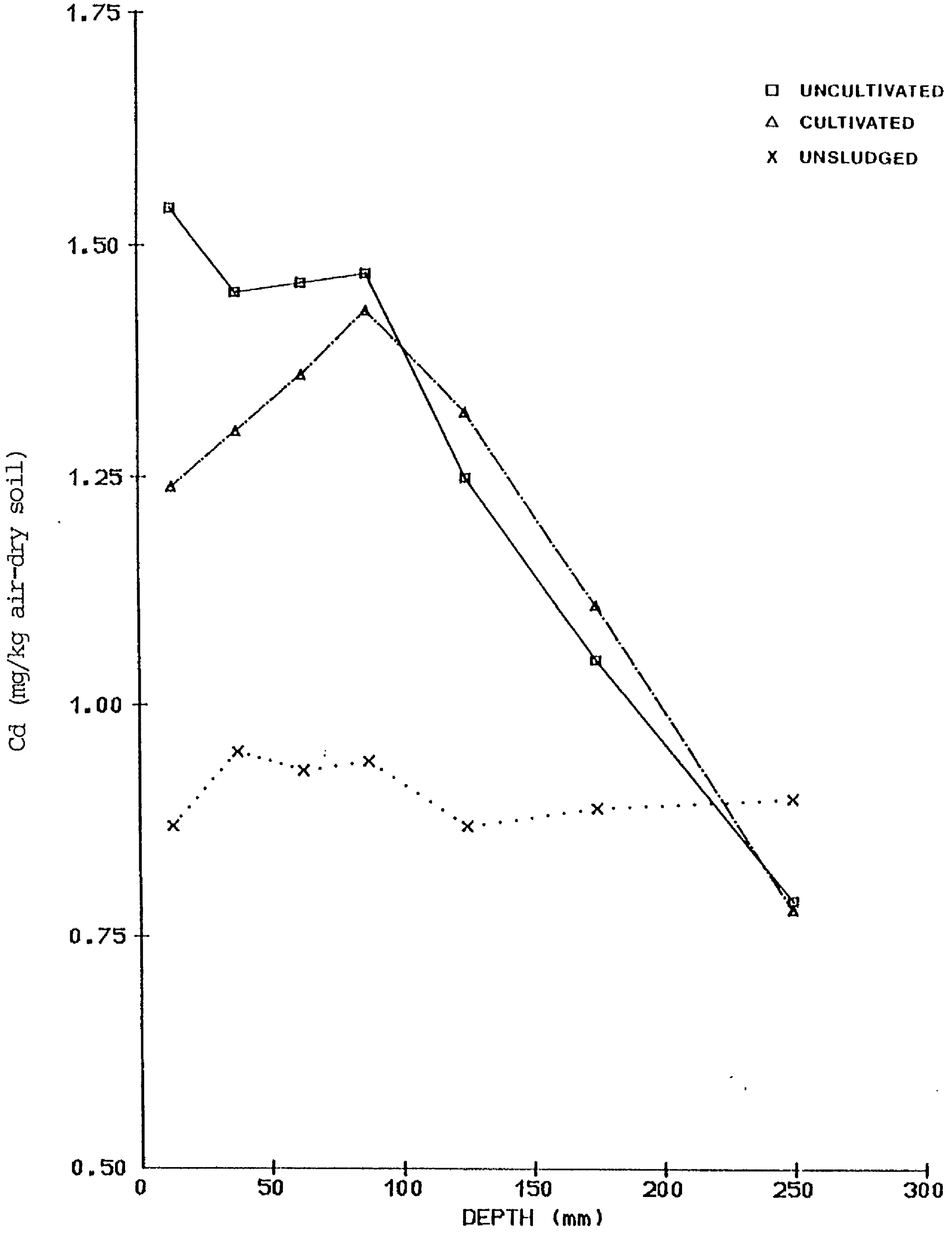


FIGURE 3.2 CHANGES IN TOTAL CHROMIUM CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES

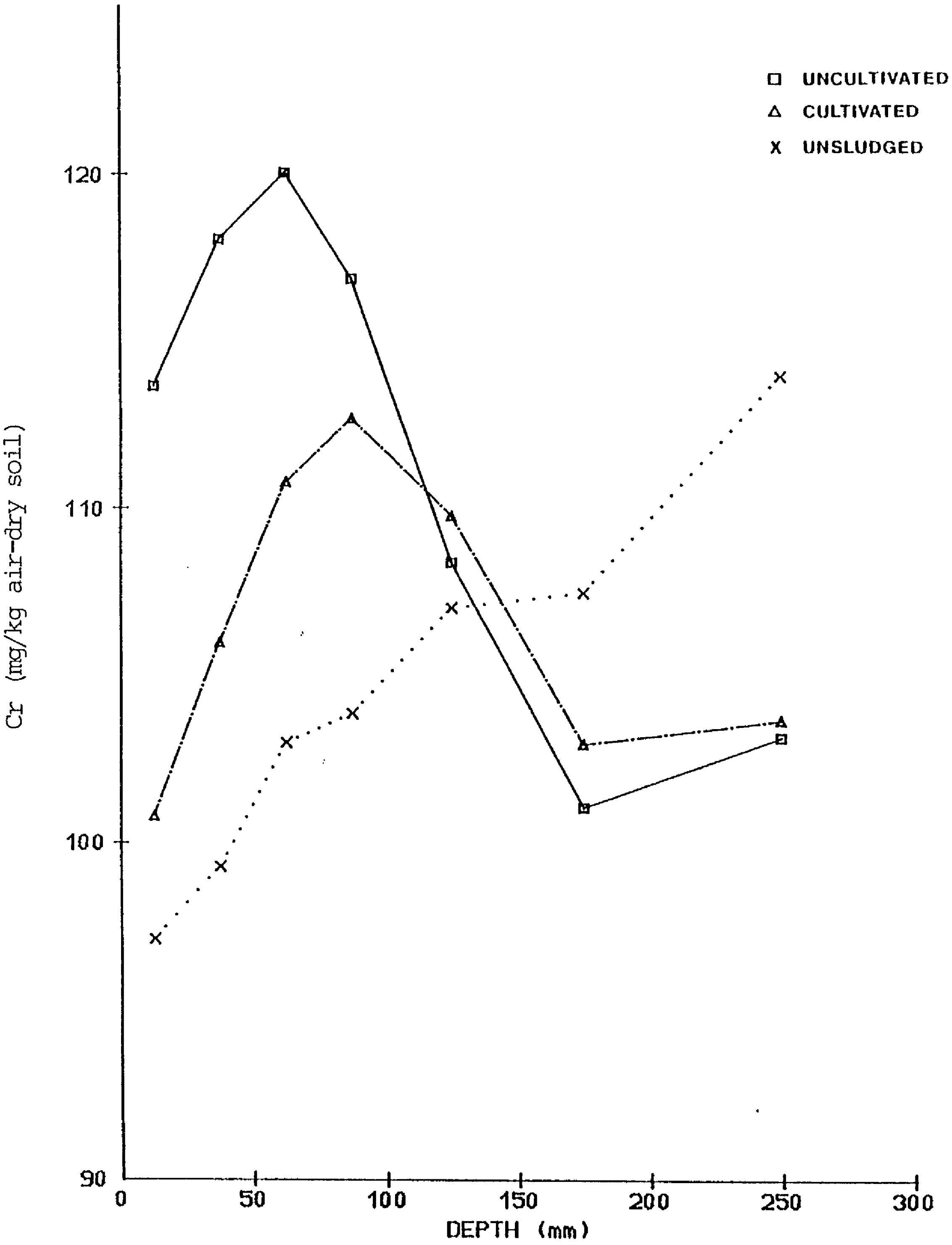


FIGURE 3.3 CHANGES IN TOTAL COPPER CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES

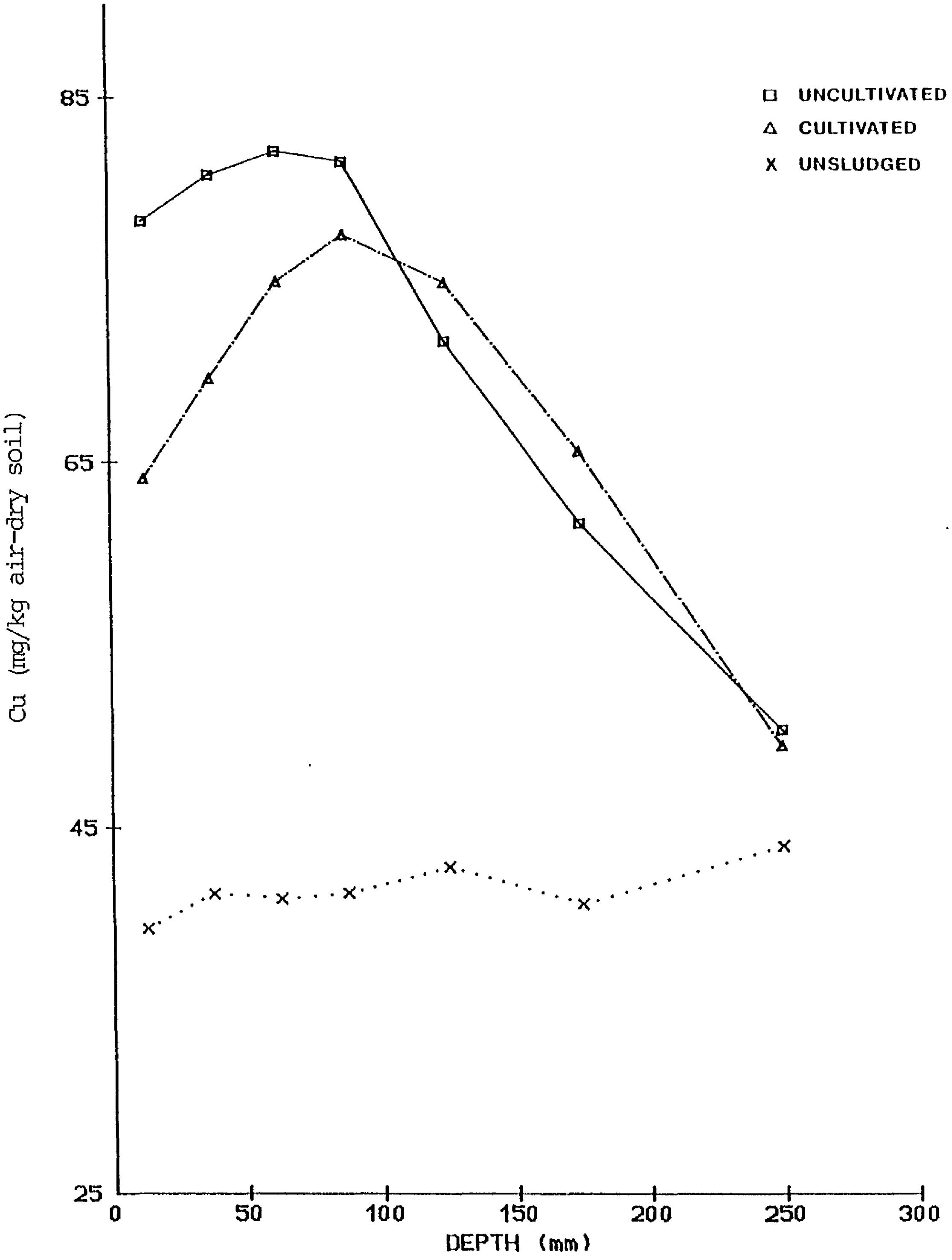


FIGURE 3.4 CHANGES IN TOTAL NICKEL CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES

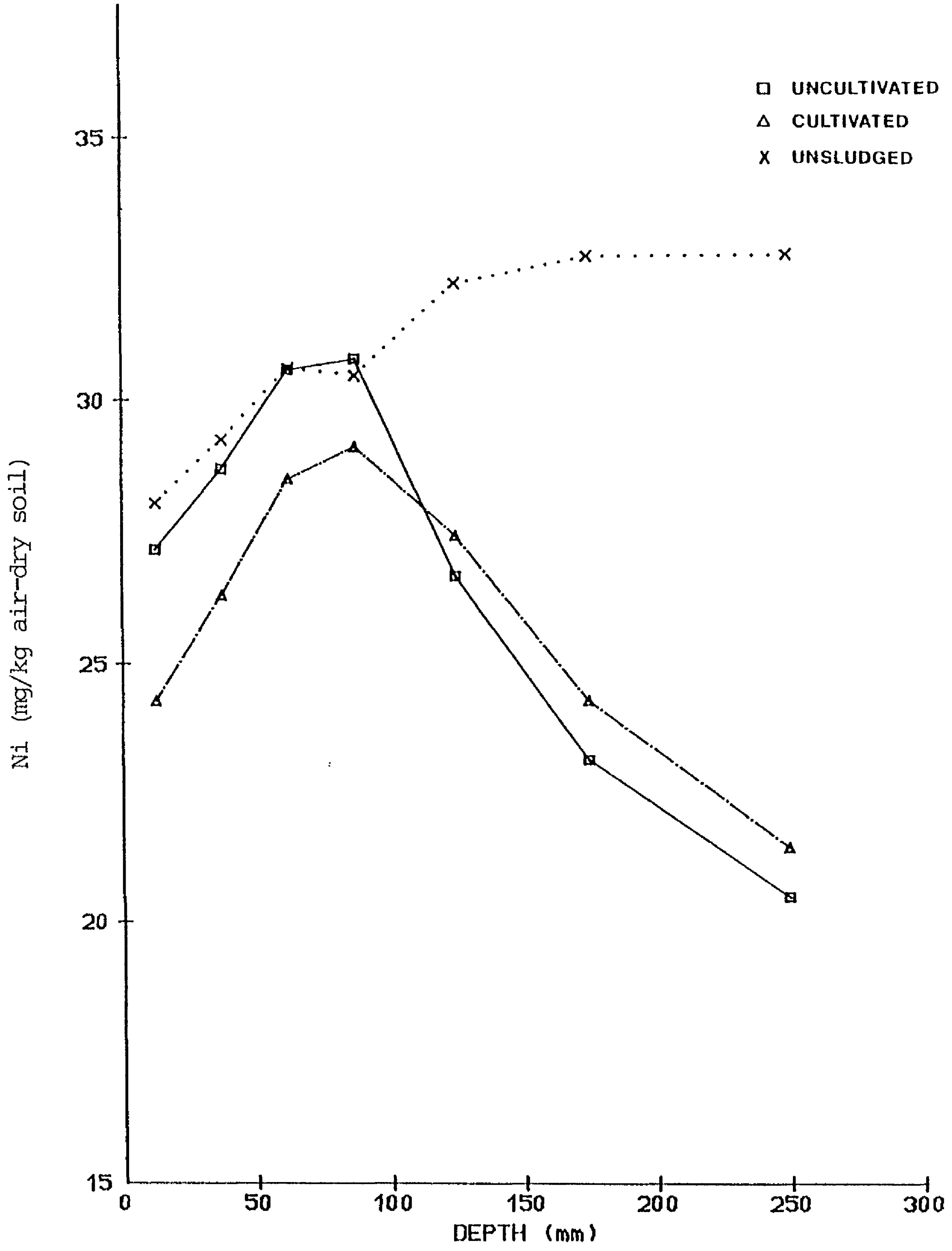


FIGURE 3.5 CHANGES IN TOTAL LEAD CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES

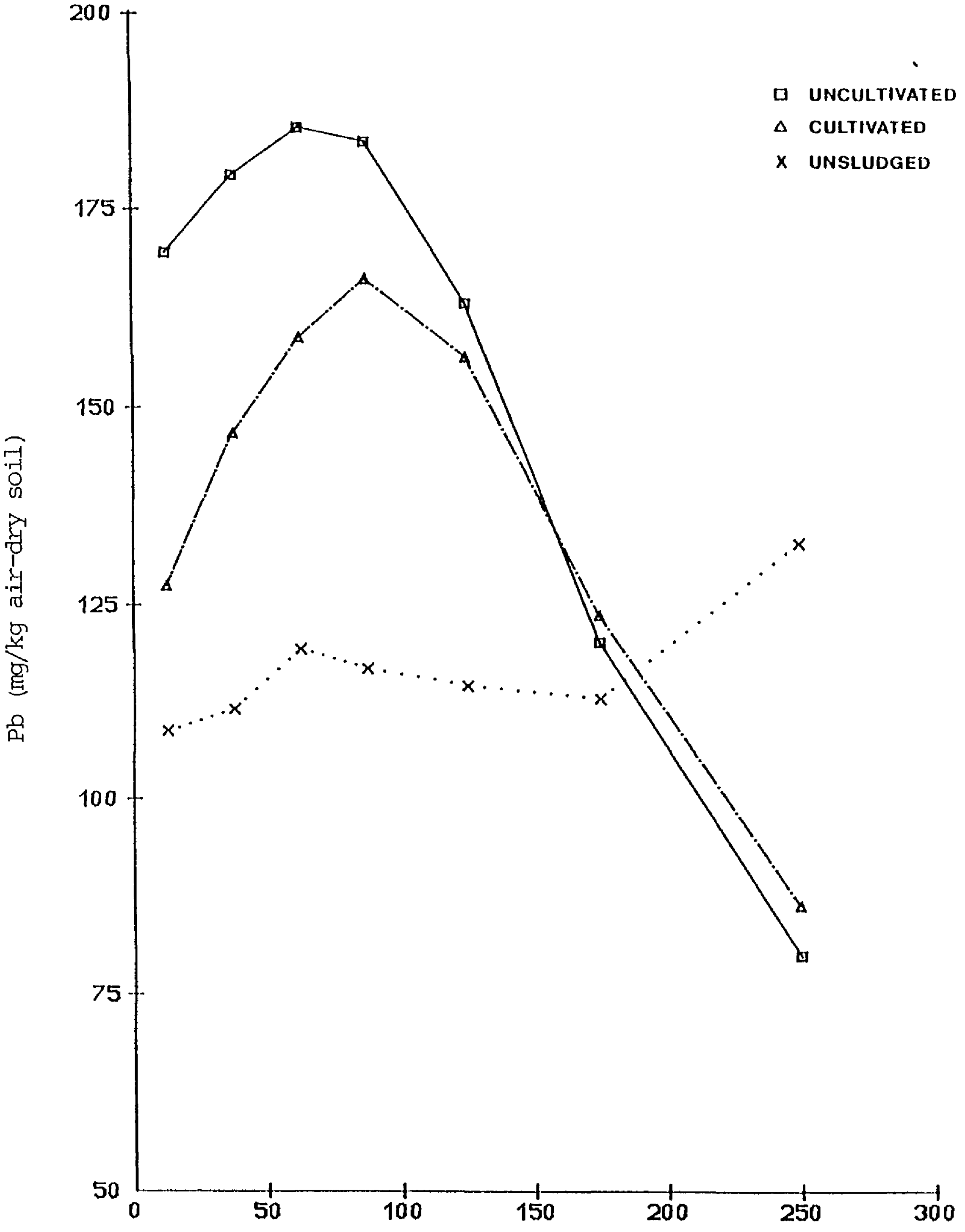
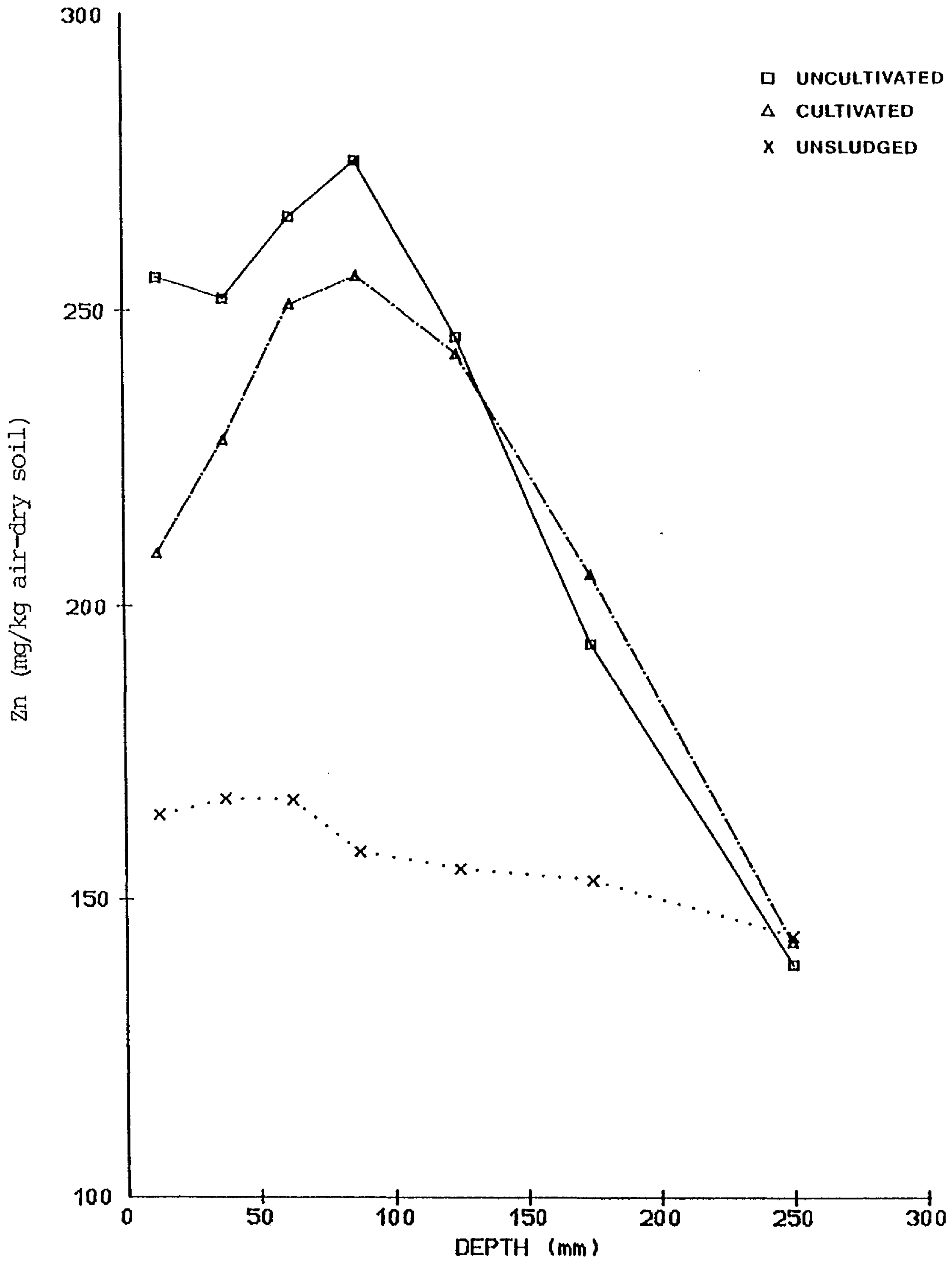


FIGURE 3.6 CHANGES IN TOTAL ZINC CONCENTRATION IN THE UNCULTIVATED, CULTIVATED AND UNSLUDGED SOIL PROFILES



described situation for the uncultivated sludged soil, the metal concentrations in the soil profile of the unsludged soil were constant with depth (Table 3.4). Chromium and Pb concentrations in each of the layers making up the 0-100mm depth in the uncultivated sludged soil were significantly ($P < .0.05$) greater than the concentrations present in the unsludged soil. This significantly ($P < 0.05$) increased metal concentration in the sludged uncultivated soil over that present in the unsludged soil profile was evident to a depth of 150mm in the case of Zn and Cd and down to 200mm for Cu. Over the 0-100mm depth the concentrations of Cu, Cd, Zn, Pb and Cr in the uncultivated sludged soil were on average 99, 61, 60, 57 and 16% respectively greater than that found in the unsludged soil. In contrast, not only were the Ni concentrations in the 0-100mm depth of the unsludged soil no smaller than those recorded in the sludged uncultivated soil, but below this depth Ni concentrations in the unsludged soil were significantly ($P < 0.05$) greater than those recorded in the sludged soil.

Mean total soil metal concentrations (i.e. the weighted mean of metal concentrations over all 7 depths) was not affected by either the application of lime or fertilizer N (Appendix C). Similarly, cultivation did not alter these total metal concentrations within plots. However, it did lead to a very significant ($P < 0.001$) change in the distribution of metals in the soil profile (Appendix C). Metal concentrations in the cultivated soil increased with depth to reach a maximum concentration in the 75 - 100mm layer (Figures 3.1 - 3.6). Below this, concentrations declined to reach a minimum in the 200 - 300mm sampling depth (Table 3.4). Metal concentrations in the surface

0-75mm were seen to be lower in the cultivated plots than in the uncultivated soil (Figures 3.1 - 3.6). Differences in total metal concentrations were generally greatest between the cultivated and uncultivated soil for the 0 - 25mm depth where, on average, the uncultivated soil contained 24, 13, 22, 12, 33 and 22% more Cd, Cr, Cu Ni, Pb and Zn respectively than did the cultivated soil (Table 3.4). This reduction in surface soil metal concentrations brought about by cultivation resulted in Cr, Pb and Zn concentrations in the 0-50mm depth of the cultivated profile being no greater than those present in the unsludged soil (Table 3.4).

Herbage metal concentrations were influenced by all three management practices investigated, i.e. cultivation, liming and fertilizer N use. In the short term, cultivation was seen to have a significant ($P < 0.05$) effect on herbage Cu, Ni and Zn concentrations (Appendix D). However, in the longer term, herbage metal concentrations were significantly ($P < 0.05$) affected by liming and fertilizer N rate (Appendix D).

Cultivation significantly ($P < 0.05$) increased herbage concentration of Cu, Ni and Zn, of both the 1986 cuts, by about 18, 43 and 14% respectively in the summer cut (1st cut) and 7, 26 and 10% respectively in the autumn cut (Table 3.5). The declining trend in the herbage metal concentration from the cultivated plots relative to the uncultivated plots led to no significant difference being recorded in any of the second year cuts, with the exception of Ni which in the spring 1987 cut, showed a significantly ($P < 0.05$) higher (18%) concentration in the herbage sampled from the cultivated plots.

TABLE 3.5 HERBAGE Cd, Cr, Cu, Ni, Pb AND Zn CONCENTRATIONS (mg/kg DRY-MATTER) FOR ALL TWELVE TREATMENTS IN ALL FIVE CUTS (CARBARNS FIELD EXPERIMENT). EACH READING IS AN AVERAGE OF 4 REPLICATES.

TABLE 3.5 (i) CADMIUM.

					CUT		
		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	0.70	0.41	0.34	0.39	0.40
		250	0.63	0.40	0.49	0.27	0.45
		500	0.58	0.45	0.61	0.31	0.49
	LIMED	0	0.55	0.39	0.33	0.33	0.37
		250	0.47	0.43	0.47	0.35	0.52
		500	0.68	0.40	0.53	0.32	0.48
CULTIVATED							
	UNLIMED	0	0.58	0.40	0.39	0.36	0.47
		250	0.65	0.44	0.48	0.34	0.47
		500	0.57	0.46	0.50	0.29	0.51
	LIMED	0	0.64	0.37	0.39	0.32	0.41
		250	0.61	0.43	0.49	0.26	0.46
		500	0.48	0.42	0.56	0.33	0.51
		S.E.	0.07	0.03	0.03	0.02	0.03

TABLE 3.5 (ii) CHROMIUM

					CUT		
		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	0.73	0.96	0.98	1.16	1.20
		250	0.79	1.72	1.07	0.81	2.03
		500	0.55	1.12	1.51	0.76	1.15
	LIMED	0	0.71	1.10	0.78	1.00	1.36
		250	0.87	1.02	1.48	1.19	1.61
		500	0.83	1.08	1.26	0.88	1.62
CULTIVATED							
	UNLIMED	0	0.87	1.27	1.09	1.20	2.19
		250	0.63	1.27	1.21	1.02	1.32
		500	0.81	1.22	1.26	0.85	1.85
	LIMED	0	0.66	0.92	1.00	0.82	1.46
		250	0.99	1.09	1.27	1.36	1.99
		500	1.00	1.15	1.45	1.15	1.71
		S.E.	0.11	0.20	0.12	0.17	0.39

TABLE 3.5 (iii) COPPER

					CUT		
		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	9.49	7.53	4.74	4.54	7.51
		250	10.08	10.25	8.96	5.42	11.39
		500	9.45	12.37	12.86	8.02	12.76
	LIMED	0	8.79	7.53	4.91	4.40	7.28
		250	9.10	9.59	8.79	4.95	11.05
		500	8.92	10.63	11.65	7.99	12.17
CULTIVATED							
	UNLIMED	0	10.31	8.47	4.97	4.54	7.44
		250	12.09	11.16	10.57	5.71	10.96
		500	10.64	13.12	13.09	8.00	14.14
	LIMED	0	11.04	7.56	4.50	4.37	6.61
		250	11.17	10.45	9.30	5.27	10.49
		500	10.79	10.96	11.64	7.52	12.45
		S.E.	0.45	0.37	0.42	0.36	0.51

TABLE 3.5 (iv) NICKEL

					CUT		
		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	4.21	4.03	4.12	4.64	3.92
		250	3.68	5.03	4.20	4.38	3.44
		500	4.22	4.65	5.48	5.30	4.14
	LIMED	0	3.95	2.94	3.20	3.15	2.31
		250	2.73	2.47	2.58	2.79	2.10
		500	3.78	3.55	3.74	4.21	3.19
CULTIVATED							
	UNLIMED	0	5.65	5.17	5.19	4.61	4.51
		250	5.86	5.51	5.56	5.58	4.45
		500	5.18	6.30	6.08	6.48	4.83
	LIMED	0	5.30	3.97	3.28	3.58	2.79
		250	4.98	3.42	3.26	3.44	2.59
		500	5.17	4.10	4.17	4.55	3.64
		S.E.	0.62	0.62	0.53	0.55	0.54

TABLE 3.5 (v) LEAD.

				CUT		
	N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED						
UNLIMED	0	1.48	1.79	1.33	1.31	1.27
	250	1.39	1.62	1.74	0.73	1.62
	500	1.60	1.77	1.99	1.12	1.58
LIMED	0	1.40	1.63	1.29	1.44	1.19
	250	1.56	1.48	1.58	0.93	1.31
	500	1.51	1.55	1.92	0.80	1.22
CULTIVATED						
UNLIMED	0	1.50	1.91	1.43	1.17	1.34
	250	1.43	1.81	1.68	1.36	1.45
	500	1.52	1.55	1.79	0.94	1.77
LIMED	0	1.50	1.51	1.37	1.10	1.22
	250	1.46	1,52	1.51	0.88	1.56
	500	1.47	1.35	1.83	1.13	1.54
	S.E.	0.07	0.15	0.10	0.14	0.15

TABLE 3.5 (vi) ZINC.

				CUT		
	N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED						
UNLIMED	0	68.41	48.81	42.16	44.12	65.55
	250	66.86	63.72	66.64	53.81	89.14
	500	83.05	84.05	95.81	88.27	98.97
LIMED	0	59.33	42.22	41.08	38.97	52.66
	250	57.28	51.91	57.33	42.50	72.94
	500	69.78	66.48	78.13	78.28	81.64
CULTIVATED						
UNLIMED	0	77.27	55.91	52.42	50.97	70.58
	250	81.59	71.80	81.94	67.47	102.59
	500	79.58	90.08	100.11	87.59	115.56
LIMED	0	80.72	49.56	41.86	45.28	59.58
	250	74.95	64.67	71.47	57.20	83.80
	500	68.59	62.59	70.63	67.81	83.86
	S.E.	5.98	4.76	5.60	5.87	6.58

Liming of the soil was particularly effective in bringing about a significant ($P < 0.01$) decrease in herbage Ni concentrations, apart from in the first cut of 1986 (Table 3.5). On average, Ni concentrations were 33% lower in herbage from limed plots compared with the unlimed plots. Similarly, Zn concentrations were significantly ($P < 0.01$) reduced by about 18% as a result of liming , although in the August 1986 cut, only an 11% reduction was observed ($P < 0.05$). A significant ($P < 0.01$) decrease in herbage Cu concentrations of approximately 9% was observed in cuts 2 and 3 (i.e. the autumn 1986 and spring 1987 cuts respectively) in the limed plots. No such effect of lime on Cu concentrations was however observed in cut 4 (i.e. the summer 1987 cut) although liming resulted in a significant ($P < 0.05$) decrease in Cu concentrations in the autumn 1987 herbage, albeit of 6%.

The single surface application of lime was seen to have had a significant ($P < 0.01$) effect on soil pH (Appendix E). In the limed soil profiles, the pH of the 0-25mm 25-50mm, 50-75mm and 75-100mm depths were on average 0.9, 0.6, 0.4 and 0.3 pH units higher respectively than those recorded in these soil layers of the unlimed plots (Table 3.6). However, it was only in the 0-25mm and 25-50mm depths that this difference in pH between the limed and unlimed soil was significant ($P < 0.05$).

Soil pH was itself seen to be highly correlated with herbage Ni, Zn and Cu concentrations (Table 3.7). In fact, using best fit regression analysis (Appendix F) to help explain the observed herbage metal concentrations as affected by management practices (i.e. liming,

TABLE 3.6 CHANGES IN SOIL pH WITH DEPTH IN THE CULTIVATION, LIME NITROGEN APPLICATION TREATMENTS (CARBARNS FIELD EXPERIMENT).

		DEPTH/mm			
		0-25	25-50	50-75	75-100
UNLIMED	UNCULTIVATED	5.66	5.44	5.34	5.47
	CULTIVATED	5.33	5.38	5.51	5.45
LIMED	UNCULTIVATED	6.48	6.03	5.72	5.70
	CULTIVATED	6.38	6.05	5.88	5.73

S.E.(1) = 0.08 (same level of cultivation and lime)

S.E.(2) = 0.11 (all other comparisons)

	DEPTH/mm			
NITROGEN	0-25	25-50	50-75	75-100
0kg/ha/yr	6.23	5.86	5.53	5.46
250kg/ha/yr	6.00	5.81	5.74	5.71
500kg/ha/yr	5.66	5.51	5.64	5.61

S.E.(1) = 0.07 (same level of nitrogen)

S.E.(2) = 0.09 (all other comparisons)

TABLE 3.7 CORRELATION (r) BETWEEN HERBAGE METAL CONCENTRATIONS AND SOIL pH, ORGANIC MATTER CONTENT, TOTAL AND EXTRACTABLE METAL CONCENTRATIONS (0 - 100mm SOIL DEPTH) (FOR CLARITY ONLY SIGNIFICANT CORRELATIONS ($P < 0.05$) ARE SHOWN).

HERBAGE	TOTAL	ACETIC ACID	EDTA	%ACETIC ACID	%EDTA	pH	O.M.
CADMIUM							
1st CUT							
2nd CUT	0.37	0.32					
3rd CUT						-0.34	
4th CUT							
5th CUT						-0.33	
COPPER							
1st CUT		0.47		0.63		-0.40	-0.48
2nd CUT	0.36	0.44	0.32			-0.50	
3rd CUT	0.33		0.30			-0.31	
4th CUT		0.33				-0.42	
5th CUT						-0.35	
NICKEL							
1st CUT	-0.41			0.31		-0.56	-0.43
2nd CUT	-0.41			0.39		-0.69	-0.43
3rd CUT	-0.35			0.49		-0.79	-0.36
4th CUT	-0.36			0.46		-0.79	-0.34
5th CUT	-0.34			0.40		-0.79	-0.33
LEAD							
1st CUT	0.40	0.36					0.29
2nd CUT		0.30					
3rd CUT							
4th CUT	0.32						
5th CUT	0.37	0.32					
ZINC							
1st CUT				0.36	0.29	-0.59	
2nd CUT		0.30	0.34	0.38	0.41	-0.70	
3rd CUT		0.33	0.37		0.30	-0.56	
4th CUT			0.28	0.36	0.38	-0.66	
5th CUT		0.28	0.33	0.40	0.43	-0.70	

FOOTNOTE: HERBAGE Cr WAS NOT SIGNIFICANTLY RELATED TO ANY OF THE PARAMETERS SHOWN.

fertilizer N and cultivation) and soil parameters (i.e. total and extractable metal concentrations, pH and organic matter content over the top 100mm of soil within which depth most grass roots are found), soil pH was seen to best explain Cd, Ni, Cu and Zn herbage concentrations. Herbage Ni, Cu, Zn and Cd concentrations were inversely proportional to soil pH, although such a relationship did not hold true for Cd concentrations in any of the herbage sampled in 1986. In this instance, Cd concentrations were seen to be directly proportional to soil pH (Appendix F).

The concentration of metal in the herbage was seen to be influenced significantly ($P < 0.05$) by the application of N fertilier (Appendix D). In particular, Cu and Zn concentrations in herbage sampled after and including October 1986 were significantly ($P < 0.05$) increased as fertilizer N rate increased (Table 3.5). Copper and Zn concentrations in the herbage from plots receiving 250kg N/ha/yr were respectively 20-97% and 23-56% greater than the concentrations recorded for herbage from the no N control. Similarly, the concentrations of Cu and Zn in herbage receiving 500kg N/ha/yr contained respectively 14-47% and 9-46% more than herbage which had received half as much N. Although increases in herbage Cd, Ni and Pb concentrations were observed as the rate of fertilizer N application increased, this effect was not as pronounced nor as frequent as that recorded for Cu and Zn. For instance, herbage Cd concentrations only exhibited a positive response to fertilizer N in the first and last 1987 cuts, whereas in the second 1987 cut, herbage from the no N control contained significantly ($P < 0.05$) higher Cd concentrations (13%) than the N treatments. The response of Pb to fertilizer N was identical to that of Cd. For its part, Ni concentration in herbage which had received no N or the low N rate were similar but significantly ($P < 0.05$) lower than that derived from the high fertilizer N rate. The influence of N application rate on herbage metal concentration is further described in the predictive equations (explained previously and given in Appendix F). In these equations fertilizer N rate was considered as 0, 1 and 2 which represented 0, 250 and 500kg N/ha/yr respectively.

Chromium concentrations in the herbage were seen to be independent of all three management practices (Appendix D) although in the spring 1987 cut, fertilizer N rate was seen to have a significant ($P < 0.001$) effect on herbage Cr concentrations (Table 3.5).

Herbage dry matter yield was seen to be significantly ($P < 0.001$) increased by the application of fertilizer N (Appendix G). In contrast, both soil cultivation and liming did not change the overall herbage dry matter yield. Nitrogen application resulted in a significantly ($P < 0.05$) greater herbage dry matter production than that obtained from the plots which had received no fertilizer N. However, the dry matter yield from herbage receiving 500kg N/ha/yr was no greater than that obtained from herbage supplied with only half the amount of N fertilizer (i.e. 250kg N/ha/yr) (Table 3.8). The effect of fertilizer N on yield became more apparent with successive cuts. Whereas no significant effect was seen in the first cut following sowing (i.e. August 1986), in the October 1986 and May 1987 cuts application of fertilizer N led to a 41% increase in herbage yield, and this increased to 64% and 85% over the two subsequent cuts, that is August 1987 and

TABLE 3.8 DRY MATTER YIELD OF EACH OF THE 5 HERBAGE CUTS (TONS/ha). EACH READING IS AN AVERAGE OF 4 REPLICATES (CARBARNS FIELD EXPERIMENT).

	NITROGEN kg N/ha/yr	CUTTING DATE 08/86	10/86	05/87	08/87	10/87
UNCULTIVATED						
UNLIMED	0	5.37	2.37	4.17	3.83	0.92
	250	5.21	3.21	6.17	6.08	1.96
	500	5.92	3.25	5.71	5.92	2.12
LIMED	0	4.33	2.42	5.04	3.75	1.17
	250	5.21	3.42	6.25	7.29	1.92
	500	4.96	3.29	5.83	5.46	2.25
CULTIVATED						
UNLIMED	0	4.83	2.00	3.83	3.42	1.00
	250	5.67	3.29	5.50	5.83	1.71
	500	5.37	3.17	5.79	5.50	1.75
LIMED	0	5.67	2.33	3.83	3.54	1.12
	250	5.54	2.87	6.46	5.83	2.17
	500	5.00	3.29	5.87	5.75	1.75
	S.E.	0.58	0.17	0.38	0.51	0.15

October 1987 respectively.

Mean uptake for each of the six metals (Cd, Cu, Cr, Ni, Pb and Zn) in each of the five cuts are reported in Table 3.9. Of the three management practices investigated, soil cultivation was seen to have little effect (Appendix H). In fact, it was only in the first cut that the Cu and Ni uptake by the herbage from the cultivated plots was 20 and 42% higher respectively, than that recorded for herbage from uncultivated plots ($P < 0.05$). In contrast, the rate of N application had a very marked affect on metal uptake. With the exception of herbage sampled in the summer of 1986 (cut 1) the application of N led to a significantly ($P < 0.05$) higher uptake of all six metals (Appendix H). In particular, the uptake of Cu and Zn was significantly ($P < 0.05$) increased as fertilizer N rate increased. The uptake of Cu and Zn by herbage receiving 250kg N/ha/yr was increased by 86-180% and 77-158% respectively, over that recorded for the herbage which had received no N. A further significant ($P < 0.05$) increase of 16-35% and 13-31% in the uptake of Cu and Zn respectively, was observed in the herbage which had received 500kg N/ha/yr over that treated with 250kg N/ha/yr. The uptake of Cd, Cr, Ni and Pb was significantly ($P < 0.05$) increased by the application of 250kg N/ha/yr compared to that recorded for the herbage which had received no fertilizer N, with increases of 54-116%, 72-100%, 42-75% and 34-119% respectively. However, increasing the application rate to 500kg N/ha/yr led to no significant increase in Cd, Cr, Ni and Pb uptake over that recorded for 250kg N/ha/yr, although in the cut taken in autumn 1987 (cut 5) Ni uptake by herbage receiving the high nitrogen treatment was 37% greater than that of herbage

TABLE 3.9 HERBAGE Cd, Cr, Cu, Ni, Pb and Zn UPTAKE (g/ha). FOR ALL TWELVE TREATMENTS IN ALL FIVE CUTS (CARBARNS FIELD EXPERIMENT). EACH READING IS AN AVERAGE OF 4 REPLICATES.

TABLE 3.9 (i) CADMIUM

		N-FERTILIZER kg N.ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	3.83	0.96	1.41	1.50	3.46
		250	3.21	1.29	3.04	1.71	0.87
		500	3.50	1.42	3.46	1.83	1.04
	LIMED	0	2.29	0.96	1.67	1.25	0.42
		250	2.42	1.46	2.96	2.50	0.96
		500	3.54	1.29	3.00	1.75	1.08
CULTIVATED							
	UNLIMED	0	2.91	0.79	1.46	1.24	0.46
		250	3.79	1.50	2.67	1.92	0.83
		500	3.08	1.46	2.92	1.58	0.92
	LIMED	0	3.71	0.87	1.46	1.12	0.46
		250	3.58	1.29	3.12	1.54	1.00
		500	2.46	1.37	3.29	1.87	0.87
		S.E.	0.62	0.12	0.23	0.18	0.08

TABLE 3.9 (ii) CHROMIUM

		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	3.87	2.29	3.96	4.37	1.08
		250	4.12	5.75	6.62	5.04	3.50
		500	3.17	3.67	8.46	4.37	2.42
	LIMED	0	3.25	2.67	3.87	3.71	1.58
		250	4.50	4.17	9.25	8.50	3.04
		500	4.04	3.50	7.29	5.17	3.62
CULTIVATED							
	UNLIMED	0	4.25	2.54	4.25	4.04	2.25
		250	3.62	4.29	6.62	5.75	2.17
		500	4.37	3.92	7.25	4.67	3.12
	LIMED	0	3.79	2.12	3.71	2.87	1.54
		250	5.62	3.12	8.12	7.83	4.12
		500	5.04	3.75	8.50	6.58	2.92
		S.E.	0.86	0.71	0.71	1.09	0.68

TABLE 3.9 (iii) COPPER

		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	51.33	17.79	19.42	17.37	6.92
		250	53.21	32.92	55.17	33.17	21.75
		500	54.83	40.00	73.00	47.00	26.62
	LIMED	0	39.42	18.17	25.12	16.46	8.62
		250	47.42	32.42	54.33	36.00	21.12
		500	43.37	34.42	67.29	44.42	27.54
CULTIVATED							
	UNLIMED	0	48.54	17.08	19.62	15.33	7.46
		250	67.12	36.12	57.83	32.92	18.79
		500	57.25	41.75	75.79	44.04	24.75
	LIMED	0	62.96	17.67	17.21	15.42	7.58
		250	59.04	29.83	60.25	30.21	22.87
		500	51.62	35.96	68.17	43.04	21.87
		S.E.	5.45	2.03	3.86	2.95	2.00

TABLE 3.9 (iv) NICKEL

		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	22.58	9.29	15.21	16.79	3.29
		250	19.29	16.25	26.04	27.17	6.17
		500	23.92	15.71	30.83	30.71	8.33
	LIMED	0	18.33	7.00	15.42	11.75	2.62
		250	13.79	8.29	16.37	20.04	3.75
		500	17.04	11.37	20.42	24.46	7.33
CULTIVATED							
	UNLIMED	0	25.29	10.58	18.25	15.79	4.33
		250	30.92	17.46	29.92	33.25	7.12
		500	27.75	20.12	35.46	37.25	8.62
	LIMED	0	29.96	9.46	11.83	12.67	3.04
		250	25.87	9.46	20.25	19.08	5.46
		500	24.08	13.21	24.62	25.54	6.50
		S.E.	3.30	1.03	1.59	4.72	1.21

TABLE 3.9 (v) LEAD

		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/87	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	8.04	4.25	5.37	5.08	1.17
		250	7.33	5.21	10.71	4.50	3.17
		500	9.62	5.67	11.12	6.33	3.42
	LIMED	0	6.04	3.96	6.50	5.37	1.37
		250	8.08	5.04	9.87	6.54	2.50
		500	7.46	5.04	11.25	4.58	2.75
CULTIVATED							
	UNLIMED	0	7.25	3.75	5.33	4.00	1.42
		250	8.17	6.12	9.25	8.08	2.54
		500	8.12	4.96	10.46	5.21	3.08
	LIMED	0	8.50	3.50	5.21	3.83	1.37
		250	8.17	4.42	9.75	5.00	3.46
		500	7.29	4.46	10.75	6.50	2.83
		S.E.	0.94	0.53	0.76	0.91	0.41

TABLE 3.9 (vi) ZINC

		N-FERTILIZER kg N/ha/yr	08/86	10/86	05/85	08/87	10/87
UNCULTIVATED							
	UNLIMED	0	369.17	114.17	168.33	162.92	56.67
		250	353.33	203.33	410.83	332.08	166.67
		500	502.08	272.92	540.83	515.83	20.71
	LIMED	0	262.50	101.25	210.00	145.83	62.08
		250	292.08	172.08	354.58	333.75	138.33
		500	339.17	213.75	448.33	441.25	185.00
CULTIVATED							
	UNLIMED	0	362.08	112.50	205.42	176.25	71.67
		250	455.42	232.08	450.42	393.33	171.67
		500	430.42	286.67	575.42	487.08	204.17
	LIMED	0	472.08	118.75	155.42	159.17	64.17
		250	399.58	184.17	454.58	333.75	181.25
		500	325.42	204.58	415.00	385.42	147.08
		S.E.	53.39	20.41	37.24	54.21	18.18

receiving 250kg N/ha/yr ($P < 0.05$). The application of lime had a significant ($P < 0.05$) influence on the uptake of Ni (Appendix H). In all but the first cut, Ni uptake by the herbage from the limed plots was 32-52% lower than that from unlimed plots. A similar significant ($P < 0.05$) reduction in Cu and Zn uptake by the herbage from limed plots was also observed (Appendix H). However, whereas a reduction in Zn uptake of 15-23% for herbage from the limed plots was recorded in cuts 1 - 3 (i.e. summer and autumn 1986 and spring 1987), it was only in the cut of autumn 1986 that lime application led to a significant ($P < 0.05$) reduction in the uptake of Cu, with the herbage from limed plots showing 10% less Cu uptake than the herbage from the unlimed plots.

Herbage metal concentrations were significantly ($P < 0.05$) correlated to acetic acid- and EDTA- extractable metal concentrations in soil (Table 3.7). However, for some metals such as Cd, these correlations were only apparent in one single cut, whereas other metals such as Zn and Ni showed significant ($P < 0.05$) correlations between herbage concentrations and percentage acetic acid- and EDTA- extractable soil concentrations, (i.e. the metal concentration extracted in acetic acid or EDTA expressed as a percentage of the total metal concentration), for all five cuts. Nevertheless, notwithstanding these significant correlations, even in the case of Zn and Ni, acetic acid- and EDTA- extractable levels did not explain the observed metal concentrations in the herbage when these were included in the predictive equations (Appendix F). In addition, unlike herbage metal concentrations, soil metals extracted by acetic acid and EDTA, whether expressed as a concentration or as a percentage of total, were independent of cultivation, lime and N fertilizer application (Appendix C).

In the soil profile, acetic acid- and EDTA- extractable metals, irrespective of whether considered as a concentration (Tables 3.10 and 3.11) or as a percentage of the total metal (Table 3.12), showed a similar distribution to that previously described for the total metal concentrations. Moreover, as was the case with total metal concentrations, the sludged soil contained significantly ($P < 0.01$) greater concentrations of acetic acid- and EDTA- extractable metals as compared to those present in the unsludged soil, except in the 200 - 300mm sampling depth (Tables 3.10 and 3.11). Furthermore , the proportion of total metal concentrations extracted by both EDTA and acetic acid was significantly ($P < 0.01$) greater in the sludged than in the unsludged soil (Appendix C). However, the proportion and concentration of metal extracted by both acetic acid and EDTA in both the cultivated and uncultivated profiles of the sludged soil were no different to those present in the unsludged soil at depths below 200mm (Table 3.12).

It should be pointed out that the acetic acid- and EDTA- extractable metal concentrations read for the second year samples were significantly ($P < 0.05$) greater than those measured in the first year (Appendix C) with the exception of EDTA- extractable Zn for which the converse was true. These differences could not be explained by either cultivation, liming or fertilizer N (Appendix C). In addition, these differences showed a significantly ($P < 0.01$) uneven distribution with depth, with no one uniform trend being common to the metals. Interestingly, there

TABLE 3.10 ACETIC ACID EXTRACTABLE Cd, Cu, Ni, Pb AND Zn CONCENTRATIONS (mg/kg AIR DRY SOIL) IN THE UNSLUDGED (UNSL.), UNCULTIVATED (UNCULT.) AND CULTIVATED (CULT.) SOIL PROFILES (CARBARNS FIELD EXPERIMENT).

		Cd			Cu	
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	0.82	0.59	0.36	5.90	6.76	1.74
25-50	0.74	0.59	0.38	7.59	7.88	1.88
50-75	0.75	0.61	0.30	8.69	8.39	1.76
75-100	0.70	0.63	0.33	9.01	8.49	1.48
100-150	0.59	0.59	0.23	8.33	8.39	1.64
150-200	0.42	0.54	0.32	7.69	7.69	1.64
200-300	0.22	0.27	0.52	6.23	5.97	1.21
S.E.D.(1)		0.10			0.87	
S.E.D.(2)		0.06			0.51	
S.E.D.(3)		0.13			1.12	

		Ni			Pb	
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	6.45	5.47	3.46	8.02	6.50	3.13
25-50	7.16	6.11	3.48	9.63	7.72	3.04
50-75	8.11	7.22	3.22	9.48	8.14	3.14
75-100	8.11	7.81	2.99	8.93	8.34	2.44
100-150	6.22	7.31	3.02	8.06	8.51	3.64
150-200	4.50	6.00	2.70	6.06	6.89	2.71
200-300	3.22	3.88	3.27	3.89	4.07	3.42
S.E.D.(1)		0.54			1.42	
S.E.D.(2)		0.32			0.83	
S.E.D.(3)		0.69			1.83	

		Zn	
DEPTH/mm	UNCULT.	CULT.	UNSL.
0-25	77.84	57.88	51.03
25-50	69.22	61.20	50.13
50-75	74.25	70.76	45.26
75-100	79.95	74.42	42.05
100-150	68.59	72.35	40.77
150-200	46.17	59.02	40.57
200-300	31.96	34.91	37.82
S.E.D.(1)		9.74	
S.E.D.(2)		5.72	
S.E.D.(3)		12.53	

FOOTNOTE: S.E.D.(1) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTHS BETWEEN EITHER UNSLUDGED VS. UNCULTIVATED OR UNSLUDGED VS. CULTIVATED.

S.E.D.(2) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTH BETWEEN CULTIVATED VS. UNCULTIVATED.

S.E.D.(3) = VERTICAL COMPARISON OF DEPTHS WITHIN UNSLUDGED.

TABLE 3.11 EDTA EXTRACTABLE Cu AND Zn CONCENTRATIONS (mg/kg AIR DRY SOIL) IN THE UNSLUDGED (UNSL.), UNCULTIVATED (UNCULT.) AND CULTIVATED (CULT.) SOIL PROFILES (CARBARNS FIELD EXPERIMENT).

	Cu			Zn		
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	39.3	31.8	16.4	75.8	50.1	52.3
25-50	41.7	34.6	16.6	63.8	53.0	49.8
50-75	42.2	37.7	16.5	67.4	62.5	45.4
75-100	41.9	39.1	16.2	72.7	67.3	41.7
100-150	36.7	37.6	17.4	59.5	65.2	40.0
150-200	29.9	32.7	16.0	37.3	51.6	39.9
200-300	24.0	22.9	16.0	25.3	27.4	34.3
S.E.D.(1)		3.45			9.02	
S.E.D.(2)		1.97			5.30	
S.E.D.(3)		4.31			11.61	

FOOTNOTE: S.E.D.(1) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTHS BETWEEN EITHER UNSLUDGED VS. UNCULTIVATED OR UNSLUDGED VS. CULTIVATED.

S.E.D.(2) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTH BETWEEN CULTIVATED VS. UNCULTIVATED.

S.E.D.(3) = VERTICAL COMPARISON OF DEPTHS WITHIN UNSLUDGED.

TABLE 3.12 CHANGES IN MEAN ACETIC ACID- AND EDTA-EXTRACTABLE METALS (EXPRESSED AS A PERCENTAGE OF TOTAL SOIL METAL CONCENTRATION) AND ORGANICALLY BOUND Cu (mg/kg AIR DRY SOIL) WITH DEPTH IN 2ND YEAR UNCULTIVATED (UNCULT.), CULTIVATED (CULT.) AND UNSLUDGED (UNSL.) SOIL PROFILES.

	Cd-% ACETIC ACID			Cu-% ACETIC ACID		
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	54.7	48.7	23.8	7.5	10.7	2.6
25-50	52.2	46.1	22.8	9.3	11.4	2.7
50-75	51.8	45.5	18.9	10.6	11.3	2.5
75-100	48.1	43.9	21.6	11.1	11.0	2.1
100-150	49.1	46.1	31.4	11.7	11.2	4.5
150-200	40.8	49.3	42.7	12.4	11.8	4.7
200-300	28.1	33.0	137.7	12.2	12.0	6.5
S.E.D.(1)		6.01			0.50	
S.E.D.(2)		13.17			1.09	
S.E.D.(3)		10.24			0.85	

	Ni-% ACETIC ACID			Pb-% ACETIC ACID		
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	23.8	22.6	7.2	4.8	5.2	1.6
25-50	25.1	23.2	7.0	5.6	5.4	1.5
50-75	26.8	25.4	6.1	5.3	5.1	1.5
75-100	26.6	27.0	5.7	5.0	5.1	1.1
100-150	23.6	26.8	10.9	5.0	5.6	3.4
150-200	19.3	24.6	9.6	5.1	5.7	2.7
200-300	15.7	18.0	23.5	4.8	4.8	5.4
S.E.D.(1)		1.04			0.35	
S.E.D.(2)		2.27			0.77	
S.E.D.(3)		1.77			0.60	

	Zn-% ACETIC ACID			CU-% EDTA		
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	30.4	27.7	17.7	50.5	49.6	24.2
25-50	27.5	26.7	17.2	51.8	49.7	23.3
50-75	28.0	27.9	15.5	51.7	50.4	23.3
75-100	29.2	29.0	15.2	51.7	50.6	22.8
100-150	28.1	29.7	30.0	51.5	50.6	46.7
150-200	23.2	28.1	29.7	48.5	49.9	45.6
200-300	22.3	23.6	59.0	47.4	46.1	83.9
S.E.D.(1)		1.15			1.36	
S.E.D.(2)		2.53			2.98	
S.E.D.(3)		1.97			2.32	

	ZN-% EDTA			ORGANICALLY BOUND Cu		
DEPTH/mm	UNCULT.	CULT.	UNSL.	UNCULT.	CULT.	UNSL.
0-25	29.8	23.9	18.0	33.4	25.1	8.5
25-50	25.4	23.1	16.8	34.2	26.7	8.6
50-75	25.5	24.8	15.4	33.5	29.4	8.6
75-100	26.6	26.3	14.9	32.9	30.6	8.6
100-150	24.3	27.0	29.2	28.3	29.3	18.3
150-200	18.8	24.6	29.0	22.3	25.1	16.8
200-300	17.6	18.4	53.1	17.7	16.9	34.4
S.E.D.(1)		1.15			1.66	
S.E.D.(2)		2.51			3.64	
S.E.D.(3)		1.95			2.83	

FOOTNOTE: S.E.D.(1) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTHS BETWEEN EITHER UNSLUDGED VS. UNCULTIVATED OR UNSLUDGED VS. CULTIVATED.

S.E.D.(2) = HORIZONTAL AND DIAGONAL COMPARISONS OF DEPTH BETWEEN CULTIVATED VS. UNCULTIVATED.

S.E.D.(3) = VERTICAL COMPARISON OF DEPTHS WITHIN UNSLUDGED.

ORGANICALLY BOUND Cu = (EDTA minus ACETIC ACID) EXTRACTABLE Cu appeared to be less of a difference between measurements taken for each sampling depth in the first and second year for the cultivated plots.

Soil organic matter (% loss on ignition), measured for the first year soil samples, was seen to be significantly ($P < 0.05$) correlated ($r = 0.41$; $n = 48$) to herbage Ni concentrations in all five cuts (Table 3.7). Inclusion of this soil parameter, together with soil pH, resulted in predictive equations which best explained the observed changes in herbage Ni (Appendix F), where soil organic matter and herbage Ni concentrations were inversely related. Similar effects on herbage Cr and Zn were also present, however, these were transient and were recorded in only one single cut.

Soil metal concentrations, both total and extractable, showed significant ($P < 0.05$) positive correlations with soil organic matter content (Table 3.13). Best fit regression analysis (Appendix I) showed that soil organic matter was a major predictor of soil total and extractable metal concentrations, with the only exceptions of EDTA-extractable Cu concentrations and acetic acid-extractable Zn concentrations, which were best explained by their total soil metal content.

A measure of Cu and Zn bound to soil organic matter could be estimated by subtracting the acetic acid extractable concentration from the concentration extracted in EDTA (Berrow, personal communication). While this was possible for Cu, Zn concentrations extracted by both EDTA and acetic acid were similar. The amount of Cu bound to organic matter was seen to be unaffected by cultivation, liming and fertilizer N

TABLE 3.13 CORRELATION (r) BETWEEN SOIL pH, ORGANIC MATTER CONTENT, TOTAL AND EXTRACTABLE METAL CONCENTRATIONS AND % EXTRACTABLE LEVELS (FOR CLARITY ONLY SIGNIFICANT CORRELATIONS ($P < 0.05$) ARE SHOWN).

	ACETIC ACID	EDTA	%ACETIC ACID	%EDTA	O.M.	pH
Cd-TOT	0.64		N.A.		0.72	
Cd-Ac			N.A.		0.65	
Cd-%Ac					0.29	
Cr-TOT					0.60	
Cu-TOT	0.65	0.94	N.A.	N.A.	0.71	
Cu-Ac		0.71	N.A.	0.31		-0.39
Cu-EDTA				N.A.	0.67	
Cu-OM	0.54	0.98		0.40	0.76	
Cu-%Ac					-0.54	-0.46
Cu-%EDTA	0.31					
Ni-TOT	0.76		N.A.		0.71	
Ni-Ac			N.A.		0.66	-0.30
Ni-%Ac					0.37	-0.45
Pb-TOT	0.87		N.A.		0.69	
Pb-Ac			N.A.		0.62	
Pb-%Ac						
Zn-TOT	0.95	0.92	N.A.	N.A.	0.71	
Zn-Ac		0.98	N.A.		0.74	
Zn-EDTA				N.A.	0.79	
Zn-%Ac		0.57		0.89	0.43	-0.33
Zn-%EDTA					0.55	0.39

FOOTNOTE: N.A. = NOT APPLICABLE.

application (Appendix C). Organically-bound Cu (Table 3.12), as was the case for the soil organic matter content (Table 3.14), exhibited an uneven distribution with depth which was significantly ($P < 0.01$) affected by cultivation. In both cultivated and uncultivated plots the changes in Cu bound to organic matter were similar to those previously described for total soil metal concentrations.

Soil organic matter was highly correlated ($r = 0.76$; $n = 336$) to the organically-bound Cu fraction in the soil (Table 3.13). However, the concentration of soil Cu bound to organic matter was best explained by total soil Cu concentration and inclusion of soil organic matter into the predictive equation did not result in any significant improvement of the latter (Appendix I).

3.3.2 A POT TRIAL INVESTIGATING THE EFFECTS OF SOIL pH ON THE UPTAKE OF METALS

Table 3.15 reports the mean herbage Cd, Cr, Cu, Ni., Pb and Zn concentrations in both cuts. With the exception of Pb, herbage metal concentrations showed a significant ($P < 0.05$) decrease as soil pH increased from 4.5 - 5.0. With a further rise in soil pH over the range of pH 5.0 - 9.0, a gradual decrease in herbage metals was observed. However, Cu concentration in the second cut herbage exhibited a significant ($P < 0.05$) increase as soil pH increased over the range 5.0 - 6.5 , although above this pH herbage Cu decreased. Herbage Pb concentration was independent of soil pH.

TABLE 3.14 CHANGES IN SOIL ORGANIC MATTER CONTENT (% LOSS ON IGNITION) WITH DEPTH (CARBARNS FIELD EXPERIMENT).

DEPTH/mm	UNCULTIVATED	CULTIVATED
0-25	19.48	11.82
25-50	17.18	12.48
50-75	15.43	13.17
75-100	14.22	13.95
100-150	12.19	14.23
150-200	9.65	12.29
200-300	8.11	9.60

S.E.(1) = 0.36 (between uncultivated and cultivated)

S.E.(2) = 0.21 (within uncultivated or cultivated)

TABLE 3.15 CHANGES IN HERBAGE METAL CONCENTRATIONS (mg/kg D.M.) WITH pH. EACH VALUE IS AN AVERAGE OF 4 REPLICATES. (POT TRIAL).

	Cd		Cr		Cu		Ni		Pb		Zn	
pH	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
4.5	0.20	0.83	1.21	3.25	4.98	3.79	3.27	6.86	0.35	0.59	30.94	69.24
5.0	0.18	0.51	0.85	1.25	3.77	3.41	1.68	5.07	0.31	0.57	23.25	42.47
5.5	0.20	0.33	0.73	0.67	3.97	3.27	1.59	2.93	0.34	0.47	23.33	31.38
6.0	0.19	0.29	0.64	0.75	4.03	3.23	0.93	1.81	0.31	0.53	22.11	30.24
6.5	0.21	0.29	0.83	1.05	3.96	3.91	0.39	1.10	0.36	0.51	16.27	27.95
7.0	0.19	0.25	0.54	0.61	4.24	3.81	0.32	0.78	0.26	0.40	15.25	20.74
7.5	0.22	0.26	0.58	0.68	4.30	3.72	0.38	0.84	0.39	0.64	15.06	20.50
8.0	0.24	0.26	0.64	0.27	3.98	3.07	0.33	0.73	0.33	0.39	14.92	18.34
8.5	0.25	0.29	0.68	0.54	4.03	3.11	0.36	0.68	0.49	0.56	15.17	17.75
9.0	0.24	0.26	0.55	0.61	3.80	2.98	0.26	0.77	0.38	0.37	13.20	17.24
S.E.	0.01	0.04	0.14	0.57	0.38	0.13	0.37	0.53	0.06	0.11	3.10	3.83

3.4 DISCUSSION

Over the years, and in particular, over the last decade, the metal load of the anaerobically digested sewage sludge disposed on to land by the Lower Carbarns Sewage Works has decreased (Table 3.16). This reduction has been due to both the recession experienced by the local steel industry and also to tighter quality standards imposed on effluents discharged by industry. At present, the only industrial effluent treated by the Lower Carbarns Works is that originating from a film processing laboratory and some light engineering concerns. In the past, the industrial load of the sludge was supplemented by a number of steel works and plating shops which contributed significantly to the Cr, Cd and Zn load of the sludge. Application of sludge from the Lower Carbarns Sewage Works on to adjacent permanent grassland for over fifty years, has led to an increased metal concentration in the treated soil. This has been confirmed by trials reported in this work on the very same soil (see Table 3.4).

In the unsludged soil, metal concentrations (mg/kg air-dry soil) were in the range 0.87-0.95 Cd, 97-114 Cr, 39-44 Cu, 28-33 Ni, 109-133 Pb and 144-167 Zn (Table 3.4). Accumulation of organic matter and/or the activities of man, particularly in industrial areas, can strongly influence the total trace element content of the A-horizon (Berrow and Ure, 1985). In fact, these authors reported an increased concentration of Pb, Hg, Cu and Zn in the A-horizon relative to the concentration found in the B-horizon due to an accumulation of organic matter in the former. Concurrently, organic matter accumulation in the A-horizon resulted in a decreased concentration of Ba, Cr, Li, Mn, Ni, Ti and V

TABLE 3.16 TOTAL Cd, Cr, Co, Cu, Fe, Mn, Ni and Zn CONCENTRATIONS (mg/kg DRY SOLIDS) IN ANAEROBICALLY DIGESTED SEWAGE SLUDGE FROM THE LOWER CARBARNS SEWAGE WORKS IN THE YEARS 1978, 1983 AND 1987 (DATA SUPPLIED BY THE LOWER CARBARNS SEWAGE WORKS).

		YEAR	
METAL	1978	1983	1987
Cd	8.4	4.9	3.7
Cr	164	71	70
Co	11	6.8	5
Cu	506	487	418
Fe	24500	19300	21800
Mn	843	554	400
Ni	174	58	35
Zn	1324	935	775

relative to that found in the B-horizon (Berrow and Ure, 1985).

Concentrations of Cu, Cd, Zn, Pb and Cr over the entire 100mm sampling depth were respectively 99, 61, 60, 57 and 16% higher in the sludged soil than those recorded in the unsludged soil (Table 3.4). However, whereas metal concentrations in the unsludged soil showed no significant changes with depth, as was also reported by Berrow and Reaves (1984) from their survey of background metal concentrations in Scottish soils, in the sludged soil metal concentrations showed an uneven distribution in the soil profile with highest concentrations of Cd, Cr, Cu, Pb and Zn being recorded over the top 100mm. Thereafter, with each subsequent increase in sampling depth (i.e. 100-150mm, 150-200mm and 200-300mm) a significant reduction in metal concentration was recorded. The marked accumulation of added sludge metals in the surface 100mm of soil has been noted by many other authors (see Section 1.3.4). Berrow and Reaves (1985) attributed this to the immobility of applied metals and their consequent retention close to the zone of incorporation and to the influence of organic matter which is known to bind several metals, especially Cu, quite strongly (see Section 1.2.3). The accumulation of metals in the surface soil layers has led to a situation where the concentrations of metals in the sludged soil are only significantly greater than those in the unsludged soil up to a depth of 100mm for Pb and Cr, 150mm for Zn and Cd and up to 200mm for Cu, and at greater depths there is no indication of any metal enrichment due to sludge disposal to land. In marked contrast, not only did the Ni concentrations in the sludged soil show a significant increase with depth over the top 100mm, but curiously, Ni concentrations in the 100-300mm layer of the unsludged soil were greater than those recorded in the sludged soil (Table 3.4).

The total metal concentrations recorded in the sludged soil were unchanged by the application of both lime and fertilizer N. Similarly, cultivation did not lead to any significant change in the mean (0-300mm) soil metal concentration. However, soil cultivation did result in a radical change in the distribution of metals in the soil profile. Whereas metal concentrations in the uncultivated plots were highest over the 0-100mm depth, in the cultivated soil the highest metal concentrations were recorded over the 75-150mm depth (Table 3.4). As traditional cultivation involves soil inversion, such a redistribution of metal concentrations is to be expected. Soil inversion led to soil with relatively lower metal concentrations being translocated from the deep layers to the surface, with respective Cd, Cr, Cu, Ni, Pb and Zn concentrations being on average, 24, 13, 22, 12, 33 and 22% lower in the 0-25mm depth of the uncultivated soil than in the cultivated soil.

From a health aspect, the significant reduction in metal concentrations of the surface soil resulting from the cultivation of soil, may be of particular relevance in determining the total metal intake of grazing animals. During grazing, involuntary ingestion of soil together with herbage is known to occur (Healy, 1974) and does not appear to be the result of a depraved appetite (Healy, 1974). In New Zealand, under intensive farming conditions, soil ingestion by grazing animals has been recorded throughout the year and although a winter peak in soil intake has been observed, ingestion during the remaining part of the year accounted for about half the total annual soil intake (Healy, 1972). In this way, soil ingestion can account for as much as 15-25%

of the total dry matter intake of grazing animals (Healy, 1972 and 1974). However, the amount of soil ingested by grazing animals varies greatly and is known to be particularly affected by soil type, stocking rate, season, stock management and earthworm activity (Fleming, 1986; Healy, 1973). For instance, under New Zealand conditions, Healy (1973) noted that whereas sheep ingested relatively small amounts of soil when availability of fresh pasture herbage per sheep was approximately 300kg/ha, when available herbage fell to approximately 100kg/ha, sheep ingested approximately 600g soil/day. Under Scottish winter conditions intake of soil by grazing sheep may exceed 400g/day (Field and Purves, 1964). Measurements by Thornton (1974) showed that in South West England soil ingestion by grazing cattle over the winter months ranged between 140 to 1400g/day.

Ingested soil may be an important source of trace elements in the diet, and perhaps the main source of elements, such as Co, which are present in relatively small amounts in the herbage as against relatively higher soil concentrations (Thornton, 1974). The beneficial effects of such ingestion were recognised very long ago when, in England, Co deficiency in sheep was successfully treated by dosing with a suspension of soil in water whereas anaemic pigs were provided with upturned sods (Fleming, 1986). On the other hand, soil ingestion may also lead to undesirable effects, e.g. excessive wear of incisor teeth in sheep caused by the physical abrasion of the teeth with ingested soil (Healy and Ludwig, 1965).

It is now recognised that ingested soil is an important pathway for metal absorption by animals especially in areas which are naturally contaminated (geochemical pollution) or where enhanced metal levels have resulted from man's activities. Ingested soil as it passes through the animals's alimentary canal is subjected to a range of conditions associated with the digestive process. In particular, differences in pH between the abomasum (c. pH 3.0) and the rumen (c. pH 7.0) as well as the effects of enzymes and microbial flora in the intestines are important and exercise significant effects on metal absorption (Fleming, 1986; Kjellstrom et al., 1984). Other processes, such as complexation, may also be involved. Soil may contribute to the pool of elements in the intestinal solution and may thus either raise concentrations, or it may compete for elements in solution and thus lower their concentrations (Healy, 1972). These changes in concentration influence animal nutrition, for it is from this pool of elements in solution that elements are absorbed into the bloodstream. Alimentary tract conditions may, therefore, increase or decrease heavy metal availability to the animal. Short-term animal studies using radio isotopes showed that ingested soil can be a source of Co, Mn, Se and Zn (Healy et al., 1970 cited by Healy, 1973). Increased retention of Ca, Mg and P following soil ingestion has also been reported (Grace and Healy, 1974) and Healy (1974) has pointed out that ingested soil may constitute a useful and significant source of Cu, Mn, Se and I.

Ingested soil may supply more of the various elements than does herbage. For instance, cattle have been reported to ingest ten times more Cu, Pb and As from soil than they do from herbage (Thornton, 1974). Even where soils do not contain anomalous levels of elements, it is thought that soil ingestion exercises some effects. Thus Stratham and Bray (1975) considered that I intake by sheep from ingestion of a clay soil was greater than that from a sandy one, as sheep grazing on the latter had more goitrous lamb than those grazing on the former. One of the most common trace element disorders associated with livestock production is that of Cu deficiency. This is frequently associated with high levels of Mo in herbage and is exacerbated by high intakes of S. Iron has now been implicated in the complex mechanisms that bring about hypocupraemia in animals (Suttle et al., 1982) and this being so, soil intake is brought into sharp focus, as it can be a major source of ingested Fe (Healy, 1973). Recently, it has been suggested that a possible mechanism for copper depletion may first involve precipitation of FeS in the rumen followed by release of sulphide in the more acid abomasum with resultant trapping of potentially absorbable Cu as insoluble CuS (Suttle et al., 1984). Other elements such as Zn and Cd may also be involved and can also act as antagonists towards Cu absorption (Little, 1981).

Soil ingestion may be of particular importance and concern when considering the intake of Pb by grazing animals. Lead is a cumulative poison and its intake and translocation into the food chain is generally controlled effectively by its immobilization in plant roots. Lead is so immobile in the soil that the only effective pathway into the food chain is via direct ingestion of soil (Dean and Suess, 1985) as this route by-passes the soil-plant barrier. Cases of Pb poisoning in cattle, at times leading to death, are still reported occasionally around historical mining sites in Derbyshire, when animals stray on to mine spoil (Thornton and Abrahams, 1984). On these historically contaminated soils, the cattle take in the majority of the Pb in the form of accidentally ingested soil (Thornton et al., 1983 cited by

Thornton and Abrahams, 1984). Soil Pb concentrations in areas of varying Pb contamination have been shown to clearly reflect the Pb concentrations found in the blood of animals grazing herbage growing on these sites (Thornton and Abrahams, 1984). Russell et al. (1985) reported that at these sites up to 87% of the Pb assimilated by cattle was attributable to ingested soil, and though this proportion was greatest on heavily contaminated land, even where the soil was only slightly contaminated, soil ingestion contributed to more than 40% of the total Pb load assimilated by the animals.

In view of the above, soil cultivation, especially of those sites where metal concentration of the surface soil has been increased following man's activity, may be desirable. Soil cultivation, in bringing soil of a lower metal load to the surface, would lead to a reduced assimilation of potentially toxic metals, such as Pb and Cd, by grazing animals. Moreover, soil ingestion may result in more subtle ill-effect on the overall health of the grazing animal and need not necessarily be reflected in increased blood and tissue metal concentrations. For example, the substantial increases in the amounts of elements like Al, Fe, Mn and Zn in rumen liquor may affect rumen microflora, perhaps by influencing their metabolic activities, and/or by changing the rumen populations (Healy, 1972). In this respect Martinez and Church (1970; cited by Healy, 1972) observed both stimulatory and inhibitory effects in *in vitro* cellulose digestion.

Cultivation of the sludged soil at Lower Carbarns although reducing the concentrations of potentially toxic metals available to the ruminant in ingested soil, increased the metal content of herbage available for animal consumption. Herbage sampled from cultivated plots contained Zn, Cu and Ni concentrations which were circa 14, 18 and 43% respectively greater than those present in herbage growing on uncultivated plots (Table 3.5). However, when considering metal uptake by herbage, cultivation was seen to have a significant effect only on the uptake of Cu and Ni, but not on the uptake of Zn (Table 3.9).

Cultivation leads to changes in the physico-chemical conditions of the soil which are both varied and complex. When grasslands are used for agriculture, there is a decline in soil aggregation and soils become more compact (Foth, 1978). Soil compaction results in an increase in micropore space, and a decrease in both macropore and total pore space. Cultivation has the immediate effect of loosening the soil and increasing soil aeration and water infiltration (Foth, 1978). All these effects permit better growth and development of plant roots. A larger root system provides a larger soil volume from which the plant can absorb nutrients. Consequently, the total availability of metals to those plants growing on the cultivated soils would be greater than their availability to plants growing on uncultivated soils. However, were the increases in metal concentrations in herbage from the cultivated plots to be due solely to this greater root development, then an increase in the concentration of all metals, and not just of Cu, Ni and Zn, would be expected. Of possibly greater significance is the increased microbial activity resulting from improved aeration and organic matter incorporation in the cultivated soil. A stimulation of the rate of microbial decomposition of soil organic matter results in an associated increase in the rate of resolubilization of those metals bound or associated with the soil organic matter fraction. The significance of this process in terms of metal availability would be greatest for those metals such as Cu and Zn, which are known to be strongly associated with organic matter in soil (see Section 1.2.3). As such, the availability for plant uptake of these metals would be determined by the rate of organic matter breakdown. However, the enhancement of soil organic matter mineralization following cultivation is transient (Jenkinson, 1988). The short term nature of this effect of cultivation on mineralization and its implications to metal availability has been noted in this work. In fact, it was only over the first two cuts following soil cultivation (i.e. cuts 1 and 2) that Zn and Cu concentrations in herbage from cultivated plots were greater than those recorded in herbage from uncultivated plots. Moreover, this effect was less pronounced at the second cut (Table 3.5).

In the case of Ni, the effect of cultivation extended into the second year of the experiment, being detected in the Spring 1987 cut (cut 3). This extended effect may have arisen from a localised decrease in soil pH associated with the decomposition of the old grass sod which took place at some depth beneath the soil surface. The pH at which the concentration of Ni present in the aqueous phase of a soil-sludge mixture shows a sharp increase, as pH decreases, is much higher than that for Cu and Zn. Whereas the concentration of Ni in the aqueous phase of a soil-sludge mixture increases sharply as pH decreases below a 'threshold' pH of 6.3, for Zn and Cu this 'threshold' is lower at pH 6.1 and 4.8 respectively (Adams and Sanders, 1984).

The absorbtion of heavy metals by plants is highly dependent on soil pH (see Section 1.2.2.), so that the practice of liming to improve growth has important additional affects on crop composition. The uptake and hence toxicity of many metals is reduced by raising soil pH (see Section 1.2.2.) although Mo and Se are notable exceptions. At Lower Carbarns, the single surface application of calcium hydroxide, aimed at raising the soil pH from a background level of 5.5 (mean for the top 100mm) to a target pH of 6.3, resulted in a 0.9, 0.6, 0.4 and 0.3 unit increase in the pH of the soil sampled at depths of 0-25mm, 25-50mm, 50-75mm and 75-100mm respectively. However, it was only over the 0-50mm depth that this increase was found to be significant (Table 3.6).

The herbage concentrations of Cd, Ni, Cu and Zn, from both limed and unlimed plots, were best explained by soil pH (Appendix F), with herbage metal concentrations inversely proportional to soil pH. A similar relationship between soil pH and herbage heavy metal concentrations was evident where *L. perenne* was grown on soil ranging in pH from 4.5 - 9.0 (Table 3.15). In this pot experiment, a significant reduction in herbage Cd, Cr, Ni, Cu and Zn concentrations was recorded as pH increased over the range 4.5-5.0. As pH increased beyond this level no further significant reduction in herbage metal concentrations was recorded, not even for Zn and Ni whose uptake is known to be significantly affected by pH up to a value of 7.5 (Rothamsted Experimental Station, 1985).

In the field situation at Lower Carbarns, liming resulted in a decreased metal concentration in herbage. Nickel, Zn and Cu concentrations in herbage from limed plots were on average 33, 18 and 9% respectively lower than those recorded in herbage from unlimed plots. However, this significant reduction in herbage Ni and Cu concentrations was not evident in the first cut following lime application (i.e. Summer 1986).

Although Cu, Ni and Zn uptake was similarly reduced as a result of liming, the effect was smaller (Table 3.9). A reduction in the uptake of Cu was, for instance, only recorded in the Autumn 1986 cut (cut 2). Similarly, a significant affect of liming on the uptake of Zn was only evident in the first three cuts and no significant reduction in Zn uptake was observed in the Summer and Autumn 1987 cuts. In contrast, lime application reduced the uptake of Ni in all but the first cut, as was the case for herbage Ni concentrations.

Application of N fertilizer may affect the trace element contents of plants in several distinct ways. In the first instance, where the amounts applied are relatively large, up to 400kg N/ha/yr, soil conditions, such as the osmotic potential, can be significantly altered (Burridge et al., 1983). Secondly, changes in the trace element content of plants can occur as a consequence of increased plant growth. Thirdly, plant uptake of trace elements may be affected by changes in the relative amounts of the major elements (and minor nutrients) available to roots. Furthermore, each of these three types of effects has a variety of causes. Thus, it is often very difficult to explain observed effects of fertilizer N on the trace element content of plants under field conditions, because single factors can seldom be isolated.

At Lower Carbarns the rate of application of fertilizer N had a profound effect on the herbage concentration of Cu and Zn (Appendix D). As fertilization rate increased so too did the concentration of Cu and Zn in herbage. In fact, not only was there a significantly higher concentration of Cu and Zn in herbage which had received 250kg N/ha/yr over that recorded in the herbage which had received no N, but a further increase in the rate at which N was applied, i.e. 500kg N/ha/yr, led to a further significant increase in herbage Cu and Zn concentrations (Table 3.5). In practice, Cu and Zn concentrations in herbage which had received no N were respectively 20-97% and 23-56% lower than those found in herbage from plots supplied with 250kg N/ha/yr. In turn, the Cu and Zn concentrations recorded in the herbage from the latter plot were 14-47% and 9-46% lower respectively than those present in the herbage which had received 500kg N/ha/yr. The Cd, Pb and Ni concentrations in the herbage sampled in 1987 were also significantly affected by fertilizer N (Appendix D). However, these effects were neither as pronounced nor as consistent as those observed with Cu and Zn. For instance, whereas Pb concentrations in herbage sampled in Spring 1987 were seen to increase with an increase in fertilizer N rate, in the next two cuts (i.e. Summer and Autumn 1987) the herbage Pb concentration, which had received no N, was greater than that present in herbage sampled from plots which had received 250 and 500kg N/ha/yr (Table 3.5). In contrast, Ni concentrations in herbage receiving no fertilizer N and that which had received 250kg N/ha/yr were similar and at the same time significantly lower than the concentration recorded in the herbage which had received 500kg N/ha/yr (Table 3.5).

Application of fertilizer N had a very marked effect on the total dry-matter yield of the herbage, which increased as N application rate increased (Table 3.8). As such, the rate at which fertilizer N was applied was seen to have a very significant effect on the uptake of all metals in all but the first cut (Summer 1986). As a rule, the uptake of Cd, Cr, Ni and Pb by the herbage which had received 250kg N/ha/yr was greater than that recorded where no N was applied. However, increasing the fertilizer N application rate to 500kg N/ha/yr led to no significant increase in the uptake of Cd, Cr, Ni and Pb than that recorded where herbage had received 250kg N/ha/yr. In contrast, not only was the uptake of Cu and Zn increased as the application rate of fertilizer N increased from 0kg N/ha/yr to 250kg N/ha/yr, but a further increase in the uptake of both these metals was achieved when the rate of N fertilizer application was increased to 500kg N/ha/yr.

Two widely recognised direct effects of fertilizers on soil conditions are (i) impurities in the fertilizers may significantly increase the total trace element content of the soil and (ii) soil pH may be changed. Both (i) and (ii) can lead to changes in the availability of trace elements to plant roots, and thus, affect their uptake. The effect (i) depends strongly on the origin of the fertilizer used and trace element impurities in inorganic NPK fertilizers are more likely to be associated with the phosphate component (Swaine, 1962). At Lower Carbarns, application of NH_4NO_3 fertilizer did not affect the total soil metal concentration, not even that of the surface 0-25mm soil layer (Appendix D). An instance of effect (ii) is provided by ammonium sulphate which is well known for its acidifying effect on soil. Heavy applications of N in this form can produce effects on trace elements opposite to those that result from liming. Application of NH_4NO_3 can equally affect the pH of the soil. All ammonium salts cause soil acidification and loss of exchangeable cations, with the loss of Ca predominating in most soils (Wild, 1988). The main cause is the oxidation of NH_4^+ to yield protons and NO_3^-. Under field conditions some of the NO_3^- produced by this nitrification, is leached out of the soil as calcium nitrate. In addition, Ca may also be lost as an associate cation if fertilizer-derived NO_3-N is leached. With ammonium sulphate, Ca is leached with the sulphate making this the most acidifying of the commonly used fertilizers (Wild, 1988). However, the loss of Ca following the application NH_4NO_3 is much smaller because, if most of the nitrate which is added or formed by nitrification is taken up by the crop, there is no excess anion to be leached which would require Ca as a counter ion (Wild, 1988). Volatilization of NH_4^+ as NH_3 may also contribute in the acidification of the fertilized soil (Rowell, 1988). However, the affect of NH_4NO_3 on the pH of the receiving soil may be much more influenced as a result of the uptake of NH_4^+ and NO_3^- by plant roots. The subject of ammonium versus nitrate nutrition has been reviewed by Haynes and Goh (1978) and Kirkby (1981). Generally, as nitrate concentrations in the soil are usually much higher than ammonium concentrations, the main source of N for non-leguminous crop plants is nitrate (Wild, 1988). In nutrient solutions most crop plants grow equally well whether supplied with nitrate or ammonium given that relatively low NH_4-N concentrations are present and that pH is adjusted. For the majority of plants, the highest growth rates are recorded when a combination of both nitrate and ammonium ions is supplied (Clarkson and Warner, 1979), although certain species of plants may show preference to ammonium as opposed to nitrate and vice versa (Haynes, 1986). Flowing solution culture studies have shown that nitrate fed plants raise pH and ammonium fed plants lower it (Rowell, 1988). In the field situation, the effects on the pH of the bulk soil are very much smaller than those observed in the rhizosphere (Marschner, 1986). Root induced changes in the rhizosphere pH are brought about by the excretion of H^+ or HCO_3^-. This excretion is related to the cation/anoin uptake ratio and is an indication of the need to maintain electrochemical balance both in root cells and in the external solution (Marschner, 1986). The form of N supply has the most prominent influence on the cation/anion uptake ratio and thus on rhizosphere pH. When the rate of NO_3-N absorption exceeds that of NH_4-N, HCO_3^- is excreted and if the opposite is true, H^+ is excreted by the root (Marschner, 1986). The changes in the root zone are at a maximum when the soil pH is about 5.3, as was the soil pH of the unlimed Carbarns soil, because at this pH, diffusion of acidity is slow (Rowell, 1988). It is from within the rhizosphere that most of the uptake of micronutrients occurs (Rowell, 1988). Had the situation arisen where NH_4^+ was preferentially absorbed as can occur under relatively low soil temperatures or in non-nitrifying soils (Haynes, 1986), this would have resulted in a decrease in rhizosphere pH and hence an increased availability of heavy metals whose availability is greatly influenced by soil pH (see Section 1.2.2).

Increased growth in response to fertilizer application, especially of N, can also affect plant trace element concentrations for a number of reasons. For example, where the increased growth of shoots is not accompanied by a comparable increase in the uptake of trace elements, a dilution effect occurs. Such an effect might have led to the situation observed at Lower Carbarns where for the fourth cut (Summer 1987) the Cd concentration in the herbage from plots which had received no N fertilizer was greater than that recorded in the herbage which had been treated with both 250 and 500kg N/ha/yr (Table 3.8). A similar situation was noted for Pb concentration in this same herbage. Lead, unlike Cd which is rapidly translocated from the roots to the leaves, tends to be immobilized in the root and only a small fraction of that taken up is actually translocated (Webber, 1980). Hence, in the case of Pb the increased dry matter production may not have been matched by an equivalent increase in Pb translocation. Nitrogen application may also alter the stage of growth and its form, for example, the leaf:stem weight ratio. Different parts of plants are known to have different concentration of heavy metals. In general, the most metabolically-active plant parts, especially the strongly transpiring plant leaves, exhibit considerably higher heavy metal contents than stems and storage organs or fruits and seeds respectively (Davis and Coker, 1980; Kloke et al., 1984). Changes in the proportion of leaf, stem, reproductive, storage and fruiting tissues of the *L. perenne* sward as influenced by fertilizer N use and season (i.e. stage of maturity) would consequently have resulted in changes in the metal concentrations.

As well as the effect of fertilizer N on promoting growth, the botanical composition of a mixed pasture may also change as a result of fertilizer treatment, an instance being the suppression of clover by high N applications. Although the experimental field at Lower Carbarns was sown out solely to *L. perenne*, this effect will be relevant to grass/clover leys. Different species of plants have different capacities to absorb and translocate metals (see Section 1.4.2). For instance, on soils low in Cu, clover generally contains less Cu than does ryegrass, whereas on soils high in Cu, the converse is true (Mitchell et al., 1957; cited by Burridge et al., 1983). Thus, a change in the relative composition of a grass sward would influence the herbage metal concentration of a mixed sward.

Nitrogen is a major constituent of all proteins. In plants, both Cu and Zn are closely associated with a number of proteins. Copper is required for the synthesis of a number of proteins amongst which are plastocyanin, superoxide dismutase, cytochrome oxidase, ascorbate oxidase, phenolase, laccase and amine oxidase (Marschner, 1986). For its part, Zn is present in a number of plant enzymes: alcohol dehydrogenase, superoxide dismutase and carbonic anhydrase and is also required for the activity of various other types of enzymes such as dehydrogenases, aldolases, isomerases, transphosphorylases and RNA and DNA polymerases (Marschner, 1986). Copper is, in fact, highly correlated with the true protein content of plant material (Rasheed and Seeley, 1966).

At Lower Carbarns the increased concentration of Cu and Zn in herbage in response to fertilizer N from 0-500kg N/ha/yr may be attributed to the increased synthesis of protein. Similar positive interactions between N application and herbage Cu concentrations have also been established by Burridge et al. (1983) on soils having an adequate supply of Cu or on deficient soils which had been treated with Cu. In fact, it is well known that the Cu concentration in herbage growing on Cu-deficient soils is decreased by application of fertilizer N (Reith, 1975).

Chromium, its uptake and concentration in the herbage, unlike the other metals, was unaffected by any of the three treatments, that is, cultivation, liming and application of fertilizer N. Only on one single occasion was a relationship shown between either the uptake or concentration of Cr in the herbage and the application rate of fertilizer N. In this instance, the herbage which had received either 250kg N/ha/yr or 500kg N/ha/yr had a higher Cr concentration and uptake than was recorded in the herbage which had received no fertilizer N. Although, it is now well established that Cr is required by animals for glucose metabolism, an essential role of Cr in plant nutrition has not as yet been established (Davies and Jones, 1988). The chemistry of Cr in soil is poorly understood. It appears, that in soil Cr is present predominantly as Cr (III) as the relatively more phytotoxic Cr (VI) is rapidly reduced to the insoluble forms of Cr (III) in aerobic soils (Davies and Jones, 1988). However, Bartlett and James (1979) have suggested that in aerobic soils with high pH and low organic matter content, Cr (III) is unstable and is oxidized to Cr (IV). Chromium is so strongly fixed in near-neutral soils that it is hardly absorbed by plants (Jaworski et al., 1984). Transfer coefficients in the range of 0.01-0.1 (plant/soil) illustrate this behaviour (Kloke et al., 1984). The mechanisms involved in the uptake and translocation of Cr in plants are not understood. There is, however, evidence that Cr (VI) is reduced to Cr (III) within the plant and that irrespective of the form in which it is supplied most of the Cr is retained in the roots (Davies and Jones, 1988). Thus, any changes in Cr uptake, as a consequence of liming, cultivation or fertilizer N application, could have been masked by immobilization of Cr in the root and, therefore, any change in Cr availability brought about by grassland management practices is difficult to elucidate.

Although herbage analysis is obviously the more direct way of establishing the effects of soil management practices on the concentrations of metals in plants and, hence, their implications to plant and animal health, there are practical reasons why soil analysis is often preferred. For instance, one of the chief reasons is the possibility of being able to sample soils at almost any time of the year; herbage sampling, on the other hand, is much more restricted (Burridge et al., 1983). A large and varied array of soil extractants have, thus, been used, with varying degrees of success, to predict the availability of a number of metals for plant uptake (see Section 2.1).

When sludges are mixed with soil, there is a substantial reduction in the concentration of metals extracted by water (Bradford, 1973; cited by Page, 1974). However, the concentrations of all trace elements in the sludge-amended soil normally exceed those commonly observed for saturation extracts from soils not treated with sewage sludge (Bradford, 1973; cited by Page, 1974) and large increases in the extractable metal concentration in soils following sludging are well documented (Andersson and Nilsson, 1972; Berrow and Webber, 1972; Andersson and Nilsson, 1974; Bolton, 1975). At Lower Carbarns, the proportion of total metal concentration in the soil extracted by both acetic acid and EDTA was significantly greater in the sludged soil than in the unsludged soil (Tables 3.12). On average, over the 0-200mm depth, the proportion of total Cd, Cu, Ni, Pb and Zn extracted by acetic acid was 84%, 225%, 214%, 155% and 33% respectively greater in the sludged soil than in the soil which had received no sludge. On the other hand, the proportions of total Cu and Zn extracted by EDTA from sludged soil were only 64% and 22% respectively greater than from the unsludged soil. The concentration of acetic acid-extractable Cd, Cu, Ni, Pb and Zn, as well as the EDTA-extractable Cu and Zn was also significantly greater in the sludged soil than in the soil which had no history of sludge disposal (Tables 3.10 and 3.11). Over the 0-200mm depth, the concentration of acetic acid-extractable Cd, Cu, Ni, Pb and Zn in the sludged soil were on average 109%, 366%, 115%, 177% and 54% respectively greater than those recorded in the unsludged soil. On the other hand, increases in EDTA-extractable Cu and Zn concentrations in the sludged soil were not as pronounced, being only 134% for Cu and 40% for Zn.

Acetic acid and EDTA extractable metals, expressed either as a concentration or as a percentage of the total metal concentration, showed significant correlations with the metal concentrations found in the herbage (Table 3.7), as reported by a number of other workers (Richardson, 1980 and Berrow and Burridge, 1983). Such significant correlations have prompted many authors to state that the fraction of soil metals available for plant uptake could quite easily be predicted by determining the acetic acid- and EDTA- extractable metal concentration in the soil. One very important consideration when assessing the validity of using any extractant to determine the plant-available metal fraction in a soil, is to compare the response of actual plant metal uptake and metal extractability under changing soil conditions. Cultivation, liming and fertilizer N application all led to significant changes in herbage metal concentrations. However, none of these three management practices resulted in any significant changes in the amounts of metal extracted by either acetic acid or EDTA. This insensitivity of acetic acid- and EDTA- extraction to soil management practices tends to discredit the use of either of these chemical extractants for predicting metal uptake by plants. The inability of both acetic acid and EDTA to reflect changes in metal toxicity as a result of liming has been commented upon by Bolton (1975). This inability of both acetic acid and EDTA to reflect changes in plant metal concentrations subsequent to changes in soil physico-chemical conditions, particularly following lime application, has been ascribed to the 'swarming' effect these extractants have on soil pH (see Section 2.1). Since soil pH is a major determinant of heavy metal availability, it is for this reason that weaker extractants such as water and calcium chloride have recently been put forward as alternatives to acetic acid and EDTA. However, chemical extractants can never mimic living systems like plants. Plants respond to soil management practices in ways which can never be mimicked by chemical extractants. For instance, an increase in herbage metal uptake could be due to increased root growth and not to pH. Equally, changes in the soil microbial biomass, herbage dry-matter production and in the micro-environment of the root, brought about by varying management practices, cannot be taken account of by choice of extractant.

Substracting the concentration of metal extracted by acetic acid from that fraction extracted by EDTA, yields a measure of how much metal is complexed with organic compounds (Berrow, personal communication). Whereas acetic acid extracted similar Zn concentrations as did EDTA, the latter extracted much more Cu than did acetic acid. The soil chemistry of Cu is intimately related to the organic fraction in soil (see Section 1.2.3) and Cu availability is largely determined by the nature of the Cu-organic compounds complexes present in soil (see Section 1.2.3). However, at Lower Carbarns the concentration of Cu in the herbage showed no relation to the Cu held by soil organic compounds

(Table 3.7). In contrast, herbage Ni concentrations were best explained by soil pH and organic matter content (Appendix F). Nickel concentrations in the herbage decreased as the soil organic matter content increased. A similar relationship was exhibited by both Zn and Cr but this appeared to be a transitory or chance relationship holding for only one cut.

The accumulation of metals in the surface 0-150mm of the soil makes the application of sewage sludge to land a long-term pollution risk to both plant and animal components of the food chain. The absorption of metals into the food chain, via plant uptake or by involuntary soil ingestion by animals, is greatly affected by grassland management practices. Herbage Cd, Cu, Ni, and Zn concentrations are reduced by liming whilst increasing the rate of fertilizer N application increases herbage Cd, Cu, Ni, Pb and Zn concentrations which could not be explained by changes in bulk soil pH. Metal concentrations extracted by both acetic acid and EDTA did not reflect these changes in metal uptake. Chromium uptake was unaffected by either liming or fertilizer N rate. Ploughing and reseeding led to short-term (i.e. over the first two cuts) increases in herbage Cu, Zn and Ni. However, the resultant decrease in metal concentrations in surface soil after ploughing could be of considerable importance in lowering the dietary metal intake of grazing animals.

CHAPTER 4

EFFECTS OF LONG TERM SLUDGE APPLICATION ON SOIL MICROBIAL BIOMASS AND NITRIFICATION

4.1 INTRODUCTION

The soil microbial biomass, consisting of soil bacteria, fungi, algae and protozoa has been termed "the eye of the needle through which all dead organic matter entering the soil must pass as it is broken down to its organic components that plants can use once again" (Jenkinson, 1977). In terrestrial ecosystems, about 90% of the energy associated with soil organic matter is utilised by decomposers (Doelman and Haanstra, 1979). Without micro-organisms, the soil would become a repository of dead organic remains with no facility for recycling vital nutrients, such as C, N, P and S for plant growth.

Typically, the biomass comprises between 2-3% total soil organic C, equivalent to about 0.3-1t microbial C/ha in arable soils and considerably more in grassland (Brookes et al., 1984). Bacteria are the most numerous soil organisms with viable populations estimated at up to 2 x 10^8 individual cells/g soil (Harris, 1988). Bacteria are also the most diverse in terms of species and function (Harris, 1988). The soil microbial population is an important labile reservoir of N, P and S (Jenkinson and Ladd, 1981) and are fundamentally involved in important transformations of soil N such as ammonification, nitrification, denitrification and N_2 fixation. In this way, up to half of the total N uptake by cereal crops can be derived from soil organic N mineralization during the growing season (Rothamsted Experimental Station, 1981).

In addition to its role in nutrient cycling, the biomass and its metabolites are important in creating and stabilising soil structure (Elliott et al., 1983; Foth, 1978) and in the breakdown of pesticides (Harris, 1988b). Of considerable interest in recent years has been the possible significance of microbial excretions which influence plant growth. These include products such as ethanoic acid, ethylene and various plant regulatory compounds with gibberelin-like or indole acetic acid-like properties (Harris, 1988b). Moreover, the production by bacteria of powerful Fe chelating compounds called 'siderophores' have been described which result in the decline of root pathogens (Emery, 1980; cited by Harris, 1988).

About half a million tonnes of sewage sludge, as dry matter, are now applied annually as organic manure to agricultural land in Britain. Soil biomass generally increases in response to inputs of decomposable organic substrates (e.g.: animal manures and plant residues) to soil (Jenkinson and Ladd, 1981; Schnurer et al., 1985). However, some sludges in Britain contain potentially toxic metals, such as Cu, Zn, Ni, Cd, Pb and Cr, which can accumulate in soil after prolonged additions (see Section 1.3.2). Recently, concern has been expressed as to the potentially harmful effects these heavy metals might have on microbial populations and processes in agricultural soils receiving sewage sludge amendments (Brookes et al., 1984; Long, 1985; Zibilske and Wagner, 1982).

Micro-organisms need some heavy metals (e.g. Co, Cu, Fe, Mn and Zn) as essential nutrients. However, in sufficiently high concentrations each metal may become toxic. The manifestations of this toxicity may vary; changes in cell morphology, changes in cell metabolism or a decrease in species diversity of bacteria (Damyanova, 1983). The effects of heavy metals on micro-organisms or microbial enzymes *in vitro* under controlled conditions are well documented (e.g. Tyler, 1981). However, to extrapolate such data to a field situation can be misleading as soil properties, particularly pH, cation exchange capacity and chelating ability, generally act to reduce the biological availability of heavy metals (Babich and Stotzky, 1976, 1977; Baroux and Sechet, 1974; Mikkelson, 1974) . For instance, whereas a Pb concentration of 375 ug/g in a sandy soil led to a 15% decrease in soil respiration, a similar reduction was only attained with 1500 ug/g in a clay (Doelman and Haanstra, 1979).

Assessment of metal toxicity on microbial numbers and activity in soil has been investigated using soils formed on parent material rich in trace elements (Troyer et al., 1980; cited by Brookes et al., 1984), on soils amended in the laboratory with soluble metal salts (Doelmnan and Haanstra, 1979a) or using soils contaminated with metals by agricultural or commercial activity (Brookes and McGrath, 1986). It has been suggested that metal pollution of soils not only adversely affects microbial biomass and activity but also brings about a change in species abundance and composition (Houba and Remacle,1980; Olson and Thornbton, 1982; Wassel and Mills, 1983; Barkay et al., 1985), and in more severely metal-polluted environments, bacterial or fungal metal tolerant populations have been identified (Timoney et al., 1978; Doelman and Haanstra, 1979; Davey and Reanney, 1981; Duxbury and Bicknell, 1983).

Microbial activity can be used as an indicator of metal toxicity in soils where rates of consumption of substrates and production of synthates, as well as the activity of specific enzymes, can be measured. For instance, exposure of soils to elevated heavy metal concentrations can result in a marked decrease in urease (Tyler, 1974) and acid phosphatase (Juma and Tabatabai, 1977) activities. Deleterious effects on soil respiration (Bhuiya and Cornfield, 1972; Bond et al., 1976; Tyler, 1974; Damyanova, 1983; Doelmnan and Haanstra, 1979a), organic matter decomposition (Ebregt and Boldewij, 1977; Doelman and Haanstra, 1979a,b), P mineralization (Tyler, 1976), N_2 fixation (Brookes et al., 1986) and ammonification (Tyler, 1975), have also been reported where soil metal content is increased. However, the activity of other enzymes such as B-glucosidase may not be affected by increased heavy metal exposure (Tyler, 1974). The particular function of bacterial enzymes may also determine their response to metals. For example, exocellular enzymes are more exposed to metals in the soil solution than endocellular enzymes. Thus, Brookes et al.,(1984) suggested that the activity of enzymes inside living cells is a better indicator of the effects of metal toxicity on microbial activity than are exocellular enzymes which are more exposed to the soil solution and are consequently more prone to metal inhibition.

Effects of metal toxicity on microbial biomass in soil have been studied by direct counting, viable counts using dilution plates or most probable number techniques (Brookes et al., 1984; Waid, 1984).

However, these approaches are very subjective and results largely depend on the method employed. The principal problem in estimating microbial biomass by direct microscopy is distinguishing living organisms from the necromass, particularly dead fungal cells, in that living and dead cells both respond to dyes (Brookes et al., 1986). In addition, viable counts may be criticised as only measuring the organisms which will grow on the specific culture medium used and, consequently, greatly underestimate total soil biomass (Jenkinson and Ladd, 1981).

Measurements of total ATP (adenosine 5-triphosphate) (Jenkinson and Oades, 1979) or, following soil fumigation, total readily respirable organic C (Jenkinson and Powlson, 1976), have each provided convincing biochemical measures of living cell components which, if used with appropriate caution and experimental controls, are thought to provide reliable estimates of total soil biomass (Waid, 1984). ATP occurs in all living cells and can be measured readily and accurately by the luciferin - luciferase system. The fact that ATP in dead cells is rapidly decomposed (Holm-Hansen and Booth, 1966), as is extracellular ATP in soil (Conklin and MacGregor, 1972) enables the living soil microbial biomass to be quantified by ATP determination. The main difficulties in using the ATP technique are in extracting it efficiently from soil and in relating the extracted amount to the amount of biomass in soil (Waid, 1984). Attempts have been made to find other specific biomass constituents that could be used to gauge soil biomass, such as muramic acid, n-acetyl-glucosamine and nucleic acid bases, but without success (Jenkinson and Ladd, 1981).

In 1976, Rothamsted Experimental Station developed a new method for measuring the microbial population of soils (Jenkinson and Powlson, 1976). The chloroform fumigation method is essentially a bio-assay technique. It involves killing the soil biomass by chloroform vapour and, after fumigant removal, the killed cells are decomposed by a recolonising population. When a soil is exposed to a volatile fumigant, the fumigant removed, and the soil incubated, oxygen consumption, over a short period, is found to be greater than that for a control unfumigated soil (Birch, 1959) and evolves more CO_2 (Powlson and Jenkinson, 1976). Jenkinson (1966) proposed that this "flush of decomposition" was due to the decomposition by the survivors (or by an inoculum) of cells of organisms killed during fumigation. It assumed that the recolonising population mineralizes the same amount of non-biomass soil organic matter in both the fumigated and unfumigated control soil. Powlson and Jenkinson (1976) and McGill et al. (1981) suggested that the cytoplasmic component of the soil microbial biomass is the fraction that is mainly mineralized during fumigation since stainable microbial cell walls are still apparent more than 50 days after aerobic incubation of a previously fumigated soil (Jenkinson et al., 1976). To provide a true measure of soil microbial biomass a near total kill is required. In this respect, chloroform fumigation can yield a 99.9% kill-off (Shields et al., 1974). The chloroform fumigation-incubation technique has been widely used for studying biomass C in agricultural systems and, with modifications, biomass N (Shen et al., 1984), biomass P (Brookes et al., 1982; Hedley and Stewart, 1982) and biomass S (Saqqar et al., 1981) can also be measured.

Chloroform fumigation and soil ATP measurements have been used to investigate short-term effects of field applications of metal contaminated sludge on soil biomass (Eiland, 1982; Zibilske and Wagner, 1982). For soils receiving sludge, there is evidence to suggest that soil metal concentrations at or below current permitted U.K. levels seem to adversely affect total soil biomass and microbial activity (Brookes and McGrath, 1984; Brookes et al., 1984).

AIMS AND OBJECTIVES

It is clear from the preceding section that heavy metals can have a marked affect on soil microbial population and activity. An experiment was therefore devised with the aims and objective of investigating the effects of past sewage sludge applications to agricultural soils in relation to:

i) soil microbial biomass as measured by the chloroform-fumigation-incubation technique,and;
ii) soil nitrification potential.

Moreover, the effect of pH, of a sludge amended soil, on the nitrification potential was also studied.

4.2 MATERIALS AND METHODS

4.2.1 SOIL DESCRIPTION

Soil samples were collected from three sites in the West of Scotland namely Lower Carbarns Farm (Nat. Grid Ref. NS 773 537), Auchterhead Farm (Allanton) (Nat. Grid Ref. NS 862 553) and Maudslie Sewage Works (Nat. Grid Ref. NS 806 505).

Details of the soils' physical and chemical properties are given in Tables 4.1 and 4.2. The sludged and control soils from Lower Carbarns Farm (referred to as Carbarns soil) are described in Section 3.2.1. The Allanton soil samples were collected from a 15.2ha permanent grassland field which has received regular sewage sludge amendments during 1978 - 1988. The control Allanton soil was collected from the boundary of this field which, being adjacent to a stream, had not received any sludge. The sludged field sampled at the Maudslie Sewage Works has in the past been used as 'sacrificial' land for disposal of sewage sludge originating from the Works. This land is now used for grazing. The Maudslie control soil was collected from the field boundary. For the nitrification experiment the control Maudslie soil was collected from the side of the field which borders the River Clyde. However, for the determination of biomass the control Maudslie soil was sampled from the field boundary furthest from the River Clyde as the original control was found to have a very different soil texture to that of the sludged area.

Thirty-two top soil cores, to a depth of 20cm, were collected from each

TABLE 4.1 DESCRIPTION OF CARBARNS, ALLANTON AND MAUDSLIE SOILS.

	pH	%LOI	C : N	AVAILABLE N mg/l	AVAILABLE P mg/l	AVAILABLE K mg/l	AVAILABLE Mg mg/l	pc	TEXTURE*
<u>CARBARNS</u>									
SLUDGED	5.49	15.6	17	93	65	76	196	4.13	CL/C
CONTROL	6.49	14.5	13	112	285	458	236	3.91	CL/C
<u>MAUDSLIE</u>									
SLUDGED	5.29	9.3	17	72	6.0	90	202	4.06	SCL
CONTROL I	5.10	9.3	10	48	7.3	73	84	4.05	SCL
CONTROL II	6.06	5.4	12	56	22	80	148	4.31	LS
<u>ALLANTON</u>									
SLUDGED	5.36	9.9	11	120	63	83	116	3.92	SL
CONTROL	5.00	13.7	13	101	6.0	100	111	4.18	SL

MAUDSLIE CONTROL I - used in biomass experiment.

MAUDSLIE CONTROL II - used in nitrification experiment

*TEXTURE : CL/C = Clay loam/clay SL = Sandy loam
SCL = Sandy clay loam LS = Loamy sand.

TABLE 4.2 TOTAL HEAVY METAL CONTENT (mg/kg AIR-DRY SOIL) OF CARBARNS, ALLANTON AND MAUDSLIE SOILS.

	HEAVY METAL CONCENTRATION					
	Cd	Cr	Cu	Ni	Pb	Zn
<u>CARBARNS</u>						
SLUDGED	1.15	109	63	24	121	196
CONTROL	0.90	104	41	31	114	159
<u>ALLANTON</u>						
SLUDGED	0.37	55	27	7	58	66
CONTROL	0.47	88	23	4	37	56
<u>MAUDSLIE</u>						
SLUDGED	1.07	107	30	31	88	96
CONTROL (1)	0.66	92	27	33	53	73
CONTROL (11)	0.30	73	14	19	55	68

MAUDSLIE CONTROL (1) = used in biomass experiment.

MAUDSLIE CONTROL (11) = used in nitrification experiment.

site (using the soil corer described in Section 3.2.2.4). Sample handling was kept to a minimum. Soils required for determination of soil biomass were fumigated on the day of sampling, whereas soils destined for the nitrification work were sampled 24 hrs before the start of the experiment during which time they were stored at 4-6°C. All the soils used during the course of this work were 'fresh as sampled', that is, none of the soil samples were air-dried as this results in a severe disruption of the native soil microbial population (Birch, 1959; McLaren and Skujins, 1967). The only treatment afforded to these soils was sieving through a 6mm sieve to remove any stones and plant material.

4.2.2 EXPERIMENT I: SOIL BIOMASS DETERMINATION BY FUMIGATION.

Microbial biomass C was measured by the chloroform fumigation-incubation procedure (Jenkinson and Powlson, 1976). Eight replicate 25g moist soil samples were placed in 50ml glass beakers. Four portions were fumigated with chloroform and four left unfumigated. The fumigations were done in a large dessicator lined with moist paper and containing a beaker with 50ml alcohol-free chloroform and a few anti-bumping granules. To obtain a near total microbial kill, two consecutive fumigations were carried out on the soil samples, permitting the entry of air into the dessicator after the first fumigation ensured a thorough penetration of the chloroform into the soil samples. The dessicator was evacuated until the chloroform boiled vigorously, and after the second fumigation the tap was closed and the dessicator placed in a dark 25°C incubator for 18 hours. The beaker of chloroform and the moist paper were then removed and the chloroform vapour was removed from the soil by repeated 3min evacuations using a high vacuum pump. Eight evacuations were usually sufficient to remove all traces of chloroform from the soil. Each portion of fumigated soil was then inoculated with 0.5g of fresh unfumigated soil derived from the same bulk soil samples as those originally fumigated. These soils were then transferred into separate 3l pyrex flasks sealed by means of a rubber bung fitted with two taps. After inoculation, sufficient distilled water was added to each soil sample to bring it to 55% water-holding capacity (WHC). The fumigated soils were then incubated at 25°C for 10 days, after which time the CO_2 concentration in the flask was measured. The taps fitted to the bungs served in creating a closed system for measuring CO_2 production using a gas analyser, preventing a drop in internal gas pressure, within the incubation flasks, which could have led to erroneous readings.

The four 'unfumigated' soil samples were kept at 25°C in a dessicator lined with moist paper for the time taken between fumigation and inoculation of the 'fumigated' samples. These unfumigated portions of soil were not inoculated prior to their transfer into the 3l flasks. After adjusting the WHC to 55%, the CO_2 production of these samples was measured after a 20 day incubation at 25°C which included aeration of the flasks after 10 days.

The amount of CO_2 evolved was determined using a URAS 3G infra-red gas analyser.

4.2.3 EXPERIMENT II: NITRIFICATION POTENTIAL OF CARBARNS, ALLANTON AND MAUDSLIE SOILS.

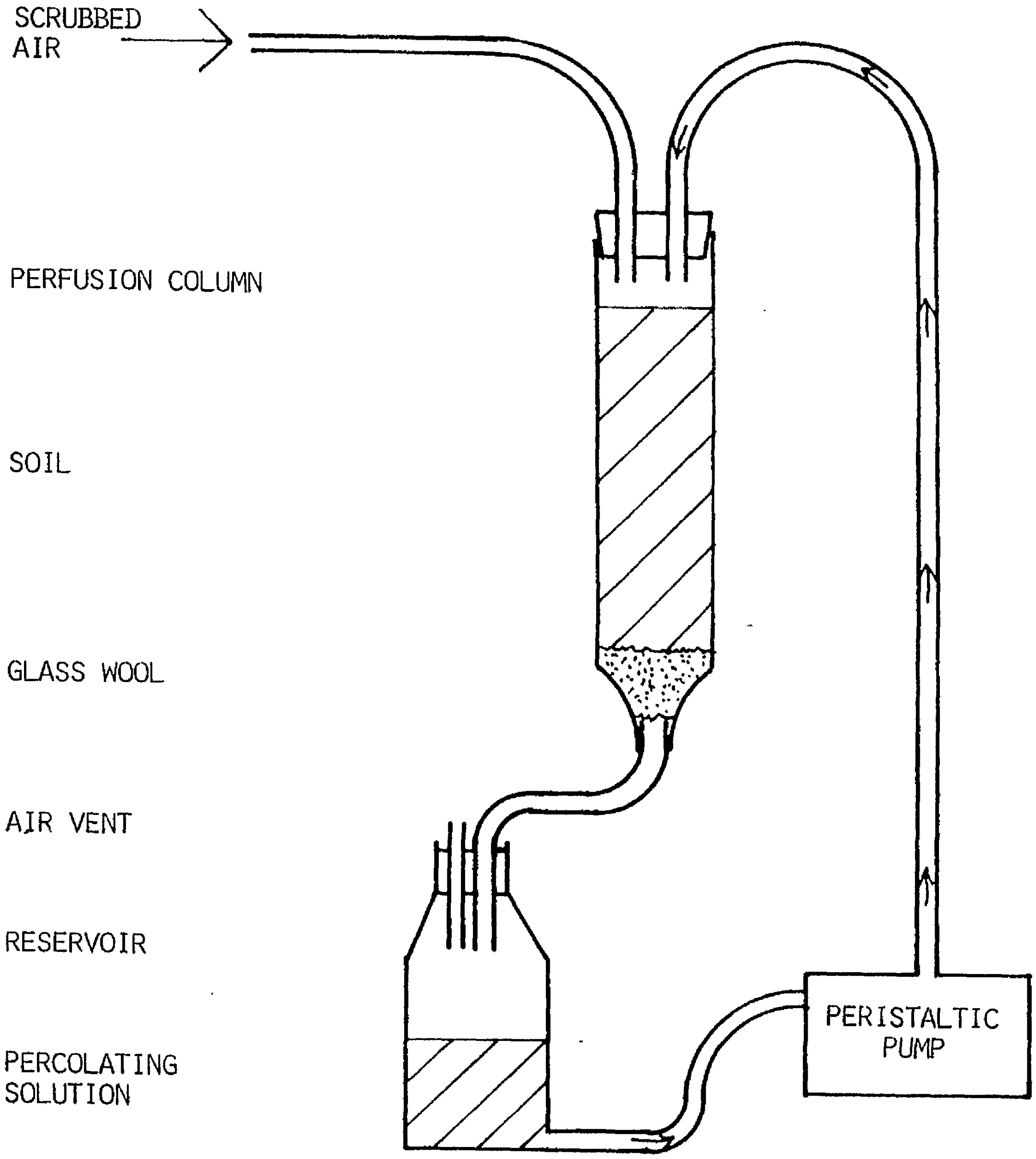
A 36 channel continuous cyclic percolation system (Plate 4.1) was used in this study. Such a system allows the study of chemical processes in soil, producing soluble intermediates or end products, without disturbance of the soil, by sampling the leachate over time.

Six replicate columns, each containing 38g fresh soil and 0.8g of medium grade perlite (to facilitate drainage and aeration) were set up for each of the six soils, that is Carbarns sludged (CS), Carbarns control (CC), Allanton sludged (AS), Allanton control (AC), Maudslie sludged (MS) and Maudslie control (MC). Each column was percolated with 150ml (initial volume) NH_4Cl solution (125mg N/l) from individual reservoirs at a delivery rate of 1ml/min. The system was kept at room temperature (20°C). A Watson-Marlow peristaltic pump fitted with a 510Z 40 channel pumphead was used to circulate the percolating solutions. 1.0mm (i.d.) silicone tubing was used on the pumphead.

All the columns were aerated with NH_3-free air (scrubbed through 0.5M H_2SO_4) to provide aerobic conditions, necessary for nitrification, within each column.

Samples of percolating solution (3ml) were collected every 2 days from each of the 36 reservoirs. To each sample, 2 drops of chloroform were immediately added to prevent any further microbial activity. The samples were stored at 2°C in a refrigerator for subsequent NH_4-N and NO_3-N determinations.

PLATE 4.1 DIAGRAM ILLUSTRATING ONE OF 36 PERCOLATING SYSTEMS USED FOR MEASURING SOIL NITRIFICATION POTENTIAL



Unfortunately, after 20 days of operation, most of the 1mm (i.d.) silicon tubing used on the pumphead snapped, forcing an abrupt end to the experiment.

4.2.4 EXPERIMENT III: EFFECTS OF SOIL pH ON THE NITRIFICATION POTENTIAL OF SLUDGED SOIL.

The soil used in this work was collected entirely from individual plots at the Carbarns site (described in detail in Chapter 3). The sampled plots were those which had either been limed or unlimed but which had remained uncultivated and had received no fertiliser-N. Each sample consisted of 5 cores, taken from the 0-20cm depth of the eight plots. Of the 8 samples collected, five were chosen to give the widest range of pH values (Table 4.3). In addition, a control soil from Carbarns which had never been sludged (taken from the same site as detailed in Section 3.2.1) was also used.

The same procedure as given in Section 4.2.3 was used to measure the nitrification potential of these six soils. To prolong the life of the pumphead delivery tubes, 2mm (i.d.) silicon tubing was used.

The percolating solution was sampled every 2 days up to day 28 and a final sample was collected on day 32. These samples were treated and stored as described in Section 4.2.3.

TABLE 4.3 MEAN pH OF MOIST CARBARNS SOIL SAMPLES USED IN EXPERIMENT III. EACH READING IS AN AVERAGE OF 7 REPLICATE MEASUREMENTS.

	pH	
SOIL	MEAN	S.E.
CONTROL	6.54	0.008
1	5.47	0.003
2	5.69	0.003
3	5.76	0.009
4	6.28	0.004
5	6.37	0.002

4.2.5 DETERMINATION OF NH_4-N AND NO_3-N IN LEACHATES

The NH_4-N and NO_3-N concentrations in the leachates was determined using a Chemlab Instrument Ltd Continuous Flow Analyser.

NH_4-N determination involves its reaction with salicylate (2-hydroxybenzoate) and dichloroisocyanurate (DIC), nitroprusside being added as a catalyst to the reaction (Chemlab, 1981). The resulting 'substituted indophenol' is measured colorimetrically at 650nm.

For the determination of NO_3-N, this is first reduced to NO_2^- via a Cd - Cu reduction column. This method of reduction overcomes any possible interference arising from the presence of Ca^{2+} and Mg^{2+} in the samples were they to be read using the Cu-hydrazine reduction method (Ananth and Moraghan, 1987). The nitrite so produced is reacted with sulphanilamide to form a diazonium salt which, in turn, couples with naphthylethylenediamine to give an azo dye which can be measured colorimetrically at 540nm (Chemlab, 1984).

In adopting the Cd column reduction method care was taken to ensure the absence of air bubbles and dislodged metallic particles from within the reduction column as these could adversely affect the precision of this method (Best, 1975).

4.3 RESULTS

4.3.1 EXPERIMENT I: EFFECTS OF SEWAGE SLUDGE APPLICATIONS ON SOIL MICROBIAL BIOMASS.

The equation used to convert the measured CO_2 concentrations in the incubation flasks into soil microbial biomass C, was that proposed by Jenkinson (1966):

$$B = F/K_c$$

where

B = soil biomass carbon (mg C/100g soil)

$F = CO_2\text{-}C_{\text{fumigated}} - CO_2\text{-}C_{\text{unfumigated}}$

i.e. CO_2-C (mg C/100g) evolved by the fumigated soil less that evolved by the unfumigated soil, incubated for the same time under the same conditions.

K_c = 0.45 at 25°C (Jenkinson and Powlson, 1976; Jenkinson and Ladd, 1981).

i.e. K_c = the fraction of biomass C mineralized to CO_2 during incubation which followed fumigation.

A single value of K_c may be applied to different soils without serious errors for the following reasons:

i) the values of K_c for different organisms, although by no means all the same, do not span a very wide range (Anderson and Domsch, 1978),

ii) for soils developed under natural conditions, there are similarities in the pattern of distribution of different types of organisms, although the population size may differ widely (Jenkinson et al., 1976).

The mean soil microbial biomass at each of the three sites (Carbarns, Allanton and Maudslie), for both sludged and control samples, are reported in Table 4.4, the full set of results being shown in Appendix J.

To enable an analysis of variance of biomass C between sludged and control samples to be carried out, the 4 replicate fumigated and unfumigated CO_2 - C readings for each individual soil were pooled. This resulted in 16 individual values (i.e. 4 x 4) for biomass C, instead of just one single measure for which a statistical analysis would have been impossible.

It was found that for all three sites, there was a significant difference ($P < 0.05$) between the sludged and control samples, with the sludged Carbarns and Allanton soils exhibiting an 18% and 24% lower soil microbial biomass C than their respective controls. In contrast, the sludged Maudslie soil showed a 27% higher soil microbial biomass C than that found in its control.

It is interesting to note that although there were significant ($P < 0.05$) differences in microbial biomass C between the sludged and control soils for both the Carbarns and Allanton sites, there was no significant difference in the 'base respiration rate' (the CO_2 produced by the unfumigated samples) of the sludged and control soils (Table 4.5). For the Maudslie sludged soil, however, a significantly ($P < 0,05$) higher 'base respiration rate' was shown over its control (Table 4.5).

TABLE 4.4 MICROBIAL BIOMASS (mg C/100g) IN SLUDGED AND CONTROL SOILS FROM CARBARNS, ALLANTON AND MAUDSLIE. EACH READING IS AN AVERAGE OF 4 REPLICATES.

	BIOMASS C		
	SLUDGED	CONTROL	POOLED S.E.
ALLANTON	26.38	34.71	0.86
MAUDSLIE	26.67	10.67	0.34
CARBARNS	33.39	40.73	1.46

TABLE 4.5 BASE RESPIRATION RATE, i.e. CO_2-C(mg C/100g FRESH SOIL) EVOLVED BY UNFUMIGATED SAMPLES OVER 10-20 DAY PERIOD. EACH READING IS AN AVERAGE OF 4 REPLICATES.

	MEAN	S.E.
<u>CARBARNS</u>		
CONTROL	19.81	0.73
SLUDGED	17.42	0.88
<u>ALLANTON</u>		
CONTROL	19.37	0.71
SLUDGED	18.17	0.51
<u>MAUDSLIE</u>		
CONTROL	13.22	0.30
SLUDGED	11.72	0.25

4.3.2 EXPERIMENT II: EFFECTS OF SEWAGE SLUDGE APPLICATION ON SOIL NITRIFICATION POTENTIAL.

The rate of NO_3-N production in the sludged and control soils from Carbarns, Allanton and Maudslie was measured. For each of the six (3x2) soils, the mean NO_3-N concentration on each of the ten sampling days is reported in Table 4.6 and represented graphically in Figure 4.1. The full set of results are given in Appendix D.

There was a near linear increase in NO_3-N concentration with time for all the soils (Table 4.7) which on linear regression (Approach 1 - see below) was shown to be significantly greater ($P<0.01$) than zero.

Three approaches can be adopted to quantify the rate of NO_3-N production:

Approach 1: A different nitrification potential for each soil is assumed;

Approach 2: An identical nitrification potential for each soil is assumed in which a single relationship between NO_3-N and time is used;

Approach 3: An identical nitrification potential is assumed but soils differ in the NO_3-N concentration at time (t) = 0.

By using each of the three approaches it was found that the least residual mean square (RMS) was generated by Approach 1 (Table 4.8) and, therefore, this proved to be the most appropriate method for quantifying

TABLE 4.6 CHANGES IN NO_3-N CONCENTRATION (mg NO_3-N/kg AIR-DRIED SOIL) WITH TIME (DAYS) FOR THE SLUDGED AND CONTROL SOILS FROM CARBARNS, ALLANTON, AND MAUDSLIE. EACH READING IS AN AVERAGE OF BETWEEN 2-6 TREATMENT REPLICATES. A POOLED S.E. FOR EACH DAY IS GIVEN.

	MEAN NO_3-N						
	CARBARNS		ALLANTON		MAUDSLIE		
DAY	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL	S.E.
2	24.9	91.0	25.8	7.9	7.9	5.2	4.4
4	46.7	115.0	34.9	11.7	14.7	14.9	2.9
6	17.3	169.2	52.3	12.5	15.7	11.1	8.0
8	106.5	213.6	62.5	12.2	20.1	27.8	7.4
10	115.8	252.7	73.1	13.6	25.6	41.8	9.1
12	119.3	276.8	93.9	17.6	33.1	57.6	12.6
14	145.4	298.5	108.9	21.0	42.2	57.8	14.2
16	173.2	253.4	127.4	28.5	57.0	87.4	17.3
18	195.8	348.3	133.7	31.9	62.8	99.0	14.5
20	251.3	433.4	158.0	43.1	82.5	94.0	14.3

FIGURE 4.1 EFFECTS OF SEWAGE SLUDGE ON THE RATE OF NO_3-N PRODUCTION IN SOIL COLUMNS

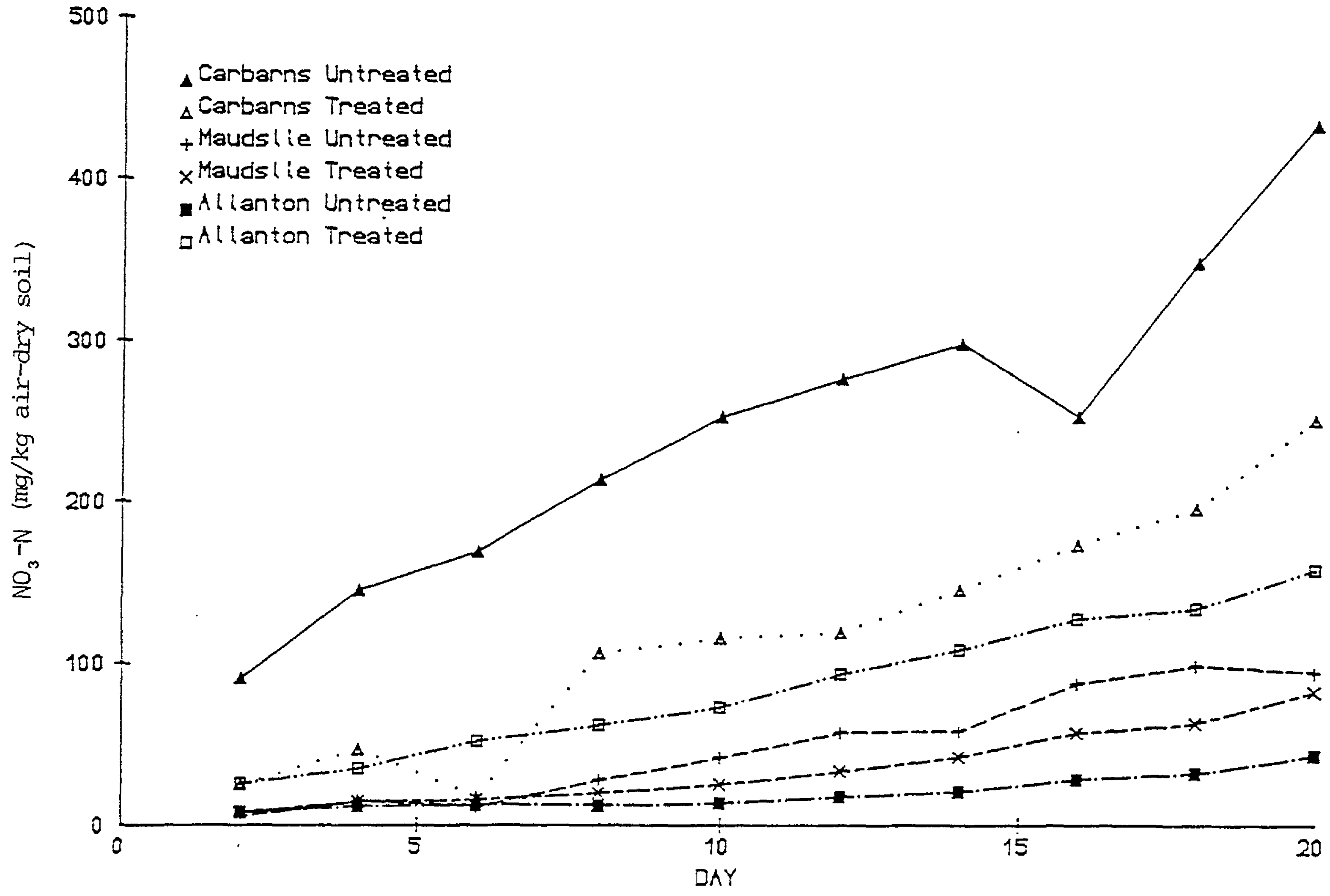


TABLE 4.7 RELATIONSHIP BETWEEN NO_3-N CONCENTRATION (mg NO_3-N/kg AIR-DRY SOIL) AND TIME (DAYS) FOR THE SOILS USED IN EXPERIMENT II.

	CARBARNS		ALLANTON		MAUDSLIE	
	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL
GRADIENT	12.06	16.41	7.33	1.72	3.90	5.68
S.E.	1.17	1.81	0.23	0.24	0.37	0.44
INTERCEPT	-13.07	64.49	6.39	1.02	-6.79	-12.84
S.E.	14.48	22.51	2.86	2.93	4.54	5.45
S_Y	21.20	32.94	4.19	4.28	6.65	7.98
R^2	0.92	0.90	0.99	0.85	0.93	0.95

TABLE 4.8 COMPARISON OF RESIDUAL MEAN SQUARES (RMS) FOR THE THREE APPROACHES USED TO ESTIMATE THE STRAIGHT-LINE EQUATIONS FOR THE CHANGES IN NO_3-N AND NH_4-N CONCENTRATIONS WITH TIME IN EXPERIMENT II.

	APPROACH		
	1	2	3
NO_3-N	781	6114	1430
NH_4-N	1070	5156	1089

APPROACH 1 - Separate slopes and intercepts.

APPROACH 2 - Common slope and intercept.

APPROACH 3 - Separate intercepts but common slopes.

NO_3-N production of each of the six soils with time.

The gradient ranged between 1.7-16.4mg NO_3-N/kg air-dried soil/day in the order, Allanton control < Maudslie sludged = Maudslie control < Allanton sludged < Carbarns sludged < Carbarns control (where '<' represents a significant difference at $P < 0.05$). The nitrification rates of soils from each site varied significantly in the order shown, but as far as sludge treatment was concerned there was no general trend in its effect on nitrification rate. A significantly ($P < 0.05$) higher rate of nitrification was shown in the control soil from Carbarns but the opposite was the case for the Allanton site. There was no significant difference between the sludge-treated and control soils from Maudslie.

The straight-line equation relating NO_3-N production and time, predicted an initial NO_3-N concentration not significantly different from zero although, in actual fact, some NO_3-N was present in each of the six soils (see Table 4.1). Moreover, the control Carbarns soil had an intercept of 64.7mg NO_3-N/kg air dry soil which was significantly different ($P<0.05$) from zero.

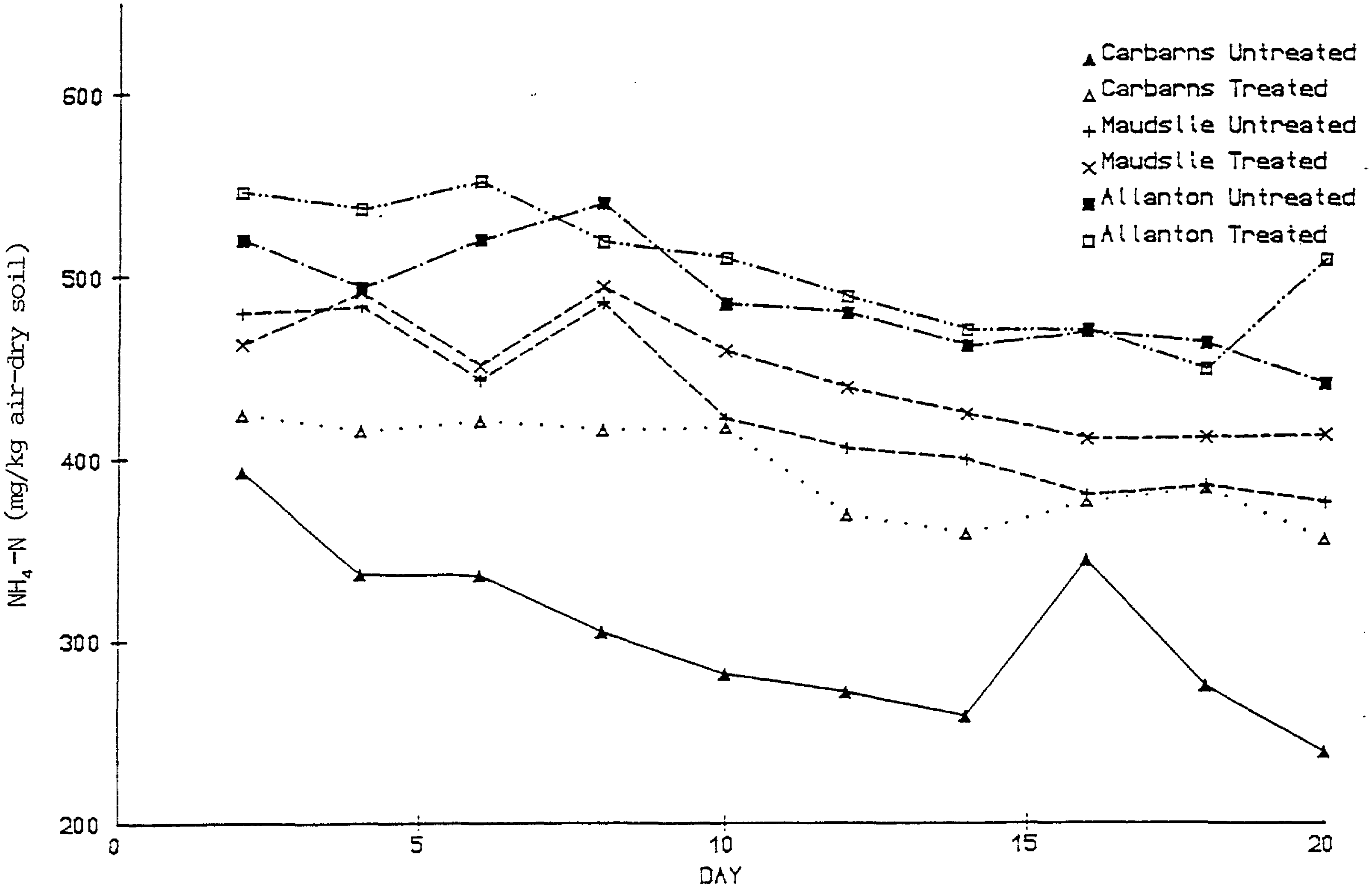
Table 4.9 reports the mean NH_4-N concentration on all ten sampling days for each of the six soils, and this data is represented graphically in Figure 4.2. The full set of results are shown in Appendix K.

NH_4-N concentration appeared to decrease linearly over time. Using the same statistical methods (i.e. Approaches 1, 2 and 3) as previously described for the analysis of the NO_3-N data, Approach 1 provided the

TABLE 4.9 CHANGES IN THE NH_4-N CONCENTRATION (mg NH_4-N/kg AIR-DRY SOIL) WITH TIME (DAYS) FOR THE SLUDGED AND CONTROL SOILS FROM CARBARNS, ALLANTON AND MAUDSLIE. EACH READING IS AN AVERAGE OF BETWEEN 2-6 TREATMENT REPLICATES. A POOLED S.E. FOR EACH DAY IS GIVEN.

	CARBARNS		ALLANTON		MAUDSLIE		
DAY	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL	S.E.
2	425.1	392.9	546.6	520.4	463.7	480.7	10.3
4	416.0	336.3	537.5	494.3	491.9	484.1	7.5
6	421.7	336.0	552.8	520.9	452.5	444.0	11.3
8	416.7	305.0	519.9	540.9	495.1	486.6	7.9
10	418.2	281.7	510.8	485.8	460.4	422.9	14.7
12	396.3	271.9	490.0	480.8	439.9	406.6	9.7
14	359.5	258.6	471.5	462.7	425.4	400.3	8.6
16	377.2	344.8	472.0	470.6	411.8	380.6	23.5
18	384.6	275.5	450.1	464.5	412.6	386.0	6.4
20	356.6	239.1	510.3	442.2	414.0	376.5	9.7

FIGURE 4.2 EFFECTS OF SEWAGE SLUDGE ON THE RATE OF NH_4-N DEPLETION IN SOIL COLUMNS



lowest residual mean square (Table 4.8). The best-fit straight-line equations were then obtained by linear regression for each of the six soils (Table 4.10). In each case, a negative gradient was obtained. The rate of NH_4-N depletion ranged between 3.88-6.71mg NH_4-N/kg air-dry soil/day in the order Maudslie control < Carbarns control < Allanton sludged < Maudslie sludged < Allanton control < Carbarns sludged. However, the only significant difference, at the 95% confidence interval, was that between the rates of NH_4-N depletion exhibited by the Maudslie control and Carbarns sludged soils.

When absolute rates of NO_3-N production and NH_4-N depletion within a given soil were compared, no significant difference was observed for the Maudslie soils. However, significantly different ($P < 0.05$) rates were seen for both the sludged and control soils from Carbarns and Allanton. More specifically, in the Carbarns soils the absolute rate was in the order:

$$\mathrm{GRADIENT}_{NO3\text{-}N} > \mathrm{GRADIENT}_{NH4\text{-}N}$$

whereas in the Allanton soils the converse was true.

Using the measured concentrations of NO_3-N and NH_4-N in each of the 36 percolating columns, the net change in mineral-N (i.e. ΔN) (Table 4.11) was calculated:

$$\Delta N = ([NH_4\text{-}N]_t + [NO_3\text{-}N]_t) - [NH_4\text{-}N]_o$$

where:

$[NH_4\text{-}N]_0$ = initial NH_4-N concentration i.e. 125mg/l at time $t = 0$.

TABLE 4.10 RELATIONSHIP BETWEEN NH_4-N CONCENTRATION (mg NH_4-N /kg AIR-DRY SOIL) AND TIME (DAYS) FOR THE SOIL USED IN EXPERIMENT II.

	CARBARNS		ALLANTON		MAUDSLIE	
	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL
GRADIENT	-3.88	-5.80	-4.57	-4.26	-4.34	-6.71
S.E.	0.85	1.86	1.21	1.00	0.99	1.00
INTERCEPT	437.14	368.03	556.45	535.13	494.46	500.67
S.E.	10.61	23.10	15.06	12.44	12.34	12.37
s_y	15.53	33.81	22.05	18.21	18.07	18.11
R^2	0.68	0.49	0.59	0.65	0.67	0.83

TABLE 4.11 CHANGES IN △ N (mg N/kg AIR—DRY SOIL) WITH TIME (DAYS), FOR THE SLUDGED AND CONTROL SOILS FROM CARBARNS, ALLANTON AND MAUDSLIE. EACH READING IS AN AVERAGE OF BETWEEN 2-6 TREATMENT REPLICATES. A POOLED S.E. FOR EACH DAY IS GIVEN.

	CARBARNS		ALLANTON		MAUDSLIE		
DAY	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL	S.E.
2	-285.3	-227.9	-102.8	-188.2	-253.5	-134.4	10.9
4	-157.9	-216.3	- 89.4	-196.2	-203.9	-108.8	8.0
6	-266.9	-178.1	- 43.1	-154.4	-228.2	-140.4	14.1
8	-167.9	-150.6	- 52.6	-120.3	-166.4	- 68.7	6.3
10	-142.5	-120.5	- 37.3	-159.7	-181.1	-106.0	11.2
12	-173.2	- 92.1	- 23.7	-146.4	-179.5	- 94.1	7.9
14	-142.2	- 69.4	- 13.8	-146.8	-170.5	-87.7	8.6
16	- 82.0	- 14.0	- 18.7	-117.1	-154.7	- 65.4	15.3
18	- 37.3	25.8	16.6	-105.4	-133.6	-36.1	11.8
20	4.9	88.7	114.7	-102.2	- 98.1	- 38.1	14.4

$[NH_4\text{-}N]_t$ = NH_4-N concentration (mg/l) at time t.

$[NO_3\text{-}N]_t$ = NO_3-N concentration (mg/l) at time t.

On linear regression of this data (Table 4.12), it was shown that there was a significant ($P<0.05$) rate of N mineralization which ranged between 4.6 and 17.1mg N/kg air dry soil/day in the order Carbarns control > Carbarns sludged > Allanton sludged > Maudslie sludged > Maudslie control > Allanton Control. There were no significant differences between the sludged and control soils from both Carbarns and Maudslie. However, there was a significant difference ($P<0.05$) between the sludged and control Allanton soil (Table 4.12). Over the first two days , for all the soils, there was a decrease in the NH_4-N concentration which could not be accounted for by nitrification, implying N immobilization (Figure 4.3). However, this proved to be very transitory because after day 2 net mineralization occurred but the rate differed between the soils.

4.3.3 EXPERIMENT III: EFFECTS OF SOIL pH ON NITRIFICATIOIN POTENTIAL IN A SLUDGED SOIL.

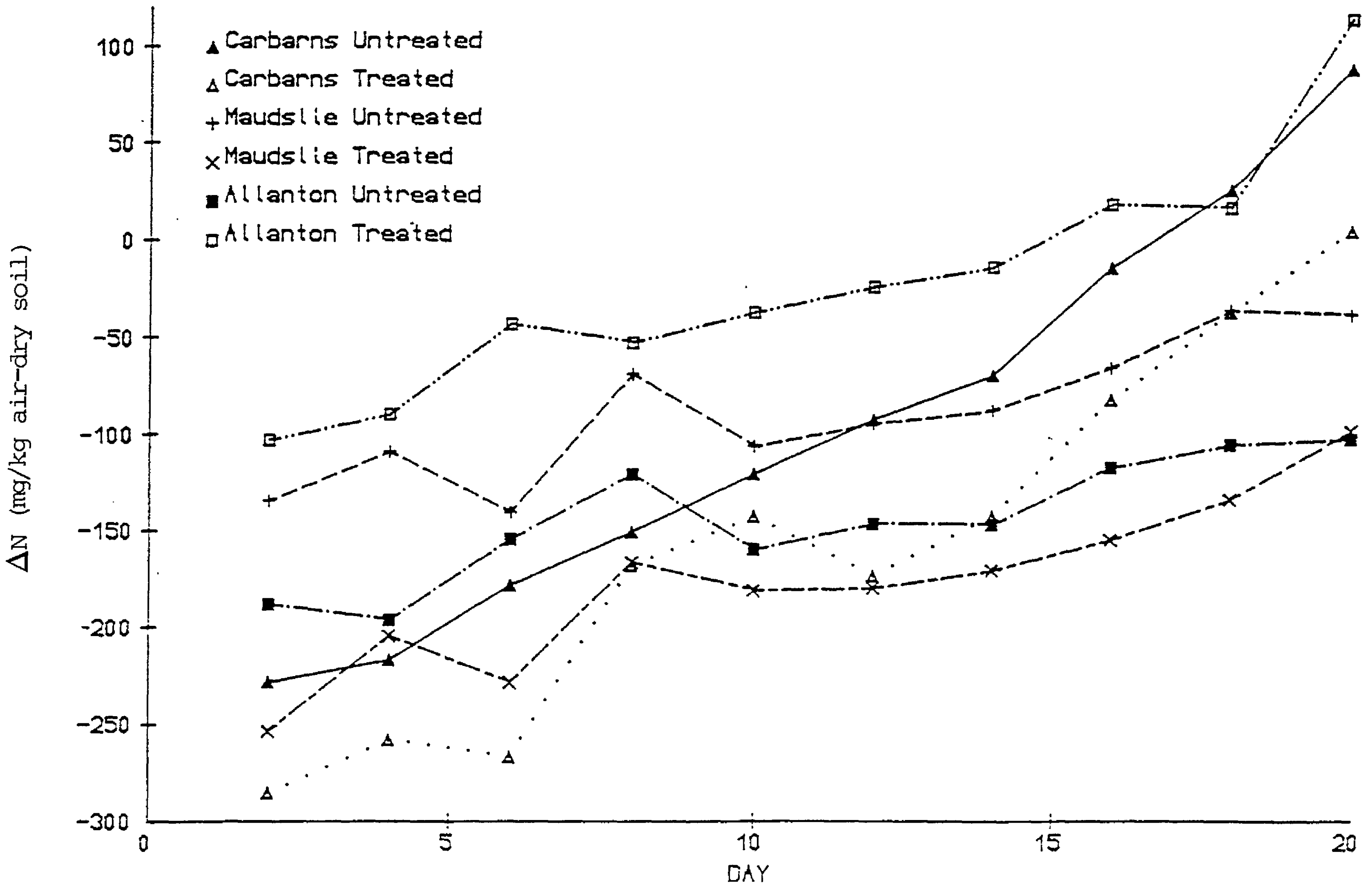
The rates of nitrification observed in the sludged and unsludged soils from Carbarns, Allanton and Maudslie (Experiment II) showed no definite relation to the concentrations of heavy metals in the soils. However, the rate of nitrification did appear to be closely associated with soil pH. It was consequently decided to conduct a second experiment to investigate the effects of soil pH on the nitrification potential of sludged soils.

TABLE 4.12 RELATIONSHIP BETWEEN ΔN CONCENTRATION (mg/kg AIR-DRY SOIL) AND TIME (DAYS) FOR THE SOILS USED IN EXPERIMENT II.

	CARBARNS		ALLANTON		MAUDSLIE	
	SLUDGED	CONTROL	SLUDGED	CONTROL	SLUDGED	CONTROL
GRADIENT	15.54^{c}	17.08^{c}	9.51^{b}	4.63^{a}	6.81^{a}	5.17^{ab}
S.E.	1.51	0.93	1.34	0.97	1.01	1.06
INTERCEPT	-325.92	-283.36	-125.84	-194.64	-251.89	-144.82
S.E.	18.71	11.59	16.61	12.00	12.49	13.10
s_y	27.39	16.97	24.31	17.57	18.28	19.17
R^2	0.92	0.97	0.85	0.71	0.83	0.72

FOOTNOTE: Values followed by the same superscript are not significantly different at the 95% confidence interval.

FIGURE 4.3 EFFECTS OF SEWAGE SLUDGE ON CHANGES IN INORGANIC N (ΔN) WITH TIME



The concentrations of NH_4-N and NO_3-N in the leachates from each of 36 soil columns on each sampling day are given in Appendix L.

When mean NO_3-N concentration (Table 4.13) was plotted against time for each pH treatment and the no sludge control treatment (Figure 4.4), a sigmoidal relationship was shown, unlike the linear relationships observed in Experiment II. These sigmoidal curves are typified by a 'delay phase', a 'maximal rate phase' and a 'retarded rate', as described by Sabey et al. (1959).

The NO_3-N data (Table 4.13) was then fitted to a model proposed by Hadas et al. (1986) which describes soil nitrification in terms of a sigmoidal relationship between soil NO_3-N production and time.

i.e. $$NO_3 = \frac{a}{1+(a/[NO_3]_0-1)\ \exp(-aK[t-t_0])} \qquad [1]$$

where:

a = asymptotic value of NO_3-N

$[NO_3]_0$ = initial value of NO_3-N (i.e. at time (t) = 0)

K = constant

t_0 = initial time (0).

This equation was derived by Hadas et al. (1986) from the Verhulst equation:

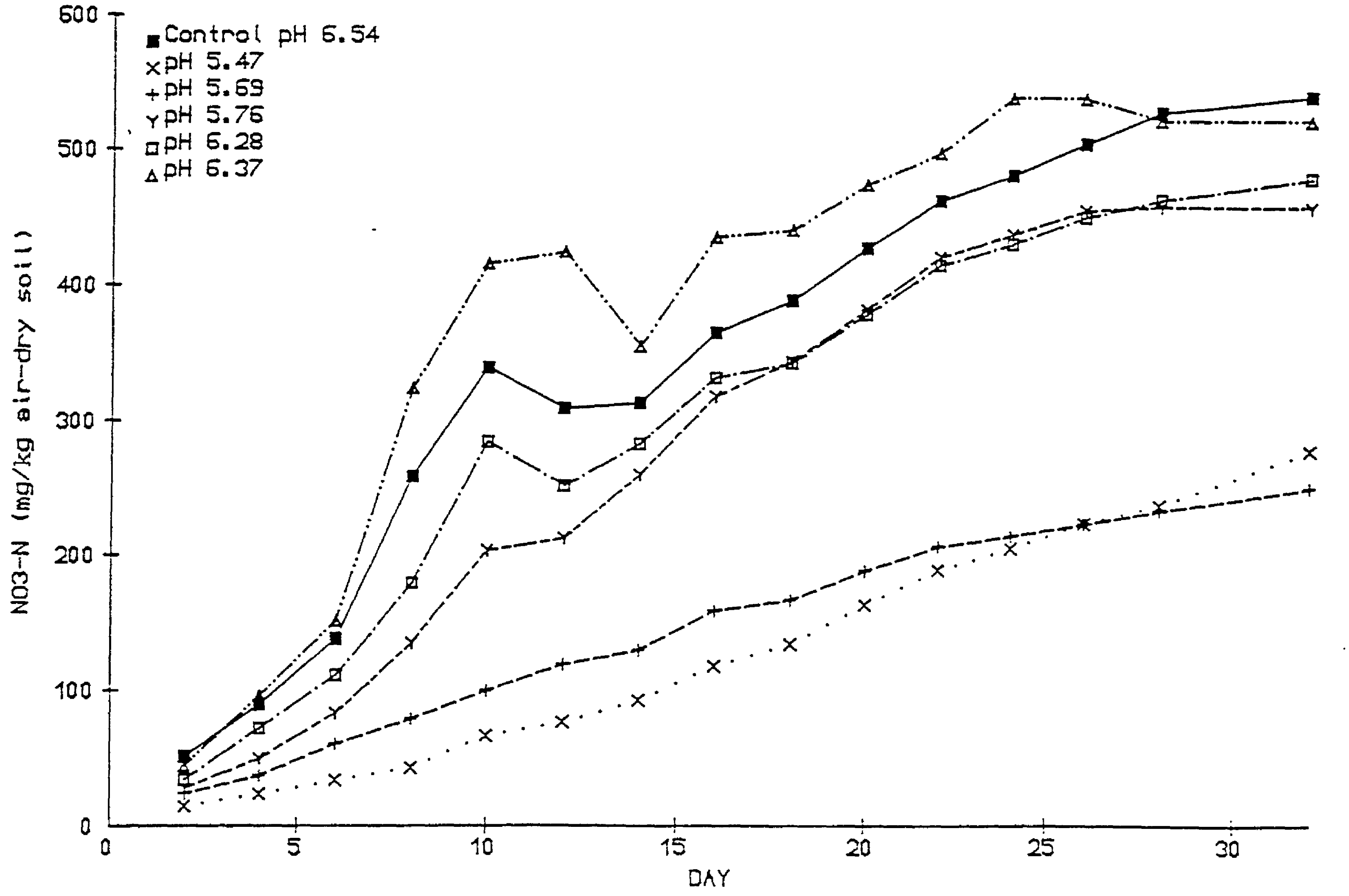
i.e. $$dN/dt = KN(a-N) \qquad [2]$$

The parameters a, K and $[NO_3]_0$ in equation [1] were calculated by least

TABLE 4.13 CHANGES IN NO_3-N CONCENTRATIONS (mg NO_3-N/kg AIR-DRY SOIL) WITH TIME (DAYS) FOR EACH OF THE SIX SOILS USED IN EXPERIMENT III. EACH READING IS AN AVERAGE OF BETWEEN 5-6 TREATMENT REPLICATES. POOLED S.E. FOR EACH DAY IS GIVEN.

	pH						
DAY	5.47	5.69	5.76	6.28	6.37	CONTROL	S.E.
2	14.3	24.0	28.2	34.2	43.9	50.8	2.0
4	23.4	37.2	49.7	72.2	96.0	89.6	2.9
6	33.3	60.1	82.9	111.2	150.8	138.2	5.4
8	42.5	78.7	135.0	179.0	323.9	258.7	12.8
10	66.3	99.8	203.5	284.3	416.2	339.4	16.2
12	77.0	119.4	212.4	251.5	424.7	309.8	25.0
14	93.2	130.1	259.8	283.2	354.9	313.6	14.0
16	117.9	158.4	318.1	332.2	435.7	364.8	9.3
18	134.0	166.4	344.2	343.0	440.3	388.4	9.5
20	163.2	188.4	382.2	378.3	474.0	427.7	12.0
22	189.1	206.7	421.0	414.8	497.6	462.4	12.3
24	205.1	213.8	437.5	430.3	538.3	480.9	17.0
26	223.4	223.0	454.9	450.2	537.9	504.3	16.9
28	237.2	232.9	457.8	462.9	521.3	527.8	16.2
32	277.3	250.2	457.1	478.3	520.9	539.6	19.6

FIGURE 4.4 EFFECTS OF pH ON THE RATE OF NO_3-N PRODUCTION IN A SLUDGED SOIL



squares fit of NO_3-N vs. time (Table 4.13) using the Genstat Statistical Package (Rothamsted Experimental Station, 1980) and are listed in Table 4.14

The maximal rate (K_{max}) was derived as described by Hadas et al. (1986) where:

$$K_{max} = K \cdot a^2/4 \qquad [3]$$

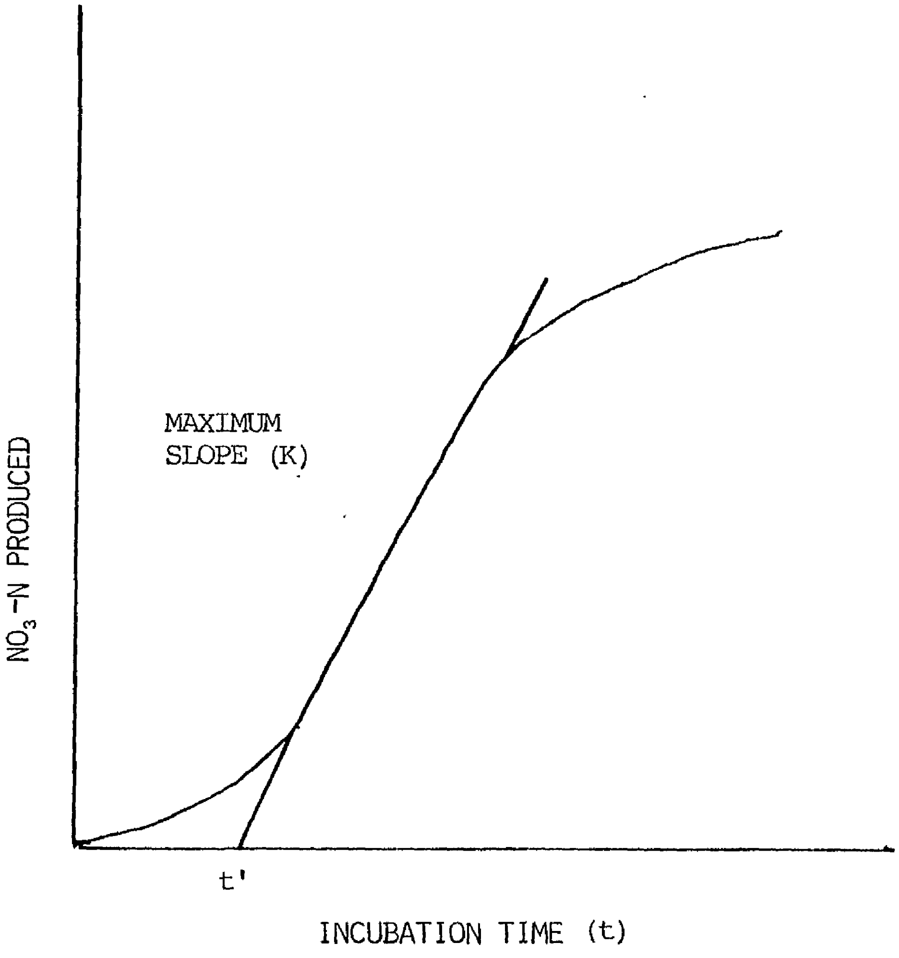
i.e. K_{max} is the maximum slope of equation [1] at the inflection point (where $[NO_3\text{-}N] = a/2$). At the same time an estimate for the delay phase (t') was obtained from:

$$t' = \frac{1}{aK} \ln\left[\frac{a}{(NO_3)_0} - 1\right] + \frac{[NO_3]_0 - a/2}{K_{max}} \qquad [4]$$

Equation [4] is given by Hadas et al. (1986) who base it upon the definition given by Sabey et al. (1959), that the delay period is the value of t at which the extrapolated maximum slope intercepts the projected initial value of NO_3-N (see Figure 4.5).

It would appear (Figure 4.4), that two distinct 'groups' of soils can be discerned on the basis of their nitrification characteristics. Soils at the higher pH (i.e. pH 5.76 and pH 6.28) including the control (pH 6.54) exhibited a maximal nitrification rate (K_{max}) which was twice as great as that of the more acid soils (i.e. pH 5.47 and 5.69). Moreover, the K_{max} for the sludged soil at pH 6.37 was 4 times as great as that recorded for the most acid soils. In addition, significantly

FIGURE 4.5 GENERAL NITRIFICATION CURVE SHOWING THE DELAY PERIOD (t') AND THE POINT OF MAXIMUM SLOPE (K) (Sabey et al., 1959)



higher ($P<0.05$) asymptotic NO_3-N concentrations characterised soils having a pH > 5.76 compared to the more acid soil. Similarly, differences were also apparent in the delay period (t') which was inversely related to pH and ranged from 7.7 days at pH 5.47 to 2.6 days at pH 6.37 for the sludged soils (Table 4.4). The delay period for the control soil was even shorter at 2.3 days.

For the soil at pH 6.37, there appears to be a stray data point on day 14. If this value is excluded from the curve fitting equation a better fit is achieved, the RMS dropping from 2215 to 1249 (Table 4.14). This results in an associated increase in K_{max} from 45.33 to 58.05 , but also in an increase in the delay period from 2.6 to 3.2 days.

When considering the residual mean squares (Table 4.14) of the other curves, it is apparent that for those soils with the relatively lower pH values a better curve fit was obtained. The fact that the leachates from the more acid soils required less dilution prior to analysis could account for this situation. In fact, these more acid soils exhibited a lower S.D. about the mean.

The mean NH_4-N concentrations in each of the six soils over time is given in Table 4.15 and represented graphically in Figure 4.6. The graph clearly shows that, whereas there was a sigmoidal relationship between NO_3-N and time, this was not the case for NH_4-N. Instead, between t = 0 and t = 4-6 days there was a rapid decrease in the NH_4-N concentrations in the percolating solutions corresponding to 290-380mg/kg air-dried soil, which was directly proportional to soil pH. The greatest decrease occurred in the control soil which could be

TABLE 4.14 ESTIMATES AND STANDARD ERROR FOR ASYMPTOTES, INITIAL VALUES AND RATE CONSTANTS FOR THE SIGMOIDAL RELATIONSHIPS DESCRIBING THE CHANGE IN NO_3-N CONCENTRATION WITH TIME (DAYS) FOR THE SOILS USED IN EXPERIMENT II.

a = Asymptotic value (mg N/kg air-dry soil).

$[NO_3]_0$ = Initial NO_3-N concentration (mg N/kg air-dry soil).

K = Rate constant. K_{max} = Maximal rate.

RMS = Residual mean square. t' = Delay period (days).

	pH					
	5.47	5.69	5.76	6.28	6.37	Control
a	311	255	472	473	495	535
S.E.	10.25	6.72	10.10	23.90	21.88	36.05
$[NO_3]_0$	15.14	25.48	29.16	50.51	31.11	72.76
S.E.	1.33	2.40	4.09	11.79	23.75	18.49
K	4.9×10^{-4}	6.4×10^{-4}	4.5×10^{-4}	4.0×10^{-4}	7.4×10^{-4}	3.2×10^{-4}
S.E.	4.0×10^{-5}	5.0×10^{-5}	4.0×10^{-5}	7.0×10^{-5}	2.7×10^{-4}	8.0×10^{-5}
K_{max}	11.85	10.40	25.06	22.37	45.33	22.90
t'	7.66	3.73	4.56	2.91	2.60	2.30
RMS	22	35	187	800	2215	1495

TABLE 4.15 CHANGES IN NH_4-N CONCENTRATION (mg NH_4-N/kg AIR-DRY SOIL) WITH TIME (DAYS) FOR EACH OF THE SIX SOILS USED IN EXPERIMENT III. EACH READING IS AN AVERAGE OF BETWEEN 5-6 TREATMENT REPLICATES. A POOLED S.E. FOR EACH DAY IS GIVEN.

	pH						
DAY	5.47	5.69	5.76	6.28	6.37	CONTROL	S.E.
2	428.7	407.9	443.1	406.5	406.3	344.6	5.5
4	306.3	264.9	310.3	291.2	268.8	245.8	17.1
6	282.1	320.2	295.9	311.8	263.6	232.8	46.9
8	375.4	353.4	367.9	327.9	292.8	264.1	8.5
10	412.3	384.2	387.3	330.0	284.9	264.5	14.2
12	386.3	354.2	327.7	294.0	236.4	243.3	12.2
14	374.4	337.8	319.2	257.7	184.4	211.5	8.0
16	366.7	333.3	285.0	236.2	170.6	187.6	8.0
18	280.5	285.9	261.4	236.0	215.0	145.0	29.4
20	349.0	320.7	234.8	185.9	121.7	140.0	5.8
22	344.1	310.1	224.6	177.7	123.8	105.4	11.0
24	321.9	283.9	201.8	152.4	86.8	94.1	10.0
26	296.4	274.8	177.9	131.2	76.9	87.4	10.1
28	250.4	267.1	173.8	123.2	63.1	49.2	15.2
32	256.4	226.2	139.6	81.2	38.2	30.4	9.3

attributed to either the fact that it had the highest pH and/or the lowest available heavy metal concentration. However, over the 6-10 day period, there was a slight recovery in the NH_4-N concentration, the extent of this recovery again depending on soil pH. At this stage, the NH_4-N concentration in the soils at pH 5.47, 5.69 and 5.76 returned to levels close to those monitored on day 2. However, for the soils at pH 6.28 and 6.37, and in particular for the control soil, the recovery was less marked.

After day 10 and for the remainder of the sampling period, a gradual linear decrease in the NH_4-N concentrations was observed. Straight-line equations generated by linear regression (Table 4.16) indicated that two groups of lines could be distinguished. Soils at pH 5.47 and 5.69 exhibited a significantly ($P<0.05$) lower rate of NH_4-N depletion than those at a higher pH including the control.

As can be seen from Table 4.16, a high RMS was associated with the equation for the soil at pH 6.37. This could, in part, be due to a stray data point on day 18. However, this high RMS could also reflect a non-linear relationship which might have been better described as quasi-sigmoidal from day 8 onwards (Figure 4.6).

Δ N (Table 4.17) was calculated, as described previously (Section 4.3.2), and represented in Figure 4.7. Over the first four days the decrease in ΔN was linked to the immobilisation of NH_4-N (Figure 4.5) which was not accounted for by an increase in NO_3-N concentration. Thereafter, and relative to the value of Δ N on day 4, there was net N

TABLE 4.16 RELATIONSHIP BETWEEN NH_4-N CONCENTRATION (mg NH_4-/kg AIR-DRY SOIL) AND (DAYS) TIME FOR ALL SIX SOILS USED IN EXPERIMENT III (ONLY DATA AFTER DAY 10 CONSIDERED).

	pH					
	5.47	5.69	5.76	6.28	6.37	CONTROL
GRADIENT	-7.18	-6.31	-10.76	-10.91	-10.59	-11.09
S.E.	0.68	0.41	0.68	0.53	0.92	0.51
INTERCEPT	482.30	437.90	466.70	419.50	354.60	367.60
S.E.	14.63	8.82	14.75	11.37	19.87	11.05
RMS	220	80	224	133	406	126
S_y	14.85	8.96	14.98	11.54	20.17	11.22
R^2	0.93	0.96	0.97	0.98	0.94	0.98

FIGURE 4.6 EFFECTS OF pH ON THE RATE OF NH_4-N DEPLETION IN A SLUDGED SOIL

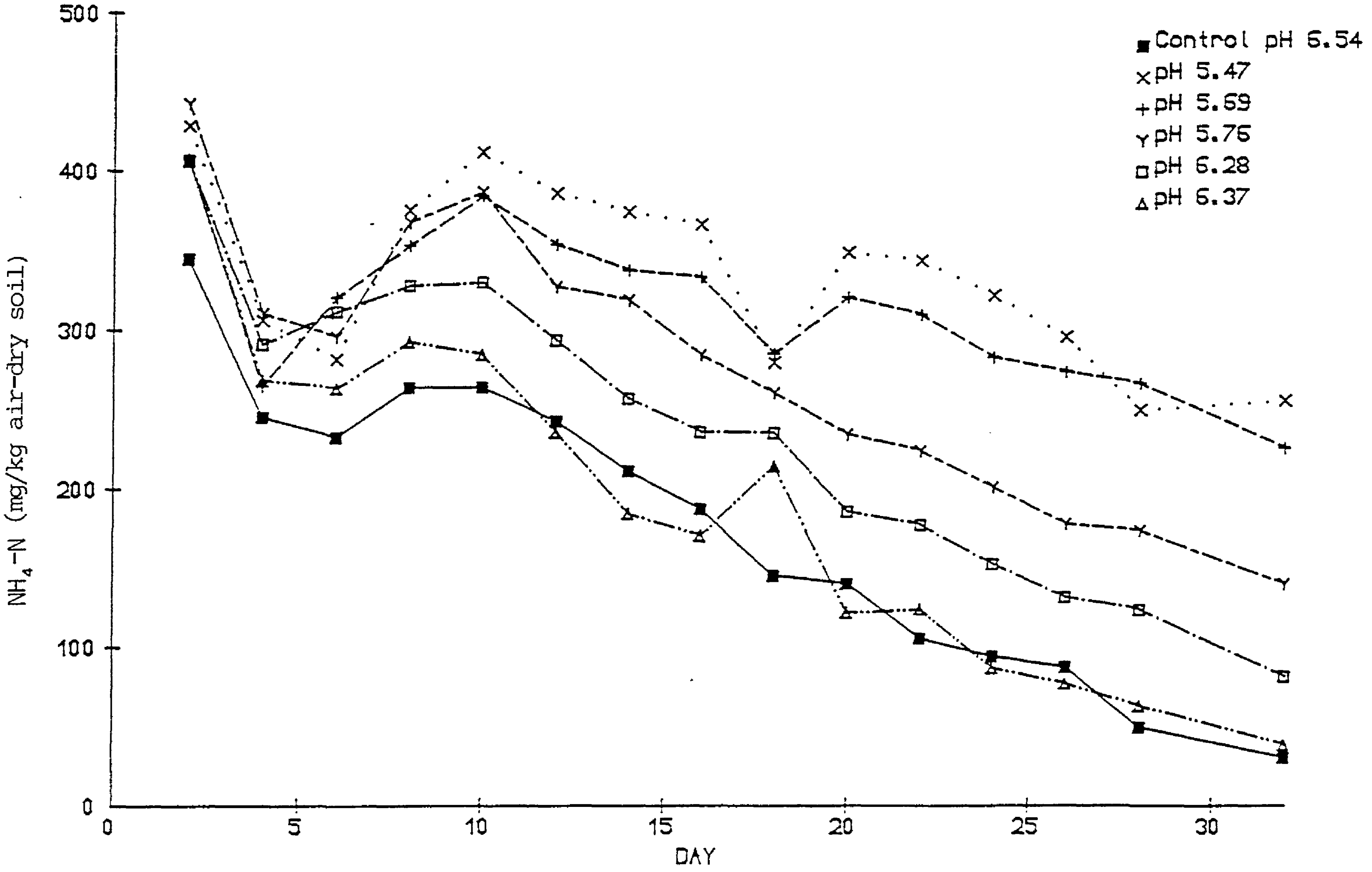
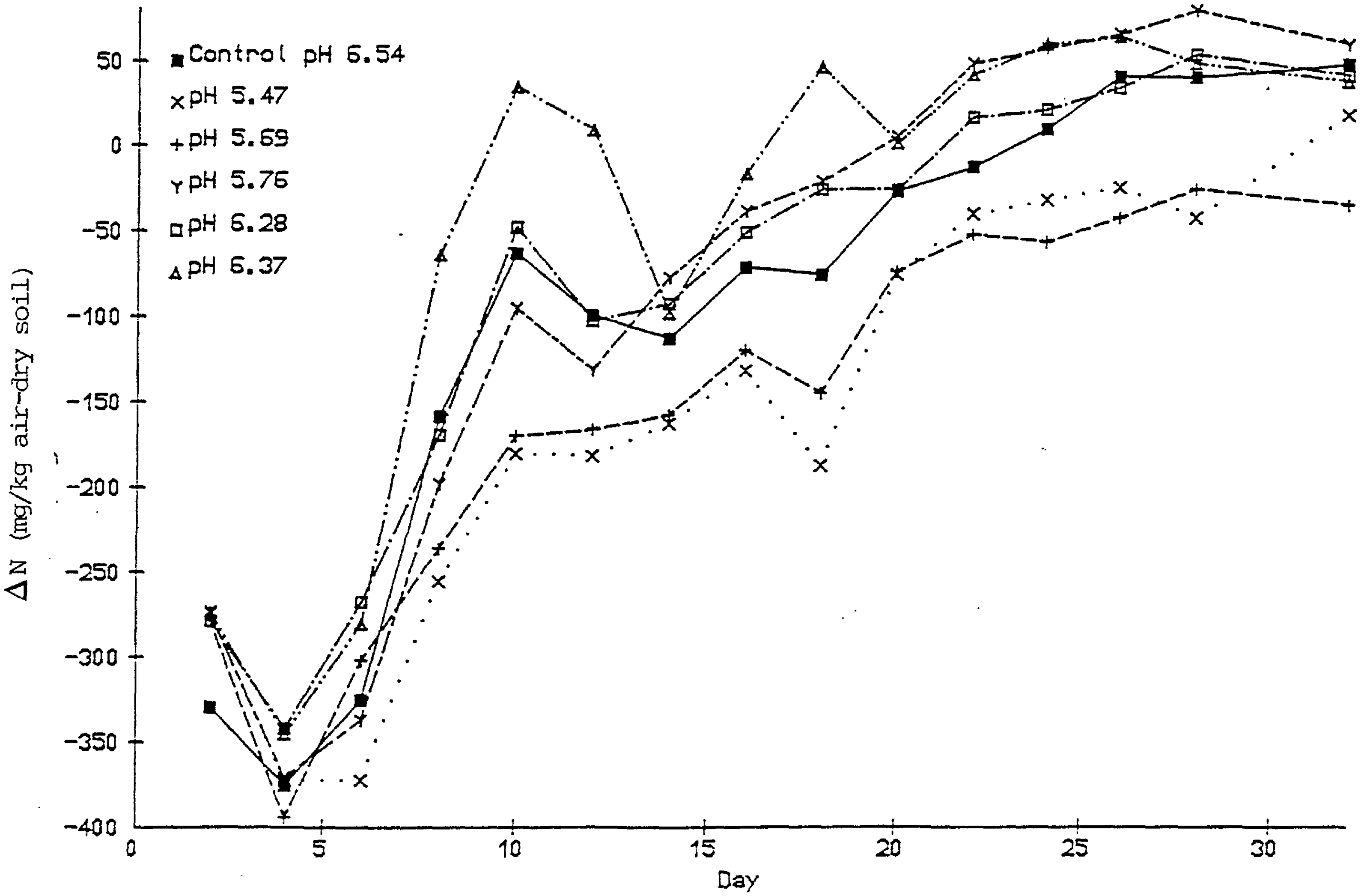


TABLE 4.17 CHANGES IN ΔN (mg N/kg AIR DRY SOIL) WITH TIME (DAYS) FOR ALL THE SIX SOILS. EACH READING IS AN AVERAGE OF BETWEEN 5-6 TREATMENT REPLICATES. A POOLED S.E. FOR EACH DAY IS GIVEN.

	pH						
DAY	5.47	5.69	5.76	6.28	6.37	CONTROL	S.E.
2	-273.5	-278.8	-274.5	-279.0	-273.9	-329.7	5.4
4	-372.5	-394.5	-370.9	-342.0	-345.0	-375.4	18.3
6	-372.4	-302.0	-337.1	-268.1	-280.8	-325.1	45.3
8	-255.6	-236.1	-198.2	-169.7	-64.0	-158.8	12.7
10	-180.6	-169.9	-95.3	-47.9	34.8	-63.1	19.4
12	-181.5	-166.1	-131.2	-102.3	9.3	-99.6	24.8
14	-162.9	-157.5	-77.3	-92.6	-98.1	-112.9	15.6
16	-131.6	-119.6	-38.3	-50.6	-16.6	-71.2	8.2
18	-187.3	-144.8	-20.9	-25.5	46.9	-75.6	30.0
20	-75.3	-73.7	5.5	-26.0	1.8	-26.9	13.5
22	-39.9	-51.8	48.9	16.6	42.1	-12.3	12.3
24	-31.8	-56.7	57.5	21.3	60.2	9.4	14.8
26	-24.8	-42.5	66.0	34.4	64.4	40.7	14.2
28	-42.6	-25.9	79.7	53.5	48.5	40.4	23.1
32	15.7	-35.3	59.7	41.2	37.7	48.0	17.3

FIGURE 4.7 EFFECTS OF pH ON CHANGES IN INORGANIC N (ΔN) WITH TIME



mineralisation which showed a sigmoidal relationship with time.

Sigmoid curves were then fitted to the data points for each pH treatment and the control over the 4 - 32 day period, using the Genstat Statistical Package (Rothamsted Experimental Station, 1980). The curves fitted to these data sets were based on the following general sigmoid equation:

$$\frac{A}{(1 + \exp[u - k.t])} \qquad [5]$$

where

A = asymptotic value of ΔN

u = initial value (at time 0) of ΔN

k = Rate constant

t = day number.

The parameters for these curves are listed in Table 4.18. No significant differences appear between the N mineralisation rates of the six soils. The asymptotic, rate constant and initial Δ N values were similar for each of the six soils. However, none of the asymptotic values (A) obtained were significantly different ($P<0.05$) from 0, and, hence over the 32 day experimental period there was no net N mineralisation relative to day 0.

The high RMS (Table 4.18) obtained for all six soils indicated a poor fit to the equation, but general trends could still be detected.

TABLE 4.18 ESTIMATES FOR ASYMPTOTE, INITIAL VALUE AND RATE CONSTANT OBTAINED FROM CURVE FITTING TO DATA FOR △ N WITH TIME (DAYS) FROM EXPERIMENT III.

A = asymptotic value (mg N/kg air dry soil)

k = Rate constant

u = Initial N concentration (mg N/kg air dry soil)

RMS = Residual mean squares.

	5.47	5.69	5.76	6.28	6.37	CONTROL
A	0	-41	61	24	22	9
S.E.	40.27	21.97	21.99	24.99	13.17	40.05
k	0.19	0.23	0.26	0.26	1.06	0.29
S.E.	0.05	0.06	0.05	0.08	0.40	0.17
u	-398	-398	-397	-397	-393	-398
S.E.	0.48	0.47	0.47	0.60	2.78	1.22
RMS	1804	1064	1409	1542	2140	2741

4.4 DISCUSSION

The chloroform fumigation method is based on a number of assumptions (Jenkinson, 1966, 1976):

i) the C in dead organisms is mineralized to CO_2 more rapidly than that in living organisms i.e. the protected substrate that is a living cell becomes available to others on its death;

ii) the kill is substantially complete (see Section 4.1);

iii) the biomass dying in the unfumigated control - soil during incubation is negligible compared to that killed by fumigation;

iv) the fraction of the killed biomass C mineralized (K_c) is the same in different soils (see Section 4.2.1), and

v) fumigation has no effect on the soil other than the killing of biomass.

The validity of these assumptions are discussed elsewhere (Brookes and McGrath, 1986; Jenkinson and Ladd, 1981), and are outside the scope of this work. When applying the fumigation method to investigate whether or not sewage sludge application results in any changes in soil microbial biomass, possibly as a result of enhanced soil metal levels, it is essential that higher soil metal concentrations do not, in any way, alter the validity of this technique. Comparative work involving ATP determination and chloroform fumigation, led Brookes and McGrath (1986) to conclude that "the fumigation method does provide a valid estimate of soil biomass in high-metal and low-metal soils alike".

Significant differences in microbial biomass C between sludged and unsludged soils at Carbarns, Allanton and Maudslie were recorded. However, no uniform effect was evident. Whereas at both Carbarns and Allanton microbial biomass C was lower in the sludged soil than that present in the unsludged (control) soil, the converse was true for the Maudslie soils. In other words, the microbial population seems to have been stimulated by sludging at Maudslie, whereas it was depressed in the sludged soils of Carbarns and Allanton. Nevertheless, at the latter sites there was no evidence that the smaller microbial populations in the sludged soils were any less effective in decomposing plant or animal residues as no root mat was present.

The positive relationship between soil microbial biomass and soil organic matter content is well known and documented. Application of sludge with the resultant introduction of organic matter to the soil, on the basis of decomposable substrate alone, would, therefore, be expected to increase the soil's microbial biomass, not only in the short term (as the more easily decomposable fractions of the sludge solids become available to the soil microflora), but also in the longer term (as the soil organic matter content increases). The higher organic matter of the unsludged Allanton soil over that present in the sludged soil could thus account for the lower biomass C recorded in the latter soil (Table 4.4). This, however, does not exclude the possibility that some fraction of the sludge applied, could have also contributed to the reduction in the microbial population.

At Maudslie the sludged soil contained an organic matter content equal to that found in the unsludged control soil (Table 4.1). Nonetheless, the higher metal content of the sludged soil could have inhibited microbial activities to some extent, as could any organic pollutants introduced into the soil via sludge. However, microbial biomass C in the sludged Maudslie soil was 27% greater than that determined in the unsludged control soil (Table 4.4). Stimulation of the microbial population, evidently not coming from an increased total organic matter content, could, nevertheless, have resulted from organic matter in the sludged soil being of a more decomposable nature. As decomposition progresses, the proportion of aromatic compounds, such as lignin and polyphenols in the organic matter fraction, increases. This is coupled with a gradual humification of soil organic matter. Both humified organic matter and aromatic compounds are relatively more resistant to microbial breakdown (Jenkinson, 1988).

A different situation prevailed at Carbarns. The sludged soil contained more organic matter than did the unsludged control but contained smaller biomass C as also reported by Brookes and McGrath (1984) using the Woburn Market Garden soil. Brookes and McGrath blamed this reduction in microbial biomass C on the toxic effects of metal concentrations in the sludged soil which was very badly contaminated with Cd, Cu, Ni and Zn, with concentrations of 8.6, 102, 27 and 289mg/kg air dry soil respectively (Brookes et al., 1986). At Carbarns the sludged soil contained higher Cd, Cr, Cu, Pb and Zn concentrations than those present in the unsludged soil (Table 4.2), although these concentrations, i.e. 1.1, 63, 24 and 196mg/kg air dry soil respectively, were much lower than those recorded at Woburn.

Attributing the effect to one particular metal or group of metals or separating the individual effects is difficult as all these metals were added together in sludge. Trying to separate possible individual metal effects by, for example, adding heavy metal salts to uncontaminated soils and monitoring possible changes in biomass or microbial activity could only be speculative as metal form might not match the chemical forms found in other recently applied sludge or sludge-derived metals which have been in the soil for a number of years. Some insight into this problem was offered by the results of the work carried out by Brookes and McGrath (1986) using metal enriched sludges which showed that whereas Cu and Ni in sludges depressed soil biomass, Zn and Cr did not. Moreover, Damyanova (1983) found Cd to be more toxic to microbial respiration than Cu. Horsfall (1956) proposed the following order of toxicity of metals for a variety of fungal species:

$$Ag > Hg > Cu > Cr > Ni > Pb > Co > Zn > Ca.$$

However, the observed depression of microbial biomass in the sludged Carbarns soil may also be attributed to persistent toxic organic compounds. No chemical tests for any such organic pollutants were carried out. However, Brookes and McGrath (1984, 1986) rejected this possibility in favour of microbial inhibition due to increased soil metal concentrations in view of the fact that depression in microbial activity was evident 20 years after the last application of sludge.

The mode of action by which metals may inhibit and/or suppress microbial populations is not known. In all probability more than one factor is involved. Some metallic ions are known to destroy the integrity of semipermeable membranes, thus allowing metabolites to leak out (Rich and Horsfall, 1963). The synthesis and function of proteins, including enzymes, is essential for active and proper growth of organisms. Heavy metals may act in reducing microbial populations by inhibiting the action of proteins thus reducing their metabolic efficiency. For instance, Pb is known to inhibit the production and action of several enzymes (Doelman and Haanstra, 1979). In general, inhibition by metals may be due to:

i) their ability to compete with essential elements (eg. Mn, Fe, Mg) for active (eg. -SH, $-NH_2$, =NH) or structural sites of the protein (Bhuiya and Cornfield, 1972).

ii) their masking of catalytically active groups (Tyler, 1981), and/or

iii) protein denaturation (Tyler, 1981).

Enzymes whose activities depend on the presence of free amino and sulfhydryl groups are inhibited by heavy metals which form stable complexes by ligand-binding with these groups. Metals, such as Cu, which form very sparingly soluble sulphides from sulfhydryl groups are the strongest inhibitors (Katz and Cowans, 1965; Hughes et al., 1969). On the other hand, Cu and Fe metallo-enzymes are less readily inhibited by metals due to the high stability of the enzymes' metal complexes (Owens, 1953; Malkin and Malmstrom, 1970).

Reference to Tables 4.4 and 4.5 suggests that more of the energy generated by respiration is required to maintain the microbial cells in the sludged Allanton and Carbarns soils than is needed in the unsludged soils. Consequently, proportionately less of the energy generated during microbial respiration is available for growth in the sludged soils, hence the reduced biomass C. Enzyme - protein inhibition and/or inactivation by heavy metals in sludged soils could help explain the hypothesis put forward by Brookes and McGrath (1984), that lower microbial biomass C in high metal sludged soils is a result of reduced microbial longevity.

Part of this increase in maintenance energy may be required not only to compensate for any enzyme/protein inhibition by the metals, but may also be required to support some resistance mechanisms which the microbes may use to overcome problems of metal toxicity. Many species of bacteria have genes that control resistances to specific toxic heavy metals. These genes are often coded on extrachromosomal DNA (plasmids) (Foster, 1983). The occurrence of these plasmids in a variety of bacteria isolated from polluted and unpolluted natural environments is well documented (Hada and Sizemore, 1981; McNicol et al., 1982). Impermeability of the plasma membrane (Kondo et al., 1974) or accumulation in the cell-wall constituents (Mitra et al., 1975) have been reported to offer two tolerance mechanisms for metals such as Cd by certain species. Commonly, resistance to heavy metal involves the production of compounds which render the metal less available for microbial metabolism. One such detoxifying mechanism is based on a high rate of H_2S production (Ashida, 1965) where, for instance, Cu-tolerant *Saccharomyces* cells deposit CuS at the cell periphery. The production of volatile compounds involving metals such as Hg, a potentially more toxic metal than Cu, reduces the metal's availability to the micro-organisms (Komura and Izaki, 1971). Other mechanisms known to occur involve energy driven efflux pumps that rapidly excrete Cd^{2+} and AsO_4^{3-} (Silver, 1984), thus keeping intracellular concentrations low. Alternatively, micro-organisms may synthesise intracellular polymers which trap the toxic metals (Doelman, 1986). Each of these mechanisms require the expenditure of energy. A comprehensive outline of resistance mechanisms is given by Silver (1984).

Work by Thornton et al. (1985) offered some proof that bacterial populations rapidly adapt to increasing metal concentrations in sludge-amended soils. If the active microbial flora develops metal tolerance, its susceptibility to metal toxicity may be circumvented. Bacterial or fungal metal tolerant populations have been identified in a number of metal-polluted environments, but Brookes et al. (1984) report that as yet no such populations have been found in sludge treated soils.

The observed reduction in biomass C in the sludged Carbans soil may be a result of a decrease in all microbial species, but it may equally be due to a decrease of particular, more sensitive species. The emergence of species more tolerant and/or resistant to metal toxicity may be favoured in metal - stressed environments even though the necessity for resistance mechanisms increases energy expenditure, hence reducing growth rate. A similar argument would likewise apply to the selection within a single species since the sensitivities within a given population follow a statistical distribution and under continuing selection pressure, tolerant and/or resistant organisms will emerge.

Bacteria have been found to be more sensitive to Cd (Gupta and Stadelmann, 1983) and Pb (Doelmnan and Haanstra, 1979) than hyphal fungi. In contrast, Brookes et al. (1984) reported that bacteria and protozoa are unaffected by metal enrichment in sludged soils and that an observed reduction in microbial biomass is probably due to a depression of the fungal population. It has also been reported that in metal-stressed environments there appears to be selection in favour of gram-negative bacteria (Barkay et al., 1985; Bramryd, 1983). The general lack of agreement in determining which group(s) of micro-organism is most sensitive to metal pollution is probably a reflection of the wide variety of micro-niches exploited by soil micro-organisms and the particular metal availability within each niche. For instance, Hattori and Hattori (1976) reported that the proportion of gram-negative bacteria to the total number of bacteria, under field conditions, was usually smaller than 10% in the outer part of soil aggregates. However, in the inner part of the aggregates this ratio was much higher and frequently exceeded 30%. In contrast, coryneform bacteria are rather drought resistant and are likely to occur in the outer part of the soil aggregate (Doelman and Haanstra, 1979). Therefore, a shift in the composition of the microflora in high metal-sludged soils does not necessarily have to be solely ascribed to the selection of tolerant population, but may also be of an indirect nature where, for instance, micro-organisms within soil aggregates are less exposed to metal pollutants.

Bacteria involved in the oxidation of nitrogen compounds in the soil, the soil nitrifiers, are an integral part of the soil microbial population. Nitrification, which is the conversion of ammonium to nitrate, is carried out entirely by micro-organisms. *Nitrosomonas* converts ammonium to nitrite and *Nitrobacter* converts nitrite to nitrate. There are reports of various other genera eg. *Nitrosocystis* and *Nitrosococcus* performing these functions in the sea. The organisms are obligate aerobes and chemoautotrophs, although some *Nitrobacter* strains have been shown to use acetate as a C and energy source (Stanier et al., 1981). However, these strains grow much more slowly with acetate than with nitrite (Stanier et al., 1981). The population of *Nitrosomonas* and *Nitrobacter* is frequently quite small and many soils, particularly those which are acid, have fewer than 100 viable cells of one or both genera per gram and, as a rule, populations in excess of 10^5 per gram are rare in unfertilised soils (Alexander, 1965). However, in fertilised soils the numbers rise and may reach values in excess of 10^6 cells per gram (Alexander, 1965).

The conversion of ammonium (oxidation state -3) to nitrite (+3) yields 65 - 66 Kcals/mole, and nitrite (+3) to nitrate (+5) about 17 - 18 Kcals/mole (Campbell, 1977), which is a low level of energy yield when compared with respiration (686 Kcals per mole of glucose) or deamination (176 Kcals per mole of ammonium). Molar growth yields are thus low, approximately 0·7g dry weight *Nitrosomonas* cell per mole ammonium oxidized and 0·3g dry weight *Nitrobacter* cell per mole nitrite oxidized (Knowles et al., 1965).

The chemistry of nitrification is not clear; some of the enzymes have not been isolated and some of the postulated intermediates cannot be detected. The first step probably involves the oxidation of ammonium to hydroxylamine using molecular oxygen. Hydroxylamine is toxic but the concentration is usually low, the equilibriumn is towards ammonium and the reaction proceeds only as hydroxylamine is oxidized to nitrite. There are intermediates, possibly the nitroxyl radicle and nitrohydroxylamine, though whether these exist in a free state is not known. Parts of the flavoprotein and cytochrome system are required for the oxidation of hydroxylamine. *Nitrobacter* then oxidizes the nitrite to nitrate in a single step with molecular oxygen as the terminal electron acceptor mediated by a cytochrome system. An in-depth study of nitrification processes is given by Jones (1982). In soil, ammonium and nitrite oxidation occurs concurrently, but the latter is more rapid, so nitrite rarely accumulates (Rodgers, 1984).

Details of the heavy metal requirements of nitrifying bacteria are unknown. Winogradsky and Omeliansky (1899) first demonstrated the need of Fe for nitrifiers. This has since been confirmed for *Nitrosomonas* in liquid culture (Lees and Meiklejohn, 1948) and for cell-free nitrification in *Nitrobacter* (Aleem and Alexander, 1958). Low concentrations of various metal salts have been reported to stimulate nitrite formation in pure cultures of *Nitrosomonas* (Khare et al., 1966). Copper is probably an essential element for *Nitrosomonas* (Lees and Meiklejohn, 1948), being involved in an oxidase system (Nicholas et al., 1962). Although Cu has also been found to inhibit nitrification (Liang and Tabatabai, 1978), the requirement for Zn and Co is more doubtful and these metals can, in fact, inhibit nitrite formation (Loveless and Painter, 1968). Silver, Hg, Cd and Cr have also been reported to inhibit the growth of nitrifying bacteria (Meyerhof, 1916;

Fargo and Fleming, 1977; Liang and Tabatabai, 1978).

The presence of metals in the soil environment can cause a variety of effects, from stimulation to inhibition. Nitrifiers, being obligate aerobes, occur primarily on the surface of soil aggregates in preference to the more anaerobic centre. As such, they are more likely to be susceptible to metal pollution. McGrath and Brookes (1986) reported a reduction in the rate of oxidation of added ammonium and nitrite in sludge-treated soil. This reduction was ascribed to a lower activity or fewer nitrifying organisms arising as a result of high metal concentrations in the sludge-treated soils (Brookes et al., 1984). However, Yamamoto et al. (1983, cited by McGrath and Brookes, 1986) pointed out that metals can inhibit the activity of nitrifying organisms without reducing their numbers.

Comparing the nitrification rates of added ammonium in sludged and unsludged soil samples from Maudslie, Carbarns and Allanton (Figure 4.1) resulted in no clear-cut conclusion as to the effects of sewage sludge or sludge metals on soil nitrification. Notwithstanding the fact that the sludged soil from all three sites had higher metal concentrations, it was only the Carbarns sludged soil that exhibited a nitrification rate which was slower than that of the unsludged sample (Table 4.7). In contrast, at Allanton a higher nitrification rate was evident in the sludged soil, whereas at Maudslie the sludged and unsludged soils exhibited similar rates of nitrification (Table 4.7).

Inhibition in soils of nitrification by heavy metals derived from sludge is difficult to interpret partly because much of the work has been done using metal salts, generally at unnaturally high concentrations, and partly because of variations in soil properties which may modify or alter the relative and absolute toxicity of metals. In particular an increase in the pH of acid soils results in decreased Cd, Cu and Zn toxicity to nitrifiers. However, an excessive increase in soil pH may likewise decrease nitrification rates (Bhuiya and Cornfield, 1972) due to resolubilisation of metals such as Zn. Soil properties such as pH, may themselves influence the activity of the nitrifying population. Nitrification occurs rapidly near neutral pH, but it is not certain how quickly it proceeds under acid conditions, such as in many moorland and forest soils, since *Nitrosomonas* and *Nitrobacter* strains that have so far been isolated do not carry out nitrification much below pH 6 (Campbell, 1977). The optimum for most *Nitrosomonas* strains tends to fall in the range between pH 7 and 9, and activity is found in even more alkaline solutions (Alexander, 1965). On the other hand, in even slightly acid conditions proliferation of the bacteria is markedly reduced. For *Nitrobacter* strains, the optimum is often in the neutral to slightly alkaline range and activity is often detectable from about pH 5 to 10 (Alexander, 1965), although above pH 8 *Nitrobacter* does not convert much nitrite to nitrate (Campbell, 1977). However, generalisations concerning a specific optimum hydrogen ion concentration are of little value. Ulyanova (1961, 1962) found that the optimum pH for activity of ammonium oxidizers was often similar to that of the environment from which they were isolated; frequently however, the pH optimum was far removed from that of the original habitat. In addition, the bacteria may occupy microecological sites having acidities far different from that suggested by pH determinations of bulked soil.

With reference to either Table 4.7 or Figure 4.1, there appears to be a positive relationship between nitrification rate and pH, in that the nitrification rate was slowest for the most acid soil (Allanton unsludged) and quickest for the soil at the highest pH (Carbarns unsludged). However, the exception to this trend was the unsludged Maudslie soil with a pH of 6.0

When soil pH was studied in detail, it was found to have a very significant influence on nitrification in the sludged Carbarns soil (Table 4.13). Increasing the pH of the high metal sludge treated soil over even a narrow range (i.e. pH 5.47 - 6.37) led to significant decreases in the delay period and increases in K_{max} (Table 4.14). In the most acid soil (pH 5.47) the delay period was of 7.66 days and K_{max} was 11.85 mg NO_3/kg air-dry soil/day. In contrast, in the least acid soil (pH 6.37) the delay period was only 2.60 days and K_{max} increased to 45.33mg NO_3/kg air-dry soil/day. In other words, as pH rose by 0.9 units the delay period decreased by 5.06 days while K_{max} increased by 33.48mg NO_3/kg air-dry soil/day. The rate of nitrification in the sludge-treated soil of pH >5.76 was, in fact, similar to that recorded in the unsludged soil. This suggests that whereas prolonged sludge application leads to a reduction in biomass C (see Table 4.4) possibly due to the accumulation of heavy metals in the soil, provided soil pH is adjusted, no such reduction in the nitrifying microbial population occurs. Any decrease in the rate of nitrification following the disposal of sludge may be attributed to the resultant decrease in pH of the treated soil.

These results seem to contradict that stated by Harris (1988): "In general over the pH range of 5.5 to 8.0 there is usually little effect of pH and there is evidence that nitrifiers in soils of low pH are adapted to those conditions". This may hold true for the oxidation of indigenous ammonium (that produced by organic matter decomposition) where ammonification can occur sufficiently slowly for the populations of nitrifying organisms to adjust to the supply of ammonium under both high and low pH, but when presented with a large input of added ammonium the existing nitrifying organisms in high pH soils are more active (or numerous) than in low pH soils. In addition, Harris's comments take no account of the relationship between pH, metal availability and nitrification given that metals are capable of inhibiting autotrophic activity.

In nitrifying bacteria the oxidation of nitrogen compounds is a growth-linked process. As such, it is expected that the concentration of products of this oxidation, such as nitrate, follow closely the growth curve of the bacterial population, thus exhibiting a sigmoid relationship when fresh substrate is supplied. Growth curves can be divided into four phases:

i) lag phase: a period of no growth and growth acceleration;
ii) log phase: a period of logarithmic growth;
iii) maximum stationery phase: i.e. when the specific growth rate (u) is equal to the death rate; and
iv) death phase: when the death rate is greater than the specific growth rate.

During the lag phase the nitrifying population multiplies and increases in size in response to the introduction of substrate into the system. The smaller the initial population size, the longer the delay period (Sabey et al., 1959). Therefore the increased delay period as pH decreases (Table 4.14) may have resulted from a smaller initial nitrifying population in the acid soils. An increase in the delay period, i.e. the time between introduction of ammonium and exponential growth of the nitrifying population in acid soils, may stem from specific inhibition of enzymes, not necessarily those solely linked directly to nitrification. In response, the nitrifying bacteria, before being able to take full advantage of the increased substrate, need first to synthesise more of the suppressed enzymes. The time required for ammonium to be oxidised to nitrite by *Nitrosomonas*, is the rate limiting step to nitrate formation. As *Nitrosomonas* requires a higher ambient pH than does *Nitrobacter* (as discussed previously), it might be the case that the increase in the delay period as pH decreases is primarily due to the inhibition of *Nitrosomonas* rather than *Nitrobacter*. However, as no separate measure of NO_2-N production was estimated this statement cannot be verified.

After the initial delay period, an exponential increase in nitrate concentration was recorded during which time the nitrifying population grew logarithmically. At this stage, K is equivalent to u. During this phase, substrate is not limiting and the nitrifying bacteria divide rapidly by binary fission. Whereas K_{max} is unaffected by the initial (at time t = 0) population size of the nitrifying bacteria (Sabey et al., 1959) it is influenced significantly by pH. Enzymes are amphoteric molecules and as such their overall charge is influenced by pH. A change in the ionization of particular amino acids can lead to significant changes in the structural configuration of enzymes and proteins. Moreover, the catalytic efficiency of enzymes could be significantly curtailed were such changes to occur at active sites, and would, consequently, decrease K_{max}. Some form of feedback control may also be involved. The overall nitrification reaction can be represented simply as:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$$

The decrease in soil pH due to nitrification is well documented (Alexander, 1965; Lance and Whisler, 1972). Hydrogen ions, being a by-product of nitrification could, theoretically, exert some control on the enzymes involved in the oxidation of ammonium. Likewise, feedback control could originate from the build-up of nitrate and K_{max} has been shown to be dependent upon the initial substrate concentration (Sabey et al., 1959). The greater the substrate concentration, the greater K_{max}, up to a point where K_{max} can increase no further.

As the population size increases, the ammonium concentration decreases (given that bacteria are being grown in batch culture, i.e. with a fixed initial mass of nutrient), at a time when the demand for oxidizable substrate increases. Consequently, a point is reached where the remaining substrate can no longer sustain the needs of the growing nitrifying population, which, thus, ceases to expand. At this point, the rate of ammonium consumption decreases and is maintained at a constant level throughout the duration of the maximum stationary phase. This could in part explain the linear decrease in ammonium concentrations after day 10, i.e. after the bacteria had experienced log growth and sufficient time had elapsed for the system to equilibrate (Table 4.6). As the ammonium was used up, the sustainable population size decreased and the death phase set in.

When all the substrate has been utilized the concentration of nitrate increases no further, reaching its asymptote. The magnitude of the asymptotic nitrate concentration is a function of the initial ammoniumn concentration. All six pH treatments were initially supplied with equal amounts of ammonium and were, thus, expected to attain similar asymptotes. This, however, was not the case with the more acid soils which apparently exhibited a lower asymptotic values. Lowering the pH lengthens the delay period and reduces K_{max}. Thus, a longer time-period would have been necessary for the more acid soils to attain asymptotes. Moreover, for the acid soils, relatively high ammonium concentrations (i.e. 58mg/ml) (Table 4.15) were still present in solution at the end of the sampling period and, consequently, true asymptote could not have been achieved. In addition, the observed rate of nitrate production was also a product of ammonification, i.e. the production of ammonium as a result of organic matter decomposition by the soil's heterotrophic population. Were ammonification to be greater in the soils at high pH this may have contributed to the higher nitrification rates recorded for these soils. Ammonification proceeds even after the exhaustion of added ammonium and, hence, a true asmptote is never reached. After the 32 day experimental period ammonium was still present in the percolating solution and, as such, still available for nitrification (Table 4.15). This unutilized fraction of ammonium was greater in the more acid soils further underlining the inhibition of nitrification at low pH.

As stated above, nitrification is a growth-linked process and as such a sigmoid relationship between nitrate concentration and time is to be expected. Where the influence of pH on soil nitrification potential was studied (Experiment III) a sigmoid response of nitrate concentration with time was, in fact, observed. However, where the effect of sludge application on nitrification potential was investigated (Experiment II) a linear rate of nitrate production was evident. Soil conditions, especially pH, affect the rate of nitrification. As verified in Experiment III, soil pH has a very significant influence on soil nitrification, where a decrease in pH decreases K_{max} while increasing the delay period. For instance, whereas a soil of pH 6.37 exhibited a delay period of 2.60 days and a K_{max} of 45.33mg NO_3/kg air-dry soil/day, a soil of pH 5.47 exhibited a delay period of 7.66 days and a K_{max} of 11.85mg NO_3/kg air-dry soil/day. Of the six soils used in Experiment II four, i.e. Allanton sludged, Allanton control, Maudslie sludged and Carbarns sludged, were quite acidic with a pH of 5.36, 5.00, 5.29 and 5.49 respectively. Therefore, due to the acidic nature of these soils, it is possible that all that was measured over the 20-day experimental period was the delay phase and the initial stages of the log phase and, thus, resulted in an apparent linear response of nitrate concentration with time. A small initial nitrifying population would have also contributed to increased delay periods, as would enzyme inhibition which also results in a decreased K_{max}. A similar explanation may account for the linear relationship observed for the Maudslie control soil. However, in this case, factors other than pH were responsible for a long delay period and a low K_{max} as the soil pH was > 6.0. As for the Carbarns unsludged soil, which was used in both Experiments II and III, the production of nitrate in both experiments was generally similar, although in Experiment II a much greater variation was present between replicates.

The absolute rate of ammonium depletion, given that no other process other than that of nitrification led to a change in NH_4-N concentration in the percolating solution, would be expected to be equal to the absolute rate of nitrate production (assuming that NO_3-N was conserved). However, over days 0-2 in Experiment II and days 0-4 in Experimnent III, the marked decrease in the concentration of NH_4-N in the percolating solution was not reflected in a comparable increase in the concentration of NO_3-N. Immobilisation of ammonium can either be chemical, biological or both. When a soil is flushed with a cationic solution, the cation can be adsorbed onto exchange sites on the soil particles and, provided it is present in sufficient concentrations, may also replace other cations held on such sites. Adsorption onto exchange sites has been used to explain such initial decreases in ammonium concentration (Hadas et al., 1986). Chemical fixation, as opposed to sorption on exchange sites was also possible, although not likely to be very significant or probable in topsoils. Substitution of ammonium within the clay lattice is a slow process which in topsoils is restricted by organic matter shielding interlammelar sites. In addition, immobilisation of NH_4-N in this manner is dependent upon clay type with illite and to a lesser extent vermiculite showing specificity to NH_4-N (Wild, 1988). Alternatively, assimilation of NO_3-N into the microbial biomass or denitrification may have masked the true rate of

NO_3-N production and thus led to the observed situation where NH_4-N depletion was greater than NO_3-N production. However, microorganisms utilize ammonium in preference to nitrate as their source of N. The concentration of ammonium in the percolating solution was relatively high and it is, thus, improbable that any significant NO_3-N assimilation occured.

In Experiment II, after day two, the rate of ammonium depletion and nitrate production in both Maudslie soils was similar (Figures 4.2 and 4.1). However, a different situation was observed in the soils from Allanton and Carbarns. Both the sludged and unsludged Carbarns soils exhibited a rate of nitrification greater than the observed rate of ammonium depletion. In contrast, the converse was true for both Allanton soils. A production of nitrate not mirrored by an equivalent decrease in ammonium indicates the presence of a mechanism which buffers ammonium. In soils, ammonium concentrations in solution could be buffered either by the rate of decomposition of organic matter liberating sufficient ammonium to mask the rate at which it was being nitrified, or alternatively by the desorption of intrinsic soil reserves of exchangeable ammonium. However, the available N recorded for the Carbarns soil was no greater than that for the Allanton soils (Table 4.1). At Allanton, the rate of ammonium depletion was greater than nitrate production, consequently ammonium must have been utilised for purposes other than nitrification or else NO_3-N was 'lost' from the system. Although every effort was done to ensure adequate aeration of the systems, it is possible that some anaerobic pockets developed in the soils. Aanaerobic conditions promote denitrification and, hence, loss of NO_3-N from the system as N_2 or N_2O.

Microbial immobilisation of ammonium, not only by nitrifying bacteria, but also by the whole microbial population, particularly the heterotrophic fraction, could have also contributed to the removal of ammonium from the percolating solution. On sampling, soil disturbance may have resulted in improved aeration and opened new surfaces for decomposition. Ammonium immobilisation by the heterotrophic microbial population is determined by the C : N ratio of the decomposable substrate. High C : N ratio plant residues such as cereal straw, can limit the availability of N as the micro-organisms draw on soil reserves to satisfy their own nutritional needs. Generally, organic residues with C : N ratios less than 30 : 1 are unlikely to make demands on soil N during the course of decomposition (Harris, 1988). The C : N ratio of both sludged and unsludged soils from Allanton and Carbarns were well below this value (30 : 1) (see Table 4.1), and, therefore, microbial immobilisation appears to be ruled out. However, laboratory determinations of soil C : N ratios yield only a gross measure of the proportion of C and N in the organic matter. Within organic matter, there are fractions which will decompose more easily than others (i.e. they will have a higher C : N ratio and/or be relatively more easily decomposed) and may, thus, contribute in reducing available soil N reserves.

Ammonium can also be lost as ammonia gas. However, at pH 7 only 1 per cent of the ammonium present in solution is present as ammonia and this decreases with decreasing pH (Wild, 1988). Therefore, due to the acidic nature of the Allanton soils, this route of ammonium loss is highly improbable.

In both Experiments II and III over the first 2-4 days a sharp decrease in the total concentration of $(NH_4 + NO_3)$-N in the percolating solutions (Δ N) was observed (Figures 4.3 and 4.7). Thereafter, Δ N increased markedly. However, whereas in Experiment II a linear increase in Δ N was recorded, a sigmoidal increase of Δ N over this same period was observed in Experiment III and in particular for those soils of pH > 5.76. In these soils, ammonium, which had initially been adsorbed onto exchange sites, was desorbed due to the diffusion gradient generated by the activity of the nitrifying bacteria, thus increasing the overall Δ N in solution. The rate of supply of NH_4-N reflects the rate of nitrification. Ammonification could have also itself contributed to this increase in Δ N. However, after day 10, by which time nitrate concentrations had attained an asymptote, the rate at which Δ N increased slowed down markedly, suggesting that nitrification and not ammonification was the primary agent in bringing about N remobilisation and, hence, an increase in Δ N.

After day 10, the decomposition of dead nitrifying bacteria could have also contributed to the remobilisation of N which had previously been immobilised in the expanding nitrifying population. However, a net gain in $(NH_4 + NO_3)$-N in the percolating solution, over and above that present initially (i.e. at time t=0), was entirely due to ammonification.

The accumulation of metals in sludged soils, even when metal concentrations are within maximum recommended levels (EEC, 1986), can result in a significant reduction of soil microbial biomass C. However, the interpretation of any such situation needs to take into consideration various soil parameters and, in particular, organic matter content. Provided the pH of the soil is maintained close to neutrality, soil nitrification may be stimulated by long term sludge disposal, despite the accumulation of metals. However, a reduction in soil pH results in increased delay-periods coupled with decreased K_{max}. Sludge application can lead to changes in the pH of the receiving soil (see Section 1.3.1) and this itself may affect nitrification.

CHAPTER 5

FACTORS AFFECTING THE ADHERENCE AND 'WASH-OFF' OF SLUDGE DERIVED METALS FROM LEAF SURFACES

5.1 INTRODUCTION

Sewage sludge contamination of leaf surfaces is an inherent problem with conventional methods of sewage sludge application to agricultural land, which generally involve spray guns or surface spreading by tanker. This adherence of sludge to the leaf has raised considerable concern in relation to human and animal health, due to the wide array of pathogenic organisms which can be present in sludge. In U.K. sludges, four main groups of potentially infectious pathogens have been identified (DoE, 1981):

i) Bacteria
ii) Eggs of parasitic worms
iii) Viruses
iv) Protozoan cysts.

The risk associated with potential infection of man or grazing animals from these pathogens in adhering sludge led to the adoption of a three-week 'no-grazing' period (DoE, 1981) after sludge application. The sludges to which this limitation applied included those produced by anaerobic digestion (> 40% reduction in organic matter), lagooning (>2yrs) or drying or de-watering, followed by stacking (>1yr overall age). Where unpasteurised milk was produced from cows grazing contaminated herbage, a 5-week no-grazing period was implemented (DoE, 1981). The adoption of this no-grazing safety period was principally a precaution against *Salmonella* infection (DoE, 1981). Where other forms of sewage sludges, which did not conform to the prerequisites of a 3-week no-grazing period, were applied to grazing land, the no-grazing period was enforced for 6 months to prevent any infection by the eggs of parasitic worms, particularly *Taenia saginata* (DoE, 1981).

In the recent E.E.C. Directive (E.E.C., 1986) which regulates sewage sludge disposal on agricultural land in Member States, a 3-week minimum no-grazing period has been adopted where sludge is applied to grazing land or to forage crops before harvest. Furthermore, the application of raw untreated sludge is prohibited except where this is injected or worked into the soil, provided that there is no associated risk to human or animal health (E.E.C., 1986).

The most common causitive organisms of clinical salmonellosis in cattle are *Salmonella dublin* and *S. typhimurium* (Bell, 1976). The former is considered to be host-specific, while the latter, which is widely distributed, is a common cause of salmonellosis in man and can therefore be expected to be present in sludge (Bell, 1976). However, in the U.K., there is no clear association between sludge disposal and salmonellosis in grazing animals (Davis, 1980). It appears that the overall numbers of *Salmonella* in sludge applied to grassland are low in relation to the known infective doses for cattle (Davis, 1980), which for *S. dublin* are around 10^4 to 10^5 per gram of grass (Bell, 1976). Mesophilic anaerobic digestion of sludge is particularly effective in reducing *Salmonella* numbers to levels which are not infective (Healey, 1984), with up to 90-99% reduction in numbers of enterobacteria being possible (Pederson, 1981). Moreover, the die-off rate of *Salmonella* in the field is very high particularly during sunny periods (Healey, 1984) and can reach undetectable levels within 42 days of application (Jones and Watkins, 1985). However, some instances of salmonellosis in cattle have been linked to the disposal of sludge to grazing land (Bicknell, 1972; Hess, 1981; Reilly et al, 1981). The possibility of infection is greatest when animals are subjected to extreme conditions of prolonged exposure to heavy contamination or when the susceptibility of cattle might be unusually high (Hall and Jones, 1978). In practice, the danger to human and animal health are minimal if proper operating practices and guidelines are followed (Jones et al, 1983).

There is no epidemological evidence to link the use of digested sludge on grassland with infection of man or animals by other pathogenic organisms such as *T. saginata* (Davis, 1980; Block, 1983; Healey, 1984). This is probably due to the effectiveness of sludge-treatment processes and, in particular, mesophilic anaerobic digestion, in killing or inactivating pathogenic organisms within the sludge (Silverman and Guiver, 1960; Healey, 1984; Evans et al, 1986). The presence of *T. saginata* in sludge is of particular interest as its life cycle involves both man and cattle. The primary host is man in whom the tapeworm develops and sheds ova which find their way into the sewage sludge. If the secondary host (cattle) ingests the ova, infection in the form of cysticerci (*Cysticercus bovis*) develops in the muscle and the cycle will be repeated if humans consume infected and inadequately cooked beef. The cycle is broken by meat inspection to detect infected carcasses and by cooking which destroys the cysticerci, although some infected carcasses may slip undetected through inspection and cause infection in humans. However, this disease is uncommon in the U.K. and although it is not a notifiable disease, usually less than 100 cases per annum come to light, two-thirds being accounted for by foreign visitors (DoE, 1981). In some cases, *Brucella abortus* can be of importance (Bell et al, 1978), where sludge-fertilized grass is grazed by brucellosis-free attested herds. However, the presence of these bacteria in sewage sludge is unlikely and their survival in sludge is limited (Bell et al, 1978; Lewin et al, 1981).

As regards viruses, these are extremely host-specific (Hudson and Fennell, 1980) and, consequently, it is very unlikely that human enteroviruses present in sewage sludges would present any hazard to animals or vice-versa. Also, the viruses which are of economic importance to the farmer, such as foot and mouth disease and swine vesicular disease, are so rigidly controlled by inspection that the chance of contamination of sewage sludge is extremely unlikely (Hudson and Fennell, 1980).

Unlike pathogenic organisms, heavy metal ingestion from contaminated leaf surfaces has received very little attention. The DoE (1981) guidelines laid down restrictions on the maximum Pb (i.e. 2000mg/kg) and F (i.e. 3500mg/kg) content of sewage sludge applied to land as a precaution against possible ingestion of sludge or sludged soil by children and grazing animals. However, very little work has been directed at studying the contribution of this adhering sludge to the net metal content of herbage. Even less attention has been paid to investigating the behaviour of these 'adhering' metals or the factors influencing their rate of removal. With this in mind, field and pot experiments were undertaken.

AIMS AND OBJECTIVES

The aims and objectives of the experimental work were:

(a) To investigate the importance of sludge-dry matter content and application rate on

  (i) the adherence of sludge metals to leaf surfaces

  (ii) the loss of adhered sludge from leaf surfaces with time.

(b) To assess the importance of the grass canopy height on (i) and (ii) as outlined above.

(c) To relate the dilution of adhering sludge to

  (i) rainfall

  (ii) plant growth.

5.2 MATERIALS AND METHODS

5.2.1 FACTORS INFLUENCING SLUDGE METALS ADHERING TO LEAF SURFACES IN A FIELD SITUATION.

A trial was carried out at Temple Field (Nat. Grid Ref. NS 381 237), which is part of the West of Scotland College farm estate. The soil in this field is an imperfectly drained loam on a reddish brown clay-till of the Bargour series. The sward consisted of perennial ryegrass (*Lolium perenne*) Cv. Springfield, which had been sown in the spring of 1986.

An 11.5m x 6m area was pegged out and divided into 3 blocks, each consisting of 6 sub-blocks of 1.5m x 1.5m on which 6 treatments were randomized (see Section 5.2.1.1). Each sub-block was, in turn, divided into 9 mini-plots of 0.5m x 0.5m each providing an area from which one of 9 sequential herbage cuts were taken over the duration of the experiment (see Section 5.2.1.1). A 0.5m discard strip separated each mini-plot.

5.2.1.1 TREATMENTS

Six treatments were devised:

Treatment 1 (T1): uncut grass, no sludge applied;

Treatment 2 (T2): cut grass, no sludge applied;

Treatment 3 (T3): uncut grass, 'normal' (2% D.S.) sludge applied at 67.5m^3/ha;

Treatment 4 (T4): cut grass, 'normal' (2% D.S.) sludge applied at 67.5m^3/ha;

Treatment 5 (T5): uncut grass, 'normal' (2% D.S.) sludge applied at 25m^3/ha;

Treatment 6 (T6): uncut grass, 'thickened' (12% D.S.) sludge applied at 67.5m^3/ha.

Sewage sludge from the Maudslie Sewage Works, which contains treated sewage of predominantly domestic origin (Table 5.1), was applied on the 24th March 1987. A watering can fitted with a spoon at the tip of the spout (to act as a splash plate) was used to apply the appropriate volume of sludge to each mini-plot. 'Normal' sludge containing 2% dry solids consisted of anaerobically digested sewage sludge taken directly from the digestor. 'Thickened' sludge containing 12% dry solids was anaerobically digested sludge which had been stored in lagoons for about 3 months.

On the 2nd March 1987, 3 weeks prior to sludge application, treatments T2 and T4, i.e. the cut grass treatments, were mown to within 4cm of ground level using a motorised 'Agria' grass cutter. The whole experimental area then received a base dressing of 50kg N/ha, 25kg P_2O_5/ha and 25kg K_2O/ha as a 20:10:10 NPK fertilizer.

A total of 9 herbage cuts were harvested weekly between 31st March - 19th May 1988 (inclusive) by random selection from each sub-block. The first cut was taken just 3hrs after sludge application. Each sample consisted of all the herbage growing in an individual mini-plot cut at a height of 2cm above ground level using hand shears to minimise soil

TABLE 5.1 MEAN (±S.D.) HEAVY METAL CONCENTRATION (mg/kg DRY SOLIDS) AND DRY SOLIDS CONTENT (%) OF SEWAGE SLUDGES ('NORMAL' AND 'THICKENED') USED IN THE TEMPLE FIELD TRIAL.

	NORMAL	THICKENED
Cd	2.7(±0.2)	3.7(±0.7)
Cr	69.3(±3.1)	72.0(±0.0)
Cu	341.0(±15.8)	346.5(±9.2)
Fe	18365.0(±441.0)	19270.0(±410.0)
Mn	951.3(±44.8)	899.0(±0.0)
Ni	39.5(±1.9)	40.8(±0.0)
Ti	2548.5(±326.0)	2849.5(±208.0)
Pb	258.5(±14.4)	312.5 (±6.4)
Zn	687.7(±40.8)	753.5(±47.4)
%D.S.	2.1(±0.1)	12.4(±6.1)

for the need to obtain sufficient herbage for heavy metal analysis.

Herbage samples were weighed, dried and analysed for their heavy metal content as described in Section 2.2. During the experiment, meteriological data (precipitation, wind speed and air and soil temperature) were recorded. This information was obtained from the meteriological station at the West of Scotland College, except for rainfall intensity which was obtained from Prestwick Airport around 2km from the site.

5.2.2 THE INFLUENCE OF 'RAINFALL' AND DRYING ON 'WASH-OFF' OF ADHERING SLUDGE.

Perennial ryegrass (*L. perenne*) was grown in 20cm diameter pots containing medium grade perlite in a growth room (25°C). To prevent any perlite from contaminating the herbage, the grass was sown and grown on nylon netting lying over each pot. Pots stood in gravel trays filled with a low-N (CF 20) nutrient solution (see Appendix B).

A 0.354g/pot seeding rate, equivalent to 112.5kg/ha , was used. However, this resulted in a very thin sward. To promote tillering and, hence, a thicker sward, the grass was cut on three occasions prior to sludge application.

Sludge from the Carbarns Sewage Works (Table 5.2) was applied on the 14th March 1988, at which time the grass had attained a height of around 12cm. Apart from the four replicate control pots which received no

TABLE 5.2 MEAN (±S.D.) HEAVY METAL CONCENTRATION (mg/kg D.M.) AND DRY SOLIDS CONTENT (%) OF SEWAGE SLUDGE USED IN THE POT TRIAL.

Cd	2.9(±0.1)
Cr	120.3(±12.0)
Cu	472.0(±8.3)
Fe	26350.0(±420.0)
Mn	1016.0(±13.5)
Ni	47.2(±1.8)
Pb	748.8(±67.8)
Zn	776.3(±10.4)
%D.S.	2.8(±0.1)

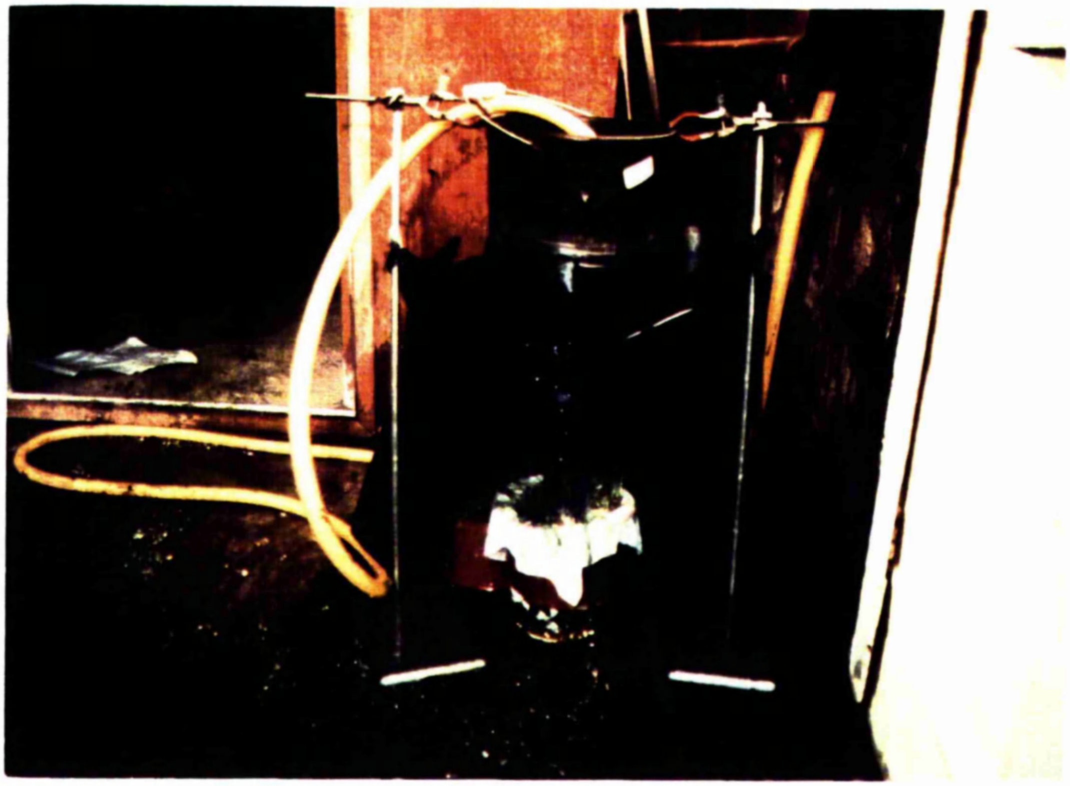
sludge, sewage sludge was applied to all the other pots using a measuring cylinder fitted with a spoon (to act as a splash-plate), at a rate of 200ml/pot (equivalent to 63.7m^3/ha). Following sludge application four replicate pots, chosen at random from a pool of 44, were subjected to different simulated rainfalls as follows:

i) Control - no sludge, 2.0mm 'rain';
ii) Sludged, 0.0mm 'rain';
iii) Sludged, 0.7mm 'rain';
iv) Sludged, 1.3mm 'rain';
v) Sludged, 2.0mm 'rain';
vi) Sludged, 2.7mm 'rain';
vii) Sludged, 3.3mm 'rain';
viii) Sludged, 2.1mm 'rain' applied as 3 x 0.7mm;
ix) Sludged, 2.0mm 'rain' applied after a 24h drying period.

Thirty minutes after sludge was applied, during which time the pots were kept in an atmosphere of 100% humidity, each pot received simulated 'rain'. The only exception to this was the 'dried sludge' treatment (i.e. Treatment ix) for which pots were kept for 24hrs in an atmosphere of 51% humidity prior to 'rain' application.

'Rain' was provided via a pail fitted with a plastic hose at its base (Plate 5.1). To ensure constant 'rainfall' intensity, the water level within the pail was maintained at a fixed level by means of a series of outlet holes drilled at a given height along its side and a constant rate of water supply to the pail. This set-up was suspended at a height of about 70cm. The pots were introduced at a fixed spot

PLATE 5.1 PHOTOGRAPH SHOWING 'RAIN' APPLICATOR FOR POT EXPERIMENT INVESTIGATING THE INFLUENCE OF 'RAINFALL' ON 'WASH-OFF' OF ADHERING SLUDGE METALS.



underneath the rainfall simulator and kept there for an appropriate period of time, during which they were rotated to ensure a uniform distribution of 'rain' over all of the pot area. A very high rainfall intensity of 6.3mm/min was used.

The entire herbage on each pot to the nylon netting surface was cut within 10mins of the 'rain' event. The fresh and dry weight yields, as well as the total Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn concentrations of these samples were determined as described in Section 2.2.

5.3 RESULTS.

5.3.1 FACTORS INFLUENCING SLUDGE METALS ADHERING TO LEAF SURFACES IN A FIELD SITUATION.

The concentration of total Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn (i.e. the sum of metal within the plant and metal from any contaminating source adhering to leaf surfaces) is reported in Table 5.3. The full sets of data are given in Appendix M. The high Fe concentrations present in the control treatments, i.e. 884 and 3398mg/kg for the uncut (T1) and cut (T2) respectively, on day 0 (i.e. the day of sludge application) indicate a high degree of contamination, probably from soil. The Fe content of plant tissue varies according to species but is usually within the range 50-250mg/kg, (Davies and Jones, 1988), much lower than the concentrations measured for the controls . In practice, the extent of any suspected soil contamination can be ascertained by titanium (Ti) determination of plant material. Titanium is prevalent in soil but is not taken up by plants and > 2mg Ti/kg dry matter in plant material indicates significant soil contamination (Scott et al, 1971). However, analysis of the sludge showed in excess of 7000mg Ti/kg dry matter (see Table 5.1), which ruled out the use of Ti as an indicator of soil contamination. The 'cut' control probably exhibited a greater degree of soil contamination compared to the 'uncut', due to its more open canopy, resulting in greater 'soil splash' during periods of rainfall. Moreover, cutting could have contributed to soil contamination by disturbing the surface soil through the effects of traffic.

During the experiment, metal uptake by the treated grass sward could

TABLE 5.3 TOTAL METAL CONCENTRATION (mg/kg DRY-MATTER) OVER TIME (DAYS) FOR TREATMENTS 1-6 (TEMPLE FIELD). EACH READING IS AN AVERAGE FOR 3 REPLICATES.

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT CONTROL (T1)								
0	0.471	4.68	8.20	884	125.3	1.76	4.88	32.00
7	0.446	3.74	9.21	601	143.7	2.22	4.93	34.67
14	0.429	5.20	9.47	1167	157.7	2.08	6.29	34.33
21	0.375	3.74	8.58	651	141.0	1.70	5.04	32.33
28	0.327	2.86	7.94	526	151.7	1.42	4.71	30.67
35	0.258	1.79	6.02	291	120.3	1.38	2.30	23.00
42	0.246	1.39	5.57	211	112.7	1.25	2.07	21.67
49	0.296	0.87	5.81	116	77.1	1.16	1.61	23.67
56	0.204	0.42	4.42	62	88.3	1.13	1.14	18.33
CUT CONTROL (T2)								
0	0.706	15.81	10.30	3398	170.7	3.13	9.51	40.67
7	0.500	10.71	11.15	2140	156.0	2.47	7.99	37.00
14	0.521	11.13	12.04	2485	181.3	2.80	10.37	40.67
21	0.317	6.19	9.68	1216	126.0	1.98	5.29	34.33
28	0.275	3.48	9.10	646	120.7	1.23	3.97	29.67
35	0.263	2.63	6.97	507	92.9	1.09	2.65	23.00
42	0.252	2.07	4.92	286	100.5	0.92	2.01	17.33
49	0.275	1.21	5.77	189	97.9	0.99	3.38	23.00
56	0.146	0.41	3.78	79	85.6	0.73	1.02	15.67
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)								
0	0.842	35.08	35.17	8151	239.7	7.55	35.27	90.67
7	0.806	15.99	40.00	3175	224.0	3.96	30.78	94.33
14	0.717	15.27	38.13	3303	252.3	4.14	29.81	92.33
21	0.479	10.78	27.43	2116	191.0	3.05	23.07	73.33
28	0.506	9.85	26.63	1921	184.0	3.57	18.24	67.00
35	0.452	7.72	20.03	1412	164.0	2.33	13.28	54.33
42	0.348	4.23	12.27	700	115.3	1.46	8.17	34.00
49	0.308	1.22	7.12	181	79.9	1.25	3.16	30.33
56	0.252	0.58	5.12	87	80.8	1.12	1.48	19.00
CUT, FULL RATE, 2% D.S. SLUDGE (T4)								
0	0.813	35.84	35.03	8646	263.3	7.90	34.95	93.33
7	0.706	21.61	32.60	4507	241.7	4.74	24.62	83.67
14	0.621	17.29	31.17	4094	237.0	4.67	24.78	80.33
21	0.477	10.18	21.83	1970	150.7	2.81	15.22	59.67
28	0.319	4.92	13.40	934	124.7	1.97	5.50	46.00
35	0.290	3.31	9.36	561	88.5	1.31	3.67	30.67
42	0.306	1.95	7.89	278	97.1	1.15	3.17	26.33
49	0.315	1.34	6.01	192	79.4	1.17	2.59	22.00
56	0.250	0.53	4.73	82	69.7	0.94	1.07	17.33
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)								
0	0.702	23.00	26.10	5203	218.3	5.05	24.88	74.00
7	0.546	10.95	26.47	2085	223.7	3.43	20.15	67.33
14	0.519	8.01	18.99	1726	170.0	2.67	15.61	55.67
21	0.438	8.05	18.27	1243	168.7	2.65	14.14	48.67
28	0.379	5.48	15.98	986	170.3	1.92	10.15	46.00
35	0.333	3.38	9.87	498	133.7	2.99	5.21	32.00
42	0.327	2.44	7.94	325	125.0	1.37	4.59	25.67
49	0.273	1.43	6.09	190	106.2	1.48	2.82	22.00
56	0.244	0.83	5.14	97	98.9	1.25	1.76	20.00
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)								
0	1.896	62.82	131.67	14720	517.0	16.95	143.46	305.33
7	2.196	63.37	177.33	13845	575.0	17.90	173.63	389.00
14	1.967	49.86	161.00	12704	559.3	15.96	164.25	359.67
21	1.704	45.52	131.67	9994	480.7	14.13	142.44	306.33
28	1.488	41.44	126.30	10031	443.0	11.22	117.31	279.67
35	1.248	35.27	100.60	7536	384.0	9.09	92.42	223.33
42	0.533	10.54	29.84	1894	205.3	3.10	24.28	76.00
49	0.319	2.14	8.75	352	90.9	1.29	5.60	28.33
56	0.350	5.19	17.12	1095	126.6	1.88	13.97	45.00
S.E.	0.058	1.96	4.10	504	17.9	0.52	3.51	8.25

have been enhanced, but such a response would have been small given the cold weather conditions in March/April, poor growth-rates and the relatively short duration of the experiment. To provide control herbage which had received comparable rates and types of metal sources as those provided to the treated plots would have been unrealistic. Moreover, within the 8-week experimental period the metal concentrations of the sludged herbage reverted to values no different from those present in the controls. In other words, although metal availability for uptake may have increased in the sludged plots, this was irrelevant to the overall metal load of the herbage.

To minimize the effect of increased nutrient availability following sludge application, all plots including the controls received a base dressing of fertilizer NPK at the start of the experiment (see Section 5.2.1.1.) thus ensuring an adequate supply of nutrients for all plots. In the abscence of a uniform dressing of fertilizer N-P-K to all plots growth of the sludge-treated sward might have been favoured over that of the controls. On the other hand, sludge adhering to the leaf surfaces of the treated herbage (i.e. T3, T4, T5 and T6) could, theoretically, have led to reduced light assimilation by the chloroplasts and consequently, the overall effect of sludge spreading on plant growth. However, there was no evidence that either metal availability, N-P-K availability or smothering led to differences in dry - matter yield between the control and treated plots.

A measure of the contribution of adhering sludge to the total metal concentration of the herbage was obtained by:

[Adhering metal] = [Total metal] - [Total control metal]

where

[Adhering metal] = The concentration of sludge metal adhering to herbage (i.e. 'net' concentration)

[Total metal] = The total metal concentration recorded in herbage

[Total control metal] = The total concentration of metal present in the control herbage.

The mean adhering metal concentration for each of the 4 treatments on each of the 9 sampling days is reported in Table 5.4. The time taken for 'adhering metals' derived from sludge to reach concentrations which were not significantly different from zero was dependent upon the rate and type of sludge applied and the grass canopy, in the order : 2% D.S. sludge, uncut, half rate (T5) < 2% D.S. sludge, cut, full rate (T4) < 2% D.S. sludge, uncut, full rate (T3) < 12% D.S. sludge, uncut, full rate (T6). Depending on the metal, between 1-4 weeks were necessary to attain these background concentrations in the 2% D.S. sludge, uncut, half-rate treatment (T5); 2-4 weeks for the 2% D.S. sludge, cut, full rate treatment (T4) and 3-7 weeks for the 2% D.S. sludge, uncut, full rate treatment (T3) (Table 5.5). In contrast, all the 'adhering metals' in the 12% D.S. sludge, uncut, full-rate treatment (T6) required a full 7 weeks to reach concentrations which were not significantly different from zero.

The changes in adhering metal concentration with time are illustrated in Figures 5.1 - 5.8. Over the first 14 days following 12% D.S. sludge

TABLE 5.4 ADHERING METAL CONCENTRATION (mg/kg DRY MATTER) OVER TIME (DAYS) IN TREATMENTS 3, 4, 5 AND 6 (TEMPLE FIELD). EACH READING IS AN AVERAGE FOR 3 REPLICATES.

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)								
0	0.371	30.40	26.97	7267	114.30	5.80	30.40	58.67
7	0.360	12.25	30.79	2574	80.33	1.74	25.85	59.67
14	0.288	10.07	28.66	2136	94.67	2.06	23.52	58.00
21	0.104	7.04	18.86	1465	50.00	1.36	18.03	41.00
28	0.179	6.99	18.69	1395	32.33	2.15	13.53	36.33
35	0.194	5.94	14.01	1120	43.67	0.94	10.98	31.33
42	0.102	0.56	6.70	488	2.63	0.21	6.10	12.33
49	0.012	0.34	1.31	65	2.83	0.09	1.55	6.67
56	0.048	0.16	0.70	26	-7.43	-0.02	0.34	0.67
CUT, FULL RATE, 2% D.S. SLUDGE (T4)								
0	0.107	20.02	24.73	5248	92.67	4.77	25.44	52.67
7	0.206	10.90	21.45	2368	85.67	2.27	16.62	46.67
14	0.100	6.17	19.13	1609	55.67	1.87	14.41	39.67
21	0.160	3.99	12.16	754	24.67	0.82	9.93	25.33
28	0.044	1.43	4.30	288	4.00	0.73	1.54	16.33
35	0.027	0.68	2.39	55	-4.43	0.21	1.01	7.67
42	0.054	-0.12	2.97	-8	-3.37	0.23	1.16	9.00
49	0.040	0.13	0.24	2	-18.47	0.18	-0.78	-1.00
56	0.104	0.12	0.95	4	-15.87	0.22	0.04	1.67
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)								
0	0.231	18.32	17.900	4319	92.96	3.29	20.01	42.00
7	0.100	7.21	17.257	1484	80.00	1.21	15.22	32.67
14	0.090	2.81	9.514	558	12.33	0.59	9.31	21.33
21	0.063	4.31	9.690	592	27.67	0.95	9.10	16.33
28	0.052	2.62	8.037	460	18.67	0.49	5.43	15.33
35	0.075	1.59	3.847	207	13.34	1.61	2.91	9.00
42	0.081	1.05	2.370	114	12.30	0.13	2.52	4.00
49	-0.023	0.55	0.283	73	29.13	0.31	1.20	-1.67
56	0.040	0.41	0.720	36	10.63	0.12	0.62	1.67
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)								
0	1.895	58.14	123.47	13836	391.6	15.19	138.58	273.3
7	1.750	59.64	168.12	13244	431.3	15.68	168.70	354.3
14	1.538	44.67	151.53	11537	401.7	13.88	157.96	325.3
21	1.329	41.77	123.09	9342	339.7	12.43	137.40	274.0
28	1.161	38.58	118.36	9505	291.3	9.79	112.60	249.0
35	0.990	33.48	94.58	7244	263.7	7.71	90.11	200.3
42	0.287	9.15	24.27	1683	92.6	1.85	22.21	54.3
49	0.023	1.27	2.94	236	13.8	0.13	3.99	4.7
56	0.146	4.78	12.70	1033	38.3	0.75	12.83	26.7
S.E.	0.058	1.96	4.10	505	17.9	0.52	3.51	8.2

TABLE 5.5 TIME (DAYS) REQUIRED FOR MEAN ADHERING SLUDGE METAL CONCENTRATION TO REACH VALUES NOT SIGNIFICANTLY ($P < 0.05$) DIFFERENT FROM ZERO (i.e. CONTROL CONCENTRATIONS).

	TREATMENT			
	2% D.S. SLUDGE			12% D.S. SLUDGE
	UNCUT, FULL RATE	CUT, FULL RATE	UNCUT, HALF RATE	UNCUT, FULL RATE
Cd	42	14	7	49
Cr	49	28	28	49
Cu	49	28	35	49
Fe	28	21	14	49
Mn	21	21	14	49
Ni	21	21	7	49
Pb	42	28	14	49
Zn	42	28	14	49

LEGEND FOR FIGURES 5.1 TO 5.16

TREATMENT 3 - UNCUT, FULL RATE, 2% D.S. SLUDGE
TREATMENT 4 - CUT, FULL RATE, 2% D.S. SLUDGE
TREATMENT 5 - UNCUT, FULL RATE, 2% D.S. SLUDGE
TREATMENT 6 - UNCUT, FULL RATE, 12% D.S. SLUDGE

FIGURE 5.1 ADHERING CADMIUM CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

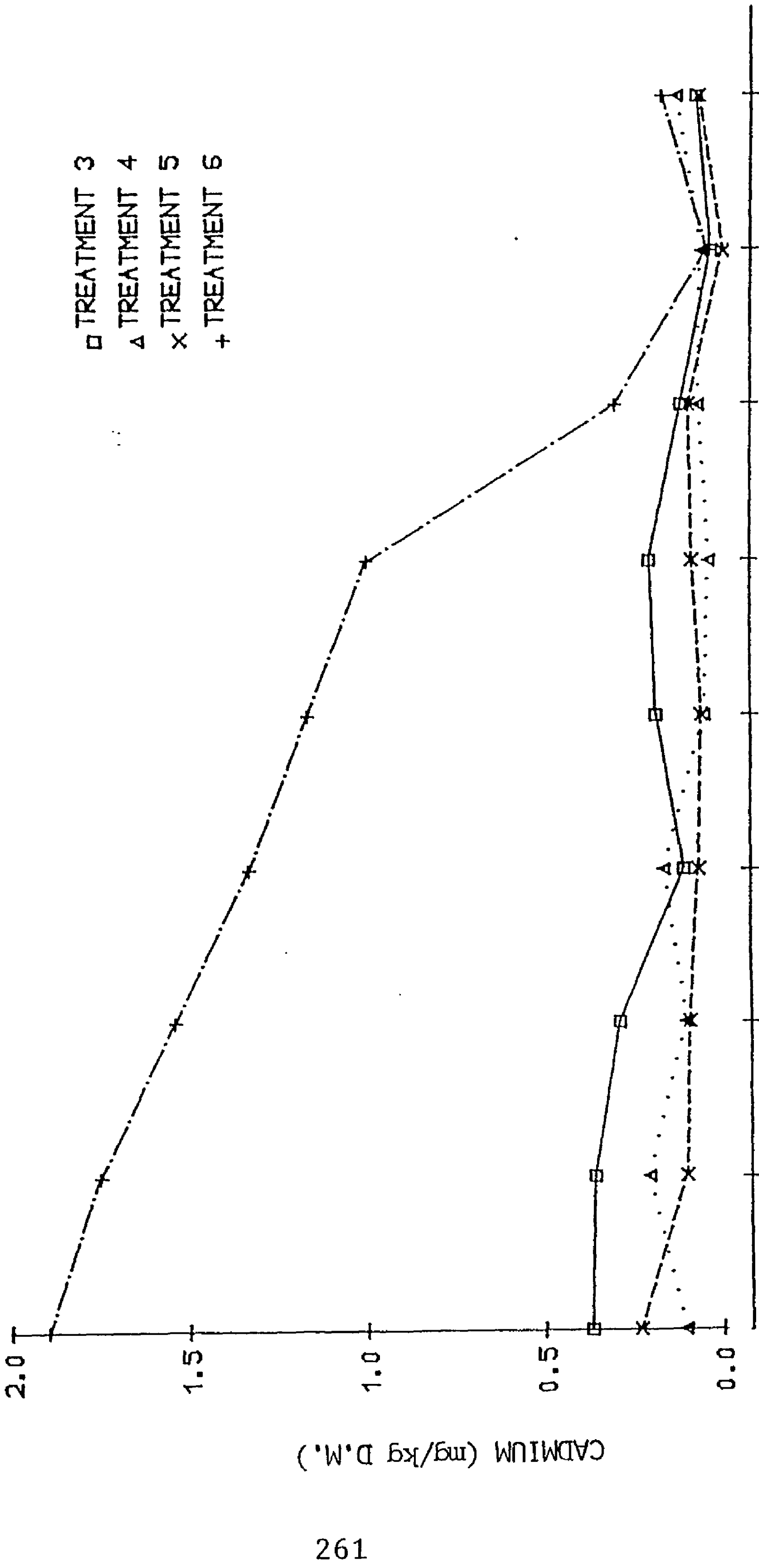


FIGURE 5.2 ADHERING CHROMIUM CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

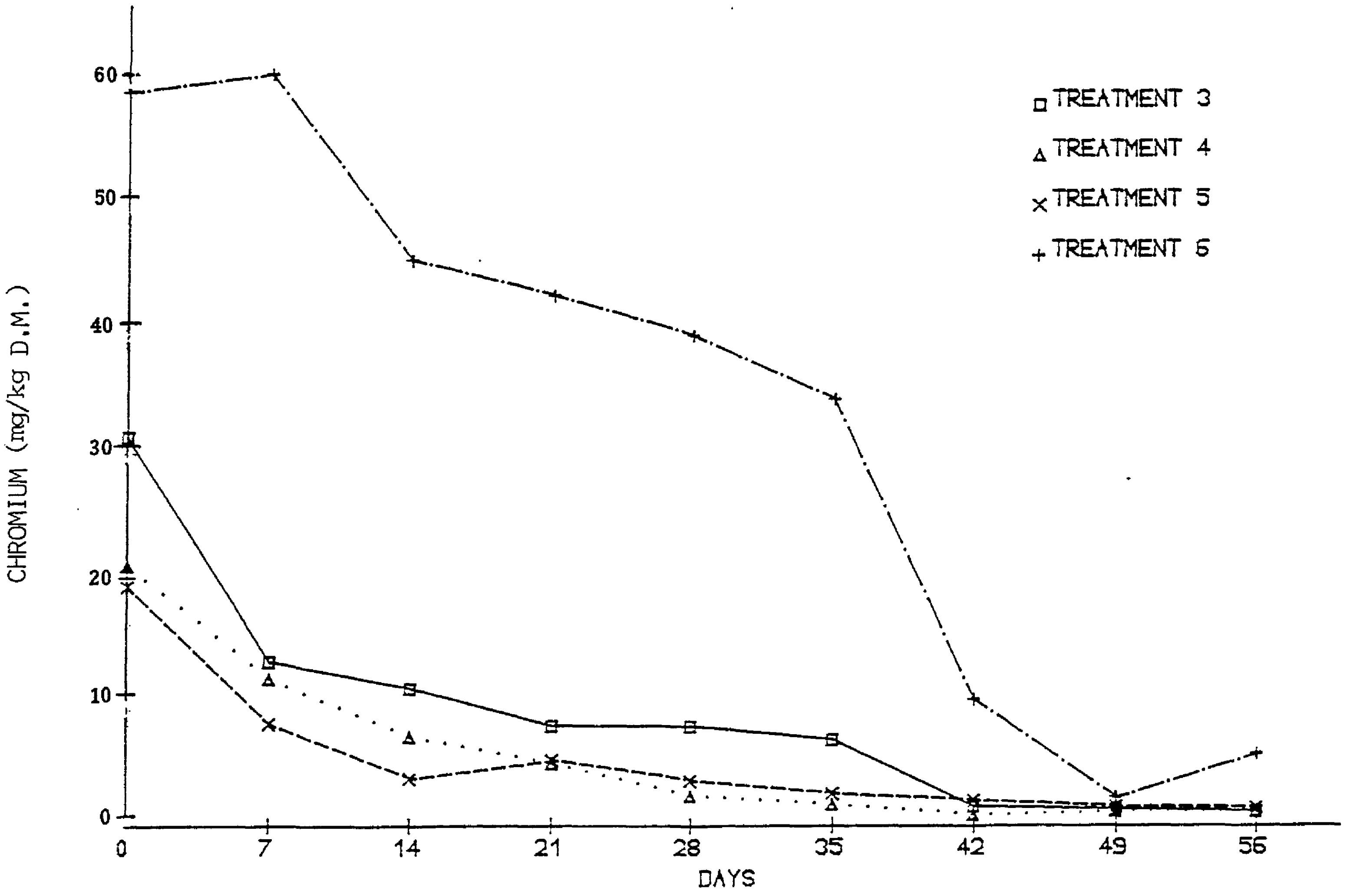


FIGURE 5.3 ADHERING COPPER CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

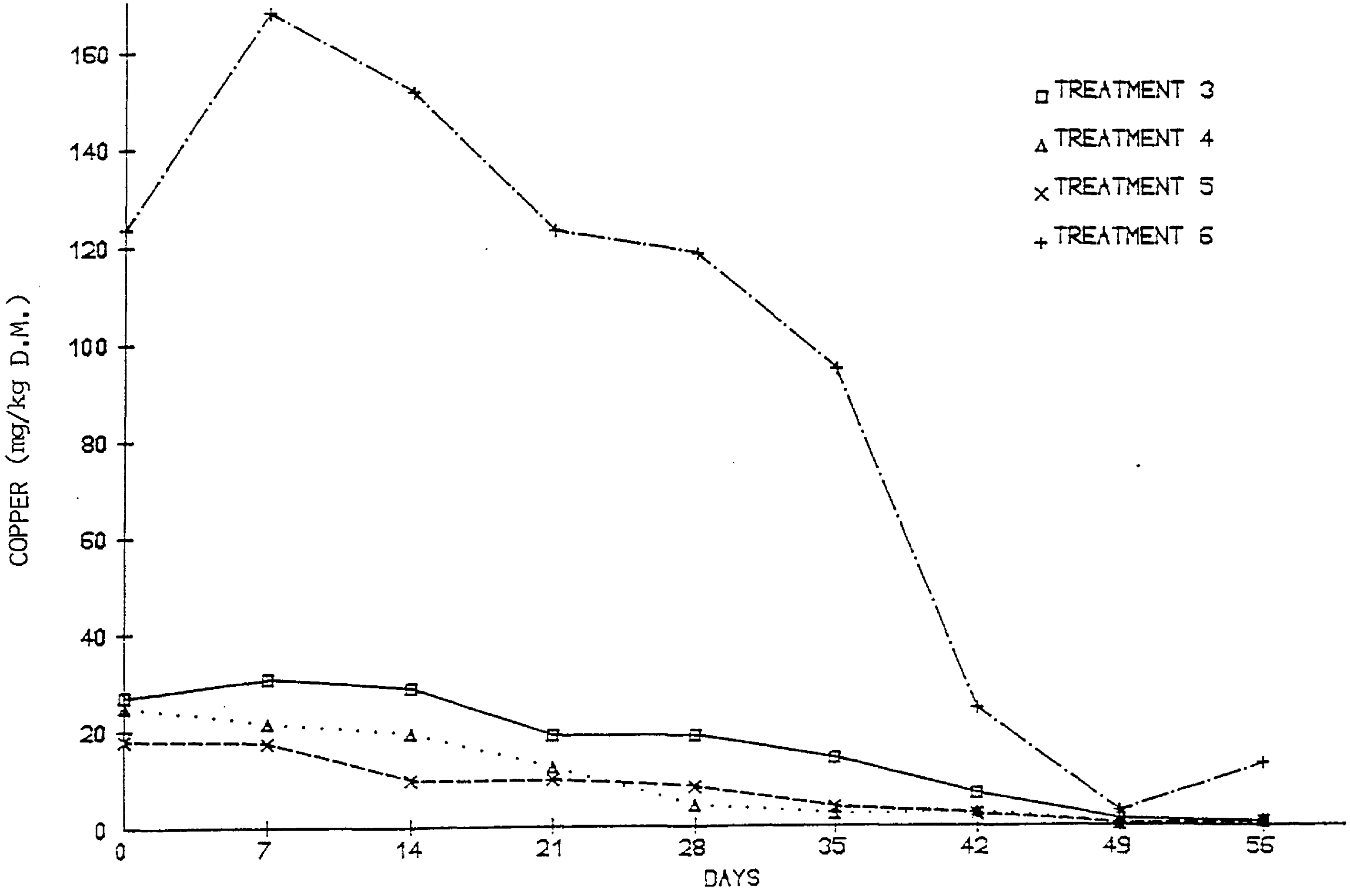


FIGURE 5.4 ADHERING IRON CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

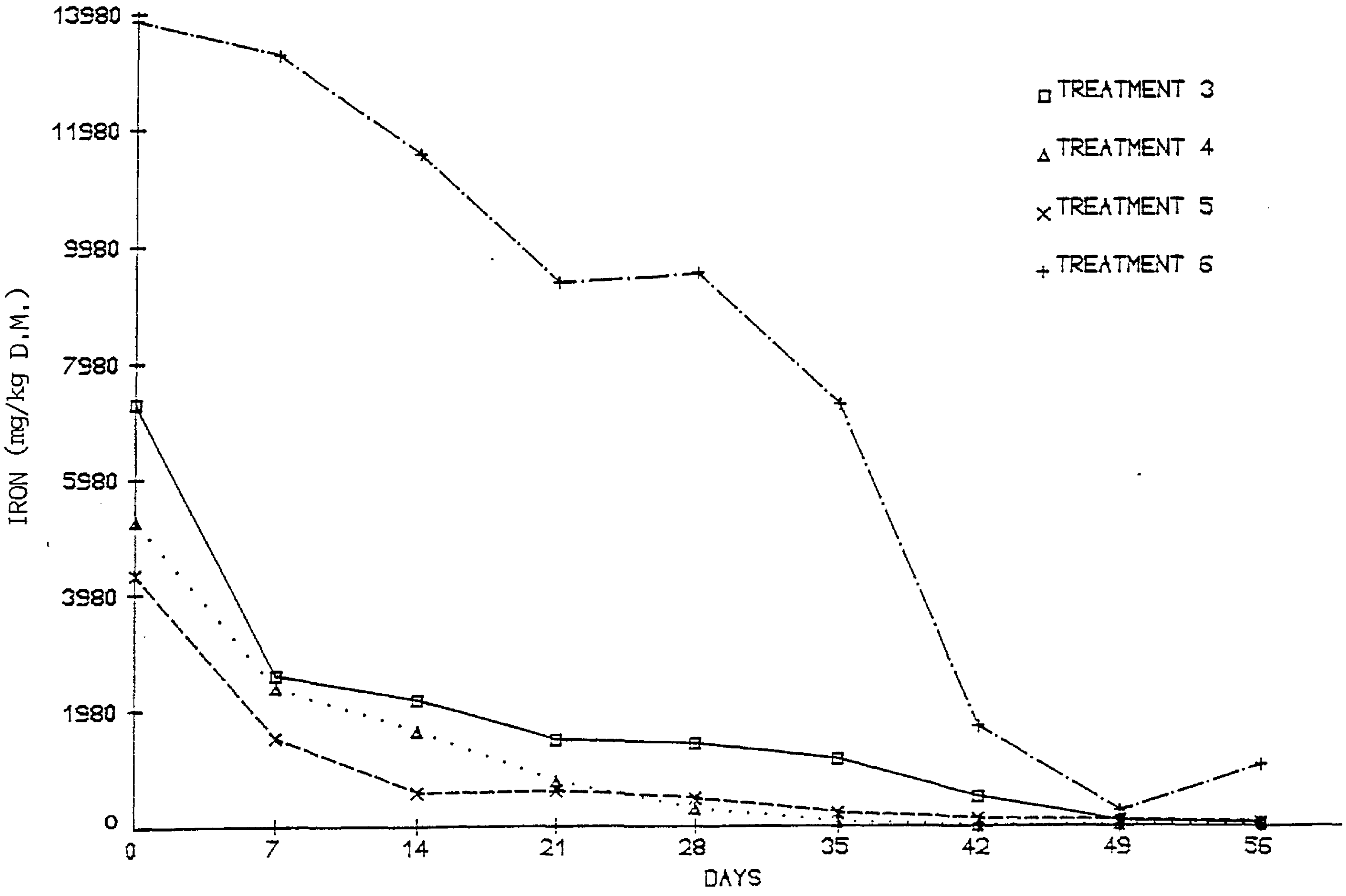


FIGURE 5.5 ADHERING MANGANESE CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

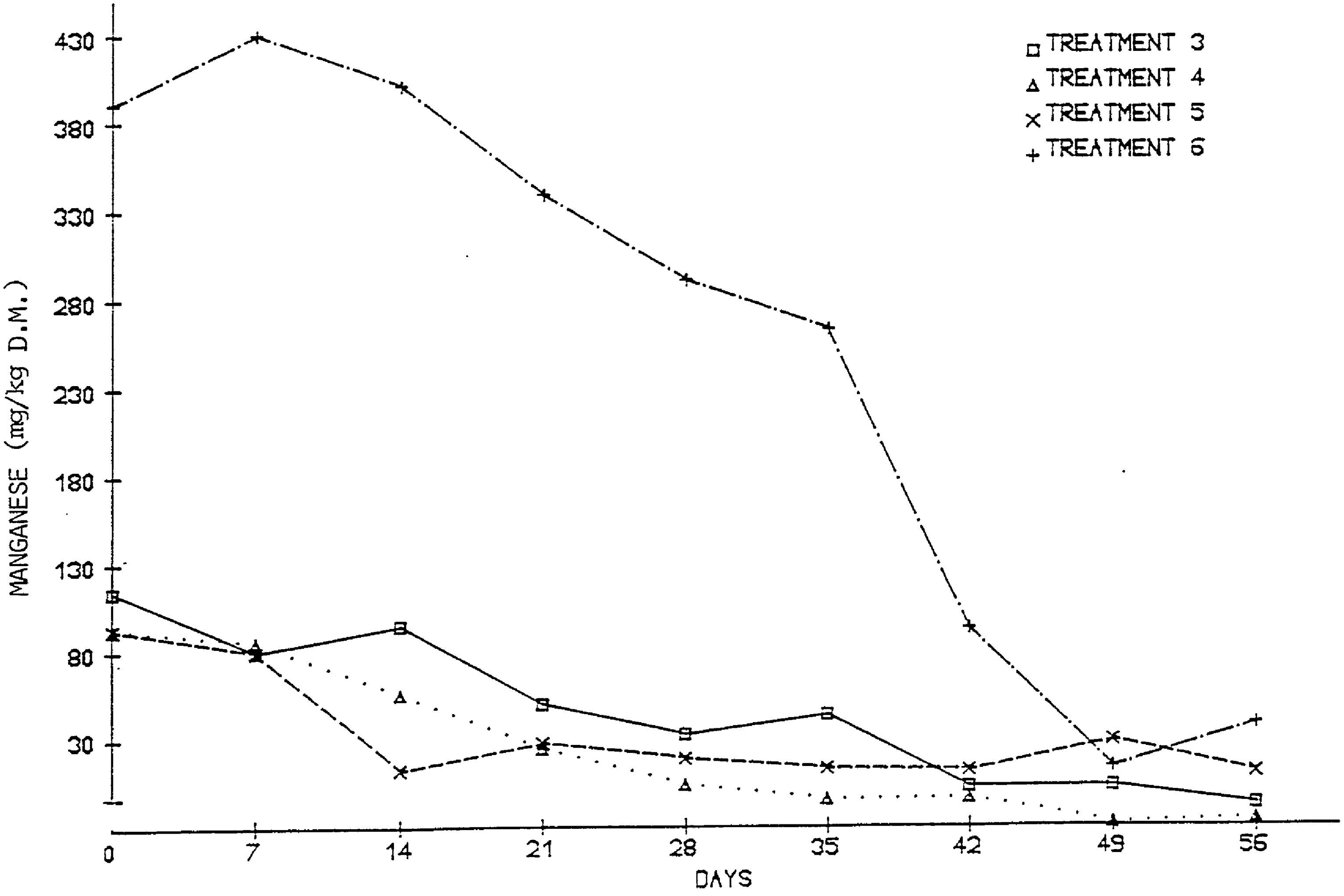


FIGURE 5.6 ADHERING NICKEL CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

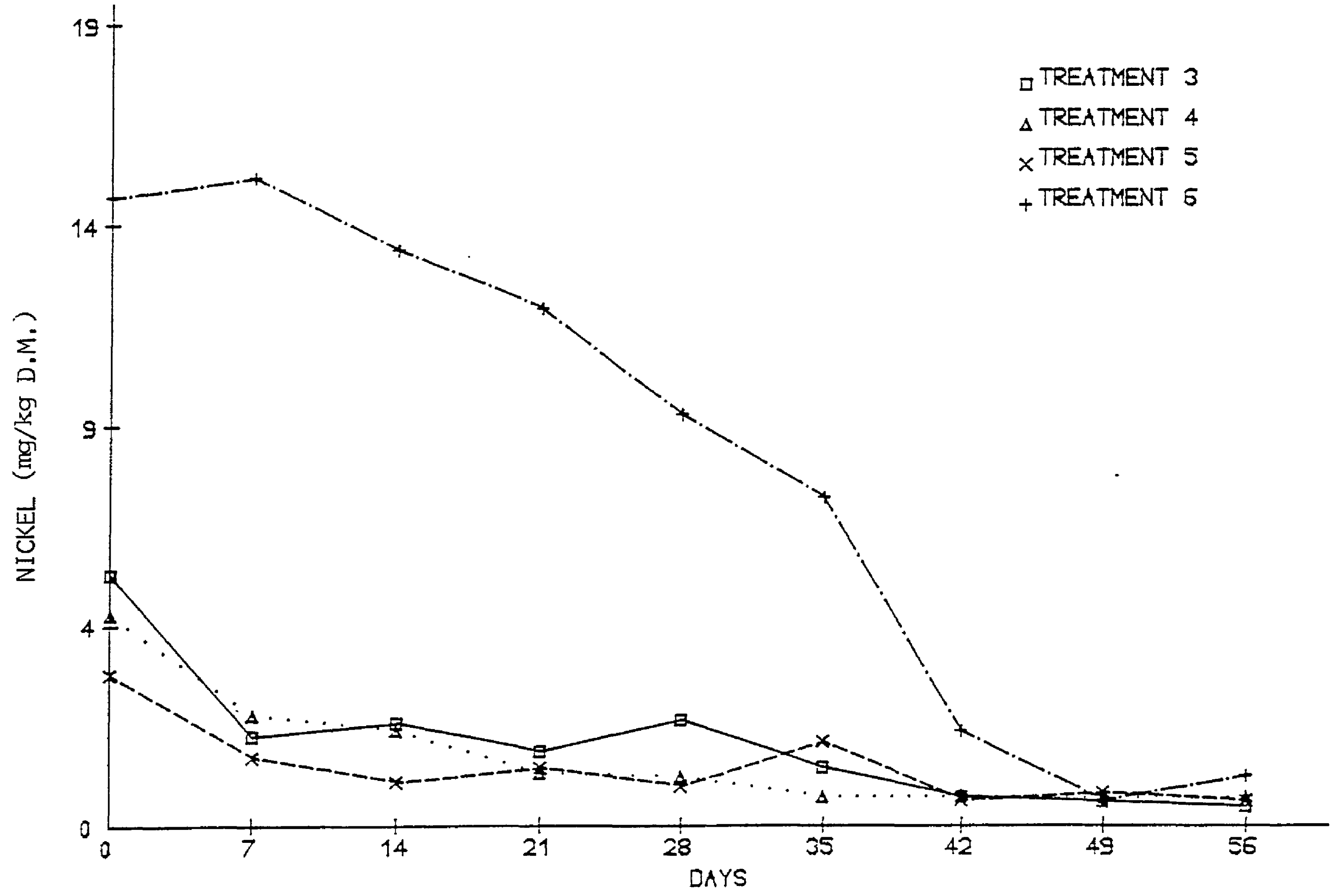


FIGURE 5.7 ADHERING LEAD CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

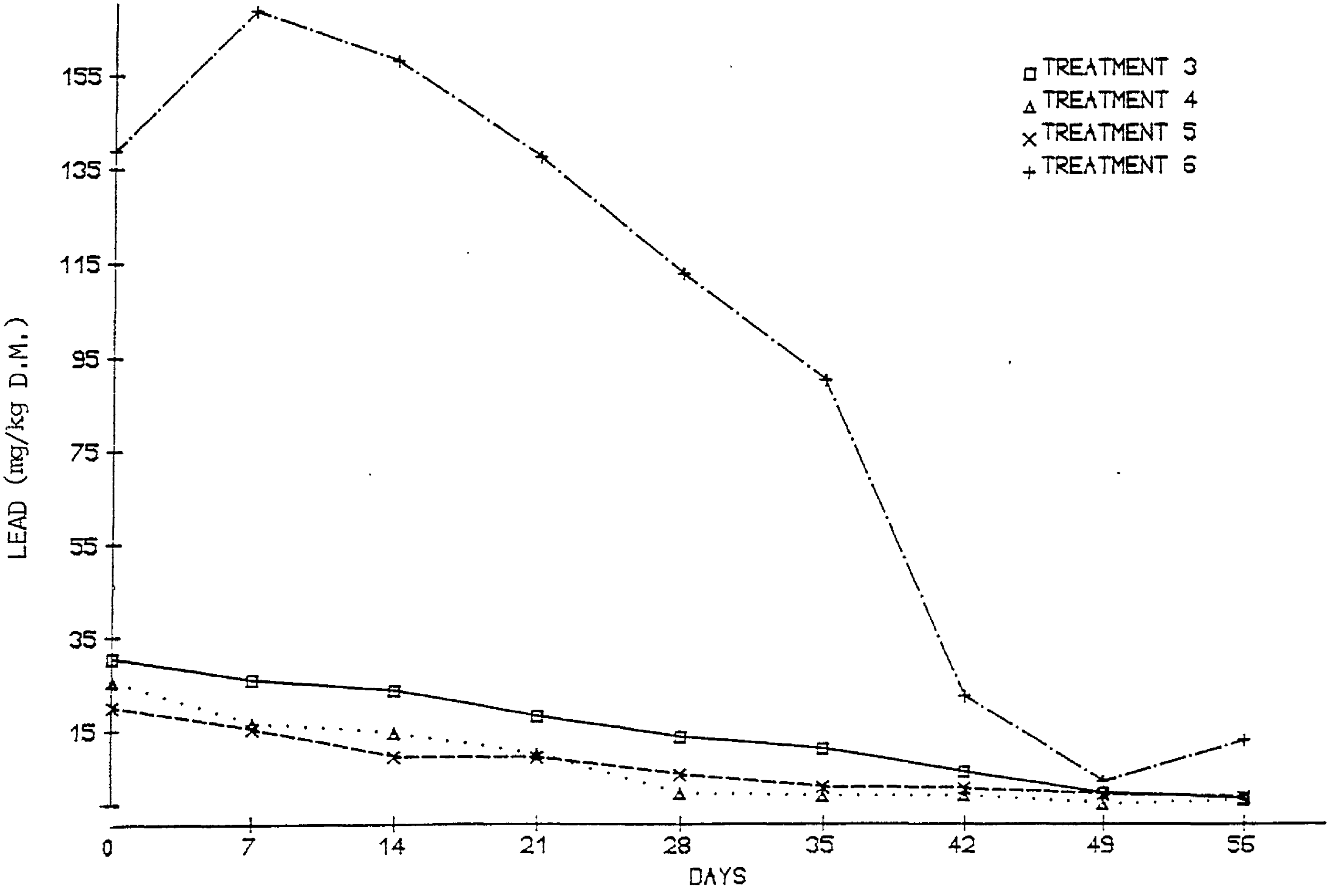
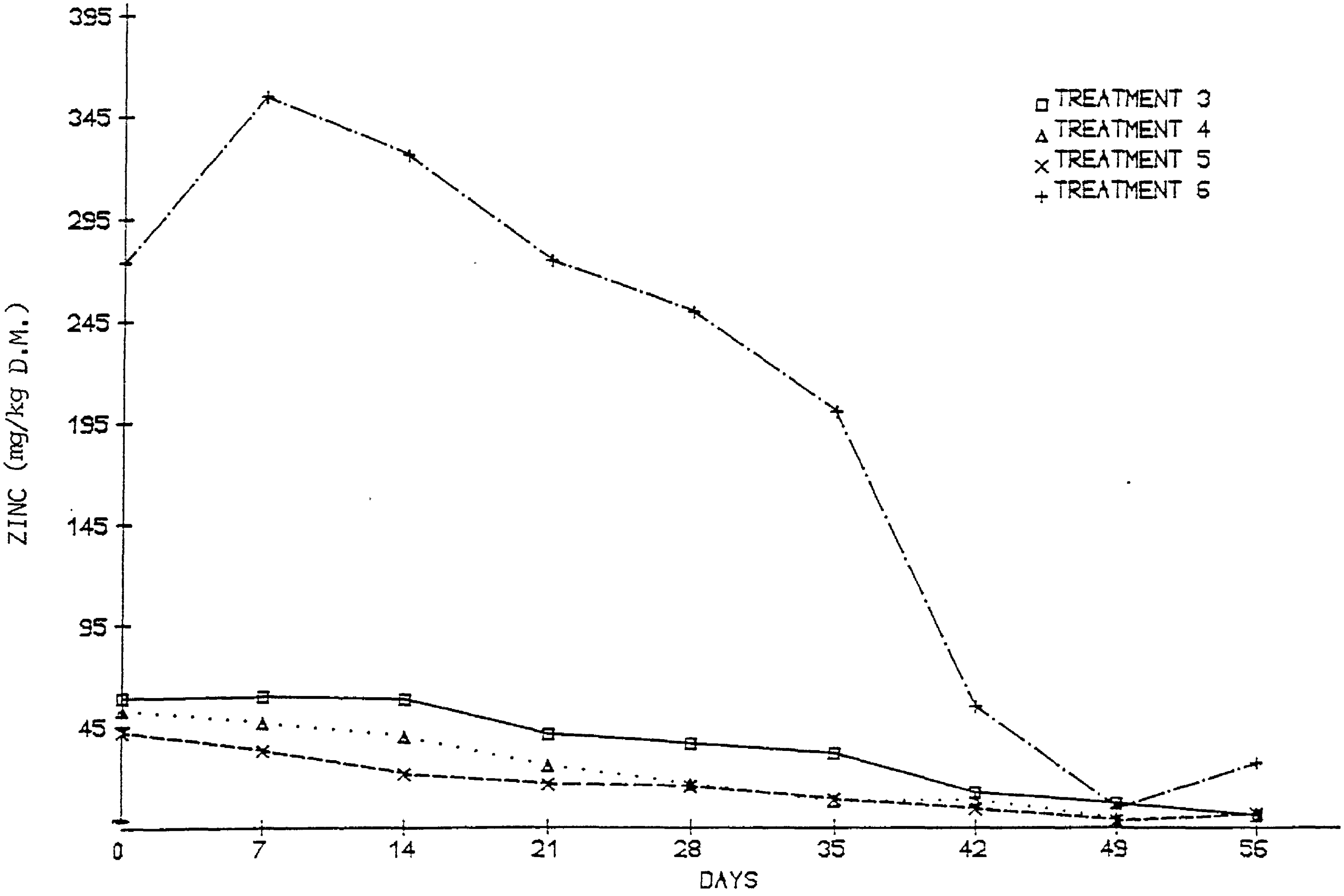


FIGURE 5.8 ADHERING ZINC CONCENTRATION THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)



application (T6), there was little change in the concentration of adhering metals. Maximum values were observed either on day of application or on day 7, which reflected heterogeneity and/or sampling error.

In all four treatments, the decrease in adhering metal concentrations could broadly be described as being linear with time (Table 5.6), the rates of which were significantly ($P < 0.05$) greater than zero for all metals, with the exception of Cd in the 2% D.S. sludge, cut, full-rate treatment (T4). Adhering Cd concentration in this latter treatment reached a value not significantly different to background levels by day 14 (Table 5.5). The rates of dilution of Cd, Cr, Cu, Ni, Mn, Fe, Pb and Zn adhering to herbage in the 12% D.S. sludge, uncut, full rate treatment (T6) were significantly greater than their respective rates in any of the other three treatments in the order 12% D.S. sludge, uncut, full rate (T6) > 2% D.S. sludge, uncut, full rate (T3) > 2% D.S. sludge, cut, full rate (T4) > 2% D.S. sludge, uncut, half rate (T5) (Table 5.6).

To enable comparisons to be made between the behaviour of adhering metals, concentrations were expressed as a percentage of adhering metal concentration on day 0 (taken as 100%), which is referred to in the text as '% adhering metal'. Table 5.7 reports the mean % adhering metals and is represented graphically in Figures 5.9 - 5.16.

When % adhering metal was related to time there was an inflection point at day 7 for all metals, in their rate of change (Figures 5.9 - 5.16). Between days 7-49 there was a linear decrease in % adhering metal, which

TABLE 5.6 RELATIONSHIPS OBTAINED BY LINEAR REGRESSION BETWEEN ADHERING METAL CONCENTRATION (mg/kg D.M.) AND TIME (DAYS) (TEMPLE FIELD).

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)								
GRADIENT	-0.006	-0.42	-0.58	-95.55	-2.17	-0.08	-0.56	-1.17
S.E.	0.001	0.09	0.06	24.51	0.24	0.02	0.02	0.10
INTERCEPT	0.36	19.98	32.45	4513	107.58	3.75	30.14	66.65
S.E.	0.04	3.15	2.02	817	8.13	0.66	0.61	3.27
R^2	0.80	0.70	0.92	0.64	0.91	0.64	0.99	0.95
S_y	0.06	5.13	3.29	1329	13.22	1.07	0.99	5.32
CUT, FULL RATE, 2% D.S. SLUDGE (T4)								
GRADIENT	-0.002	-0.30	-0.48	-76.21	-2.13	-0.07	-0.45	-1.01
S.E.	0.001	0.07	0.06	19.07	0.27	0.02	0.07	0.10
INTERCEPT	0.14	13.33	23.20	3280	84.09	3.15	20.32	50.40
S.E.	0.03	2.27	2.08	636	9.17	0.53	2.36	3.26
R^2	0.22	0.70	0.88	0.65	0.88	0.68	0.83	0.93
S_Y	0.05	3.69	3.39	1034	14.91	0.86	3.85	5.30
UNCUT, HALF RATE, 2% D.S.SLUDGE (T5)								
GRADIENT	-0.003	-0.23	-0.33	-53.90	-1.18	-0.04	-0.33	-0.73
S.E.	0.001	0.07	0.03	17.66	0.42	0.01	0.04	0.08
INTERCEPT	0.15	10.84	17.05	2381	66.09	2.01	16.66	36.05
S.E.	0.03	2.29	1.14	588	14.11	0.46	1.38	2.63
R^2	0.53	0.57	0.92	0.51	0.46	0.44	0.89	0.91
S_y	0.05	3.73	1.86	957	22.96	0.76	2.24	4.28
UNCUT, FULL RATE, 12% D.S.SLUDGE (T6)								
GRADIENT	-0.036	-1.11	-2.91	-267	-8.00	-0.32	-3.13	-6.31
S.E.	0.003	0.12	0.54	28	1.06	0.03	0.49	1.10
INTERCEPT	2.01	63.58	172.00	14987	476.00	17.48	181.00	372.50
S.E.	0.11	4.07	17.88	933	35.29	1.08	16.24	36.64
R^2	0.94	0.91	0.78	0.92	0.88	0.92	0.84	0.80
S_y	0.18	6.62	29.08	1517	57.42	1.75	26.42	59.62

TABLE 5.7 ADHERING SLUDGE METAL (%) (TEMPLE FIELD).
EACH READING IS AN AVERAGE OF 3 REPLICATES.

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7	109.4	42.3	116.6	36.9	67.3	31.4	87.0	104.1
14	88.8	35.4	108.8	30.8	82.4	39.1	78.3	102.4
21	33.7	24.2	72.4	21.0	41.4	24.7	61.4	74.3
28	46.4	24.3	68.6	20.2	25.7	40.6	43.7	61.2
35	60.3	21.1	52.6	16.4	37.0	16.6	37.0	55.0
42	31.4	9.9	24.6	7.2	2.5	3.6	19.9	21.4
49	2.8	1.2	4.6	1.0	1.7	0.5	5.0	13.1
56	14.7	0.5	2.6	0.4	-9.1	-1.8	1.0	1.8
CUT, FULL RATE, 2% D.S. SLUDGE (T4)								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7	255.6	61.6	86.3	52.5	92.5	49.8	66.0	89.0
14	94.9	35.5	77.4	36.8	60.4	45.5	57.6	75.4
21	152.6	19.5	50.5	14.2	26.7	15.6	38.5	50.4
28	23.0	5.4	17.8	3.3	4.2	16.0	5.8	32.0
35	24.2	2.8	10.0	-1.1	-4.6	4.2	3.9	16.2
42	58.8	0.2	11.8	0.3	-2.6	6.2	4.8	17.9
49	36.4	-1.3	1.1	-1.5	-20.2	3.0	-3.1	-0.9
56	75.8	1.1	3.9	0.1	-16.9	5.0	0.2	4.1
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7	49.2	43.8	94.9	39.0	82.8	35.0	79.0	77.3
14	44.1	20.6	50.4	18.9	13.2	19.4	53.3	50.1
21	31.7	25.9	52.7	17.3	16.5	27.0	46.6	39.0
28	29.4	16.7	43.6	13.3	19.6	17.3	28.7	36.4
35	40.3	11.2	21.3	6.6	24.8	42.7	15.9	23.0
42	39.7	7.2	12.4	3.6	13.6	5.4	12.6	10.9
49	-8.7	3.9	1.1	2.3	22.7	6.9	6.6	-2.5
56	21.2	2.7	4.0	1.4	7.6	1.8	3.6	4.5
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7	125.5	102.6	139.7	96.1	110.2	103.5	121.9	131.9
14	111.8	77.0	128.0	83.8	102.8	92.2	114.5	121.8
21	94.7	71.8	102.1	67.5	87.1	81.7	99.9	101.8
28	81.4	66.3	96.3	68.4	73.6	64.3	81.2	91.3
35	70.7	57.8	79.2	52.6	67.4	51.0	65.4	74.9
42	22.1	16.2	23.0	12.4	24.7	12.9	17.2	22.6
49	1.2	2.1	1.8	1.7	2.8	0.6	2.6	1.2
56	9.1	8.1	9.7	7.4	8.9	4.6	9.0	9.3
S.E.	31.7	5.1	9.9	4.7	12.4	10.1	8.9	9.6

POOLED S.E. FOR COMPARISON BETWEEN METALS= 14.32

FIGURE 5.9 % ADHERING CADMIUM THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

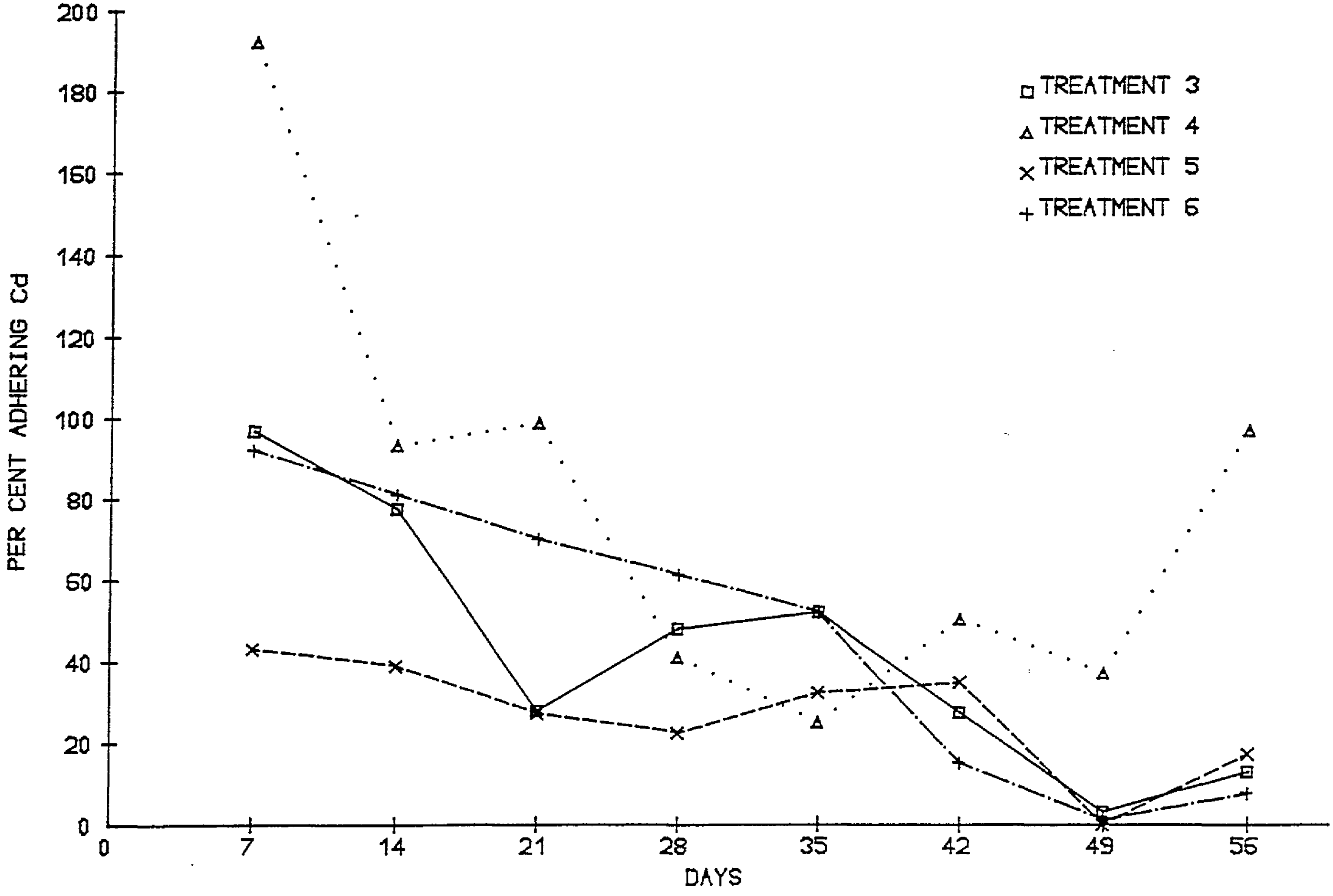


FIGURE 5.10 % ADHERING CHROMIUM THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

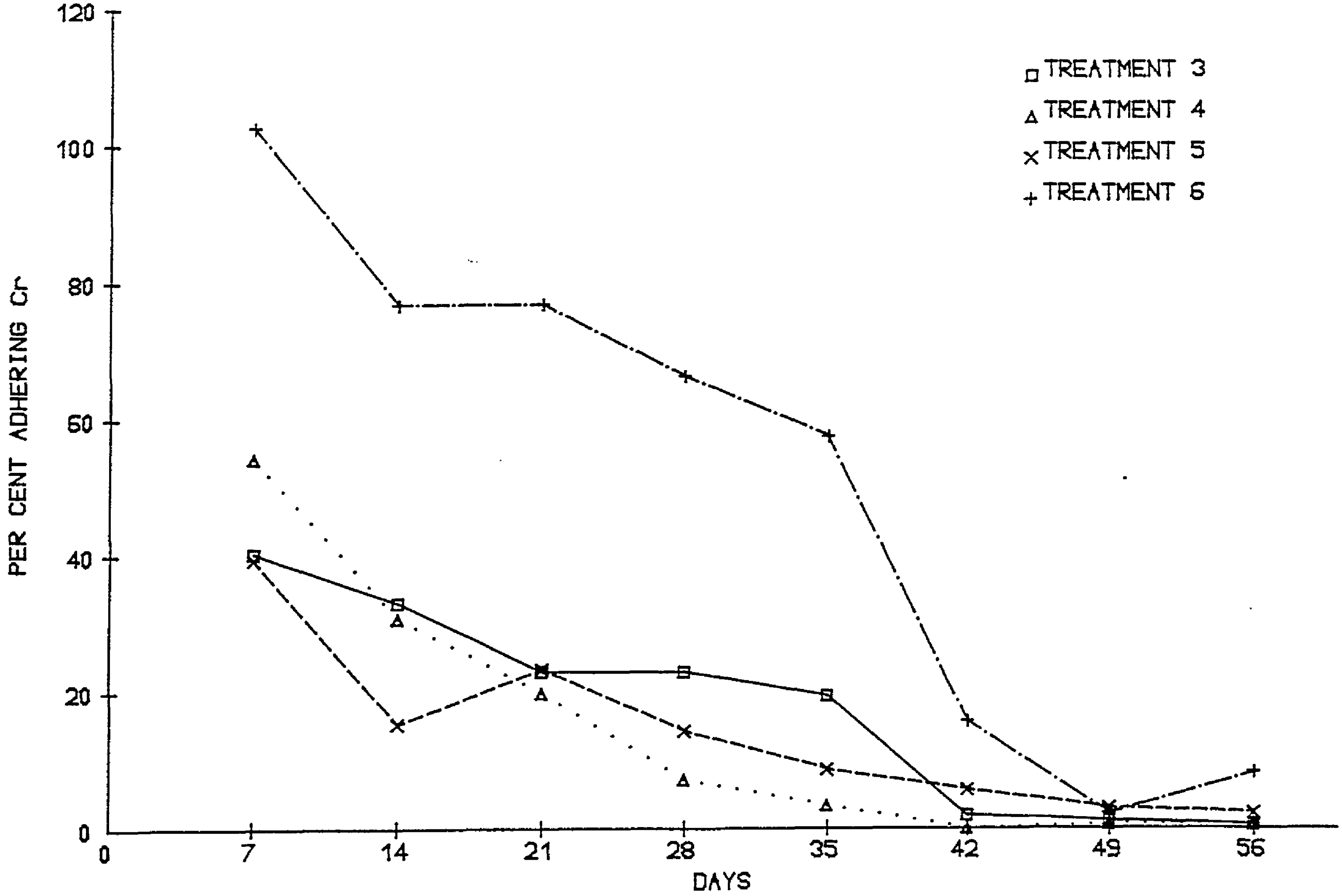


FIGURE 5.11 % ADHERING COPPER THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

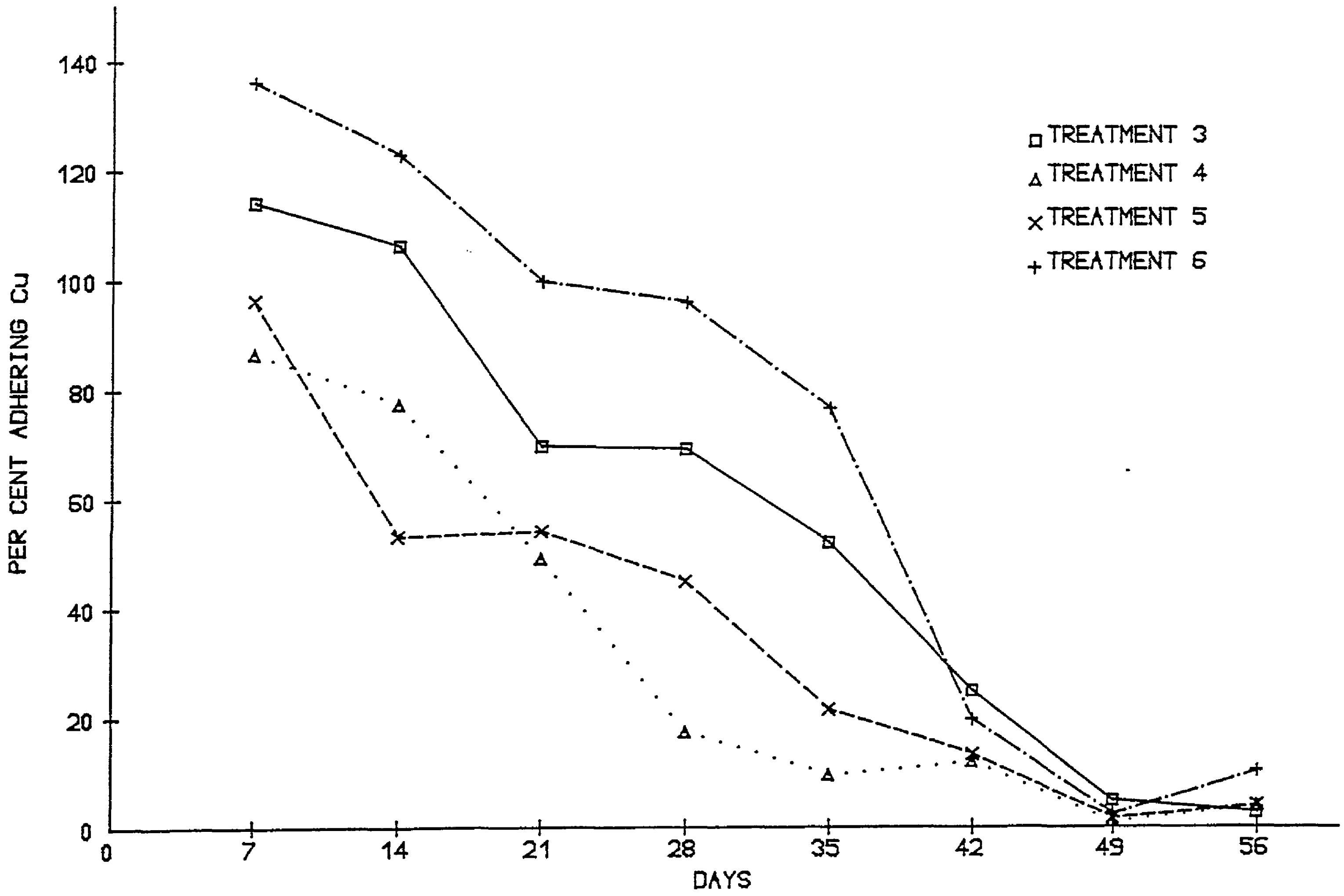


FIGURE 5.12 % ADHERING IRON THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

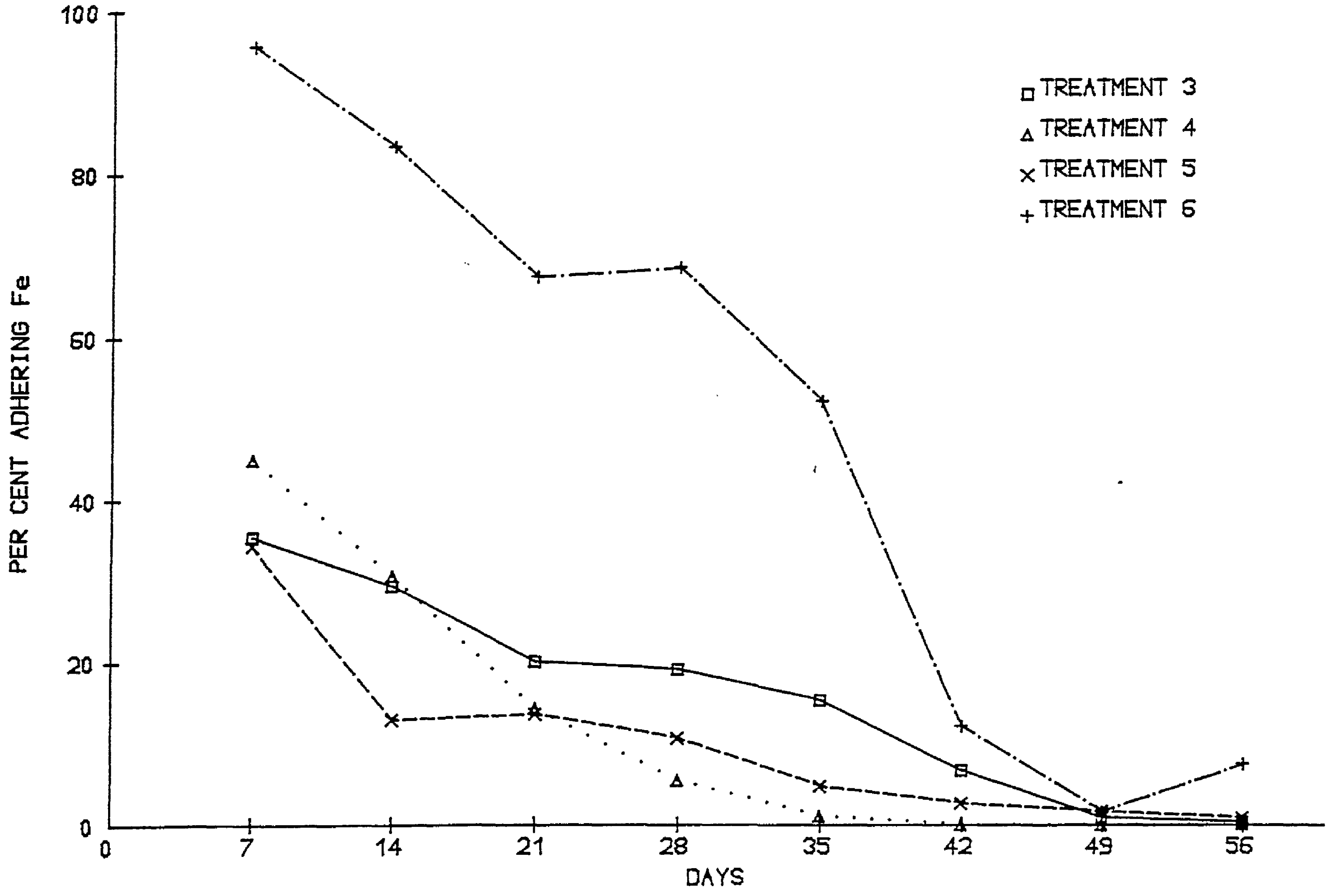


FIGURE 5.13 % ADHERING MANGANESE THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

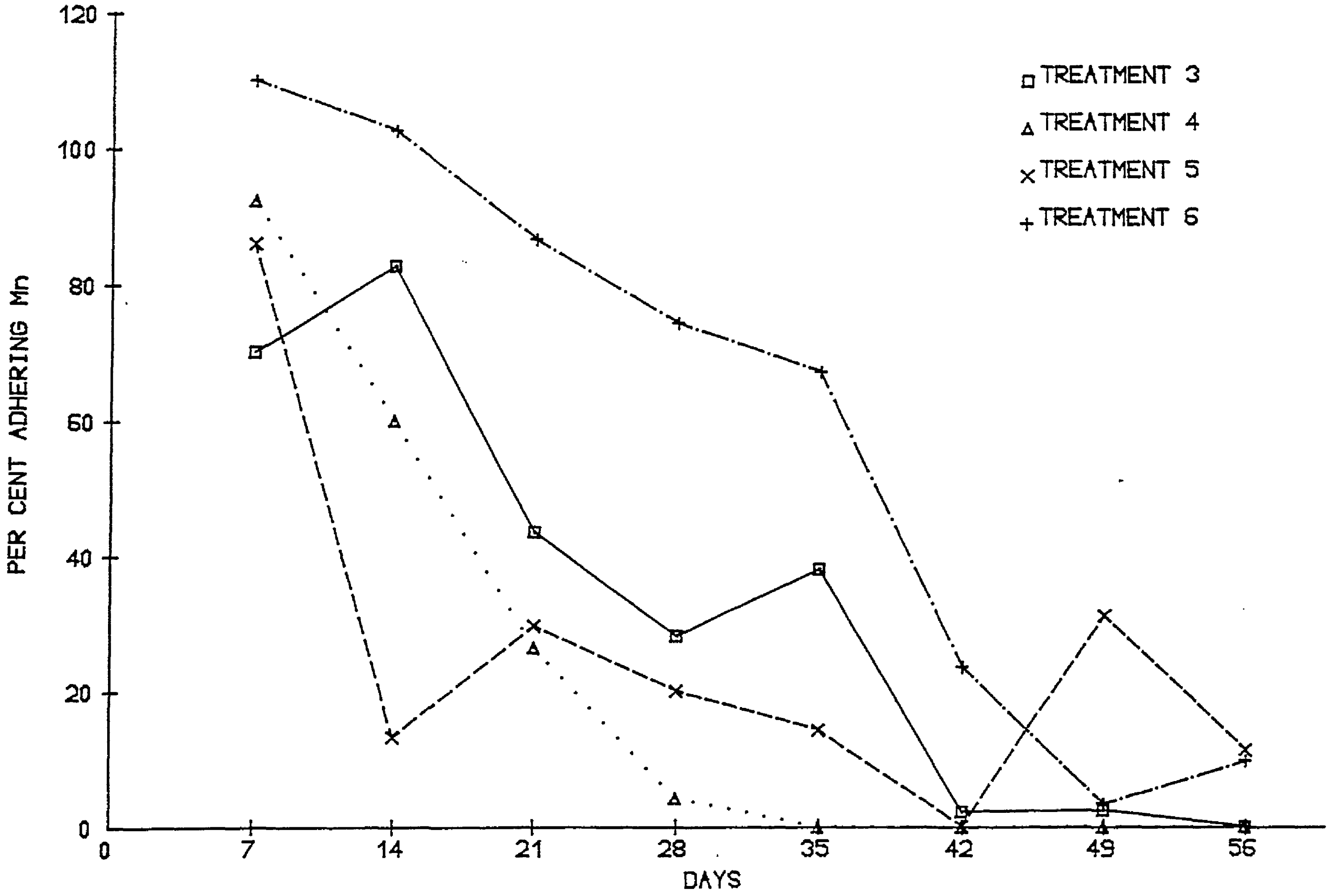


FIGURE 5.14 % ADHERING NICKEL THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

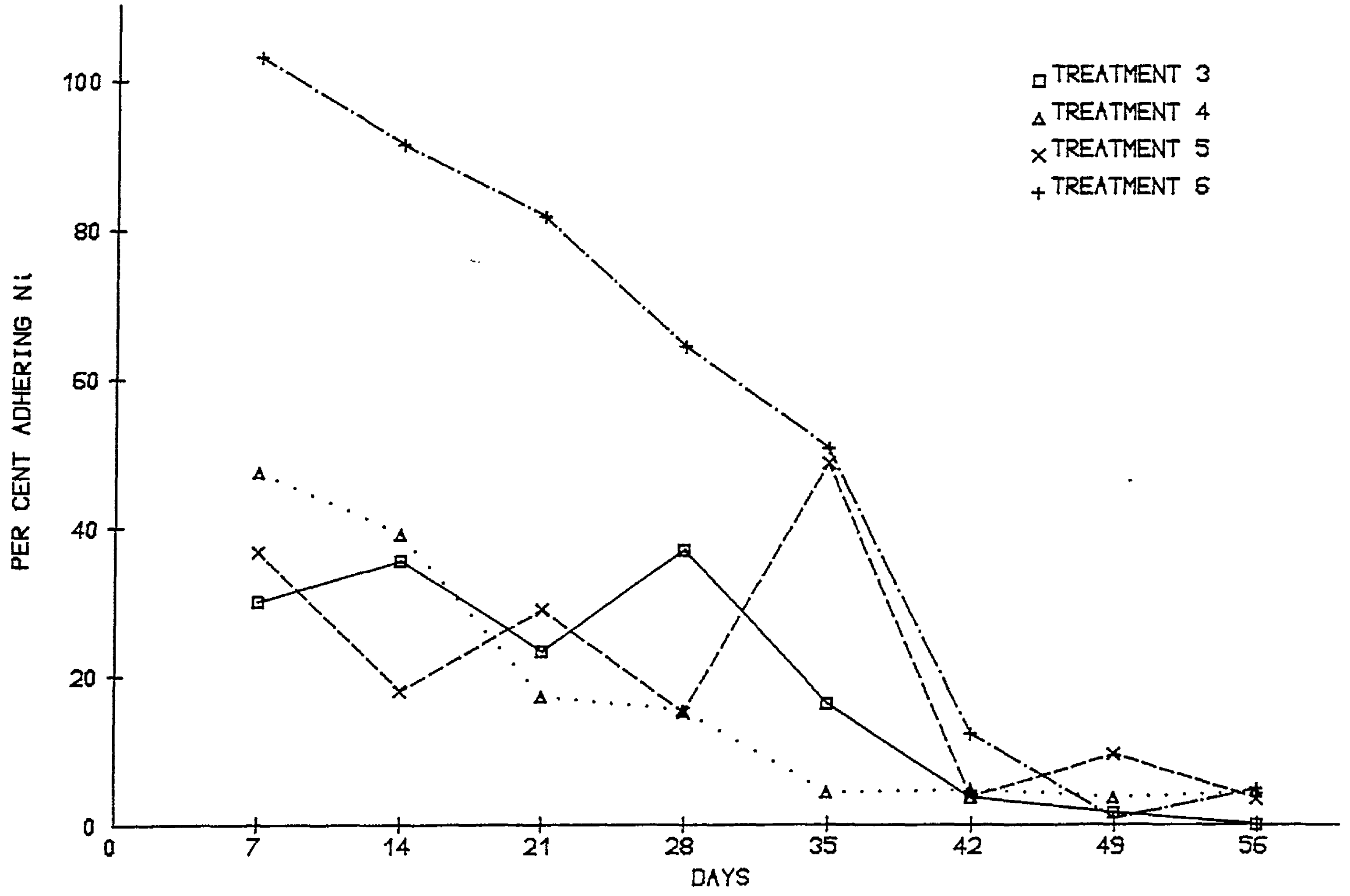


FIGURE 5.15 % ADHERING LEAD THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)

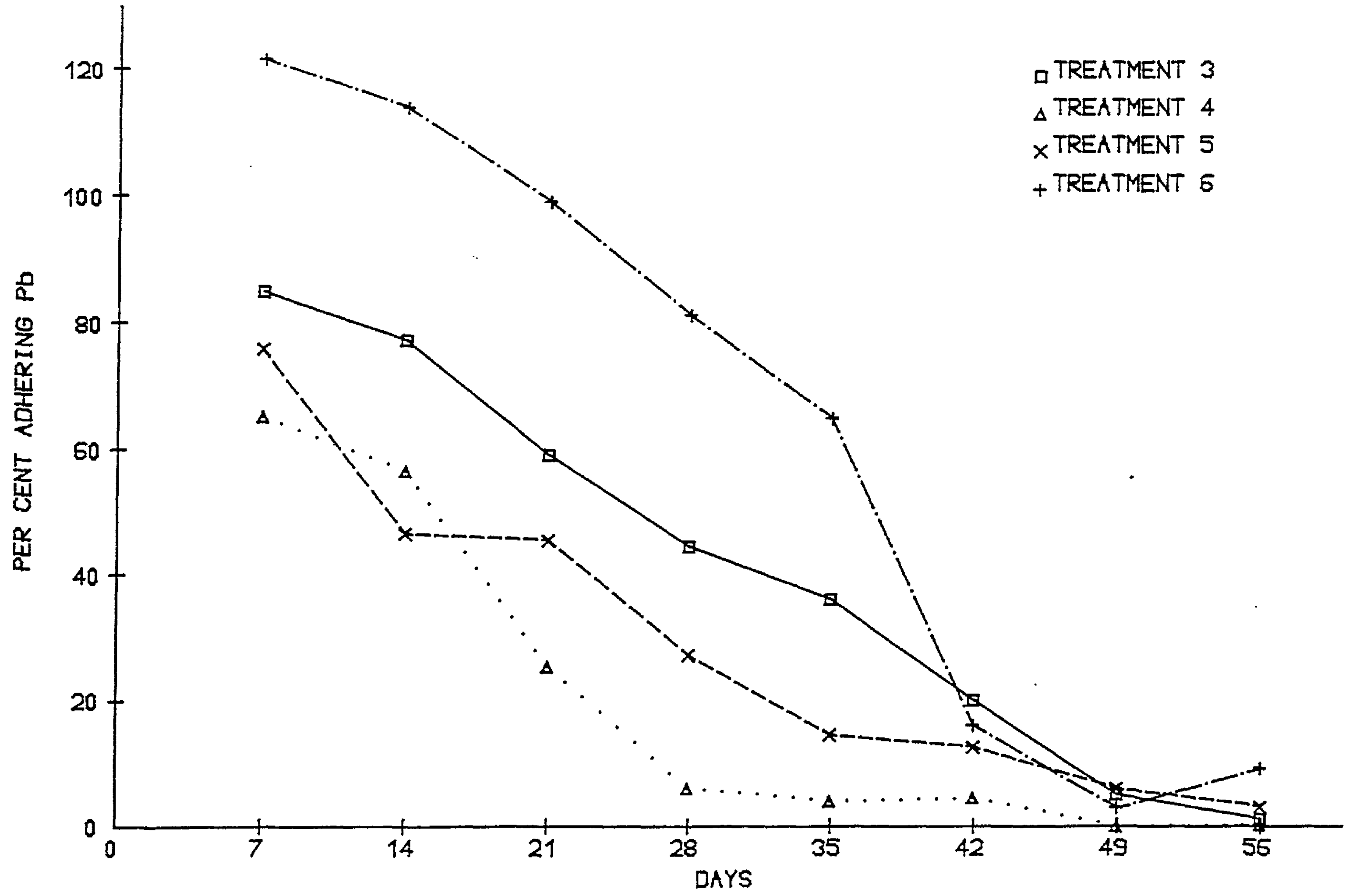
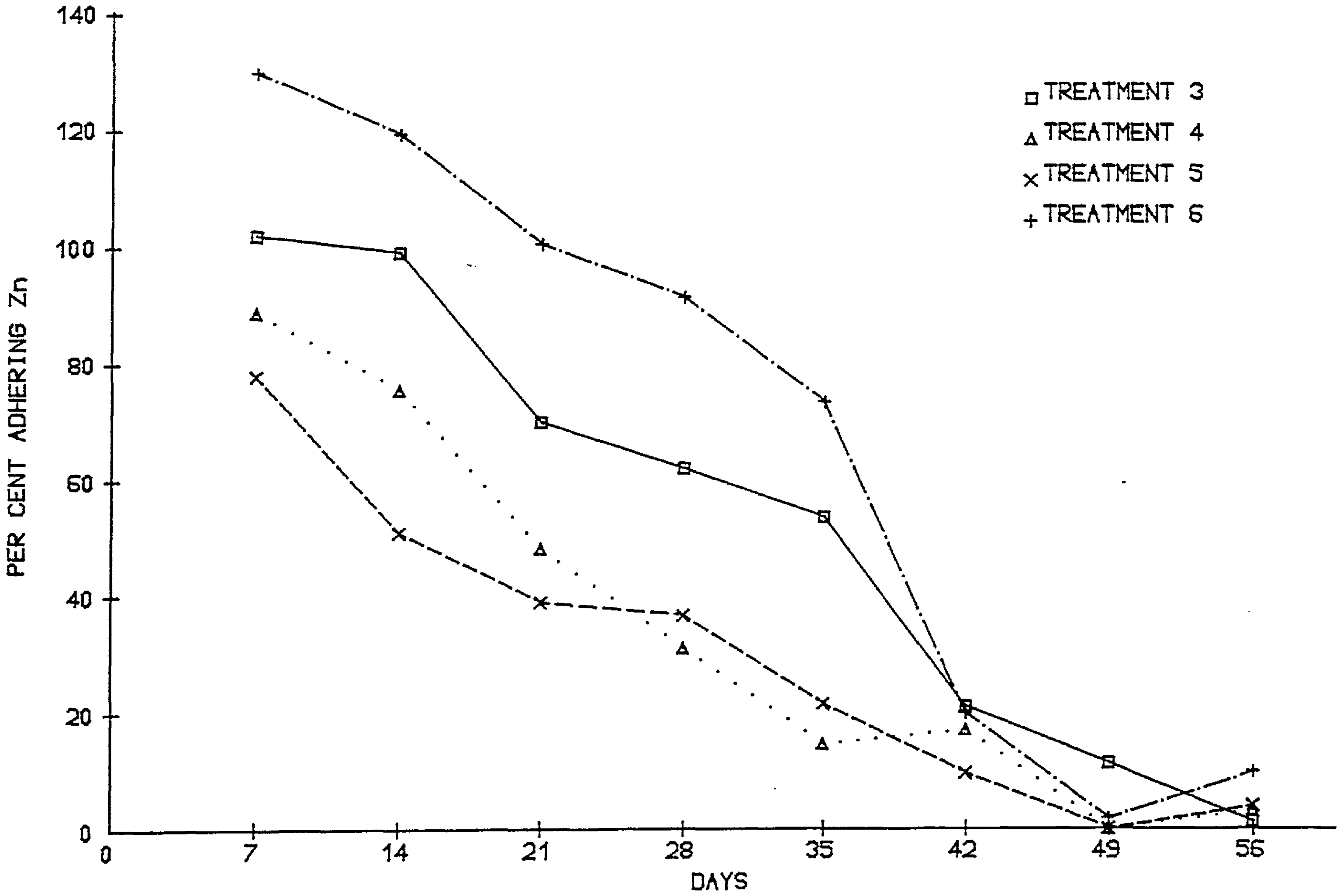


FIGURE 5.16 % ADHERING ZINC THROUGH TIME FOR TREATMENTS 3-6 (TEMPLE FIELD)



on linear regression was shown to be significantly ($P < 0.05$) greater than zero (Table 5.8). However, slopes were not significantly different from zero for % adhering Mn and Ni in the 2% D.S. sludge, uncut, half-rate treatment (T5) and for % adhering Cd in the 2% D.S. sludge, cut, full rate treatment (T4).

Where 12% D.S. sludge was applied (T6), the % adhering metals on day 7 were generally greater than that recorded on day 0. In the case of % adhering Cd, a peak on day 7 of 256% was observed in the 2% D.S. sludge, cut, full-rate treatment (T4), and probably reflects the fact that Cd concentrations were very close to the detection limit of the atomic absorption (see Table 2.1). In the treatments which received 2% D.S. sludge (i.e. T3, T4 and T5), the % adhering Cr, Fe and Ni showed a very marked decrease over the initial 7 day period compared to the other metals. Moreover, after day 7 the % adhering Fe, Cr and Ni exhibited a significantly ($P < 0.05$) smaller rate of change than any of the other metals (Table 5.8). This distinct behaviour of Cr, Fe and Ni was not evident, however, in the 12% D.S. sludge, uncut, full-rate treatment (T6), where the dilution rate of all eight metals was similar but at the same time significantly ($P < 0.05$) greater than their respective rates in any of the other three sludge treatments (T3, T4 and T5).

Although linear regression gives an overall picture of the dilution behaviour of adhering metals, in certain cases it does not provide an accurate description of change. For example, the change in % adhering metals in the 12% D.S. sludge, uncut, full rate treatment (T6) was

TABLE 5.8 RELATIONSHIPS OBTAINED BY LINEAR REGRESSION BETWEEN % ADHERING METAL AND TIME (DAYS),(TEMPLE FIELD).

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)								
GRADIENT	-1.85	-0.86	-2.45	-0.73	-1.71	-0.78	-1.83	-2.20
S.E.	0.42	0.09	0.18	0.05	0.28	0.18	0.08	0.15
INTERCEPT	106.60	44.90	132.70	39.10	87.50	43.10	98.70	121.90
S.E.	14.90	3.30	6.20	1.80	9.80	6.20	2.70	5.30
R^2	0.72	0.92	0.97	0.97	0.84	0.73	0.99	0.97
S_y	19.14	4.24	7.96	2.32	12.58	8.01	3.50	6.80
CUT, FULL RATE, 2% D.S. SLUDGE (T4)								
GRADIENT	-3.11	-1.01	-1.84	-0.88	-1.75	-0.90	-1.37	-1.84
S.E.	1.40	0.22	0.32	0.19	0.44	0.18	0.29	0.23
INTERCEPT	188.30	46.30	90.10	39.70	78.20	45.30	63.30	92.80
S.E.	49.50	7.70	11.30	6.80	15.40	6.50	10.40	8.20
R^2	0.36	0.75	0.82	0.74	0.69	0.77	0.75	0.90
S_y	63.59	9.86	14.46	8.73	19.75	8.30	13.30	10.54
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)								
GRADIENT	-0.72	-0.65	-1.79	-0.56	-0.90	-0.54	-1.40	-1.49
S.E.	0.32	0.14	0.24	0.13	0.51	0.32	0.19	0.17
INTERCEPT	55.70	34.40	92.40	27.90	54.00	37.50	73.10	76.70
S.E.	11.30	4.80	8.50	4.50	18.00	11.30	6.60	6.10
R^2	0.37	0.76	0.89	0.72	0.23	0.21	0.89	0.91
S_y	14.51	6.19	10.93	5.81	23.14	14.51	8.44	0.77
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)								
GRADIENT	-2.71	-2.09	-2.96	-2.06	-2.37	-2.32	-2.74	-2.86
S.E.	0.27	0.25	0.33	0.24	0.25	0.21	0.28	0.31
INTERCEPT	150.00	116.50	163.80	113.30	134.40	124.20	149.80	158.30
S.E.	9.50	8.90	11.70	8.60	8.80	7.60	10.10	11.10
R^2	0.94	0.91	0.92	0.91	0.93	0.94	0.93	0.92
S_y	12.93	11.46	15.03	11.03	11.30	9.73	12.91	14.22

gradual over days 7-35 but, thereafter, showed a sharp decrease. In addition, the dilution pattern for the adhering metals in the 2% D.S. sludge, cut, full-rate treatment (T4), led to them reaching 0% (see Table 5.7) within 35 days. For example in the case of Mn, over the initial 28 days the true dilution rate was relatively high (4.5% day^{-1}) compared to the computed value of 1.75% day^{-1} by linear regression during days 7 - 56.

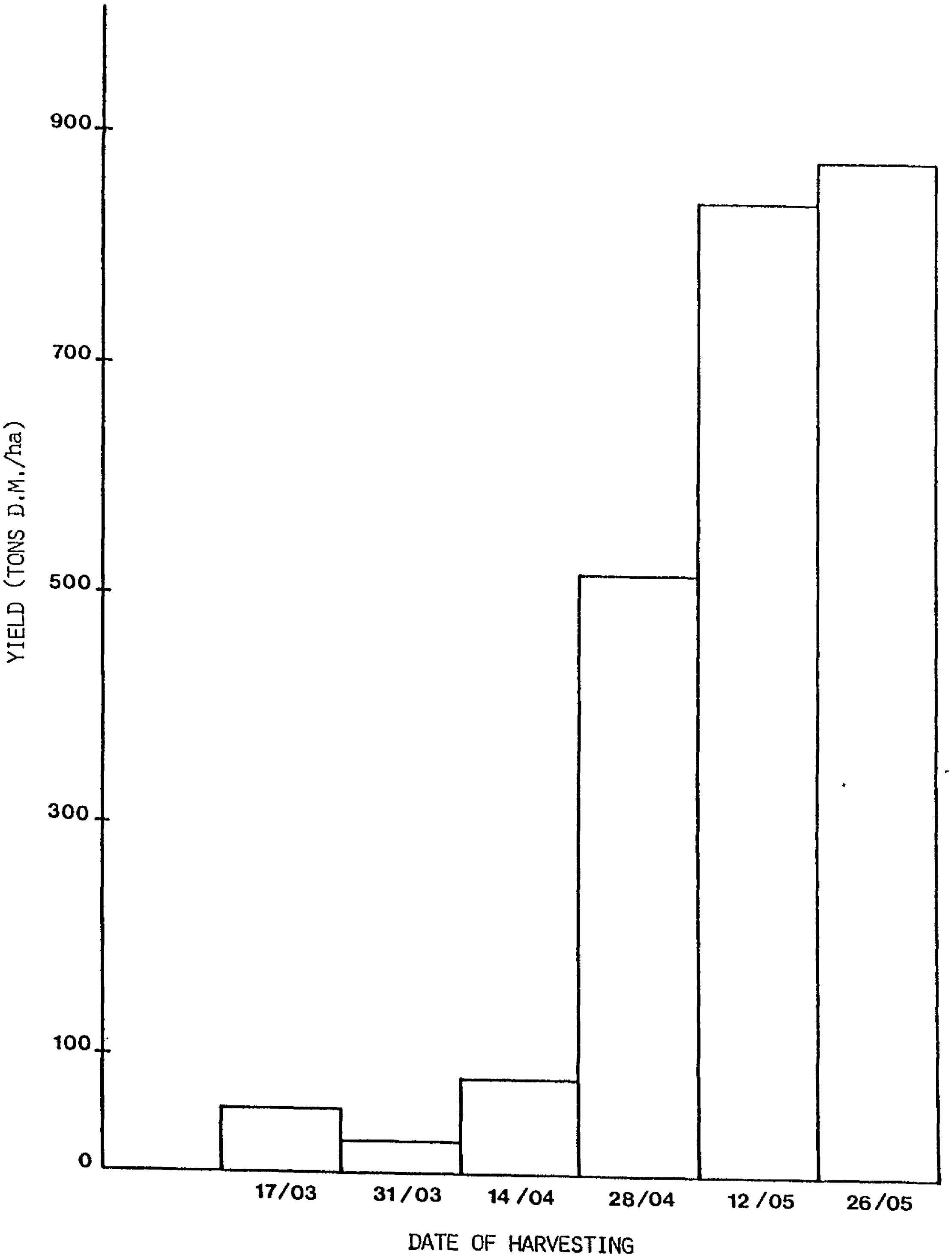
Over the experimental period, the dry-matter yield of each treatment was recorded each week (Table 5.9). The pattern of dry-matter yield of the no-sludge controls, over the 8-week period, can be broadly divided into two groups, in which the yields obtained over weeks 4-8 was greater than those obtained over weeks 1-4, although this difference was not significant. This could, in part, be explained by the small size (0.5m x 0.5m) of the mini-plots i.e. the smaller the plot the greater the dry-matter coefficient of variation for treatment replicates. Randomizing the nine mini-plots per replicate treatment within the whole replicate block could have produced a more precise estimate of yield, but such an approach would have been impractical.

The yield of *L. perenne* Cv. Perma, growing over the same period of time, in an adjacent site to Temple Field, was kindly provided by the Botany Department of the West of Scotland College (Figure 5.17). There was a large increase in dry-yield production between 14/04/87 and 28/04/87 which corresponded to weeks 3-5 of the experiment. This lends evidence to the characterization of yield data into a latent growth period, i.e. weeks 1-4, and an active growth period, i.e. weeks 4-8, as previously suggested. As shown in Figure 5.17 over the first 3 weeks there was

TABLE 5.9 DRY MATTER (g) PRODUCTION (±S.D.) OF GRASS (TEMPLE FIELD). EACH READING IS AN AVERAGE OF 3 REPLICATES.

	WEEK								
	0	1	2	3	4	5	6	7	8
UNCUT CONTROL (T1)									
	32.73	24.53	30.40	24.83	40.77	37.93	41.30	33.93	41.40
	(±11.09)	(± 5.95)	(± 8.70)	(± 3.87)	(±16.38)	(± 0.65)	(± 2.43)	(± 4.68)	(± 3.15)
CUT CONTROL (T2)									
	22.83	14.97	27.77	50.67	25.57	35.37	40.50	34.37	40.96
	(± 6.77)	(± 2.15)	(± 0.67)	(±39.33)	(± 4.01)	(± 2.40)	(± 1.71)	(± 2.53)	(± 1.93)
UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)									
	50.20	32.47	41.83	31.27	43.83	35.77	39.10	31.60	36.30
	(±24.71)	(± 6.33)	(± 5.85)	(±11.66)	(± 1.89)	(± 2.80)	(± 1.35)	(± 2.71)	(± 1.47)
CUT, FULL RATE, 2% D.S. SLUDGE (T4)									
	27.47	12.33	16.03	34.30	17.83	31.83	36.37	32.17	38.73
	(± 4.11)	(± 2.29)	(± 5.64)	(±12.98)	(± 2.48)	(± 1.78)	(± 2.77)	(± 2.74)	(± 5.10)
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)									
	33.03	25.97	35.07	46.50	40.97	34.30	41.23	36.90	39.23
	(±21.88)	(± 6.85)	(±13.49)	(±22.40)	(±16.09)	(± 4.97)	(± 3.56)	(± 3.10)	(± 3.46)
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)									
	71.47	56.40	68.00	30.03	68.03	48.93	36.70	32.50	42.43
	(±12.96)	(±18.54)	(±13.45)	(±16.00)	(±10.44)	(± 1.42)	(± 5.41)	(± 1.40)	(± 1.58)

FIGURE 5.17 THE SEASONAL DISTRIBUTION OF DRY-MATTER PRODUCTION BY SWARDS OF *L. perenne* Cv. PERMA AT AUCHINCRUIVE IN 1987



little or no growth. This was followed by a sharp increase in dry yield production over the next 2 weeks, which was maintained, all be it to a lesser degree , over the remaining 3 weeks of the experimental period.

The meterological data collected during the experimental period is reported in Appendix N. Figure 5.18 is a graphical representation of the rainfall over this time. There was high precipitation during the first 7 days of the experiment. In fact, the total rainfall over this time was approximately equal to the total rainfall over the remaining 7 weeks. The driest period occurred between days 28-35 with only 0.3mm rain.

An 'Adherence Index', i.e. the sludge metal adhering to the herbage on day of application expressed as a percentage of the total sludge metal applied, was determined for each sludged treatment (Table 5.10), where:

$$\text{Adherence Index} = \frac{\text{weight of metal adhering}}{\text{weight of metal applied}} \times 100$$

Where 2% D.S. sludge was applied (i.e. T3, T4 and T5), Cr and Fe exhibited a significantly ($P < 0.05$) greater Adherence Index than did any of the other six metals. Moreover, application of 2% D.S. sludge at full rate to a cut sward resulted in a significant ($P < 0.05$) reduction in all the Adherence Indices compared to those recorded, where 2% D.S. sludge was spread on to an uncut canopy (T3 and T5). This distinctive behaviour of Cr and Fe was not observed where 12% D.S. sludge had been applied (T6). In this instance, all eight metals

FIGURE 5.18 WEEKLY AND CUMULATIVE RAINFALL (mm) OVER THE 8 WEEK EXPERIMENTAL PERIOD (TEMPLE FIELD)

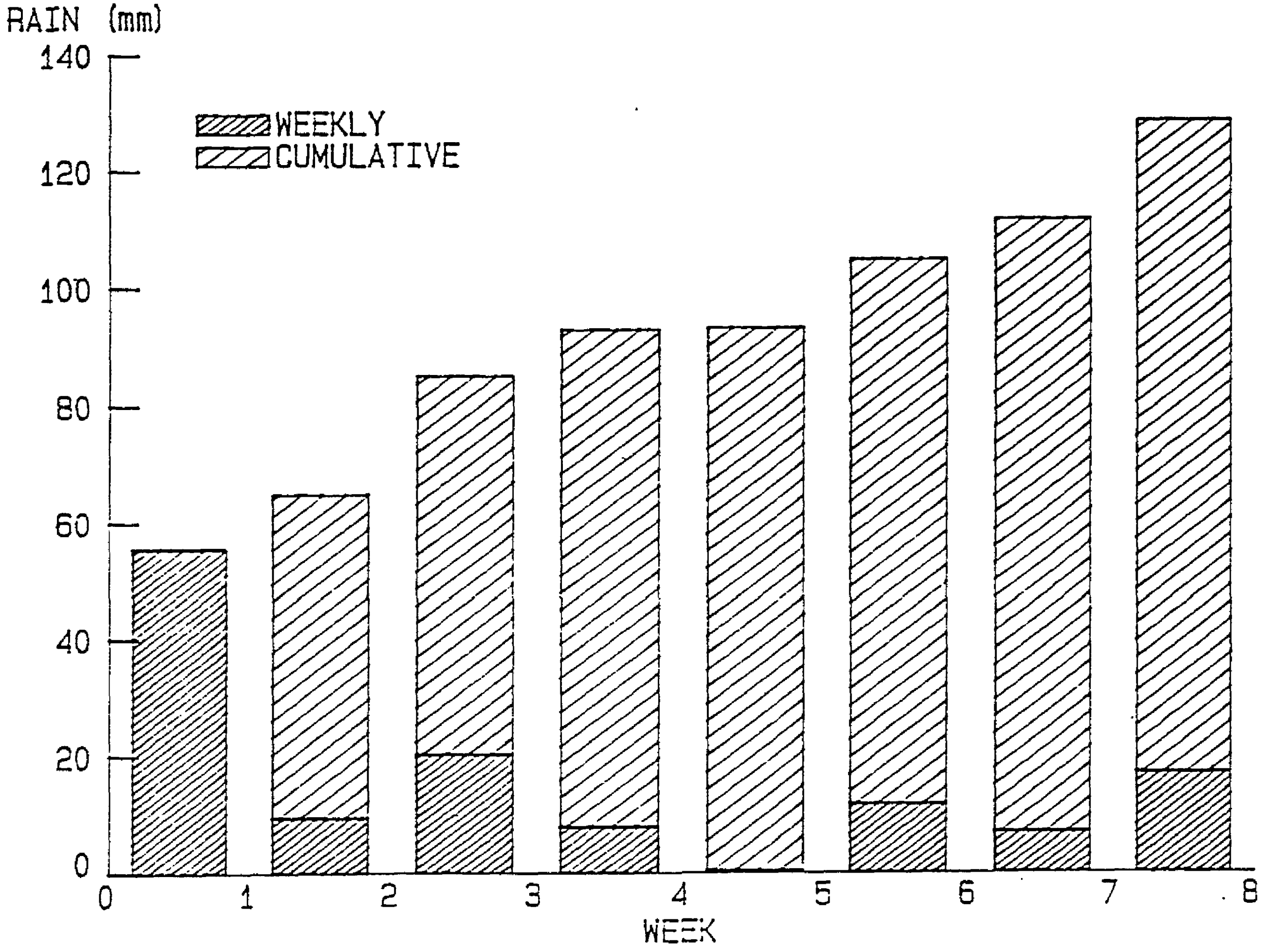


TABLE 5.10 ADHERENCE INDICES FOR SLUDGE METALS ON THE DAY OF APPLICATION (TEMPLE FIELD). EACH READING IS AN AVERAGE OF 3 REPLICATES.

	TREATMENT				
	2% D.S. SLUDGE			12% D.S. SLUDGE	
	UNCUT, FULL RATE	CUT, FULL RATE	UNCUT, HALF RATE	UNCUT, FULL RATE	S.E.
Cd	21.67	3.07	19.63	13.07	4.82
Cr	66.97	23.83	54.97	27.70	16.22
Cu	11.43	5.63	12.63	11.93	2.65
Fe	59.87	23.67	47.37	24.73	14.51
Mn	17.70	7.60	21.13	14.73	5.45
Ni	23.23	9.80	18.70	12.73	6.19
Pb	17.57	7.73	17.47	15.07	4.11
Zn	12.10	5.83	14.37	12.17	3.12

Pooled S.E. for comparison between means = 8.50

exhibited a similar Adherence Index which was not significantly different to that of Cd, Cu, Mn, Ni, Pb and Zn in Treatments 3 and 5 (i.e. 2% D.S. sludge, uncut, full-rate and 2% D.S. sludge, uncut, half-rate respectively). Application of 12% D.S. sludge resulted in a significantly ($P < 0.05$) lower Adherence Index of both Cr and Fe than that obtained where 2% D.S. sludge was applied to an uncut sward.

5.3.2. THE INFLUENCE OF RAINFALL AND DRYING ON 'WASH-OFF' OF ADHERING SLUDGE.

Table 5.11 reports the total heavy metal concentrations of herbage (the full set of data are shown in Appendix O). It is very evident that drying prior to rainfall resulted in no reduction in the concentration of metals adhering to herbage. In contrast, where rain was applied to wet adhering sludge, a near total wash-off of Zn, Ni, Cu, Cr and Cd was achieved when only 0.7mm rain was given. Iron and Pb required 1.3mm rain for total wash-off, whereas Mn required 2.0mm rain. A split 3 x 0.7mm rain event resulted in a significantly ($P < 0.05$) lower wash-off of Zn, Ni and Cu compared to a single 2.0mm application.

An Adherence Index was also computed for the sludged control (i.e. 0mm rainfall) (Table 5.12), following the procedure described in Section 5.3.1. A very low Adherence Index (0.2 - 3.9%) was recorded for all eight metals in the order Fe < Cr < Pb < Cd < Ni < Cu < Zn < Mn.

TABLE 5.11 TOTAL METAL CONCENTRATION (mg/kg DRY MATTER) IN HERBAGE FROM POT TRIAL. EACH READING IS AN AVERAGE OF 4 REPLICATES.

RAINFALL TREATMENT /mm	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0.0	0.83	9.10	85.27	3861.6	520.9	4.41	47.62	206.1
0.7	0.59	3.77	44.97	1441.6	466.6	1.39	12.47	138.4
1.3	0.42	3.09	36.37	802.9	491.4	0.49	7.06	145.2
2.0	0.48	4.07	27.59	474.5	347.4	0.55	6.04	94.6
NO SLUDGE (CONTROL)	0.65	2.43	30.80	393.5	294.9	0.66	4.24	88.4
DRIED SLUDGE	1.12	11.58	104.42	4828.4	534.2	5.33	56.34	239.9
2.7	0.41	2.41	38.23	1101.7	436.2	0.53	5.12	136.8
3.3	0.41	3.53	36.47	918.5	387.9	0.75	4.80	121.2
3x0.7	0.86	4.61	54.79	854.7	433.0	3.21	6.40	152.6
S.E.	0.08	0.66	4.58	182.0	43.0	0.28	1.65	13.2

TABLE 5.12 ADHERENCE INDEX RECORDED FOR SLUDGE METALS IN THE CONTROL (NO RAIN) TREATMENT (POT TRIAL). EACH READING IS AN AVERAGE OF 3 REPLICATES.

Cd	1.34^{bc}
Cr	0.97^{ab}
Cu	2.01^{cd}
Fe	0.23^{a}
Mn	3.94^{e}
Ni	1.38^{bc}
Pb	1.01^{ab}
Zn	2.66^{d}

Pooled S.E. = 0.25

FOOTNOTE = Different superscripts denote significant difference at 95% confidence interval.

5.4 DISCUSSION.

The surface spreading of sludges and slurries to grass swards results in the retention of solids by either adherence to leaf surfaces, or settlement on to the soil surface and, as such, the metals they contain may become directly available to animals which consume the contaminated forage and soil. For example, Price and Suttle (1975) state that the main risk in applying Cu- rich slurries to pastures lies in the ingestion of Cu as a foliar contaminant by grazing sheep.

A black colouration of herbage was noted immediately after the application of sludge to the plots of perennial ryegrass and this hue was darkest where sludge with 12% D.S. content was spread. Examination of the grass revealed a thin film of sludge adhering to the forage. This black colour, which is generally ascribed to FeS in anaerobic sludges, remained apparent for two days. Thereafter, even though the herbage reverted to its normal green colour, close inspection showed sludge-solids still adhering to the leaf surfaces.

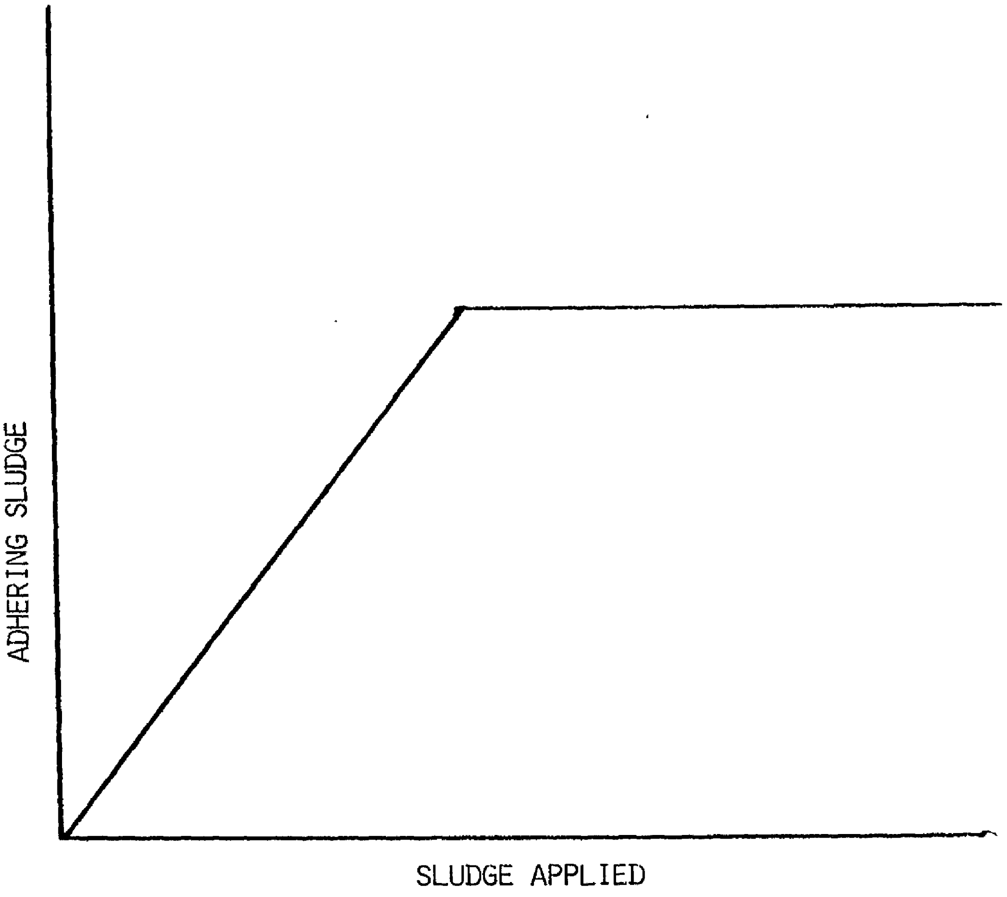
The application of sludge containing 2% D.S. at $67.5m^3$/ha (T3) (i.e. 1150kg D.S./ha) which is slightly above the recommended maximum permissible rate of $55m^3$/ha (SAC, 1986) led to as much as 61% of the applied sludge-dry solids remaining on the herbage four hours after application. In terms of herbage dry-matter yield, sludge contributed 35% of the total harvested weight on the day of sludge application. The proportion of sludge - dry solids contributing to the total dry matter yield of herbage was markedly reduced by applying the sludge to a mowed sward (T4) or reducing the rate of sludge application to $25m^3$/ha

(T5). When sludge is sprayed on to a mowed field, it adheres to less grass than when an intact, uncut canopy is sprayed. In fact, on day of application the cut control (T2) yielded, on average, 30% less dry matter than the uncut control (T1). Similarly, the application of 2% D.S. sludge at 67.5m^3/ha to a ryegrass sward which had been either cut prior to sludge application (T4) or left uncut (T3), resulted in herbage containing 17% compared to 35% sludge-dry solids, respectively. Reducing the rate of application led to sludge-dry solids accounting for 27% of the total herbage dry-matter yield. Thus, by either applying sludge to a mowed sward or reducing the application rate, a decrease in the proportion of sludge solids contained in the total herbage dry-matter yield can be obtained and can thus minimize the risk of adherinng sludge entering the food chain. Chaney and Lloyd (1979) arrived at similar conclusions regarding the effects of application rate from their work involving the spreading of anaerobic digested sludge (5.9% D.S.) to a tall fescue (*Festuca dinacea* Schreb.) sward, where reducing the application rate from 103m^3/ha to 51m^3/ha led to a corresponding decrease of sludge solids in herbage from 32% to 24%. However, they observed no significant decrease in adhering sludge solids following the mowing of the grass sward prior to sludge application.

Whereas only 13% of the total sludge-dry solids applied adhered to the leaf surfaces where the 2% D.S. sludge was spread on the cut sward (T4), a total recovery (99.8%) of sludge solids was recorded, where 2% D.S. sludge was applied to an uncut sward at a rate of 25m^3/ha (T5). In the case of both the uncut treatments to which 2% D.S. sludge was applied (i.e. T3 and T5), the grass canopies were similar and thus afforded the same leaf area for sludge adherence. Although the results from Temple Field are neither exhaustive nor conclusive they indicate what could be termed a 'threshold' effect of sludge solids adhering to the leaves (Figure 5.19). As the rate of sludge application increases up to a threshold value, at which point the herbage is saturated with sludge-dry solids, all the sludge solids are retained on the leaves. The 99.8% recovery for the half-rate treatment (T5), equivalent to 499kg dry solids/ha, implies that at this rate of application the sludge solids had not reached saturation point. However, applying 2% D.S. sludge to an uncut canopy at 67.5m^3/ha (T3) resulted in a recovery of only 61% of the sludge-dry solids applied, equivalent to 700kg dry solids/ha. Consequently, assuming this threshold hypothesis to hold, 100% saturation of the leaves (i.e. the threshold) would be attained when 35m^3/ha of 2% D.S. sludge were to be applied to this canopy. The cut grass with its reduced leaf area and, hence, its reduced threshold, would thus explain the observed lower recovery of sludge solids recorded for the cut treatment (T4).

The application of sludge containing 12% D.S. reduced the threshold application rate at which runoff from leaf surfaces took place. Application of 12% D.S. sludge to an uncut sward at a rate of 67.5m^3/ha (T6), i.e. 8370kg D.S./ha, resulted in 18.5% of the total dry solids applied remaining on leaf surfaces four hours after application, which was equivalent to 1548kg/ha. Hence, whereas the threshold application rate for 2% D.S. sludge was 35m^3/ha, 100% saturation of the leaf surfaces by sludge solids would result from the application of only 12.5m^3/ha of 12% D.S. sludge. However, owing to its much greater dry-solid content than the 2% D.S. sludge, this low-solids recovery

FIGURE 5.19 'THRESHOLD' ADHERENCE OF APPLIED SEWAGE SLUDGE SOLIDS



translated to as much as 54% of the total herbage dry → matter yield on the day of application being attributable to sludge solids.

The adherence of sludge metals to the grass sward led to elevated metal concentrations in/on the treated herbage. This increase in metal concentrations in the treated herbage over that found in the control plant material could not, in the first instance (i.e. on the day of application), be attributable to root uptake. The extent by which herbage metal load is increased following the spreading of sludge was seen to be influenced by:

i) the rate of sludge application,
ii) the dry solid content of the sludge applied,
iii) the height and/or leaf area of the receiving canopy, and
iv) the concentration of metal in the sludge.

The percentage recovery of applied sludge metals on the herbage, i.e. their Adherence Index, was lower and unrelated to those factors influencing adhered sludge-dry solids. The Adherence Index of Cd, Cu, Mn, Ni, Pb and Zn where sludge was applied to an uncut canopy, irrespective of sludge dry solid content (i.e. T3, T5 and T6), was similar and ranged between 11-23%. In contrast, Cr and Fe exhibited an Adherence Index much greater than that of the other metals. This greater adherence of Cr and Fe was only true in the case where 2% D.S. sludge was spread (T3, T4 and T5). When 2% D.S. sludge was applied to a cut sward (T4), the Adherence Index of all eight metals was lower than that recorded in the uncut treatments (T3 and T5), probably reflecting the reduced leaf area available for sludge adherence. In contrast, when the thicker 12% D.S. sludge was applied to an unmowed sward the Adherence Index for Cr and Fe was no different to that of Cu, Cd, Mn, Ni, Pb and Zn.

Metal speciation in the sludge and, hence, its chemical and physical behaviour, may be of importance in helping to explain the observed Adherence Indices. However, information in this field of work is very limited. As described in Section 1.1.3, metals are associated predominantly with the solid fraction of the sludge and are generally present as inorganic precipitates, although there is now mounting evidence to suggest that organic matter adsorption of metals and metal-organic interactions or absorption by the biomass are important removal mechanisms (Lester et al., 1983). The fact that the percentage recovery of metals was lower than that for the solids implies that the sludge metals were bound to a fraction of the sludge solids which were preferentially lost from the leaf on sludge application. Sludge solids may be divided into two fractions, depending on size, i.e.:

i) particulate fraction consisting of larger size solids and
ii) colloidal fraction consisting of smaller size solids.

Following sludge application and considering only physical forces, the smaller colloidal fraction of the sludge solids would be lost more easily from the leaf surface than the particulate fraction which, being larger and heavier, would require a greater force to be dislodged and removed from the leaf surface. Hence, were the sludge metals to be primarily associated with the colloidal fraction of the sludge solids or on the smaller sized particles of the particulate fraction, this could account for the lower percentage recovery exhibited by the metals as opposed to the sludge-dry solids. In fact, on a weight to weight basis, the smaller the particle the greater its ability to adsorb metals, since the surface area to weight ratio increases as particles size decreases.

Sludge metals need not necessarily be associated with a single fraction of the sludge solids. A higher Adherence Index would indicate association with larger sized particles. It is possible that Cr and Fe, in the 2% D.S. sludge, were held by particles which were larger than those retaining Cd, Cu, Mn, Ni, Pb and Zn. The 2% D.S. sludge was collected directly from the digestion tanks, whereas the 12% D.S. sludge was sampled from storage lagoons in which the digested sludge had been stored for several months, during which time it underwent further oxidation. This might have resulted in the breakdown of that sludge-solid component to which Cr and Fe was held. This breakdown could thus have resulted in Cr and Fe being associated with a solid fraction of comparable physical size/properties as that to which the other metals were held leading to similar adhering properties.

The failure of these marked differences in the Adherence Index of Cr and Fe on the one hand and Cd, Cu, Mn, Ni, Pb and Zn on the other to show up in the pot trial could have arisen from the fact that this trial was carried out at 100% humidity. Such a high humidity precludes the drying out of the applied sludge which, therefore, remained in a very fluid state, favouring the enhanced wash-off of sludge solids and their associated metals. In fact, the Adherence Indices exhibited by all the metals were very low (0.2 - 3.9%), indicating a general lack of metal retention by the leaf surfaces.

Sludge adhering to the forage crops carries all of its constituents, such as heavy metals, pathogens and organic compounds. Adhering sludge metals, having by-passed the soil-plant barrier (Logan and Chaney, 1983) which normally limits plant uptake of many metals to concentrations substantially below those which would be toxic to animals (Dean and Suess, 1985), could adversely affect grazing animals. Studies have been conducted to investigate the effects of sludge metal residues added to diets of cattle (Kienholz et al., 1977) or sheep (Smith et al., 1977). Depending on the metal contents of sludges and its percentage of an animal's diet, increases in the metal content of animal tissue have been found. At Temple Field, application of anaerobically digested sludge to a ryegrass sward led to a situation where on the day of application (when the metal concentrations were at their highest) and for a number of weeks thereafter, herbage metal concentrations, with the exception of Cr, Mn and Ni, were above those values known to give rise to toxic problems in grazing animals. In the case of Cr, Mn and Ni, not even the most contaminated herbage (that receiving 12% D.S. sludge at 67.5m^3/ha (T6)), provided concentrations which were above 50mg Cr/kg D.M. (Davis, 1980), 1000mg Mn/kg D.M. (Webber et al., 1983) and 50mg Ni/kg D.M. (MacNicol and Beckett, 1985), known to be toxic to sheep and cattle.

Zinc is considered to be relatively non-toxic. Diets supplying ten times the recommended dietary allowances (i.e. 400mg Zn/kg D.M.) are not considered to cause any health problems to animals (MAFF, 1984). Excess Zn, like Ni, is rapidly excreted in faeces (MAFF, 1984) thus maintaining body levels within safe limits. Adopting the value of 400mg Zn/kg D.M. for all ruminants would, therefore, place Zn in the same group as Cr, Mn and Ni as being unlikely to cause animal health problems to ruminants grazing the most heavily contaminated sward in the Temple Field study. However, if a lower tolerance of 300mg Zn/kg D.M. is assumed for sheep (Webber et al., 1983), then herbage concentrations of Zn remained above this safe dietary load for 3 weeks following the application of 12% D.S. sludge at 67.5m^3/ha to an uncut sward (T6).

The toxicity of an element is influenced by various factors including the quantity of the element the animal ingests, the balance of other elements in its diet and its general state of health (Davis and Carlton-Smith, 1980). It is particularly difficult to assign threshold figures for Cd and Pb which are cumulative poisons (Bowen, 1966). It is arguable that any increase in Pb and Cd concentrations above background levels is unacceptable for plant components of the human and animal diet, although in the latter case, due to the relatively short life of farm animals, the concentrations of Pb and Cd in the plant component of the diet may be less critical (Davis and Coker, 1980).

Lead poisoning is characterised by stiffness of gait, fractures, osteoporosis and hydronephrosis (CAB, 1980). Lead poisoning associated with pastures whose Pb content ranged between 160-760mg/kg D.M. has been described in Derbyshire, the northern Pennines and the Scottish Borders (Clegg and Rylands, 1966; Stewart and Allcroft, 1956; Butler et al., 1957; all cited by CAB, 1980). Increases in the levels of Pb in the bones and livers of grazing animals following direct ingestion of sludge have also been reported (Dean and Suess, 1985). The definition of a threshold zootoxic Pb concentration is subject to much discussion and has ranged from 60-100mg Pb/kg D.M. (CAB, 1980), to 30mg Pb/kg D.M. (Webber et al., 1983) and 15mg Pb/kg D.M. (Davis, 1980).

When 12% D.S. sludge was applied at a rate of 7.5m^3/ha to an uncut ryegrass sward (T6) the herbage was highly contaminated with Pb. In fact, over the initial 35 days, Pb concentrations were well in excess of the most liberal maximum dietary tolerable concentration of 60-100mg Pb/kg D.M. suggested by CAB (1980). Thereafter, over the next seven days, the Pb concentrations fell markedly and attained concentrations of less than 30mg/kg D.M., dropping to values below 15mg/kg D.M. by week 7. In none of the three treatments which received 2% D.S. sludge (i.e. T3, T4 and T5) was the Pb contamination problem anything as pronounced as that resulting from the spreading of 12% D.S. sludge. It was only over the first seven days of the trial that total Pb herbage concentrations remained in excess of 30mg/kg D.M., where 2% D.S. sludge was applied at 7.5m^3/ha to both a cut and uncut sward. Concentrations then decreased to below 15mg Pb/kg D.M. by day 21 for the cut swards and by day 35 for the uncut sward. Reducing the rate of application to 25m^3/ha (T5) resulted in total Pb concentrations not exceeding 30mg/kg D.M. and which required only 14 days to decrease to concentrations lower than 15mg/kg D.M., the most conservative estimate of maximum tolerable Pb dietary concentration proposed by Davis (1980).

The acute toxicity of Cd came to the forefront of world attention when

Cd was linked to the notorious Itai-Itai disease which was first reported in Toyama, Japan, shortly after World War II. A number of toxic effects due to Cd have since been encountered and acute toxicity conditions can be created in a variety of laboratory animals fed on diets containing Cd in the range 60-400mg/kg D.M. (Purves, 1985). For instance, 10-40mg Cd/kg in drinking water supplied to pregnant mice has led to various degrees of fetal growth retardation (Webster, 1978).

Davis and Coker (1980) report that for farm animals to suffer toxic effects the Cd concentration in their diet needs to be substantially in excess of 3mg Cd/kg. Cattle fed on diets containing 7.3mg Cd/kg D.M. exhibited no ill-effects on body weight, milk yield or health (Rosenberger et al., 1976; and Nelmes et al., 1974). On the other hand, lambs are particularly sensitive to Cd and Davis and Coker (1980) recommended that their maximum tolerable dietary intake should not exceed 1.0-1.5mg Cd/kg. A more conservative threshold limit of 0.5mg Cd/kg D.M. has been suggested by Webber et al. (1983) who do not distinguish any differences in susceptibility to Cd between cattle and sheep.

Application of sludge containing 2% D.S. at either 67.5m^3/ha or 25m^3/ha to both a mowed or unmowed sward did not result in a total herbage Cd concentration in excess of the 1.0mg Cd/kg D.M. safety limit recommended by Davis and Coker (1980). However, were the more conservative threshold value of 0.5mg Cd/kg D.M. to be adopted than the total herbage Cd concentration remained above this limit for 4 weeks where 2% D.S. sludge was applied to an uncut sward at a rate of 67.5m^3/ha (T3). This unsafe period was reduced to 14 days where 2%

D.S. sludge was applied to either a cut sward at 67.5m^3/ha (T4) or to an uncut sward at a rate of 25m^3/ha (T5). In contrast, application of sludge with a dry solid content of 12% at a rate of 67.5m^3/ha to an uncut sward (T6) resulted in a total Cd herbage concentration in excess of 1.5mg/kg D.M. for 3 weeks following the spreading of sludge. In this instance, it was only after day 42 that the Cd concentration dropped to concentrations below 0.5mg/kg D.M.

Interest in Fe requirements of ruminants centers primarily on the needs of young animals maintained on milk or milk substitutes, particularly housed lambs and young calves. Adequate dietary Fe is essential for the synthesis of haemoglobin and myoglobin and for incorporation into various enzymes. When Fe is deficient, haemoglobin production is first affected and an important consequence of developing anaemia is a reduction in appetite, contributing to a state of poor growth. However, excessive dietary intakes of Fe can lead to metabolic upsets within the animal. Diets supplying > 500mg Fe/kg have been reported to result in high blood haemoglobin concentrations and decreased food intake and weight gain by cattle (Standish et al., 1969, 1971; Koong et al., 1970; both cited by CAB, 1980). Higher rates of Fe dietary intake (> 1000mg/kg) lead to increased Fe contents of the liver, spleen, kidney and heart, whereas P and Cu liver and plasma contents decrease (Standish et al., 1969, 1971; cited by CAB, 1980). Therefore, a maximum dietary intake of 500mg Fe/kg D.M. is generally recommended (CAB, 1980; MAFF, 1984).

Total Fe concentrations of herbage were greatly increased by sewage sludge application and the consequent adherence of sludge solids.

Grass swards, irrespective of whether cut or uncut, which had been treated with 67.5m^3/ha of 2% D.S. sludge (i.e. T3 and T4) contained > 8000mg Fe/kg D.M. on day of application. In fact, Fe concentrations persisted above the recommended maximum tolerable value of 500mg/kg D.M. for 5 weeks following the application of sludge. Although a reduction in the degree of Fe contamination of the herbage was achieved by decreasing the application rate to 25m^3/ha (T5), total Fe concentration nevertheless remained well in excess of 500mg/kg D.M. for between 28-35 days after sludging. A much more serious Fe contamination problem of the herbage was encountered when sludge containing 12% D.S. was applied at 67.5m^3/ha to an uncut sward (T6). Not only did the total herbage Fe concentration persist above the recommended maximum tolerable level of 500mg/kg D.M. over all eight weeks of the experiment, but moreover, for the first 28 days following sludge disposal, Fe concentrations of the herbage were above 10000mg/kg D.M. i.e above 1% of total dry-matter yield.

Of particular interest to ruminants is the Cu content of herbage. Sheep are more susceptible than cattle to Cu poisoning (Klessa et al., 1985). Cattle, once weaned, are relatively resistant to Cu toxicity (Hill, 1975) although calves have been seen to develop Cu toxicosis when given milk substitutes containing 50-100mg Cu/kg (Shand and Lewis, 1957; Weiss and Baur, 1968 both cited by Hill, 1975). The occurrence of chronic Cu toxicity is normally only a major problem in the intensive rearing of sheep and primarily in lambs being fattened. The proportion of lambs affected is usually small (5-10%), but the financial loss can be substantial because death quickly follows the haemolytic crisis, and this occurs towards the end of an expensive fattening peiod (Hill, 1975). There is usually no clinical evidence of the impending danger in a group of lambs before the first death occurs, as although a gradual Cu accumulation in the liver can take place (Klessa et al., 1985), reflected in increases in the plasma concentration of a number of tissue enzymes, no obvious adverse effects on growth rates, food intake or disease incidence may be manifested. Thereafter, death quickly follows as blood Cu concentration increases with its associated implications to haemolysis followed by methaemoglobinaemia and haemoglobinuria (Klessa et al., 1985).

The concentration of Cu in the diet of sheep above which toxicity sets in, is highly influenced by breed, age, stress and in particular composition of the diet (Klessa et al., 1985). Buck (1970; cited by CAB, 1980) reported Cu poisoning of sheep fed on diets containing only 8mg Cu/kg. However, this is apparently quite an extreme incident and 20-25mg Cu/kg D.M. has been recommended as the maximum tolerable dietary concentration for sheep (Luke and Macquering, 1972, cited by Hill, 1975; Dalgarno and Mills, 1975; Bremner, 1981).

Sludge adhering to leaf surfaces following the spreading of 2% D.S. sludge at a rate of 67.5m^3/ha to an uncut ryegrass sward (T3) resulted in total herbage Cu concentrations in access of the suggested upper dietary limit for sheep (20mg/kg D.M.). This degree of contamination persisted for 4 weeks following the spreading of sludge. A reduction in the amount of herbage Cu contamination as a result of sludge adherence was achieved by applying the sludge to a cut sward, and more so by applying the sludge at a reduced rate of 25m^3/ha. In the former case, Cu concentrations remained above the maximum recommended for 3 weeks after sludge application, whereas in the latter instance this unsafe period was reduced to only 2 weeks. Hemingway and McPherson (1967, cited by CAB, 1980) estimated that a daily intake of 38mg Cu for 16-20 weeks would be necessary to increase liver concentrations to 1000mg Cu/kg D.M. and, thus, place the animal at risk. On average, sheep have a daily dry-matter intake of 1.5 kg (McPherson, personal communication). Therefore, a daily intake of 38mg Cu would require a Cu concentration of 25mg/kg D.M. in the diet. Although Cu concentrations were not maintained at these levels (> 25mg/kg D.M.) for such prolonged periods (i.e. 16-20 weeks) total Cu concentrations of herbage which had received the thickened sludge (i.e. 12% D.S.) at a rate of $67.5m^3$/ha (T6) remained at levels > 100mg/kg D.M. for 5 weeks, and only reached values < 20mg/kg D.M. by the seventh week. Consequently, over these initial 5 weeks, sheep grazing this treated herbage would have ingested in excess of 800mg Cu, the intake of which is greater than the total Cu intake of sheep fed 38mg Cu per day for 16-20 weeks.

Ingestion of sludge adhering to forage can supply the animal with not only potentially toxic elements, but also with a variety of other elements which may alter the toxicant's availability and, hence, its toxicity. Dietary composition is particularly important when assessing Cu toxicity. Copper metabolism is complicated by the antagonistic effects of Mo, S, Fe, Cu and Zn (Hill, 1975; Mills, 1975; COSAC/SARI, 1982), which reduce Cu retention by the animal. Molybdenum is thought to be of greatest importance and Lamand (1981) suggested that a ceiling of 20mg Cu/kg in the diet of sheep be maintained when Mo is above 0.2mg/kg and when the Mo content is low (< 0.2mg/kg), a lower limit of 15mg Cu/kg be applied. It has been suggested, that where liver damage is occuring as a result of Cu toxicity in sheep, their diet should be supplemented with daily rations of Mo and S (Lamand, 1981). However, Hill (1975), in view of the very complex relationship between Cu, Mo and S, suggested that the use of Zn, a relatively non-toxic element, may be more appropriate in safeguarding sheep and in particular lambs against any possible Cu toxicity. A very substantial control of the haemolytic crisis due to Cu poisoning in sheep-fed diets, containing 20-25mg Cu/kg D.M., was achieved when the Zn concentration of the diet was raised to 200mg/kg D.M. (Mills, 1975). However, a higher dietary Zn content (> 400mg/kg) may result in complicatory problems of reduced Fe availability (Mills, 1975). Copper accumulation in the liver, as well as that of Zn, is also affected by the Cd content of the diet, and it has been suggested that in order to prevent a marked depletion of tissue Cu reserves the dietary Cd : Cu ratio should not exceed 0.7 (Mills, 1974). On the other hand, Powel et al. (1964) reported that when the diet of calves contained > 100mg Zn/kg, there was no adverse effects on health even when the diet contained 40mg Cd/kg. Many other interactions are known (Mills, 1974), but existing knowledge on the nature and extent of these interactions is far from complete.

A reduction in the total metal concentration of the herbage may be brought about by either, or a combination of, two ways:

i) plant growth, or

ii) adhering sludge metal particles being physically removed from the herbage by an external factor.

The concentration of adhering metals is expressed as the weight of metal present per unit weight of dry plant material. Provided the total weight of adhering metal is constant, (i.e. there is no input or output of metal), with an increase in plant dry-matter yield, as a consequence of plant growth , the adhering metal concentration will decrease proportionately:

$$\text{Adhering metal concentration } \alpha \quad \frac{1}{\text{plant dry matter yield}}$$

This relationship holds true, irrespective of the element involved.

However, results obtained from the field trial revealed that the dilution rate of a particular metal (when expressed as the change in the initial herbage metal concentration, with time) differed between treatments. In addition, where 2% D.S. sludge had been applied (i.e. T3, T4 and T5), differences in the dilution rates of the eight metals within a single treatment were also observed. Similar dilution rates for all eight metals within a single treatment were, however, recorded in the herbage treated with the thicker 12% D.S. sludge (T6). Taken in isolation, this latter treatment (i.e. T6) may lead one to conclude that plant growth is the predominant factor bringing about a dilution of adhering sludge metals. Such a conclusion was drawn by Chaney and Llyod (1979) from their work involving the application of 5% D.S. sludge to a fescue sward. However, plant growth per se cannot explain the differences observed in the treatments receiving 2% D.S. sludge, nor for that matter, the inter-treatment differences in the dilution rates of individual metals.

In addition plant growth fails to explain the observed decrease in adhering sludge metal concentration in all four sludge treatments over the initial 3 weeks of the experiment, during which time no significant increase in herbage dry-matter yield was recorded. In contrast, between day 35 and day 42, a marked reduction in adhering metal concentrations was observed in herbage treated with 12% D. S. sludge at 67.5m^3/ha (T6), this reduction coincided with a large increase in dry matter production (Figure 5.17).

Hence, the inability to explain dilution rates of adhering metals, solely on the basis of plant growth, indicates the presence of another factor(s) which influences adhering metal concentration and foremost amongst these is rainfall.

The power of rain to dislodge, suspend and/or dissolve adhering metals, and, thereafter, to remove these metals from the plants, depends on the intensity and duration of the rain. An attempt was made to statistically relate the characteristics of the rainfall events at Auchincruive (i.e. intensity, duration, etc.) to the observed dilution of sludge metals. Unfortunately, this proved to be futile due to the limited number of rainfall events. Nonetheless, general trends in the data may still be observed. Chaney and Lloyd (1979) stated that rainfall had little effect on the dilution of adhering metals except when it fell immediately after the application of sludge. The importance of this statement is clearly illustrated by the results obtained from the pot experiment. Permitting the applied sludge to dry out (the sludged herbage being kept at 50% humidity for 24 hrs) prior to 'rain', resulted in no adhering sludge metals being removed even with the unnaturally high intensity of the rain applied. In contrast, in the field trial, the first rain fell only a short time after the sludge had been applied. This allowed no time for the sludge to dry out and a marked reduction in adhering metal concentrations was observed over the first week, when precipitation totalled 55mm - slightly less than the total precipitation recorded over the following seven weeks! This period of high rainfall was particularly effective in bringing about a marked reduction in the concentration of Cr, Fe and Ni adhering to herbage treated with 2% D.S. sludge, where a decrease of around 55% was registered over the concentrations recorded on the day of sludge application. On the other hand, over this intial seven day period, the reduction in the concentration of Cd, Cu, Mn, Pb and Zn adhering to the herbage was not as pronounced, ranging between 0 - 30% of the concentration present on day of application. Moreover, where herbage had received 12% D.S. sludge (T6), there was no recorded reduction in metal concentration as a result of the week's high rainfall, not even in the case of Cr, Fe and Ni, once again suggesting that in the thickened 12% D.S. sludge Cr and Fe are present in forms similar to those of the other metals. The increased breakdown of solids in the thickened sludge may have also contributed to the higher overall dilution rates observed for the metals derived from the 12% D.S. sludge (T6).

The distinctive behaviour of Cr and Fe originating from 2% D.S. sludge, vis-a-vis their Adherence Indices (and, together with Ni, their dilution over the first 7 days of the experiment), was further illustrated by their dilution rate over the rest of the experimental period (i.e. day 7 - 56), which was lower than that of the other metals. A proper explanation for these observed differences in the dilution pattern of the metals requires an in-depth knowledge and understanding of the chemical and physical forms of these metals in the sludge and their interactions with the leaf surface following sludge application. There is a lack of such information and one can only hypothesise as to the reasons behind their distinctive behaviours. but the possible uneven distribution of metals between the solid size fractions (as postulated earlier) could explain the differences in dilution rates.

For a particle to be dislodged and, thereafter, removed from the herbage, rain drops need have enough initial momentum to overcome the inertia of the sludge particles and, subsequently, sufficient energy to completely remove the dislodged particle from the leaf surface. The larger the particle, the greater its weight and thus its inertia, hence the greater the energy required by the raindrop. The important influence of rain intensity and, consequently, its energy, is very evident from the results of the pot trial, in which a total precipitation of only 0.7mm, but at a high intensity, was sufficient to achieve a near total wash-off of the adhering Zn, Ni, Cu, Cr and Cd. Adhering Fe, Pb and Mn were somewhat more resistant and required 1.3 - 2.0mm of 'rain' for total wash-off at this high intensity. Over the first week of the field trial, total rainfall was well in excess of 2.0mm, although its intensity was much less than that used in the pot work. However, the rainfall profile with time was shown to be very important and had a marked effect on the degree of metal wash-off.

Whereas 2.0mm rain applied as a one-off event led to the total wash-off of all eight metals in the pot experiment, where 2.1mm was applied over three separate doses (0.7 x 3), a significant reduction in the wash-off of Zn, Ni and Cu was noted. Sufficient rain intensity is required to dislodge the adhering metals, but equally important is the duration of the rainfall event which determines the extent of removal of the dislodged particles. Incomplete removal results in reduced dilution. Therefore, the temporal distribution of rain in the field trial was important in determining the pattern of adhering metal wash-off over the first week. It might have been the case that most of the recorded reduction in adhering metal concentrations, particularly for Cr, Fe and Ni, occurred over the first 24 hours when total precipitation was 15.5mm (Figure 5.18). During the second day of the trial only 1.8mm rain was recorded, possibly permitting a limited drying-out of the sludge, resulting in increased sludge resistance to wash-off.

The fate of sewage sludge-borne metals applied to a ryegrass sward can be viewed as being a 3-stage process:

Stage 1 - their initial adherence to the leaf surfaces following sludge application (the Adherence Index)

Stage 2 - the dilution of the adhering metals over the first week following the application of sludge

Stage 3 - the dilution of those metals still adhering to the leaf surfaces after Stage 2.

In stage 1, the initial adherence of applied sludge metals (the Adherence Index), differed considerably between the two sludges applied.

Where 12% D.S. sludge was applied Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn all exhibited similar Adherence Indices. In contrast, application of 2% D.S. sludge led to Cr and Fe exhibiting Adherence Indices much greater than those of the other six metals. To explain this latter situation, it was proposed that a large proportion of sludge Cr and Fe was bound to the particulate fraction of sludge solids, whereas Cd, Cu, Mn, Ni, Pb and Zn were preferentially held on the smaller colloidal fraction of sludge solids (vide page 297). The smaller (lighter) the particle, the easier its removal from the leaf surfaces by the liquid phase of the applied sludge whereby it is carried in suspension, and, consequently, the lower the Adherence Index of the associated metals. Following this initial stage, it is hypothesised that the metals which were left adhering to the leaf surfaces were those associated with the larger particulate fraction of sludge solids, the lighter colloidal fraction having been removed in stage 1. Over the next week (stage 2), in the treatments which had received 2% D.S. sludge, the greater dilution of adhering Cr, Fe and Ni over that shown by Cd, Cu, Mn, Pb and Zn could have arisen were Cr, Fe and Ni bound to adhering particulate solids of a smaller size than the particulate solids with which adhering Cd, Cu, Mn, Pb and Zn were associated. Dilution of adhering metals during this period has been attributed principally to the action of rain (vide page 309). The smaller (lighter) the adhering solid particle, the greater the facility with which it is dislodged and removed from the leaf surface by the action of rain, hence, the greater the dilution rate of the metal(s) associated to this fraction. At the end of stage 2 , what metal is left on the leaf, was bound to the larger, more recalcitrant, fraction of the adhering sludge particulate solids.

Consequently, over stage 3 the dilution of metal required a greater energy input on the part of rain for it to be dislodged and removed. Interestingly, during this third stage adhering Cr, Fe and Ni from the herbage which had received 2% D.S. sludge exhibited slower dilution rates than did the other metals. At this point in time, other factors may have come into play in determining the behaviour of adhering metals.

Chemical and/or electrostatic bonds between the negatively charged leaf surface and the sludge metals may have been involved. The smaller the size of the adhering solids, the greater its charge per unit area and consequently, the stronger the bonds established between this fraction and the leaf surface. Direct uptake of adhering metals into the leaf tissue is also possible, thus decreasing their availability for wash-off. In fact, whereas Batey et al. (1972) and Gracey et al. (1976) reported that swine manure adhering to leaf surfaces was easily removed by gentle washing with water, Chaney and Lloyd (1979) were unable to remove any adhering sludge metal by washing the contaminated herbage with a detergent, suggesting some sort of strong binding of the sludge metals by the leaf surfaces.

From this work, it appears that the behaviour of adhering metals following the application of sewage sludge to a grass sward can be predicted by monitoring one or two 'typical' metals. Where 12% D.S. sludge was applied, all eight metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) exhibited similar initial adherence and subsequent dilution. Consequently, any one of these metals could have been used to predict the response of the other metals. However, where 2% D.S. sludge was applied, the distinct response of Cr Fe and Ni on the one hand and Cd, applied, the distinct response of Cr Fe and Ni on the one hand and Cd, Cu, Mn, Pb and Zn on the other, means that two metals (one from each group) need be monitored to gauge and predict on the short term the degree of leaf contamination by these metals.

Plant growth and rainfall can together help explain much, but not all, of the observed responses of adhering sludge metals. Dilution of adhering metal still occurred during periods of no-growth or little rainfall. Other factors, such as wind, could play some part in removing sludge metals from plant surfaces. Weather conditions, such as rainfall, ambient air/soil temperature, hours of sunshine, humidity etc. will influence plant growth and, thus, affect the rate of dilution of adhering metals. Any observation and conclusions derived from a particular experiment can only be confidently applied to plants of a similar species, i.e. with similar leaf morphology, and to sludge of similar composition.

Contamination of leaf surfaces associated with surface spreading of anaerobically digested sludge to grasslands can be minimized by:

i) applying sludge to recently mown fields,
ii) applying sludge at reduced rates, and
iii) reducing the sludge-dry solid content.

Cutting the grass prior to sludge application may be unacceptable to the farmers as it results in a sward which is too short for grazing cattle, cattle preferring a longer sward. In practice, in March when sludge is applied to the best effect (SAC, 1986) a grazed grass sward in the West of Scotland is relatively very short and generally 2 - 3cm tall (Tiley, personal communication). Reducing the application rate of sludge by tanker essentially requires an increased tractor speed, but the sludging equipment's operating rate is relatively inflexible, and is, therefore, not a favoured option.

Economic considerations have led sewage plant operators to favour the thickening of sludge prior to its disposal to land. The reduction in the water content of thickened sludge makes its transport and spreading on land cheaper. However, the application of thickened sludge (i.e. higher dry-solid content) results in increased metal contamination of the herbage, thus making the latter increasingly unacceptable for animal consumption. Limiting the sludge metal load to levels found to be safe in practice, by controlling metal discharges at source, may prove to be an alternative way of reducing food chain contamination associated with sludge disposal to land. Injection of sludge directly into soil, a recent innovation to the sludge disposal industry, does away with any possible leaf contamination by adhering sludge. However, the use of such a method for disposal is comparatively expensive and not widely used.

Further work in the field of sludge metal adherence to leaf surface is essential to understand and predict this important source of herbage and food-chain contamination. Monitoring adhering sludge metal concentrations will always remain an integral part of any management of sludge disposal.

CHAPTER 6

GENERAL DISCUSSION

Each year a total of just under 7Mt sewage sludge dry solids are produced by the 16 major European countries (Charnock, 1984). This is expected to rise to 11.5Mt dry solids per year as the proportion of the population served by sewage treatment increases (Charnock, 1984). Based on 1977 estimates, 1.3×10^6 tonnes sludge dry solids are produced annually in Britain (DoE, 1981). The safe disposal of these vast quantities of sludge is a matter of great concern.

In the past, sea dumping of sludge was a favoured option but it is now frowned upon by many nations. Most European countries and the U.S.A., which in the past had practised disposal to sea in relatively small quantities, are now phasing this out, primarily as a result of international conventions which control dumping at sea. Britain is widely out of step with the rest of the world in its adherence to the practice of dumping sludge at sea. Thirty per cent of the sludge produced in Britain is taken out in ships and disposed at 13 designated dumping grounds (Pearce, 1982). In 1974, Britain signed the Oslo convention which allows sea dumping only when no other realistic option exists. The U.K is isolated amongst the EEC countries in using the sea for sludge disposal and consequently the environmental acceptability of this outlet is viewed with increasing scepticisim by its European partners. Britain is now under intense pressure to end its unique reliance on sea dumping.

Some form of disposal to land is now the only real alternative for large-scale dumping of sludge. The use of soil as a disposal medium for sewage sludge has been increasingly emphasized in recent years. By far the most economical way of getting rid of sewage sludge is to put it to agricultural land. Within Europe, just under 40% of the sludge produced goes on farmland (Charnock, 1984). In Britain, based on 1980/81 data, about 39% of all sewage sludge produced is utilized on agricultural land, of which 14% goes on grassland (Davis, 1984).

The EEC Directive (EEC, 1986) issued by the European Parliament is the first international legislation controlling the agricultural use of sewage sludge (see Section 1.5.3). By not later than June 1989, each of the twelve member states, including Britain, are required to "bring into force the laws, regulations and administrative provisions necessary to comply with this Directive" (EEC, 1986). The Directive is well-intentioned, and springs from a climate of increasing public concern about the need to protect the environment (Otter, 1986). However, international legislation is the result of scientific, technical and political compromise and this inevitably means that it is often criticized. The stated aim of the EEC Directive is "to prevent harmful effects on soil, vegetation, animals and man, while encouraging its (sewage sludge) correct use" (EEC, 1986). It lays down maximum permissible limits for heavy metal concentrations in sludge treated soils and loadings, the limits of which cannot be exceeded as a result of sludge disposal practices.

The results from Lower Carbarns presented in this thesis are interpreted in the light of the EEC Directive. Even though

i) soil Cu, Pb and Zn concentrations (see Table 3.4) were greater than the lower limits set out in the EEC guidelines (Table 1.7), and

ii) the soil at Carbarns had a pH of 5.3, (i.e. lower than the EEC stipulated minimum of 6.0 for soil receiving sludge for which the above mentioned heavy metal limits apply),

herbage Cu, Ni, Pb and Zn concentrations (see Table 3.5) remained well below those considered to be either phytotoxic or zootoxic (Table 6.1). Therefore, the limits on soil Cu, Pb and Zn concentrations as set out in the 1986 EEC Directive seem to offer adequate protection to the food chain. Grassland management practices can have a significant effect on herbage metal concentrations (see Chapter 3), whereby soil cultivation and application of fertilzer N leads to increased metal uptake, but lime application has the opposite effect. However, even herbage from cultivated, unlimed and high-N plots, Cu, Ni, Pb and Zn (Table 3.5) contained concentrations within safe limits.

The Cd concentration in the Carbarns soil (1.21 mg/kg air dry soil) was well within the permissible limits for Cd concentrations in sludged soils as defined in the EEC Directive, i.e. 1-3 mg Cd/kg air dry soil. However, the concentration of Cd in the herbage (see Table 3.5) approached and occasionally exceeded those values thought to be zootoxic (see Table 6.1), particularly in herbage from the unlimed, N fertilized plots. The potentially hazardous Cd concentrations which were recorded in the herbage, may partly be ascribed to the low soil pH

TABLE 6.1 ZOOTOXIC (WEBBER et al, 1986) AND PHYTOTOXIC (MACNICOL AND BECKETT, 1985) CONCENTRATIONS (mg/kg D.M.) OF Cd, Cr, Cu, Ni, Pb AND Zn IN HERBAGE.

ELEMENT	CATTLE	SHEEP	RYEGRASS
Cd	0.5	0.5	30 - 35
Cr	3000	3000	1
Cu	100	25	30 - 40
Ni	50	50	120 - 150
Pb	30	30	
Zn	500	300	370 - 560

which, as previously stated, was below that recommended by the EEC, Cd availability being strongly influenced by soil pH (see Section 1.2.2). In fact, the Directive does include a proviso that "where sludge is used on soils of which pH is below 6, Member States shall take into account the increased mobility and availability to the crop of heavy metals and shall, if necessary, reduce the limit values they have laid down ....". Whereas this provides some safeguard against the increased availability of metals such as Cd in soils presently at pH <6.0 it assumes the limit imposed to be satisfactory for soils presently at pH >6.0. There is, however, no guarantee that sludged fields which are currently maintained at pH >6.0 will in future remain at this pH were land use priority to change. Consequently, whereas the EEC Directive offers adequate protection against excessive Cu, Pb and Zn uptake even in the eventuality of soil pH decreasing to values <6.0, this is not the case for Cd. There thus appears to be a case for more stringent limits to be imposed, vis-a-vis permissible Cd concentrations in sludged soils.

The EEC Directive failed to fix any limits on the permissible Cr concentrations in sludged soils. Toxic effects due to Cr in sewage sludge have not been observed in plants or in animals comsuming crops grown on sludged land (DoE, 1981). Chromium is usually quite tightly fixed in soils (Kloke et al., 1984) and, in addition, very little of the Cr absorbed by plants is translocated to the above-ground (edible) parts (Baldi et al., 1984). Consequently, high soil concentrations >500mg Cr/kg air-dry soil are required to result in any phytotoxic effects (Chumbley, 1971). Moreover, although Cr (VI) is much more toxic to toxic to plants than Cr (III) it will be reduced to Cr (III) after addition to aerobic soils (Davies and Jones, 1988).

Herbage sampled from Lower Carbarns, where the soil Cr concentration totalled 109 mg/kg air-dry soil, contained between 0.55 - 2.19 mg Cr/kg D.M. (see Table 3.5). Generally, background Cr concentrations in plants are reported to range between 0.1 - 1 mg/kg D.M. (Richardson, 1980; Jell et al., 1983); lower than those recorded at Lower Carbarns. Determining what may be considered to be an acceptable soil Cr concentration depends on establishing a phytotoxic threshold concentration for Cr, it being much more toxic to plants than animals (Jell et al., 1983). Accepting what may be considered to be quite a liberal estimate of the Cr phytotoxic threshold, i.e. 20 mg Cr/kg D.M. proposed by Jell et al. (1983), and judging the situation observed at Lower Carbarns, suggests that soil Cr concentration of 109 mg/kg air-dry soil, even at pH 5.3, poses no risk to the health and productivity of a grass sward. Thus, the 150-250mg/kg tolerable soil Cr concentration limit recommended by Williams (1988), in his report to the Commission of the European Communities, offers a satisfactory safeguard against possible Cr phytotoxicity arising from sludge application on land. If, on the other hand, a much more conservative estimate of 1 mg Cr/kg D.M. is accepted as being the Cr phytotoxic threshold (Beckett and Macnicol, 1985), then at Lower Carbarns, Cr reached levels which may have led to reductions in herbage yield. However, the fact that the soil chemistry of Cr is poorly understood and that phytotoxic effects may also occur when Cr concentrations are within normal levels, complicates the definition of what constitutes an acceptable Cr concentration in soil.

Total soil metal concentrations alone is tackled in the EEC Directive and no mention is made of extractable metal levels. Several authors have argued the case for using a particular extractant to predict metal availability (see Section 2.1). In the U.K., the DoE guidelines (DoE, 1981), which remain in force in England and Wales up to the time when the U.K. formally adopts the EEC Directive, include the use of acetic acid and EDTA for estimating plant available Cu, Ni and Zn. The results from the Carbarns work clearly indicate that whereas acetic acid- and EDTA- extractable metal concentrations may reflect herbage metal concentrations, they are insensitive to soil management practices such as cultivation, liming and fertilizer N application which, in contrast, have a very significant affect on plant metal uptake. The use of weaker extractants, such as ammonium chloride, or water extracts may well be found to respond to changes in metal availability following changes in soil management practices. However, ultimately, it is only by monitoring the total concentrations that future land use may be secured. Moreover, determination of total metal levels is less susceptible to local soil and laboratory conditions and thus offers a more uniform and internationally acceptable measure of soil metal status.

To achieve the condition set out in Article 5 paragraph 1 of the EEC Directive, i.e. maintaining soil heavy metal concentrations within predetermined limits, the said article provides two options to Member States, either

a) upper limits can be set on the maximum quantity of sewage sludge which may be applied per unit area per year while observing the limits for metal concentrations in sludge selected from the ranges laid down in the Directive (see Table 1.7), or

b) the limits on metal addition per unit area per year, as laid down in the Directive, can be applied (see Table 1.7).

In formulating 'option a', there appears to have been no consideration given to the potentially hazardous levels of heavy metals which could be assimilated by grazing animals as a direct result of their ingesting sludge adhering to leaf surfaces. In a situation where 2% D.S. sludge, containing the maximun permissible metal concentrations (EEC, 1986), is applied to a sward during late March in the West of Scotland, and extrapolating from the Temple Field Study (see Chapter 5), the resultant herbage concentrations of Cd, Pb and Cu (the maximum permissible Fe and Mn load of sludge not being specified) due to sludge adhering to the leaf surfaces, would remain above zootoxic levels (see Table 6.1 and Section 5.4) for between 6 - 7 weeks. The EEC Directive does require Member States to set a minimum period of not less than three weeks after sludge has been spread, before grazing or harvesting can take place. However, for the specified limit values of heavy metal concentrations in sludge, this period is inadequate to ensure the concentrations of potentially toxic elements associated with the treated herbage to decrease back to acceptable safe levels. Establishing a safe minimum no-grazing period applicable in all 12 EEC Member States is no easy task. The wash-off of adhering sludge metals is largely dependent on plant growth and rainfall patterns following the application of sludge (see Section 5.4). The EEC spans a number of climatic zones, from the warm temperate mediterranean climate of Italy and Greece to the cool temperate western margin climate of Britain and Ireland. In Scotland, assuming the conditions of Temple Field to have been typical of those for the West of Scotland, a minimum no-grazing period of seven weeks should be imposed where sludge containing the maximum permissible metal load was applied. In dryer climates, longer no-grazing periods will be necessary. Alternatively, tighter limits could be imposed on the maximum permissible Cu, Cd and Pb concentrations in sludge for use on grassland so as to permit a no-grazing period of three weeks.

Consideration also needs to be given to the maximum permissible dry solid content of the applied sludge. The work described in this thesis highlighted the very significant increase in the concentration of adhering sludge metals on leaf surfaces where 12% D.S. sludge was applied. However, these results unless backed by further research work do not permit the formulation of any firm conclusions as to the relationships between metal adherence and sludge dry solid content. In addition, more work is required on the modelling of the wash-off of sludge particles and their metals from leaf surfaces.

A second equally important route of heavy metal entry into the food chain which by-passes plant uptake is via direct ingestion of sludge contaminated surface soil by grazing animals. As discussed previously (Section 3.4), soil ingestion may contribute as much as 25% of the total dry matter intake by grazing animals during unfavourable periods. More importanly, ingested soil constitutes a significant fraction of the total metal intake of grazing animals. For instance, as much as 40-90%

of the Pb assimilated by cattle has been ascribed to soil ingestion (Russell et al, 1985). Hence, heavy metal enrichment of the surface soil by the application of sewage sludge may lead to undesirable health effects in grazing animals. Unfortunately, limiting permissible soil metal concentrations to samples taken over a depth of 25cm (EEC, 1986), or where the surface soil is shallower than 25cm, to a smaller sampling depth, which in no case must be less than 10cm (EEC, 1986), does not provide adequate consideration of the potentially harmful ingestion of surface-contaminated soils. Imposing limits on the maximum concentration of metals, such as Pb and Hg, in soil to safeguard the health of plants, animals and man seems fruitless when such metals are known to be concentrated during uptake in shallow plant roots and, hence, not translocated to the edible plant parts, unless this is coupled with some control as to the maximum permissible metal concentrations in the ingested surface soil. A sampling depth of 25mm, in addition to the 25cm depth specified in the Directive, thus appears to offer a more effective control on the uptake of these into the food chain under grazing. In addition, work needs be undertaken to right the evident lack of published information on the availability of ingested metals in the rumen of grazing animals; the risk of metal toxicity arising from soil ingestion ultimately depends on how much of the metals go into solution, once ingested.

In temperate grasslands, the distribution of grass roots in the soil profile is very uneven, with the highest root densities in the surface 10 - 15cm, root density decreasing rapidily beyond this depth (Gregory, 1988). Consequently, a soil sampling depth of 25cm (EEC, 1986) when applied to grassland soils is too generous a margin for determining whether soil metal concentrations are such as to potentially give rise to phytotoxic effects; more so when it is realised that applied sludge metals accumulate in the surface 15cm of receiving soil (see Section 1.3.4 and Section 3.4), with little translocation of these metals to greater depths. Hence, sampling to a depth of 25cm would tend to underestimate the actual concentraton of heavy metals available to the grass sward as it includes the relatively less contaminated 15 - 25cm soil depth. A sampling depth of 15cm thus appears to be more in keeping with the conditions encountered in a grassland soil, offering a more representative measure of the total soil metal concentration potentially available for uptake into the foodchain. However, as the sampling depth is reduced, so too is the capacity of the soil to receive sludge, as the difference in soil metal concentration and the maximum permissible soil metal concentration decreases. For instance, at Lower Carbarns, by reducing the sampling depth from 25cm to 10cm, results in a 7 - 24% increase in the recorded total metal concentrations. In this respect, soil cultivation, provided it is effected to a depth greater than that of sampling, may be used in reducing the total soil metal concentration in the sampled soil, thus increasing the capacity of the soil to receive sludge. However, in the short term, i.e. over a period of 4 - 6 months, soil cultivation may result in an increased uptake of Cu, Zn and Ni (see Section 3.4). In addition cultivation by reducing the metal load of the surface 25mm of soil, may also reduce the quantity of metal assimilated by grazing animals, attributable to the involuntary ingestion of soil.

In the past it was thought that heavy metals had little effect on soil microbial activity. It was felt that metals were so tightly bound to soil particles that they would not interfere with the microbial biomass. The first clear alarm bells were sounded by researchers at Rothamsted (Brookes and McGrath, 1984) who found only half the expected amount of biomass in the metal contaminated Woburn Market Garden soil which had received sludge between 1942 and 1961. At Lower Carbarns a lower soil microbial biomass was recorded in the sludged soil than in the unsludged control soil (Table 4.4), even though the metal concentrations in the sludged soil were well within the maximum permitted by the EEC (EEC, 1986). In contrast, soil nitrification was not apparently affected by the high metal concentration in the sludged soil, but was very influenced by soil pH. In fact, when the pH of the sludged metal contaminated soil was adjusted, the nitrification potential in this soil was greater than that recorded in the unsludged control soil. However, there is a very evident lack of comparable information on the effects of metals, at or below EEC permissible concentrations, on the soil microbial population. Furthermore, the results from Allanton and Maudslie (see Chapter 4) underline the very intricate relationships that exists between sludge application and soil microbial biomass. At these latter sites, notwithstanding a higher soil metal concetration in the sludged soil compared to that present in the unsludged control soils, a greater microbial population was recorded in the sludged soils (Table 4.4).

At this stage, until further, more extensive research programmes are conducted to elucidate the effects of sludge application and the resultant increase in soil heavy metal concentration on soil microbial biomass, advice on 'safe' soil metal concentrations is purely speculative. In fact, the EEC Directive steered well clear of this question and made no mention of potential toxic effects of sludge metals on the soil microbial population. Nonetheless, the permissible metal concentrations as defined in the Directve do, on occasions, lead to a reduction in the soil microbial biomass. Soil metal contamination, being a relatively irreversible process, needs to be checked before causing irreparable damage to the ecologically important soil microbial population.

The EEC Directive is a much needed and long awaited piece of international legislation which brings into world focus the potentially very harmful effect of sludge application arising from heavy metal accumulation in the receiving soil, while at the same time promoting its safe use. It signifies an important step forward in international co-operation in the protection of the environment with twelve of the major western industrialised nations agreeing to a joint policy regulating the disposal of sludge.

The limits on the maximum permissible Cu, Ni, Pb and Zn concentrations in sludged soils as defined in the Directive (EEC, 1986) do represent realistic values which safeguard the health of plants, animals and man. However, there is a case for implementing more stringent limits on the maximum permissible Cd concentrations in sludged soil which as they stand at present, may lead to zootoxic Cd concentrations in plants. Equally, some consideration may also be given to the potentially significant ingestion of surface soil by grazing animals and the consequent need for control on metal concentrations in the surface soil.

Urgent revision of the limit values for Cu, Pb and Cd concentrations in sludge for use in agriculture (EEC, 1986) or alternatively of the three-week minimum no-grazing period is called for, since at present, these pose a serious risk of metal toxicity in grazing animals arising from the ingestion of sludge adhering to leaf surfaces. Some consideration needs also to be given to the potentially toxic effects of metals on the soil microbial population in sludge soils even when these are within the maximum permissible metal concentrations as set in the Directive. Ultimately, soil metal contamination is relatively irreversible , it is thus better to err on the safe side.

APPENDIX A

DETERMINATION OF METAL CONTAMINATION OF SOIL SAMPLES FOLLOWING SOIL MILLING.

Air-dried soil was milled using an electric mill based on that designed at the Rukuhia station (N.Z.D.S.I.R.) mill (Bascomb and Bullock, 1974).

A possible source of metal contamination of the soil samples, could be the mild steel drums used for milling. To investigate this possibility, 'Analar' grade aluminium oxide (see Table A.1) was milled through each of the three milling drums used. The milled sample were then analysed for total metal content (see Table A.1).

The procedure followed to measure the total Cd, Cr, Cu, Ni, Pb and Zn content of the Al_2O_3 is that previously described for the determination of total soil heavy metal concentration (see Section 2.2.1.13.).

The results clearly indicate that sample comtamination is negligible, with the possible exceptions of Cu and Zn. However, the scale of Zn and Cu contamination observed was very small when compared to soil metal concentrations and amounted to only 0.4 mg Cu/kg and 0.65 mg Zn/kg. Consequently this contamination of soil samples, was not considered when measuring total metals in soil.

TABLE A.1 TOTAL METAL CONCENTRATIONS (mg/kg) (± S.D.) IN ANALAR GRADE ALUMINIUM OXIDE (Al_2O_3) AND IN THE SAME OXIDE ONCE MILLED THROUGH THREE INDIVIDUAL DRUMS.

METAL	ANALAR Al_2O_3	DRUM 1	DRUM 2	DRUM 3
Cd	N.D.	N.D.	N.D.	N.D.
Cr	N.D.	N.D.	N.D.	N.D.
Cu	0.70 (0.10)	1.00 (0.00)	1.07 (0.12)	1.23 (0.40)
Ni	N.D.	N.D.	N.D.	N.D.
Pb	N.D.	N.D.	N.D.	N.D.
Zn	1.80 (0.17)	2.73 (0.55)	2.23 (0.59)	2.40 (0.27)

N.D. :- not detectable.

APPENDIX B

COMPOSITION OF LIQUID FEEDS

STANDARD FEED

SOLUTION A

	g/l
$Ca(NO_3)_2.4H_2O$	90.9

SOLUTION B

K_2SO_4	55.4
KH_2PO_4	17.7
$NH_4H_2PO_4$	9.9
$MgSO_4.7H_2O$	46.2
FeNaEDTA	3.27
$MnSO_4.4H_2O$	0.82
H_3BO_3	0.172
$(NH_4)Mo_7O_2.4H_2O_4$	0.005

Equal volumes of solutions A and B are mixed and diluted 1 : 100 to give a cF value of 20.

HIGH N SOLUTION

SOLUTION A

	g/1
$Ca(NO_3)_2.4H_2O$	59.0

SOLUTION B

KNO_3	79.0
$NH_4H_2PO_4$	12.0
NH_4NO_3	11.0
$MgSO_4.7H_2O$	25.0
FeNaEDTA	1.75
$MnSO_4.4H_2O$	0.40
H_3BO_3	0.17
$(NH_4)Mo_7O_2.4H_2O_4$	0.005
$CuSO_4.5H_2O$	0.04
$ZnSO_4.7H_2O$	0.04

Equal volumes of solutions A and B are mixed and diluted 1 : 100 to give a cF value of 20. This diluted solution will contain approximately 225 mg/1 N.

LOW N SOLUTION

SOLUTION A

	g/1
$Ca(NO_3)_2.4H_2O$	59.0

SOLUTION B

K_2SO_4	46.0
$NH_4H_2PO_4$	14.0
KNO_3	24.0
$MgSO_4.7H_2O$	25.0
FeNaEDTA	1.75
$MnSO_4.4H_2O$	0.40
H_3BO_3	0.17
$(NH_4)_6Mo_7O_2.4H_2O_4$	0.005
$CuSO_4.5H_2O$	0.04
$ZnSO_4.7H_2O$	0.04

Equal volumes of solutions A and B are mixed and diluted 1 : 100 to give a cF value of 20. This diluted solution will contain approximately 120 mg/1 N.

APPENDIX C

CARBARNS FIELD EXPERIMENT.

MEAN SQUARES (MS) AND VARIANCE RATIOS (F) FROM ANALYSIS OF VARIANCE OF:

i) TOTAL METAL CONCENTRATIONS FROM FIRST AND SECOND YEAR SAMPLES.

ii) TOTAL, ACETIC ACID- AND EDTA- EXTRACTABLE METAL CONCENTRATIONS, PERCENTAGE ACETIC ACID- AND EDTA- EXTRACTABLE METALS AND COPPER BOUND TO ORGANIC MATTER (O.M. Cu) FOR SECOND YEAR SOIL SAMPLES.

iii) TOTAL, ACETIC ACID- AND EDTA- EXTRACTABLE METAL CONCENTRATIONS FROM SECOND YEAR SOIL SAMPLES AND UNSLUDGED SOIL (UNSL.).

TABLE C (i)

SOURCE OF DIFFERENCE	df	TOTAL Cd		TOTAL Cr		TOTAL Cu		TOTAL Ni		TOTAL Pb		TOTAL Zn	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
LIME	1	0.137	0.46	698.83	0.25	4.07	0.02	3.95	0.06	3913.7	0.55	2360.6	0.63
CULT.	1	0.210	0.71	888.37	0.32	133.15	0.57	53.36	0.84	2893.8	0.41	576.9	0.16
NITRO.	2	0.028	0.09	119.85	0.04	172.63	0.74	17.67	0.28	74.3	0.01	983.1	0.26
LIME.CULT.	1	0.376	1.27	279.41	0.10	1068.66	4.60	303.86	4.76	5157.9	0.72	3163.1	0.85
LIME.NITRO.	2	0.528	1.79	795.11	0.29	13.71	0.06	46.08	0.72	3208.0	0.45	936.9	0.25
CULT.NITRO.	2	0.178	0.60	1636.73	0.59	193.90	0.83	131.10	2.05	2899.6	0.41	731.5	0.20
LIME.CULT.NITRO.	2	0.398	1.35	503.98	0.18	119.17	0.51	79.28	1.24	909.9	0.13	1642.8	0.44
RESIDUAL	33	0.295	7.34	2765.49	31.01	232.43	5.68	63.89	5.98	7136.2	7.49	3728.3	5.46
DEPTH	6	0.071	1.77	620.55	6.96	107.45	2.62	15.02	1.41	1106.1	1.16	583.6	0.86
LIME.DEPTH	6	0.031	0.77	98.14	1.10	33.47	0.82	10.18	0.95	1551.5	1.63	234.1	0.34
CULT.DEPTH	6	0.042	1.04	73.84	0.83	211.87	5.17	15.19	1.42	1183.2	1.24	1774.2	2.60
NITRO.DEPTH	12	0.051	1.26	114.34	1.28	36.01	0.88	9.97	0.93	2049.3	2.15	1044.5	1.53
LIME.CULT.DEPTH	6	0.056	1.39	99.50	1.12	28.60	0.70	12.67	1.19	2814.0	2.95	252.0	0.37
LIME.NITRO.DEPTH	12	0.080	2.00	23.76	0.27	51.99	1.27	6.31	0.59	1244.5	1.31	567.8	0.83
CULT.NITRO.DEPTH	12	0.043	1.07	95.90	1.08	32.30	0.79	10.01	0.94	740.7	0.78	835.8	1.23
LIME.CULT.NITRO.DEPTH	12	0.050	1.23	32.86	0.37	45.95	1.12	6.99	0.66	412.7	0.43	528.8	0.78
RESIDUAL	216	0.040		89.18		40.96		10.68		953.4		682.5	

SOURCE OF DIFFERENCE	df	ACETIC ACID Cd		ACETIC ACID Cu		ACETIC ACID Ni		ACETIC ACID Pb		ACETIC ACID Zn	
		MS	F	MS	F	MS	F	MS	F	MS	F
LIME	1	0.244	1.63	0.067	0.02	5.008	1.33	1.850	0.11	467.61	1.27
CULT.	1	0.221	1.48	0.172	0.04	3.284	0.87	8.477	0.52	2.62	0.01
NITRO.	2	0.199	1.33	22.132	5.19	4.772	1.26	83.902	5.10	142.34	0.39
LIME.CULT.	1	0.035	0.23	8.285	1.94	16.965	4.50	5.178	0.32	1236.56	3.36
LIME.NITRO.	2	0.093	0.62	12.672	2.97	12.780	3.39	13.166	0.80	806.70	2.19
CULT. NITRO.	2	0.025	0.17	8.970	2.10	1.012	0.27	26.121	1.59	516.18	1.40
LIME.CULT.NITRO.	2	0.190	1.27	13.049	3.06	1.817	0.48	60.874	3.70	140.19	0.38
RESIDUAL	33	0.150	5.63	4.267	3.95	3.774	5.30	16.444	6.88	368.32	6.38
DEPTH	6	0.115	4.33	11.805	10.93	14.792	20.78	30.321	12.69	399.02	6.91
LIME.DEPTH	6	0.032	1.20	3.730	3.45	0.973	1.37	1.986	0.83	67.63	1.17
CULT.DEPTH	6	0.014	0.52	4.885	4.52	0.572	0.80	7.514	3.15	247.65	4.29
NITRO.DEPTH	12	0.031	1.18	0.982	0.91	0.990	1.39	2.468	1.03	27.88	0.48
LIME.CULT.DEPTH	6	0.016	0.61	0.731	0.68	0.943	1.33	0.712	0.30	60.27	1.04
LIME.NITRO.DEPTH	12	0.008	0.29	1.386	1.28	1.339	1.88	2.069	0.87	58.25	1.01
CULT.NITRO.DEPTH	12	0.017	0.65	1.398	1.29	0.676	0.95	1.003	0.42	64.39	1.12
LIME.CULT.NITRO.DEPTH	12	0.034	1.26	1.481	1.37	0.433	0.61	5.014	2.10	17.76	0.31
RESIDUAL	216	0.027		1.080		0.712		2.389		57.71	

TABLE C (i) (Contd)

SOURCE OF DIFFERENCE	df	EDTA Cu MS	EDTA Cu F	EDTA Zn MS	EDTA Zn F
LIME	1	7.73	0.09	224.00	0.82
CULT.	1	291.41	3.58	20.98	0.08
NITRO.	2	78.97	0.97	65.62	0.24
LIME.CULT.	1	343.87	4.23	1151.46	4.20
LIME.NITRO.	2	39.54	0.49	213.10	0.78
CULT.NITRO.	2	1.29	0.02	99.26	0.36
LIME.CULT.NITRO.	2	13.44	0.17	209.05	0.76
RESIDUAL	33	81.39	11.09	274.33	5.65
DEPTH	6	31.74	4.32	303.67	6.25
LIME.DEPTH	6	2.63	0.36	122.69	2.52
CULT.DEPTH	6	49.47	6.74	421.58	8.68
NITRTO .DEPTH	12	8.22	1.12	18.46	0.38
LIME.CULT.DEPTH	6	7.83	1.07	49.31	1.02
LIME.NITRO.DEPTH	12	6.43	0.88	57.44	1.18
CULT.NITRO.DEPTH	12	2.12	0.29	47.43	0.98
LIME.CULT.NTRO.DEPTH	12	7.31	1.00	16.54	0.34
RESIDUAL	216	7.34		48.60	

TABLE C (ii)

SOURCE OF		TOTAL		TOTAL		TOTAL		TOTAL		TOTAL		TOTAL	
		Cd		Cr		Cu		Ni		Pb		Zn	
DIFFERENCE	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
CULT.	1	0.355	1.13	2056.88	4.48	1556.82	1.29	65.18	0.91	23020.8	1.01	14289.3	0.59
LIME	1	0.437	1.39	795.51	1.73	860.11	0.72	97.34	1.35	5079.3	0.22	18018.0	0.74
NITRO.	2	0.710	2.25	835.32	1.82	3057.17	2.54	263.23	3.66	55608.8	2.45	52047.4	2.15
LIME.CULT.	1	0.284	0.90	93.95	0.21	102.02	0.09	20.71	0.29	6698.7	0.29	2440.3	0.10
CULT.NITRO.	2	0.027	0.09	48.47	0.11	994.14	0.83	58.58	0.81	18932.2	0.83	23902.6	0.99
LIME.NITRO.	2	0.228	0.73	498.77	1.09	3697.60	3.07	241.52	3.36	49961.4	2.20	69693.3	2.87
LIME.CULT.NITRO.	2	0.236	0.75	926.78	2.02	790.66	0.66	28.11	0.39	10009.0	0.44	13244.7	0.55
RESIDUAL	33	0.315		459.22		1202.69		71.99		22692.9		24256.5	
DEPTH	6	2.750	91.12	1334.06	34.89	5191.99	138.18	491.50	115.29	52133.2	93.39	89287.7	144.24
DEPTH.CULT.	6	0.207	6.87	500.87	13.10	577.02	15.36	37.01	8.68	4050.7	7.26	4588.6	7.41
RESIDUAL	276	0.030		38.23		37.57		4.26		558.2		619.0	

SOURCE OF		ACETIC ACID		ACETIC ACID		ACETIC ACID		ACETIC ACID		ACETIC ACID	
		Cd		Cu		Ni		Pb		Zn	
DIFFERENCE	df	MS	F	MS	F	MS	F	MS	F	MS	F
CULT.	1	0.301	1.71	0.03	0.00	0.00	0.00	25.99	0.54	499.5	0.21
LIME	1	0.109	0.62	2.74	0.15	2.82	0.56	0.78	0.02	871.7	0.36
NITRO.	2	0.552	3.14	31.50	1.76	11.04	2.19	150.49	3.11	3456.1	1.43
LIME.CULT.	1	0.082	0.47	0.15	0.01	5.75	1.14	0.10	0.00	360.8	0.15
CULT.NITRO.	2	0.051	0.29	40.73	2.28	8.12	1.61	66.04	1.37	3023.5	1.25
LIME.NITRO.	2	0.072	0.41	79.42	4.40	24.55	4.87	185.56	3.84	6266.2	2.69
LIME.CULT.NITRO.	2	0.095	0.54	6.41	0.36	4.08	0.81	34.65	0.72	1498.1	0.62
RESIDUAL	33	0.176		17.89		5.04		48.34		2410.6	
DEPTH	6	1.303	58.64	53.23	45.84	109.81	144.45	147.71	70.77	10848.8	156.88
DEPTH.CULT.	6	0.178	8.01	2.52	2.17	13.63	17.93	13.70	6.56	1275.3	18.44
RESIDUAL	276	0.222		1.16		0.76		2.09		69.2	

TABLE C (ii) (Contd)

SOURCE OF DIFFERENCE	df	EDTA Cu MS	EDTA Cu F	EDTA Zn MS	EDTA Zn F	%EDTA Cu MS	%EDTA Cu F	%EDTA Zn MS	%EDTA Zn F	O.M. Cu MS	O.M. Cu F
CULT.	1	634.0	2.13	1060.0	0.55	67.10	1.01	0.00	0.00	642.4	3.21
LIME	1	196.3	0.66	261.4	0.13	0.08	0.00	92.46	2.29	245.5	1.23
NITRO.	2	867.7	2.92	2944.3	1.51	45.63	0.69	43.91	1.09	591.8	2.96
LIME.CULT.	1	46.9	0.16	388.1	0.20	12.89	0.20	5.81	0.14	41.8	0.21
CULT.NITRO.	2	294.7	0.99	2085.0	1.07	78.54	1.19	53.37	1.32	126.6	0.63
LIME.NITRO.	2	599.2	2.02	2847.6	1.46	34.00	0.51	7.08	0.18	292.8	1.46
LIME.CULT.NITRO.	2	160.2	0.54	1891.2	0.97	42.54	0.64	77.83	1.93	114.3	0.57
RESIDUAL	33	297.0		1945.1		66.18		40.31		200.2	
DEPTH	6	1736.0	191.30	11061.4	170.70	124.03	9.86	473.30	58.35	1321.0	190.34
DEPTH CULT.	6	185.0	20.38	1966.9	30.35	14.37	1.14	162.46	20.03	207.6	29.92
RESIDUAL	276	9.1		64.8		12.58		8.11		6.9	

% ACETIC ACID

SOURCE OF DIFFERENCE	df	Cd MS	Cd F	Cu MS	Cu F	Ni MS	Ni F	Pb MS	Pb F	Zn MS	Zn F
CULT.	1	253.2	0.27	34.13	3.06	79.69	1.57	3.38	0.58	25.78	0.69
LIME	1	2475.1	2.67	24.19	2.16	5.37	0.11	0.54	0.09	36.24	0.97
NITRO.	2	956.3	1.03	9.04	0.81	18.62	0.37	1.95	0.34	16.12	0.43
LIME.CULT.	1	1542.5	1.66	0.00	0.00	19.81	0.39	8.90	1.53	5.25	0.14
CULT.NITRO.	2	317.3	0.34	21.82	1.96	22.94	0.45	2.91	0.50	34.84	0.93
LIME.NITRO.	2	287.8	0.31	40.58	3.64	91.35	1.80	3.66	0.63	27.26	0.73
LIME.CULT.NITRO.	2	624.4	0.67	3.02	0.27	12.29	0.24	16.84	2.89	7.50	0.20
RESIDUAL	33	928.2		11.14		50.84		5.83		37.48	
DEPTH	6	2326.1	12.08	54.00	25.32	539.92	60.78	2.72	3.10	246.45	24.08
DEPTH.CULT.	6	429.8	2.23	24.73.	11.60	87.01	9.80	1.16	1.33	67.03	6.55
RESIDUAL	276	192.6		2.13		8.88		0.88		10.23	

TABLE C (iii)

SOURCE OF DIFFERENCE	df	TOTAL Cd MS	TOTAL Cd F	TOTAL Cr MS	TOTAL Cr F	TOTAL Cu MS	TOTAL Cu F	TOTAL Ni MS	TOTAL Ni F	TOTAL Pb MS	TOTAL Pb F	TOTAL Zn MS	TOTAL Zn F
UNSL.	1	3.829		675.11		25884.83		648.79		27510.0		144586.2	
UNSL..BLOCK	3	2.367		3586.73		5078.48		496.73		129326.4		177297.9	
TOTAL	4	2.733	95.55	2858.82	74.99	10280.07	278.79	534.74	128.36	103872.3	178.97	169120.0	280.96
LIME	1	0.437	1.52	795.51	1.73	860.11	0.79	97.34	1.46	5079.3	0.24	18018.0	0.80
CULT.	1	0.355	1.24	2056.89	4.48	1556.82	1.42	65.18	0.98	23020.8	1.07	14289.3	0.64
NITRO.	2	0.709	2.47	835.32	1.82	3057.17	2.79	263.23	3.94	55608.8	2.57	52047.4	2.31
LIME.CULT	1	0.284	0.99	93.95	0.21	102.02	0.09	20.71	0.31	6698.7	0.31	2440.3	0.11
LIME.NITRO.	2	0.228	0.80	498.77	1.09	3697.60	3.37	241.52	3.61	49961.4	2.31	69693.3	3.10
CULT.NITRO.	2	0.027	0.10	48.47	0.11	994.14	0.91	58.58	0.88	18932.2	0.88	23902.6	1.06
LIME.CULT.NITRO.	2	0.236	0.82	926.78	2.02	790.66	0.72	28.11	0.42	10009.0	0.46	13244.7	0.59
RESIDUAL	37	0.287	10.03	458.82	12.04	1095.91	29.72	66.84	16.04	21611.2	37.24	22512.5	37.40
TOTAL	48	0.294	10.27	511.28	13.41	1253.06	33.98	79.98	19.20	22988.2	39.61	24697.6	41.03
DEPTH	12	1.378	48.17	746.56	19.58	2601.27	70.55	254.00	60.97	26220.8	45.18	44826.1	74.47
LIME.DEPTH	6	0.021	0.74	10.87	0.29	39.75	1.08	3.44	0.82	250.4	0.43	482.7	0.80
CULT.DEPTH	6	0.207	7.25	500.87	13.14	577.02	15.65	37.01	8.88	4050.7	6.98	4588.6	7.62
NITRO.DEPTH	12	0.033	0.14	61.31	1.61	38.73	1.05	6.33	1.52	1166.0	2.01	699.3	1.16
LIME.CULT.DEPTH	6	0.028	0.98	9.63	0.25	41.39	1.12	5.18	1.24	917.5	1.58	757.0	1.26
LIME.NITRO.DEPTH	12	0.036	1.26	28.67	0.75	14.30	0.39	2.81	0.67	199.7	0.34	277.8	0.46
CULT.NITRO.DEPTH	12	0.030	1.05	33.44	0.88	17.24	0.47	3.45	0.83	503.2	0.87	676.1	1.12
LIME.CULT.NITRO.DEPTH	12	0.034	1.19	20.41	0.54	27.93	0.76	2.11	0.51	278.6	0.48	178.3	0.30
RESIDUAL	240	0.029		38.12		36.87		4.17		580.4		601.9	
TOTAL	318	0.083		72.21		142.11		14.14		1607.0		2324.9	

TABLE C (iii) (Contd)

SOURCE OF DIFFERENCE	df	ACETIC ACID Cd MS	ACETIC ACID Cd F	ACETIC ACID Cu MS	ACETIC ACID Cu F	ACETIC ACID Ni MS	ACETIC ACID Ni F	ACETIC ACID Pb MS	ACETIC ACID Pb F	ACETIC ACID Zn MS	ACETIC ACID Zn F
UNSL.	1	1.623		1150.15		302.67		605.02		11180.59	
UNSL..BLOCK	3	0.395		217.46		41.19		297.74		16959.26	
TOTAL	4	0.702	30.29	450.63	444.68	106.56	165.95	374.56	183.71	16514.59	234.83
LIME	1	0.109	0.69	2.74	0.17	2.82	0.62	0.78	0.02	871.65	0.37
CULT.	1	0.301	1.89	0.03	0.01	0.00	0.00	25.99	0.56	499.47	0.21
NITRO.	2	0.552	3.47	31.50	1.97	11.04	2.41	150.49	3.27	3456.14	1.47
LIME.CULT	1	0.082	0.52	0.15	0.01	5.75	1.26	0.10	0.00	360.80	0.15
LIME.NITRO.	2	0.072	0.45	79.42	4.96	24.55	5.36	185.56	4.03	6266.21	2.67
CULT.NITRO.	2	0.051	0.32	40.73	2.54	8.12	1.77	66.04	1.43	3023.49	1.29
LIME.CULT.NITRO.	2	0.095	0.60	6.41	0.40	4.08	0.89	34.65	0.75	1498.14	0.64
RESIDUAL	37	0.159	6.87	16.02	15.80	4.58	7.13	46.08	22.60	2350.32	35.57
TOTAL	48	0.165	7.12	18.99	18.74	5.70	8.88	54.28	26.62	2441.29	36.95
DEPTH	12	0.672	29.00	26.74	26.39	55.10	85.81	74.27	36.42	5488.03	83.07
LIME.DEPTH	6	0.042	1.81	3.58	3.53	0.23	0.36	2.54	1.25	39.28	0.59
CULT.DEPTH	6	0.178	7.68	2.52	2.48	13.63	21.22	13.70	6.72	1275.27	19.30
NITRO.DEPTH	12	0.033	1.43	1.04	1.02	1.74	2.71	2.25	1.10	62.97	0.95
LIME.CULT.DEPTH	6	0.013	0.58	0.33	0.33	0.18	0.29	0.62	0.31	64.56	0.98
LIME.NITRO.DEPTH	12	0.007	0.29	1.34	1.32	1.36	2.11	2.00	0.98	85.58	1.30
CULT.NITRO.DEPTH	12	0.016	0.68	1.82	1.80	1.03	1.60	1.46	0.71	97.42	1.48
LIME.CULT.NITRO.DEPTH	12	0.051	2.19	0.41	0.40	0.57	0.88	2.99	1.47	23.15	0.35
RESIDUAL	240	0.023		1.01		0.64		2.04		66.07	
TOTAL	318	0.051		2.07		3.01		4.99		293.14	

TABLE C (iii) (Contd)

SOURCE OF DIFFERENCE	df	EDTA Cd MS	EDTA Cd F	EDTA Zn MS	EDTA Zn F
UNSL.	1	11127.67		4805.44	
UNSL..BLOCK	3	1599.04		12171.10	
TOTAL	4	3981.20	462.30	10329.69	161.46
LIME	1	196.33	0.72	261.42	0.13
CULT.	1	633.99	2.32	1060.47	0.54
NITRO.	2	867.69	3.17	2944.34	1.49
LIME.CULT	1	46.91	0.17	388.13	0.20
LIME.NITRO.	2	599.17	2.19	2847.63	1.44
CULT.NITRO.	2	294.74	1.08	2085.02	1.06
LIME.CULT.NITRO.	2	160.24	0.59	1891.15	0.96
RESIDUAL	37	273.59	31.77	1973.71	30.85
TOTAL	48	309.24	35.91	1964.04	30.70
DEPTH	12	868.56	100.86	5628.01	87.97
LIME.DEPTH	6	12.20	1.42	54.39	0.85
CULT.DEPTH	6	184.95	21.48	1966.93	30.75
NITRO.DEPTH	12	6.97	0.81	67.97	1.06
LIME.CULT.DEPTH	6	11.09	1.29	86.48	1.35
LIME.NITRO.DEPTH	12	9.38	1.09	65.63	1.03
CULT.NITRO.DEPTH	12	4.10	0.48	68.59	1.07
LIME.CULT.NITRO.DEPTH	12	8.75	1.02	30.72	0.48
RESIDUAL	240	8.61		63.98	
TOTAL	318	44.31		309.22	

APPENDIX D

MEAN SQUARES (MS) AND VARIANCE RATIOS (F) FROM ANALYSIS OF VARIANCE OF HERBAGE METAL CONCENTRATIONS AND CULTIVATION, LIME AND NITROGEN APPLICATION (CARBARNS FIELD EXPERIMENT).

Cd

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	0.002	0.09	0.001	0.24	0.000	0.10	0.003	1.04	0.005	1.38
LIME	1	0.025	1.37	0.006	1.90	0.002	0.43	0.001	0.52	0.001	0.21
NITRO	2	0.007	0.40	0.007	2.40	0.142	35.44	0.010	4.23	0.035	9.45
LIME.CULT	1	0.005	0.28	0.001	0.31	0.009	2.37	0.003	1.36	0.004	0.99
CULT.NITRO.	2	0.033	1.77	0.002	0.57	0.008	2.02	0.000	0.16	0.005	1.34
LIME.NITRO.	2	0.012	0.68	0.003	1.00	0.000	0.02	0.006	2.32	0.005	1.23
LIME.CULT.NITRO.	2	0.041	2.24	0.000	0.14	0.005	1.20	0.011	4.50	0.002	0.54
RESIDUAL	33	0.018		0.003		0.004		0.002		0.004	

Cr

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	0.081	1.84	0.003	0.02	0.013	0.23	0.121	1.06	0.796	1.30
LIME	1	0.158	3.56	0.486	2.96	0.003	0.06	0.123	1.07	0.000	0.00
NITRO	2	0.027	0.61	0.190	1.16	0.702	12.85	0.148	1.30	0.158	0.26
LIME.CULT	1	0.000	0.00	0.000	0.00	0.014	0.25	0.002	0.02	0.051	0.08
CULT.NITRO.	2	0.056	1.27	0.092	0.56	0.050	0.91	0.090	0.78	0.549	0.90
LIME.NITRO.	2	0.155	3.50	0.180	1.10	0.152	2.78	0.430	3.76	0.245	0.40
LIME.CULT.NITRO.	2	0.063	1.43	0.255	1.55	0.153	2.80	0.040	0.35	1.147	1.87
RESIDUAL	33	0.044		0.164		0.055		0.114		0.613	

APPENDIX D (Contd)

Cu

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	34.754	42.82	4.889	8.89	1.577	2.32	0.002	0.00	0.001	0.00
LIME	1	1.690	2.08	12.729	23.14	6.453	9.13	1.012	1.94	5.728	5.42
NITRO	2	2.506	3.09	65.742	119.52	230.872	326.72	50.503	96.70	132.835	125.75
LIME.CULT	1	1.584	1.95	0.637	1.16	1.313	1.86	0.069	0.13	1.086	1.03
CULT.NITRO.	2	0.341	0.42	0.190	0.35	1.507	2.13	0.309	0.59	2.140	2.03
LIME.NITRO.	2	1.032	1.27	2.609	4.74	1.405	1.99	0.089	0.17	0.623	0.59
LIME.CULT.NITRO.	2	0.474	0.58	0.182	0.33	0.188	0.27	0.070	0.14	0.233	0.22
RESIDUAL	33	0.812		0.550		0.707		0.522		1.056	

Ni

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	30.500	19.64	11.219	7.40	5.915	5.34	4.735	3.90	4.548	3.93
LIME	1	2.767	1.78	34.967	23.06	36.010	32.49	28.714	23.68	24.941	21.57
NITRO	2	0.882	0.57	1.840	1.21	4.777	4.31	6.597	5.44	2.739	2.37
LIME.CULT	1	0.055	0.04	0.191	0.13	1.103	0.99	0.293	0.24	0.252	0.37
CULT.NITRO.	2	1.195	0.77	0.185	0.12	0.307	0.28	0.589	0.49	0.054	0.22
LIME.NITRO.	2	0.569	0.37	1.425	0.94	0.325	0.29	0.375	0.31	0.436	0.38
LIME.CULT.NITRO.	2	0.073	0.05	0.640	0.42	0.170	0.15	0.457	0.38	0.043	0.04
RESIDUAL	33	1.553		1.516		1.109		1.213		1.156	

APPENDIX D (Contd)

Pb

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	0.001	0.05	0.010	0.11	0.018	0.45	0.018	0.23	0.157	1.65
LIME	1	0.000	0.00	0.515	7.49	0.073	1.79	0.043	0.57	0.324	3.40
NITRO	2	0.019	1.02	0.098	1.13	1.105	27.01	0.391	5.08	0.340	3.57
LIME.CULT	1	0.000	0.00	0.044	0.50	0.002	0.04	0.045	0.59	0.090	0.95
CULT.NITRO.	2	0.015	0.80	0.111	1.28	0.058	1.41	0.276	3.59	0.061	0.64
LIME.NITRO.	2	0.031	1.69	0.006	0.06	0.024	0.59	0.028	0.37	0.050	0.53
LIME.CULT.NITRO.	2	0.015	0.78	0.020	0.23	0.005	0.11	0.362	4.70	0.055	0.58
RESIDUAL	33	0.019		0.087		0.041		0.077		0.095	

Zn

SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
CULT.	1	1121.4	7.84	466.8	5.16	463.4	3.69	307.5	2.23	1011.2	5.84
LIME	1	708.2	4.95	1972.4	21.80	2058.7	16.39	1289.1	9.35	3882.4	22.41
NITRO	2	111.9	0.78	2848.1	31.48	7074.1	56.32	5377.7	38.99	4725.9	27.27
LIME.CULT	1	105.0	0.73	8.3	0.09	167.7	1.34	28.7	0.21	75.8	0.44
CULT.NITRO.	2	432.8	3.03	90.3	1.00	267.7	2.13	397.0	2.88	38.3	0.22
LIME.NITRO.	2	87.3	0.61	291.5	3.32	346.4	2.76	90.0	0.65	158.6	0.92
LIME.CULT.NITRO.	2	32.9	0.23	56.1	0.62	31.3	0.25	34.3	0.25	70.6	0.41
RESIDUAL	33	143.1		90.5		125.6		137.9		173.3	

APPENDIX E

MEAN SQUARES (MS) AND VARIANCE (F) FROM ANALYSIS OF VARIANCE OF SOIL pH, OVER A DEPTH OF 100mm, AND CULTIVATION, LIME AND NITROGEN APPLICATION (CARBARNS FIELD EXPERIMENT).

SOURCE OF DIFFERENCE	MS	F
LIME	14.493	51.14
CULT	0.017	0.06
NITROGEN	0.936	3.30
LIME.CULT	0.094	0.33
LIME.NITROGEN	0.068	0.24
CULT.NITROGEN	0.424	1.50
LIME.CULT.NITROGEN	0.066	0.23
RESIDUAL	0.283	3.89
DEPTH	1.401	19.21
LIME.DEPTH	1.085	14.89
CULT.DEPTH	0.291	3.99
NITROGEN.DEPTH	0.468	6.42
LIME.CULT.DEPTH	0.026	0.36
LIME.NITROGEN.DEPTH	0.091	1.25
CULT.NITROGEN.DEPTH	0.048	0.65
RESIDUAL	0.071	

APPENDIX F

BEST-FIT REGRESSION EQUATIONS FOR HERBAGE Cd, Cr, Cu, Ni, Pb AND Zn CONCENTRATIONS IN ALL FIVE CUTS (CARBARNS FIELD EXPERIMENT).

CADMIUM

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	s_Y	R^2(adj.)
CUT 1	CONSTANT	-0.663	0.369	-1.80	0.160	19.4%
	O.M.	0.019	0.009	2.15		
	pH	0.185	0.064	2.90		
	LIME	-0.163	0.058	-2.80		
CUT 2	CONSTANT	-0.230	0.141	-1.63	0.060	35.3%
	CD-TOT	0.093	0.031	3.02		
	pH	0.092	0.024	3.77		
	LIME	-0.078	0.022	-3.56		
	NITRO.	0.026	0.011	2.43		
CUT 3	CONSTANT	0.677	0.134	5.06	0.071	55.3%
	pH	-0.053	0.023	-2.30		
	NITRO.	0.088	0.013	6.93		
CUT 4	CONSTANT	0.528	0.105	5.02	0.056	10.3%
	pH	-0.032	0.018	-1.78		
	NITRO.	-0.023	0.010	-2.33		
CUT 5	CONSTANT	0.674	0.130	5.17	0.069	25.0%
	pH	-0.044	0.022	-1.99		
	NITRO.	0.041	0.012	3.32		

CHROMIUM

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	s_y	R^2(adj.)
CUT 1	CONSTANT	0.005	0.339	0.01	0.216	11.8%
	Cr-TOT	0.006	0.003	2.04		
	LIME	0.098	0.063	1.56		
	CULT.	0.139	0.068	2.04		
CUT 2	CONSTANT	1.260	0.084	15.06	0.410	3.9%
	LIME	-0.201	0.118	-1.70		
CUT 3	CONSTANT	0.995	0.059	16.91	0.258	28.5%
	NITRO.	0.203	0.046	4.45		
CUT 4	CONSTANT	3.102	0.861	3.60	0.394	9.6%
	pH	-0.392	0.157	-2.49		
	LIME	0.317	0.143	2.22		
CUT 5	CONSTANT	2.425	0.663	3.66	0.817	1.19%
	O.M.	-0.054	0.044	-1.23		

COPPER

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	s_y	R^2(adj.)
CUT 1	CONSTANT	4.929	0.974	5.06	1.356	45.3%
	Cu-%Ac	0.454	0.097	4.69		
	CULT.	1.045	0.416	2.51		
CUT 2	CONSTANT	16.040	1.861	8.62	0.916	80.1%
	Cu-TOT	0.022	0.008	2.66		
	pH	-1.662	0.296	-5.61		
	NITRO.	1.752	0.168	10.45		
CUT 3	CONSTANT	11.758	1.837	6.40	0.972	91.3%
	pH	-1.151	0.314	-3.67		
	NITRO.	3.660	0.174	21.00		
CUT 4	CONSTANT	7.170	2.065	3.47	0.819	77.2%
	Cu-%Ac	0.145	0.061	2.38		
	pH	-0.772	0.293	-2.64		
	NITRO	1.641	0.147	11.14		
CUT 5	CONSTANT	14.240	2.006	7.10	1.062	83.5%
	pH	-1.155	0.343	-3.37		
	NITRO	2.725	0.190	14.32		

APPENDIX F (Contd)

NICKEL

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	S_y	R^2(adj.)
CUT 1	CONSTANT	27.058	3.363	8.05	1.439	56.0%
	Ni-TOT	-0.135	0.049	-2.76		
	pH	-3.515	0.578	-6.09		
	LIME	1.605	0.523	3.07		
	CULT.	1.244	0.430	2.85		
CUT 2	CONSTANT	26.066	2.681	9.72	1.394	58.4%
	O.M.	-0.286	0.076	-3.76		
	pH	-3.074	0.446	-6.89		
CUT 3	CONSTANT	27.561	2.276	12.11	1.184	68.8%
	O.M.	-0.221	0.065	-3.41		
	pH	-3.508	0.379	-9.26		
CUT 4	CONSTANT	28.558	2.470	11.56	1.285	66.7%
	O.M.	-0.213	0.070	-3.03		
	pH	-3.675	0.411	-8.94		
CUT 5	CONSTANT	24.173	2.090	11.57	1.087	67.3%
	O.M.	-0.177	0.059	-2.98		
	pH	-3.160	0.348	-9.08		

LEAD

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	S_y	R^2(adj.)
CUT 1	CONSTANT	1.361	0.046	29.55	0.126	13.8%
	PB-TOT	0.001	0.001	2.92		
CUT 2	CONSTANT	1.577	0.113	13.96	0.263	26.2%
	Pb-Ac	0.032	0.012	2.73		
	LIME	-0.226	0.076	-2.98		
	NITRO.	-0.104	0.047	-2.19		
CUT 3	CONSTANT	1.359	0.047	28.64	0.208	51.6%
	NITRO.	0.263	0.037	7.15		
CUT 4	CONSTANT	1.377	0.117	11.82	0.306	13.2%
	Pb-TOT	-0.001	0.001	-1.84		
	NITRO.	-0.107	0.055	-1.94		
CUT 5	CONSTANT	1.142	0.124	9.19	0.308	21.3%
	Pb-TOT	0.002	0.001	2.47		
	LIME	-0.172	0.089	-1.94		
	NITRO.	0.106	0.056	1.89		

ZINC

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	S_y	R^2(adj.)
CUT 1	CONSTANT	190.800	23.860	8.00	13.000	40.3%
	pH	-21.519	4.136	-5.20		
	CULT.	9.261	3.753	2.47		
CUT 2	CONSTANT	195.920	17.700	11.07	8.968	78.1%
	Zn-Ac	0.392	0.087	4.53		
	O.M.	-2.451	0.708	-3.46		
	pH	-23.621	2.956	-7.99		
	NITRO.	10.299	1.625	6.34		
CUT 3	CONSTANT	177.390	22.430	7.91	11.870	73.8%
	pH	-22.642	3.830	-5.91		
	NITRO.	18.806	2.128	8.84		
CUT 4	CONSTANT	201.440	21.770	9.25	11.520	73.3%
	pH	-27.357	3.717	-7.36		
	NITRO.	15.302	2.066	7.41		
CUT 5	CONSTANT	262.530	24.700	10.63	12.520	75.2%
	Zn-Ac	0.509	0.121	4.21		
	O.M.	-3.316	0.988	-3.35		
	pH	-31.584	4.126	-7.66		
	NITRO.	12.467	2.269	5.50		

APPENDIX G

MEAN SQUARES (MS) AND VARIANCE (F) FROM ANALYSIS OF VARIANCE OF HERBAGE DRY MATTER YIELD AND CULTIVATION AND LIME AND NITROGEN APPLICATION. (CARBARNS FIELD TRIAL).

		1ST CUT		2ND CUT		3RD CUT		4TH CUT		5TH CUT	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
CULT.	1	0.020	0.26	0.016	2.54	0.065	1.89	0.108	1.80	0.012	2.29
LIME	1	0.058	0.75	0.002	0.25	0.091	2.65	0.022	0.36	0.016	3.20
NITRO.	2	0.030	0.38	0.272	42.29	0.931	27.22	1.751	29.03	0.244	47.39
LIME.CULT.	1	0.107	1.38	0.001	0.23	0.000	0.01	0.002	0.03	0.001	0.14
CULT.NITRO.	2	0.032	0.41	0.003	0.48	0.039	1.14	0.037	0.61	0.016	3.08
LIME.NITRO.	2	0.027	0.34	0.004	0.60	0.010	0.30	0.033	0.55	0.001	0.21
LIME.CULT.NITRO.	2	0.063	0.80	0.011	1.77	0.045	1.32	0.056	0.93	0.008	1.52
RESIDUAL	33	0.078		0.006		0.034		0.060		0.005	

APPENDIX H

MEAN SQUARES (MS) AND VARIANCE RATIOS (F) FROM ANALYSIS OF VARIANCE OF HERBAGE METAL UPTAKE AND CULTIVATION, LIME AND NITROGEN APPLICATION (CARBARNS FIELD EXPERIMENT).

CADMIUM

SOURCE OF		1st	CUT	2nd	CUT	3rd	CUT	4th	CUT	5th	CUT
DIFFERENCE	df	MS	F	MS	F	MS	F	MS	F	MS	F
LIME	1	445.8	1.27	37.5	0.31	20.8	0.41	4.2	0.17	12.5	1.92
CULT	1	49.9	0.14	0.0	0.07	37.5	0.66	120.8	3.82	4.2	0.45
NITROGEN	2	12.5	0.03	316.7	27.21	3162.5	58.37	412.5	13.29	354.2	54.60
LIME.CULT	1	399.9	1.14	4.2	0.45	91.7	1.73	33.3	1.02	0.0	0.09
LIME.NITROGEN	2	12.5	0.03	4.2	0.29	12.5	0.26	41.7	1.32	4.2	0.86
CULT.NITROGEN	2	645.8	1.84	8.3	0.72	0.0	0.02	24.9	0.75	12.5	2.06
LIME.CULT.NITRO.	2	545.8	1.56	16.6	1.27	79.2	1.48	170.8	5.52	0.0	0.27
RESIDUAL	33	349.9		12.5		54.2		29.2		8.3	

CHROMIUM

SOURCE OF		1st	CUT	2nd	CUT	3rd	CUT	4th	CUT	5th	CUT
DIFFERENCE	df	MS	F	MS	F	MS	F	MS	F	MS	F
LIME	1	620.8	0.91	1158.3	2.32	1033.3	2.10	3270.8	2.94	404.2	0.90
CULT	1	1104.2	1.62	204.2	0.41	66.7	0.14	24.9	0.02	66.7	0.15
NITROGEN	2	445.8	0.65	3145.8	6.31	18591.7	37.67	8808.3	7.92	2937.5	6.48
LIME.CULT	1	216.7	0.32	8.3	0.02	54.2	0.11	37.5	0.03	0.0	0.00
LIME.NITROGEN	2	795.8	1.17	854.2	1.72	1549.9	3.14	3324.9	2.99	187.5	0.42
CULT.NITROGEN	2	166.7	0.25	324.9	0.65	108.3	0.22	495.8	0.44	133.3	0.29
LIME.CULT.NITRO.	2	220.8	0.33	212.5	0.43	854.2	1.73	366.7	0.33	1108.3	2.45
RESIDUAL	33	679.2		499.9		491.7		1112.5		454.2	

APPENDIX H (contd)

COPPER

SOURCE OF DIFFERENCE	df	1st MS	CUT F	2nd MS	CUT F	3rd MS	CUT F	4th MS	CUT F	5th MS	CUT F
LIME	1	64779	2.27	23824	6.06	5666	0.40	1433	0.11	879	0.23
CULT	1	259933	9.12	574	0.15	1683	0.12	14445	1.14	6837	1.77
NITROGEN	2	40570	1.42	429420	109.27	2630266	184.48	789341	62.39	324579	83.81
LIME.CULT	1	71570	2.51	2645	0.67	3645	0.26	687	0.05	37	0.01
LIME.NITROGEN	2	26345	0.92	9387	2.39	3324	1.40	899	0.07	1841	0.48
CULT.NITROGEN	2	13824	0.49	1237	0.32	16799	1.18	533	0.04	3591	0.93
LIME.CULT.NITRO.	2	52020	1.83	2695	0.69	7695	0.54	3720	0.29	4691	1.21
RESIDUAL	33	28508		3929		14258		12649		3874	

NICKEL

SOURCE OF DIFFERENCE	df	1st MS	CUT F	2nd MS	CUT F	3rd MS	CUT F	4th MS	CUT F	5th MS	CUT F
LIME	1	34174	3.27	75124	18.84	174937	17.84	180324	8.44	6670	4.87
CULT	1	191133	18.31	12199	3.06	20599	2.10	13049	0.61	258	0.76
NITROGEN	2	2412	0.23	35524	8.91	157279	16.04	234641	10.98	18283	13.35
LIME.CULT	1	12649	1.21	174	0.04	3941	0.40	9029	0.42	83	0.06
LIME.NITROGEN	2	9641	0.92	9612	2.41	16029	1.64	11079	0.52	279	0.20
CULT.NITROGEN	2	10624	1.02	941	0.24	6304	0.64	3749	0.18	629	0.46
LIME.CULT.NITRO.	2	4374	0.42	887	0.22	3254	0.33	5524	0.26	216	0.16
RESIDUAL	33	10437		3987		9804		21379		1370	

APPENDIX H (contd)

		LEAD									
SOURCE OF DIFFERENCE	df	1st CUT MS	1st CUT F	2nd CUT MS	2nd CUT F	3rd CUT MS	3rd CUT F	4th CUT MS	4th CUT F	5th CUT MS	5th CUT F
LIME	1	724.9	0.84	995.8	3.70	87.5	0.15	154.2	0.19	24.9	0.16
CULT	1	74.9	0.09	312.5	1.16	1354.2	2.35	8.3	0.01	8.3	0.06
NITROGEN	2	445.8	0.52	2024.9	7.52	30433.3	52.92	2204.2	2.76	3429.2	21.57
LIME.CULT	1	1170.8	1.36	1516.7	0.56	4.2	0.00	512.5	0.64	254.2	1.61
LIME.NITROGEN	2	883.3	1.03	108.3	0.40	95.8	0.17	87.5	0.11	112.5	0.70
CULT.NITROGEN	2	733.3	0.85	1749.9	0.65	12.5	0.02	1391.7	1.74	24.9	0.15
LIME.CULT.NITRO.	2	945.8	1.10	229.2	0.85	404.2	0.70	4054.2	5.08	195.8	1.23
RESIDUAL	33	858.3		270.8		574.9		799.9		158.3	

		ZINC									
SOURCE OF DIFFERENCE	df	1ST CUT MS	1ST CUT F	2ND CUT MS	2ND CUT F	3RD CUT MS	3RD CUT F	4TH CUT MS	4TH CUT F	5TH CUT MS	5TH CUT F
LIME	1	12170416	4.45	4077499	10.18	7829166	5.88	5764166	2.04	790416	2.50
CULT	1	8113749	2.97	280416	0.70	1219166	0.92	1249	0.00	51666	0.16
NITROGEN	2	1223749	0.45	17445416	43.54	100037498	75.13	86209165	30.55	16370416	51.67
LIME.CULT	1	5922083	2.16	40833	0.10	790416	0.59	627499	0.22	9166	0.03
LIME.NITROGEN	2	4184166	1.53	1089583	2.72	4099583	3.08	1389999	0.49	394583	1.25
CULT.NITROGEN	2	6614166	2.42	71249	0.18	1757083	1.32	1392916	0.49	484999	1.53
LIME.CULT.NITRO.	2	2675416	0.98	132499	0.33	1601249	1.20	222916	0.08	333749	1.05
RESIDUAL	33	2736666		400833		1331666		2822083		316666	

APPENDIX I

BEST— FIT REGRESSION EQUATIONS FOR TOTAL AND EXTRACTABLE METAL CONCENTRATIONS, % EXTRACTABLE METAL AND ORGANIC MATTER BOUND Cu. (2ND YEAR).

	PREDICTOR	COEFFICIENT	S.E.	t-RATIO	S_Y	R^2(adj.)
Cd-ToT	Constant	0.239	0.056	4.28	0.261	51.5%
	O.M.	0.077	0.004	18.88		
Cd-Ac	Constant	-0.137	0.043	-3.23	0.194	47.4%
	Cd-ToT	0.254	0.041	6.24		
	O.M.	0.030	0.004	6.84		
Cd-%Ac	Constant	26.053	3.698	7.05	17.340	7.9%
	O.M.	1.482	0.272	5.44		
Cr-ToT	Constant	79.990	2.184	36.63	10.240	36.2%
	O.M.	2.223	0.161	13.82		
Cu-ToT	Constant	19.826	2.857	6.94	13.390	49.7%
	O.M.	3.834	0.210	18.22		
Cu-Ac	Constant	3.364	0.364	9.24	1.596	61.5%
	Cu-ToT	0.149	0.007	22.83		
	O.M.	-0.469	0.035	-13.26		
Cu-EDTA	Constant	0.274	0.709	0.39	3.372	88.6%
	Cu-ToT	0.497	0.010	5.98		
Cu-O.M.	Constant	-1.210	0.664	-1.82	3.162	85.7%
	Cu-ToT	0.409	0.009	44.78		
Cu-%Ac	Constant	26.481	1.392	19.03	1.793	49.1%
	O.M.	-0.428	0.042	-10.20		
	pH	-1.717	0.235	-7.31		
Ni-ToT	Constant	12.055	0.806	14.96	3.779	50.1%
	O.M.	1.089	0.059	18.35		
Ni-Ac	Constant	5.674	0.857	6.62	1.061	63.4%
	Ni-ToT	0.223	0.017	12.97		
	O.M.	0.061	0.027	2.24		
	pH	-1.049	0.136	-7.74		
Ni-%Ac	Constant	43.255	2.974	14.55	3.946	22.5%
	O.M.	0.253	0.082	3.09		
	pH	-3.892	0.503	-7.74		
Pb-ToT	Constant	-51.380	11.760	-4.37	55.110	47.3%
	O.M.	15.044	0.866	17.38		
Pb-Ac	Constant	1.026	0.225	4.56	1.901	75.5%
	Pb-ToT	0.044	0.001	32.12		
Zn-ToT	Constant	-2.310	12.750	-0.18	59.770	50.5%
	O.M.	17.390	0.939	18.52		
Zn-Ac	Constant	-6.669	1.327	-5.03	8.550	90.3%
	Zn-ToT	0.307	0.005	55.86		
Zn-EDTA	Constant	-6.617	1.575	-4.20	10.150	84.2%
	Zn-ToT	0.276	0.007	42.23		
Zn-%Ac	Constant	36.640	2.409	15.21	3.197	23.1%
	O.M.	0.400	0.066	6.05		
	pH	-2.533	0.408	-6.22		
Zn-%EDTA	Constant	36.504	2.575	14.18	3.416	38.4%
	O.M.	0.649	0.071	9.17		
	pH	-3.612	0.436	-8.29		

APPENDIX J

CO_2 EVOLVED (mg CO_2-C/100g FRESH SOIL) BY FUMIGATED (0-10 DAY INCUBATION) AND UNFUMIGATED (10-20 DAY INCUBATION) SAMPLES USED FOR ESTIMATION OF SOIL MICROBIAL BIOMASS IN CARBARNS, ALLANTON AND MAUDSLIE SOILS.

	CO_2 EVOLVED	
	FUMIGATED	UNFUMIGATED
<u>CARBARNS</u>		
CONTROL	42.04	19.82
	34.84	19.82
	39.64	18.00
	36.04	21.62
SLUDGED	31.26	19.22
	33.64	16.22
	32.44	18.62
	32.44	15.62
<u>ALLANTON</u>		
CONTROL	33.64	20.42
	35.44	17.42
	34.84	20.42
	36.04	19.22
SLUDGED	28.84	18.62
	31.24	19.22
	28.84	16.82
	31.24	18.02
<u>MAUDSLIE</u>		
CONTROL	16.82	13.22
	16.82	13.22
	19.22	13.22
	19.22	13.22
SLUDGED	23.42	12.02
	24.62	11.42
	22.82	11.42
	24.02	12.02

APPENDIX K

EXPERIMENT II: EFFECTS OF SEWAGE SLUDGE APPLICATION ON SOIL NITRIFICATION POTENTIAL.

TABLE K (1) CHANGES IN NH_4-N (mg N/Kg AIR DRY SOIL) WITH TIME (DAYS) IN ALL 36 PERCOLATING SYSTEMS (6 COLUMNS X 6 SOILS)

DAY									
2	4	6	8	10	12	14	16	18	20
CARBARNS UNSLUDGED									
373.58	308.06	293.03	291.21	268.25	262.41	240.55	188.06	244.92	239.09
369.02	321.46	319.27	291.21	276.63	262.41	240.55	297.77	306.15	239.09
448.75	388.43	389.25	376.86	368.84	344.42	328.75	548.52	*	*
437.36	321.46	319.27	308.34	*	*	*	*	*	*
396.36	339.32	349.89	305.13	276.63	276.77	*	*	*	*
332.57	339.32	345.51	256.95	217.95	213.21	224.51	344.78	*	*
CARBARNS SLUDGED									
423.53	434.66	393.04	421.34	*	*	*	*	*	*
418.82	421.98	402.07	398.12	415.62	372.71	364.42	388.52	379.48	362.73
435.29	364.33	397.55	415.81	428.61	372.71	364.42	388.52	387.39	362.73
442.35	417.36	402.07	393.69	389.65	338.82	331.29	331.86	371.58	316.42
409.41	426.59	406.59	429.08	419.95	381.18	364.42	388.52	387.39	370.45
421.18	431.20	528.56	442.35	437.27	381.18	372.71	388.52	397.27	370.45
MAUDSLIE UNSLUDGED									
472.38	473.63	453.48	470.16	325.03	375.12	387.75	358.45	360.12	*
464.44	480.44	442.05	477.62	467.46	418.00	408.71	382.35	393.46	377.59
476.35	487.24	461.10	500.01	452.85	435.86	426.17	396.00	403.47	384.10
474.36	498.91	434.43	496.28	441.89	418.00	391.24	385.76	386.79	367.82
468.41	483.84	434.43	486.48	422.72	*	*	*	*	*
527.95	480.44	438.24	488.81	427.28	385.84	387.75	*	*	*
MAUDSLIE SLUDGED									
396.75	504.78	445.48	501.62	448.26	438.52	441.02	*	*	*
482.60	496.82	441.02	488.54	448.26	438.52	424.69	415.03	420.97	426.17
468.68	496.82	454.39	497.26	443.99	430.16	420.60	415.03	409.28	410.95
477.96	500.23	454.39	497.26	448.26	455.22	436.94	415.03	428.77	414.76
473.32	493.41	449.93	488.54	435.45	430.16	408.35	407.05	389.79	391.93
482.60	459.30	467.75	497.26	537.91	446.87	420.60	407.05	414.15	426.17
ALLANTON UNSLUDGED									
518.15	494.31	519.43	543.10	489.35	482.84	476.15	457.44	*	*
522.74	480.83	510.63	530.16	480.92	466.34	451.94	457.44	450.65	458.72
525.03	494.31	523.84	534.47	489.35	482.84	476.15	481.10	473.76	368.48
527.32	485.32	519.43	543.10	476.70	458.08	468.08	461.38	469.91	451.20
519.30	512.28	532.64	551.72	497.79	499.35	439.83	485.04	454.51	466.24
510.13	498.80	519.43	543.10	480.92	495.22	464.04	481.10	473.76	466.24
ALLANTON SLUDGED									
546.63	550.52	551.73	528.05	524.77	490.03	486.74	460.81	457.36	503.16
548.79	525.11	585.96	519.93	510.85	490.03	471.53	*	*	*
544.47	539.94	551.73	511.80	516.82	482.25	463.93	460.81	442.84	503.16
542.31	529.35	555.88	519.93	524.77	490.03	471.53	490.54	450.10	517.34
548.79	542.05	551.73	519.93	477.06	497.80	463.93	475.68	450.10	517.34
548.79	537.82	519.58	*	*	*	*	*	*	*

* Denotes missing value.

TABLE K (ii) CHANGES IN NO$_3$-N (mg N/Kg AIR DRY SOIL) WITH TIME (DAYS) IN ALL 36 PERCOLATING SYSTEMS (6 COLUMNS X 6 SOILS)

DAY 2	4	6	8	10	12	14	16	18	20
CARBARNS UNSLUDGED									
143.05	150.01	196.37	216.69	233.04	308.34	336.77	308.74	361.26	445.30
76.54	131.26	184.56	208.98	253.16	305.06	333.56	310.31	335.23	421.39
89.29	141.98	192.44	196.99	197.83	185.33	183.62	141.05	*	*
78.36	140.19	83.10	181.58	*	*	*	*	*	*
78.36	142.87	148.70	213.48	252.74	276.77	*	*	*	*
80.18	163.41	209.93	263.80	326.92	308.34	339.97	253.35	*	*
CARBARNS SLUDGED									
21.65	39.66	20.33	106.50	*	*	*	*	*	*
26.59	55.34	16.49	107.93	131.61	132.14	159.02	192.64	210.30	280.25
23.29	38.74	27.56	97.32	109.53	98.26	124.24	150.55	169.19	233.84
32.00	67.33	14.00	166.77	154.99	191.44	225.28	254.16	283.03	330.32
23.06	41.04	13.10	82.72	92.65	89.79	112.64	139.22	154.96	213.01
23.06	37.82	12.54	77.85	90.05	84.71	106.01	129.51	161.28	199.12
MAUDSLIE UNSLUDGED									
8.53	23.68	10.29	35.08	67.93	117.54	57.81	150.21	169.39	*
4.17	15.76	5.24	35.82	35.06	30.72	31.44	27.99	29.68	31.49
4.57	9.92	12.19	16.42	31.41	42.16	65.67	86.71	99.70	128.25
5.36	12.25	15.24	24.63	35.79	47.52	63.58	84.66	97.03	122.39
5.76	14.93	7.62	27.75	41.77	*	*	*	*	*
2.98	13.03	16.01	26.87	38.71	50.02	70.56	*	*	*
MAUDSLIE SLUDGED									
6.26	11.82	11.58	16.58	17.93	25.06	32.67	*	*	*
7.42	13.87	12.47	18.32	21.35	26.73	35.12	43.90	52.23	72.30
7.08	13.19	11.58	16.58	18.78	24.22	29.40	42.30	42.88	56.32
7.89	14.78	12.47	19.19	21.35	26.73	35.94	45.49	53.79	69.25
9.51	18.99	24.95	27.04	41.84	57.63	75.14	95.78	100.57	127.09
9.05	15.69	21.38	22.68	32.45	38.42	44.92	57.47	64.71	87.52
ALLANTON UNSLUDGED									
7.80	10.78	12.33	12.07	12.66	15.68	19.37	25.24	*	*
8.25	11.91	15.85	18.10	19.41	23.94	29.05	37.07	40.83	53.39
7.11	10.78	12.33	10.34	13.50	15.68	19.37	25.24	26.96	36.85
9.17	10.11	11.45	10.34	10.97	12.38	16.14	20.51	23.11	30.08
7.11	10.56	11.45	12.07	14.34	19.81	21.79	35.49	39.29	54.52
7.80	15.84	11.45	10.34	10.97	18.16	20.18	27.60	29.27	40.61
ALLANTON SLUDGED									
27.66	36.42	54.76	60.93	69.17	91.00	108.00	122.26	130.67	150.24
28.30	38.96	51.80	62.15	73.05	93.92	108.85	*	*	*
26.36	36.42	52.27	69.05	80.31	105.01	122.45	142.70	148.82	183.55
23.98	31.34	48.12	56.87	62.42	86.34	98.49	118.92	123.41	144.57
24.95	34.73	54.76	61.74	80.31	93.34	106.47	125.61	131.76	153.78
23.55	31.34	51.96	*	*	*	*	*	*	*

* Denotes missing value.

APPENDIX L

EXPERIMENT III: EFFECTS OF SOIL pH ON NITRIFICATION POTENTIAL IN A SLUDGED SOIL.

TABLE L (i) CHANGES IN NH_4-N (mg N/Kg AIR DRY SOIL) WITH TIME (DAYS) IN ALL 36 PERCOLATING SYSTEMS (6 COLUMNS * 6 SOILS).

DAY	REPLICATE 1	2	3	4	5	6
UNSLUDGED SOIL						
2	329.47	364.27	352.67	352.67	334.11	334.11
4	295.59	272.85	241.02	222.83	218.28	222.83
6	102.46	106.91	269.51	271.74	307.38	338.56
8	216.46	268.81	272.08	268.81	282.44	275.89
10	194.78	270.02	256.15	270.02	305.78	290.30
12	197.33	253.71	253.71	240.66	253.71	260.50
14	176.61	225.61	215.41	215.41	220.51	215.41
16	153.14	191.55	191.55	182.08	191.55	215.50
18	121.81	168.58	149.58	149.58	149.58	131.07
20	109.40	146.02	146.02	127.95	146.02	164.57
22	53.36	133.64	80.28	97.91	124.83	142.46
24	52.03	130.30	60.63	78.27	113.11	130.30
26	104.92	131.81	42.32	64.36	70.97	110.21
28	41.21	63.96	46.36	34.77	57.52	51.08
32	13.78	35.08	13.78	27.15	35.08	57.63
pH 5.47						
2	417.27	440.20	417.27	435.61	435.61	426.44
4	355.00	337.03	323.55	274.12	278.61	269.62
6	145.27	151.87	158.47	396.18	409.38	431.39
8	362.06	369.07	376.07	396.55	344.82	403.55
10	381.25	381.25	394.96	445.59	441.90	428.71
12	316.74	396.18	402.88	402.88	396.18	402.88
14	377.79	387.37	368.21	358.12	358.12	396.96
16	359.35	359.35	369.21	364.28	378.57	369.21
18	360.62	351.47	351.47	249.40	166.59	203.18
20	343.10	369.89	343.10	343.10	343.10	352.03
22	343.45	338.86	334.73	343.45	325.56	378.75
24	317.42	300.44	326.37	308.93	326.37	351.85
26	287.07	293.60	288.38	288.38	272.69	348.06
28	293.09	275.27	298.18	265.52	279.09	91.19
32	253.39	244.72	*	236.06	236.06	311.99
pH 5.69						
2	409.40	409.40	413.95	423.05	409.40	382.11
4	320.97	314.28	271.93	267.48	267.48	147.11
6	139.74	301.32	329.70	347.17	397.39	406.13
8	342.08	345.28	345.28	342.08	379.49	366.13
10	368.28	334.80	378.22	396.53	415.36	411.70
12	346.97	360.27	360.27	343.90	366.93	346.97
14	336.25	336.25	355.27	307.23	326.75	365.28
16	347.19	328.61	337.90	310.03	337.90	337.90
18	330.05	302.82	311.90	311.90	211.11	247.41
20	322.19	322.19	331.05	304.47	322.19	322.19
22	332.07	314.33	305.69	262.02	332.07	314.33
24	306.47	289.62	247.04	247.04	289.62	323.77
26	270.52	280.46	279.60	235.52	283.92	298.61
28	268.03	277.29	263.40	217.96	281.08	294.96
32	251.37	246.87	236.63	207.57	202.65	212.07

TABLE L.(i) (Contd)

pH 5.76

2	429.59	453.46	453.46	467.78	434.37	420.05
4	392.94	343.82	294.70	285.35	285.35	259.62
6	155.80	146.63	355.13	284.11	380.33	453.65
8	358.95	344.37	387.55	384.19	394.84	337.64
10	354.61	344.18	386.44	404.01	412.79	421.58
12	219.63	336.69	357.64	357.64	336.69	357.64
14	312.41	302.43	342.86	312.41	332.89	312.41
16	276.06	276.06	276.06	290.43	295.56	295.56
18	259.62	269.64	240.57	259.62	346.32	192.46
20	234.84	234.84	253.44	234.84	234.84	216.25
22	229.12	229.12	183.29	210.98	229.12	265.87
24	201.05	196.40	178.71	196.40	205.70	232.23
26	178.66	164.61	148.74	164.61	180.93	229.90
28	195.16	162.04	130.69	154.98	181.47	218.56
32	171.41	127.16	102.24	124.58	120.29	192.03

pH 6.28

2	396.16	386.95	416.89	414.59	419.19	405.37
4	320.52	302.46	291.18	252.81	248.29	331.81
6	141.51	289.66	300.71	355.99	391.37	391.37
8	318.81	318.81	318.81	332.34	346.41	332.34
10	325.27	261.17	318.91	345.93	359.17	369.77
12	285.03	291.77	298.50	285.03	311.98	291.77
14	281.74	262.48	243.22	243.22	262.48	252.85
16	218.88	247.11	228.29	237.70	266.42	218.88
18	213.79	222.98	204.12	357.44	167.36	250.55
20	199.26	163.37	163.37	181.31	199.26	208.70
22	194.40	176.89	159.39	176.89	199.00	159.39
24	155.40	155.40	137.88	137.88	172.47	155.40
26	118.60	136.10	111.59	133.47	151.42	136.10
28	123.14	126.13	106.10	115.47	142.32	126.13
32	71.72	82.50	63.85	81.26	98.67	89.14

pH 5.63

2	407.88	398.61	407.88	417.15	417.15	389.34
4	308.88	295.25	254.37	254.37	249.83	249.83
6	124.59	102.34	287.00	347.07	347.07	373.77
8	282.11	261.41	275.57	320.78	320.78	296.27
10	269.71	228.67	259.05	320.88	310.22	320.88
12	240.38	197.10	226.83	253.42	260.20	240.38
14	176.41	166.21	176.41	220.25	146.84	220.25
16	162.43	124.57	143.50	210.27	210.27	172.40
18	130.92	121.67	102.69	364.52	205.38	364.52
20	118.77	100.24	127.80	127.80	127.80	127.80
22	177.98	97.80	80.19	142.29	120.05	124.68
24	78.18	60.56	60.56	112.98	95.35	112.98
26	57.68	76.18	52.40	86.30	78.82	110.08
28	66.03	48.45	48.45	71.60	60.88	83.17
32	32.12	44.63	17.52	40.05	40.05	54.65

TABLE L (ii) CHANGES IN NO_3-N (mg N/Kg AIR DRY SOIL) WITH TIME (DAYS) IN ALL 36 PERCOLATING SYSTEMS (6 COLUMNS * 6 SOILS).

			REPLICATE			
DAY	1	2	3	4	5	6
UNSLUDGED SOIL						
2	69.61	48.72	50.93	46.87	48.72	40.14
4	93.68	90.72	93.23	93.68	90.04	76.17
6	180.42	137.21	140.32	132.31	127.41	111.37
8	335.00	264.33	277.42	259.10	248.63	167.50
10	434.17	332.99	343.24	320.19	321.47	284.32
12	504.29	287.33	292.34	268.96	263.11	242.23
14	404.27	323.42	330.77	287.89	287.89	247.46
16	440.58	368.74	385.50	337.61	335.22	320.85
18	460.73	381.22	411.62	360.17	366.01	350.81
20	490.86	415.52	473.74	401.82	399.54	384.70
22	559.07	432.11	516.75	409.84	441.02	415.41
24	588.53	408.28	560.30	450.63	445.20	432.17
26	596.71	418.97	558.63	490.91	482.45	478.22
28	618.10	502.72	576.89	480.06	490.36	498.60
32	601.39	573.33	533.23	475.10	521.21	533.23
pH 5.47						
2	17.20	13.99	13.41	13.07	13.07	14.90
4	33.48	22.36	21.57	20.67	21.35	20.67
6	34.12	29.71	28.61	35.22	36.32	35.88
8	50.00	40.52	37.93	43.96	37.93	44.83
10	75.93	64.12	58.22	67.50	69.18	62.86
12	75.93	77.59	74.28	77.59	80.89	75.93
14	111.37	94.42	89.58	89.58	89.58	84.74
16	134.87	120.67	118.30	113.57	106.47	113.57
18	152.53	134.04	127.11	131.73	129.42	129.42
20	182.74	182.74	148.90	157.92	153.41	153.41
22	217.90	198.09	161.77	191.49	189.29	176.08
24	248.93	228.55	150.22	214.60	210.30	178.12
26	265.55	246.73	230.00	217.46	196.55	184.00
28	285.03	258.56	223.95	244.31	232.09	179.16
32	340.71	305.06	*	277.33	265.44	198.09
pH 5.69						
2	26.16	22.06	21.61	26.16	21.61	26.61
4	40.12	34.33	32.99	41.46	34.33	39.68
6	62.88	57.64	52.40	70.74	54.15	62.88
8	75.26	65.85	69.27	94.07	75.26	92.36
10	107.14	84.54	93.74	97.93	97.51	118.02
12	114.63	108.08	112.99	138.38	111.36	131.01
14	132.10	120.09	120.09	146.51	115.29	146.51
16	157.26	143.18	154.92	183.08	143.18	169.00
18	168.51	144.44	160.49	183.41	155.90	185.70
20	192.47	183.52	181.28	203.66	172.33	196.95
22	213.98	205.25	198.70	216.16	200.88	205.25
24	229.92	215.02	183.08	229.92	210.76	213.95
26	236.47	221.95	221.95	238.37	207.43	211.58
28	242.37	234.29	250.44	234.29	230.25	206.01
32	275.12	259.40	259.40	243.68	253.50	210.27

TABLE L.(ii) (Contd)

pH 5.76

2	33.41	28.40	29.83	24.58	25.78	27.45
4	64.79	50.99	46.08	44.67	44.67	46.78
6	94.40	96.23	86.15	62.32	72.40	86.15
8	145.37	142.23	134.61	136.40	129.22	122.04
10	272.71	250.31	176.97	176.97	170.39	173.90
12	161.53	230.26	223.39	228.54	213.08	217.37
14	279.75	282.27	252.03	259.59	238.17	246.99
16	330.04	336.20	317.73	315.26	311.57	298.02
18	360.86	368.08	344.02	322.37	339.21	330.79
20	403.93	413.33	385.15	393.37	385.15	312.34
22	433.03	453.65	421.58	429.59	414.70	373.46
24	428.91	504.86	431.14	457.95	433.37	368.59
26	439.68	522.39	467.97	478.85	452.74	367.85
28	411.15	529.83	462.01	487.45	474.73	381.48
32	379.42	544.38	461.90	532.01	453.65	371.17

pH 6.28

2	33.86	29.83	34.32	34.78	33.86	38.81
4	71.10	67.04	69.52	77.65	69.97	78.10
6	114.09	97.29	108.79	118.52	105.25	122.94
8	162.81	143.76	163.68	173.21	160.21	270.20
10	302.59	163.59	298.78	312.76	289.88	338.19
12	255.39	228.02	255.39	261.19	238.80	270.31
14	311.33	267.55	285.79	272.41	267.55	294.30
16	349.41	309.01	337.53	326.83	320.89	349.41
18	369.15	319.23	359.86	315.75	325.04	369.15
20	414.75	305.97	396.62	373.96	381.89	396.62
22	445.54	395.79	422.33	400.21	402.43	422.33
24	487.22	413.92	449.50	409.61	407.46	413.92
26	508.34	432.72	470.53	432.72	432.72	424.32
28	521.55	449.97	486.78	421.33	439.74	458.15
32	569.15	453.72	493.52	437.80	453.72	461.68

pH 6.37

2	47.62	46.81	48.67	38.93	38.24	43.34
4	93.80	111.74	108.11	84.03	86.53	91.53
6	156.63	162.86	167.75	133.49	137.05	146.84
8	324.15	352.91	352.91	287.56	304.55	321.54
10	409.36	445.18	450.30	376.10	394.01	422.16
12	516.22	534.99	522.48	308.69	325.38	340.39
14	369.54	406.25	401.35	336.50	249.62	365.87
16	449.63	454.41	422.13	452.02	408.97	426.91
18	446.18	448.52	446.18	412.31	433.33	455.53
20	474.33	490.29	478.89	467.49	453.80	478.89
22	502.80	498.35	498.35	511.70	467.21	507.25
24	572.66	524.94	524.94	533.62	540.13	533.62
26	604.48	557.98	511.48	528.39	500.91	524.16
28	559.76	506.25	502.14	547.41	506.25	506.25
32	572.66	512.59	484.56	544.63	510.59	500.58

APPENDIX M

EXPERIMENT 5.I FACTORS INFLUENCING SLUDGE METALS ADHERING TO LEAF SURFACES IN A FIELD SITUATION. (TEMPLE FIELD TRIAL).

CHANGES IN THE TOTAL HERBAGE Cd, Cr, Cu, Fe, Mn, Ni, Pb AND Zn CONCENTRATIONS (mg/kg DRY MATTER) WITH TIME (DAYS) IN ALL 3 REPLICATES PER TREATMENT.

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT CONTROL (T1)								
(REPLICATE 1)								
0	0.450	3.375	7.26	634.0	96.1	1.488	3.169	28.90
7	0.331	2.844	8.37	432.0	137.0	2.300	3.050	35.30
14	0.331	2.875	8.16	636.0	133.0	1.719	3.963	33.80
21	0.331	2.238	9.05	436.0	107.0	1.713	3.713	32.20
28	0.213	1.381	5.72	202.0	123.0	1.081	2.038	24.30
35	0.200	1.769	5.34	255.0	104.0	1.381	1.800	23.00
42	0.288	0.394	6.04	44.9	83.1	1.238	1.050	23.90
49	0.306	0.363	6.86	85.2	70.6	1.425	1.025	32.70
56	0.200	0.431	4.53	55.2	88.6	1.138	1.250	17.90
(REPLICATE 2)								
0	0.438	3.888	7.54	685.0	152.0	1.544	5.019	28.40
7	0.469	4.719	9.06	703.0	162.0	2.569	6.306	32.80
14	0.469	5.556	9.56	1153.0	158.0	2.094	7.481	33.80
21	0.256	4.319	6.95	697.0	144.0	1.450	5.131	25.60
28	0.250	2.913	7.41	544.0	185.0	1.325	5.875	27.80
35	0.281	2.094	6.15	318.0	125.0	1.344	2.500	22.90
42	0.200	2.369	4.71	392.2	106.0	1.081	2.713	17.70
49	0.269	0.806	4.89	74.1	70.6	0.838	1.988	17.60
56	0.163	0.344	3.66	66.9	88.7	1.138	1.169	14.20
(REPLICATE 3)								
0	0.525	6.781	9.80	1332.0	128.0	2.238	6.444	40.20
7	0.538	3.650	10.20	668.0	132.0	1.800	5.438	37.00
14	0.488	7.159	10.70	1713.0	182.0	2.431	7.431	37.70
21	0.538	4.675	9.73	820.0	172.0	1.938	6.275	40.20
28	0.519	4.281	10.70	833.0	147.0	1.856	6.225	41.90
35	0.294	1.494	6.57	301.0	132.0	1.419	2.600	24.00
42	0.250	1.406	5.95	196.9	149.0	1.419	2.450	25.60
49	0.313	1.450	5.68	189.0	90.1	1.219	1.825	22.20
56	0.250	0.475	5.07	63.4	87.5	1.125	1.000	24.40
CUT CONTROL (T2)								
(REPLICATE 1)								
0	0.550	11.194	7.86	2419.0	211.0	2.263	7.103	32.70
7	0.538	9.369	8.86	1876.0	205.0	1.850	7.675	32.90
14	0.456	8.813	8.22	1887.0	180.0	2.281	7.938	31.30
21	0.369	3.988	7.92	696.0	105.0	1.356	4.413	27.70
28	0.250	3.013	6.06	490.0	116.0	0.894	2.381	21.80
35	0.263	2.519	5.93	361.0	89.6	0.719	1.788	19.50
42	0.250	1.263	4.22	150.7	84.5	0.606	1.650	15.10
49	0.225	0.644	4.25	126.7	79.3	0.769	1.706	21.90
56	0.125	0.588	3.31	76.1	85.0	0.581	1.056	13.30
(REPLICATE 2)								
0	0.544	14.913	9.95	3017.0	148.0	2.988	8.856	36.80
7	0.475	6.044	10.70	1164.0	144.0	1.850	6.850	35.70
14	0.675	13.375	10.50	2878.0	172.0	3.063	9.675	39.90
21	0.231	5.338	9.51	958.0	143.0	2.075	4.613	34.40
28	0.263	2.725	8.55	531.0	135.0	1.331	3.331	27.40
35	0.231	3.663	6.83	662.0	104.0	1.250	3.081	24.90
42	0.269	1.800	4.36	226.9	104.0	0.806	1.869	15.60
49	0.294	0.950	4.56	122.5	103.3	0.919	3.031	20.20
56	0.119	0.300	3.70	64.9	70.8	0.650	0.975	14.30
(REPLICATE 3)								
0	1.025	21.331	13.10	4757.0	153.0	4.125	12.581	54.00
7	0.488	16.706	13.90	3379.0	119.0	3.713	9.456	44.80
14	0.431	11.200	17.40	2690.0	192.0	3.056	13.494	52.30
21	0.350	9.244	11.60	1993.0	130.0	2.519	6.844	42.60
28	0.313	4.706	12.70	917.0	111.0	1.475	6.188	41.90
35	0.294	1.700	8.14	497.0	85.1	1.313	3.094	26.20
42	0.238	3.156	6.17	480.6	113.0	1.338	2.500	22.50
49	0.306	2.025	8.51	319.2	111.0	1.294	5.388	28.00
56	0.194	0.338	4.33	94.8	101.0	0.956	1.031	20.50

APPENDIX M (contd)

UNCUT, FULL RATE, 2% D.S. SLUDGE (T3)

(REPLICATE 1)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.700	31.013	31.40	7344.0	233.0	6.113	28.844	81.91
7	0.825	16.413	39.10	3300.0	215.0	3.650	29.880	92.40
14	0.763	14.650	34.80	3285.0	258.0	4.444	26.825	91.50
21	0.525	11.769	29.50	2266.0	180.0	3.325	24.169	84.10
28	0.400	7.519	20.40	1371.0	132.0	1.975	13.638	52.40
35	0.494	9.194	19.30	1622.0	137.0	2.156	13.138	52.20
42	0.356	3.131	10.80	486.1	111.0	1.275	6.569	31.80
49	0.294	0.656	5.95	131.6	71.6	1.050	2.669	36.40
56	0.250	0.556	4.83	52.6	69.0	0.875	1.050	17.50

(REPLICATE 2)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.881	29.906	41.00	6911.0	229.0	6.538	39.694	106.00
7	0.888	16.200	40.30	3181.0	176.0	3.975	30.906	97.00
14	0.694	17.625	36.60	3504.0	228.0	3.988	29.813	89.30
21	0.413	10.006	25.20	2055.0	158.0	2.506	21.469	65.20
28	0.606	12.581	31.90	2530.0	185.0	5.713	22.363	81.50
35	0.394	7.888	21.50	1440.0	158.0	2.181	13.906	57.50
42	0.388	5.531	14.30	975.6	121.0	1.556	10.156	39.30
49	0.331	1.800	7.97	269.4	75.6	1.081	3.969	28.30
56	0.306	0.450	5.18	84.1	53.5	0.906	1.419	19.30

(REPLICATE 3)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.944	44.319	33.10	10197.0	257.0	10.006	37.281	85.10
7	0.706	15.356	40.60	3045.0	281.0	4.263	31.550	94.10
14	0.694	13.538	43.00	3120.0	271.0	4.000	32.800	97.10
21	0.500	10.569	27.60	2027.0	235.0	3.338	23.556	71.90
28	0.513	9.450	27.60	1863.0	235.0	3.013	18.731	68.30
35	0.469	6.081	19.30	1173.0	197.0	2.638	12.800	54.70
42	0.300	4.025	11.70	638.2	114.0	1.544	7.794	32.10
49	0.300	1.194	7.43	143.0	92.6	1.631	2.844	27.40
56	0.200	0.738	5.36	124.4	120.0	1.569	1.963	21.30

CUT, FULL RATE, 2% D.S. SLUDGE (T4)

(REPLICATE 1)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.763	24.569	36.50	5354.0	257.0	5.281	34.325	96.30
7	0.794	16.769	35.80	3326.0	233.0	3.400	26.363	84.90
14	0.575	14.131	31.30	3215.0	238.0	3.981	23.781	79.30
21	0.419	7.681	19.70	1428.0	151.0	2.175	13.044	56.20
28	0.244	3.700	10.90	661.0	120.0	1.294	4.000	34.20
35	0.275	3.013	8.34	383.0	94.1	1.119	3.156	26.90
42	0.306	2.131	7.99	269.6	123.0	1.025	3.669	28.40
49	0.294	0.694	5.73	106.5	72.3	0.831	3.644	21.80
56	0.138	0.556	4.09	80.6	73.5	0.769	1.050	14.50

(REPLICATE 2)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.844	53.313	32.50	13733.0	267.0	11.156	37.131	88.50
7	0.619	27.631	28.50	6001.0	213.0	5.538	21.844	81.60
14	0.613	19.863	29.60	4852.0	194.0	4.938	20.894	76.40
21	0.631	13.750	26.60	2648.0	156.0	3.456	19.350	71.90
28	0.356	6.944	14.80	1448.0	109.0	1.794	7.375	50.50
35	0.300	4.381	10.60	773.0	85.0	1.375	4.313	33.60
42	0.325	1.431	6.79	182.7	64.4	0.950	1.988	23.60
49	0.331	2.188	6.46	334.5	56.9	1.013	2.463	22.40
56	0.350	0.419	4.98	86.4	45.4	0.850	1.094	18.60

(REPLICATE 3)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0	0.831	29.625	36.10	6851.0	266.0	7.256	33.394	96.40
7	0.706	20.425	33.50	4194.0	279.0	5.281	25.638	86.00
14	0.675	17.888	32.60	4215.0	279.0	5.081	29.650	86.30
21	0.381	9.119	19.20	1833.0	145.0	2.788	13.269	52.90
28	0.356	4.100	14.50	694.0	145.0	2.806	5.138	54.20
35	0.294	2.525	9.14	527.0	86.3	1.425	3.531	33.80
42	0.288	2.300	8.88	382.7	104.0	1.463	3.850	28.80
49	0.319	1.125	5.85	133.9	109.0	1.663	1.669	23.40
56	0.263	0.625	5.13	79.1	90.3	1.213	1.050	20.10

APPENDIX M (contd)

DAY	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
UNCUT, HALF RATE, 2% D.S. SLUDGE (T5)								
(REPLICATE 1)								
0	0.713	28.638	24.40	6856.0	244.0	5.594	26.769	71.00
7	0.413	8.875	18.70	1742.0	207.0	2.494	15.000	57.00
14	0.388	3.269	7.46	652.0	152.0	1.456	3.531	30.50
21	0.369	7.800	13.40	821.0	143.0	2.875	9.406	39.40
28	0.256	3.481	9.84	544.0	128.0	1.094	5.394	30.70
35	0.206	1.806	6.10	241.0	93.0	0.806	2.363	23.40
42	0.231	0.856	5.15	51.3	100.0	0.731	1.581	19.90
49	0.238	0.694	4.53	82.3	90.3	0.894	1.206	21.40
56	0.131	0.481	4.24	41.8	82.6	0.950	0.981	16.20
(REPLICATE 2)								
0	0.650	16.744	24.60	3620.0	169.0	3.638	20.338	69.00
7	0.650	11.294	28.20	2140.0	174.0	2.813	21.400	65.90
14	0.613	10.225	23.30	2226.0	163.0	2.656	24.263	68.10
21	0.513	8.344	18.50	1513.0	126.0	2.038	14.175	51.50
28	0.506	5.900	18.10	1097.0	159.0	2.000	11.613	53.00
35	0.469	4.544	12.70	672.0	148.0	1.831	6.813	40.70
42	0.406	2.806	8.56	348.6	119.0	1.525	4.306	28.80
49	0.306	1.794	6.59	233.4	72.4	1.113	3.519	22.80
56	0.325	0.944	5.79	185.5	85.1	1.044	2.406	21.80
(REPLICATE 3)								
0	0.744	23.606	29.30	5134.0	242.0	5.906	27.544	82.90
7	0.575	12.675	32.50	2374.0	290.0	4.988	24.063	80.40
14	0.556	10.531	26.20	2299.0	195.0	3.900	19.025	69.20
21	0.431	8.006	22.90	1395.0	237.0	3.050	18.831	56.20
28	0.375	7.050	20.00	1318.0	224.0	2.650	13.431	55.30
35	0.325	3.775	10.80	581.0	160.0	6.325	6.456	33.30
42	0.344	3.663	10.10	575.5	156.0	1.863	7.888	30.60
49	0.275	1.788	7.16	253.3	156.0	2.419	3.725	23.80
56	0.275	1.056	5.39	64.1	129.0	1.756	1.881	23.10
UNCUT, FULL RATE, 12% D.S. SLUDGE (T6)								
(REPLICATE 1)								
0	1.638	58.356	105.00	13471.0	475.0	14.856	134.000	256.00
7	2.188	58.081	165.00	12860.0	531.0	16.288	154.690	369.00
14	2.163	48.975	169.00	12290.0	536.0	15.744	164.560	357.00
21	1.569	41.244	114.00	8816.0	438.0	12.250	129.500	271.00
28	1.200	37.263	98.90	8133.0	365.0	9.513	96.438	227.00
35	1.181	35.356	94.80	7230.0	364.0	8.450	85.938	213.00
42	0.694	17.375	47.90	3281.0	244.0	4.231	42.419	114.00
49	0.313	0.800	5.30	99.4	72.4	0.738	3.513	20.40
56	0.206	2.438	9.06	451.3	86.8	1.175	6.200	26.30
(REPLICATE 2)								
0	2.181	64.194	164.00	14592.0	573.0	17.975	162.880	365.00
7	2.269	69.744	192.00	14945.0	632.0	20.125	193.880	420.00
14	1.906	52.159	161.00	13337.0	607.0	16.869	174.690	374.00
21	1.769	46.694	137.00	10348.0	498.0	15.281	145.380	320.00
28	1.650	45.025	149.00	10682.0	518.0	12.725	134.250	321.00
35	1.288	35.294	101.00	7628.0	418.0	9.213	97.688	229.00
42	0.375	1.413	8.22	121.4	169.0	1.725	1.694	30.30
49	0.400	4.869	15.70	870.3	135.0	2.175	11.488	46.20
56	0.519	8.506	24.80	1801.0	177.0	2.569	22.219	62.50
(REPLICATE 3)								
0	1.869	65.900	126.00	16098.0	503.0	18.019	133.500	295.00
7	2.131	62.294	175.00	13730.0	562.0	17.281	172.310	378.00
14	1.831	48.456	153.00	12485.0	535.0	15.269	153.510	348.00
21	1.775	48.606	144.00	10817.0	506.0	14.844	152.440	328.00
28	1.613	42.031	131.00	11279.0	446.0	11.406	121.250	291.00
35	1.275	35.156	106.00	7749.0	370.0	9.606	93.620	228.00
42	0.531	12.825	33.40	2280.0	203.0	3.344	28.720	84.50
49	0.244	0.750	5.25	87.3	65.2	0.956	1.810	19.30
56	0.325	4.638	17.50	1032.0	116.0	1.894	13.481	47.60

APPENDIX N

EXPERIMENT 5.I FACTORS INFLUENCING SLUDGE METALS ADHERING TO LEAF SURFACES IN A FIELD SITUATION. (TEMPLE FIELD EXPERIMENT).

METEOROLOGICAL DATA (PRECIPITATION, TEMPERATURE AND WIND) RECORDED AT AUCHINCRUIVE DURING THE 56 DAYS OF THE EXPERIMENT.

DAY	PRECIPITAION		TEMPERATURE (°C)				WIND	
	DAILY TOTAL	DURATION	AIR		SOIL (cm)		D[1]	RUN
	/mm	/hrs.	Max.	Min.	10	20		/Km
0	15.5	10.9	6.6	0.0	1.9	3.3	E	290
1	1.8	1.1	7.8	3.8	4.4	4.2	W	410
2	14.3	12.5	10.4	2.1	4.0	4.1	WSW	497
3	18.1	9.3	10.2	4.0	7.2	5.5	SSW	729
4	0.0	-	7.6	2.9	4.8	5.0	NW	621
5	1.3	1.1	7.4	1.1	1.9	3.6	NNW	404
6	4.5	4.2	10.3	3.1	5.6	5.0	SW	423
7	2.9	5.0	11.1	6.6	6.5	5.9	SE	325
8	4.4	15.6	9.7	2.8	4.9	5.5	N	114
9	0.0	-	6.1	1.6	4.5	5.0	NW	187
10	0.0	-	8.4	3.0	4.0	4.8	NE	475
11	0.1	0.3	7.8	5.2	5.1	5.3	NE	589
12	1.1	5.6	6.1	5.2	5.0	5.2	NE	373
13	0.9	0.3	9.4	4.4	4.5	5.0	NE	291
14	5.2	13.0	11.6	4.9	5.4	5.2	NE	283
15	1.0	2.6	8.3	4.0	5.4	5.9	NE	191
16	4.7	3.8	10.4	3.7	6.1	5.5	SE	327
17	6.7	6.5	9.2	2.6	5.0	5.1	SE	579
18	0.0	-	9.9	2.7	4.4	5.2	NW	237
19	2.2	3.6	10.4	-0.8	3.1	4.6	SW	327
20	0.4	*	11.5	6.9	7.7	6.6	SW	253
21	0.0	-	12.4	4.0	6.7	6.1	SE	330
22	0.0	-	10.5	8.4	8.6	7.9	S	201
23	0.0	-	12.8	5.3	8.4	7.5	SW	146
24	0.0	-	18.1	4.8	7.9	7.6	E	164
25	0.3	0.3	14.9	10.3	10.0	9.1	S	473
26	7.1	10.7	12.1	7.6	8.9	8.9	S	505
27	0.4	0.6	10.1	5.6	7.9	8.0	W	401
28	0.0	-	12.4	7.3	9.1	8.5	SW	254
29	0.3	0.7	15.1	6.2	9.2	8.3	E	214
30	0.0	-	15.1	10.1	9.6	8.9	E	166
31	0.0	-	17.7	5.4	8.8	8.4	calm	138
32	0.0	-	20.2	8.3	9.9	9.3	E	154
33	0.0	-	16.9	6.4	10.3	9.6	calm	127
34	0.0	-	17.2	7.1	10.7	9.9	W	119
35	0.0	-	19.8	8.7	11.4	10.2	E	145
36	0.5	0.8	18.5	9.3	11.5	10.6	E	238
37	5.3	6.3	16.1	10.7	11.8	11.2	SE	377
38	3.2	1.8	10.8	5.5	8.9	9.9	W	534
39	2.6	1.2	10.8	2.4	7.9	8.9	W	317
40	0.0	-	10.4	3.2	7.0	7.7	NNW	298
41	0.0	-	11.0	5.1	8.5	8.3	W	381
42	0.0	-	13.3	8.0	9.2	8.9	W	325
43	0.0	-	14.3	5.6	10.2	9.5	NW	164
44	0.0	-	16.6	3.0	10.8	9.6	W	172
45	0.0	-	15.4	5.2	12.2	10.7	W	266
46	0.2	0.2	11.2	7.4	11.2	11.1	WNW	394
47	3.8	5.8	12.1	4.5	9.3	9.7	W	496
48	2.8	3.3	12.1	7.2	10.4	10.2	W	409
49	1.7	2.2	11.7	6.2	9.2	9.5	W	389
50	5.4	5.8	10.8	4.6	8.4	8.6	W	330
51	0.5	0.5	11.1	5.4	9.2	9.3	N	208
52	0.0	-	11.6	0.9	8.5	8.2	N	214
53	6.9	7.6	11.9	0.9	8.4	8.4	N	214
54	2.4	1.9	11.6	7.2	9.7	10.2	W	204
55	0.0	-	13.1	4.3	10.0	9.5	N	262
56	0.0	-	15.4	7.4	11.2	10.1	W	153

FOOTNOTE: '1' WIND DIRECTION
'*' missing value

APPENDIX O

EXPERIMENT 5.2 THE INFLUENCE OF RAINFALL AND DRYING ON 'WASH-OFF' OF ADHERENCE SLUDGE.

TOTAL HERBAGE Cd, Cr, Cu, Fe, Mn, Ni, Pb AND Zn CONCENTRATIONS (mg/kg D.M.) IN ALL 4 REPLICATES PER TREATMENT.

RAINFALL TREATMENT /mm	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
0.0	0.47	11.85	85.74	3834.5	454.0	4.96	47.95	204.2
	1.01	9.15	89.80	4239.8	530.6	4.41	51.71	217.1
	0.87	8.32	78.86	3479.0	540.8	3.84	42.06	199.3
	0.96	7.08	86.68	3893.5	558.3	4.44	48.77	203.7
0.7	0.74	3.52	44.27	1192.9	430.1	1.58	12.43	138.2
	0.63	6.25	43.47	1803.2	501.8	1.04	11.92	147.5
	0.54	3.22	42.82	1379.8	439.2	1.29	11.75	124.4
	0.45	2.10	49.31	1390.7	495.1	1.65	13.79	143.1
1.3	0.45	2.79	34.55	677.7	456.3	0.50	5.45	144.5
	0.39	3.02	33.37	586.1	476.4	0.34	4.96	158.3
	0.36	2.87	39.24	969.3	561.1	0.25	7.14	141.3
	0.50	3.70	38.31	978.5	472.1	0.89	10.67	136.6
2.0	0.53	4.80	35.68	618.2	430.7	0.50	5.36	128.4
	0.53	3.28	33.34	607.8	451.1	0.59	5.45	108.1
	0.37	2.22	34.19	505.8	442.7	0.23	5.82	118.8
	0.49	6.00	7.16	166.2	65.0	0.88	7.52	22.9
NO SLUDGE (CONTROL)	0.80	2.49	45.36	161.3	425.7	0.74	5.21	118.5
	0.91	2.28	40.13	361.3	391.2	0.91	5.83	127.6
	0.70	1.73	35.33	1034.6	342.3	0.65	4.75	99.8
	0.20	3.21	2.39	16.7	20.3	0.36	1.19	7.6
DRIED SLUDGE	0.99	11.67	104.54	4285.9	545.4	4.59	50.89	213.6
	1.26	13.18	104.40	5004.5	510.0	5.38	56.24	253.8
	1.23	11.40	114.75	5715.5	573.3	6.47	67.27	281.9
	0.99	10.09	94.02	4307.9	507.9	4.88	50.94	210.3
2.7	0.36	2.35	35.37	752.2	421.6	0.41	5.56	122.5
	0.41	1.24	34.19	867.8	463.6	0.72	4.04	143.8
	0.43	1.19	36.17	1236.9	504.8	0.38	5.45	137.7
	0.47	4.86	47.18	1549.9	355.1	0.61	5.42	143.2
3.3	0.37	3.59	38.20	1320.1	407.7	0.89	4.41	127.7
	0.51	3.19	35.57	651.8	369.4	0.54	4.75	108.6
	0.48	2.72	37.28	856.6	404.0	0.51	5.00	129.2
	0.28	4.64	34.83	845.6	370.4	1.05	5.02	119.4
3x0.7	0.93	5.56	70.07	588.4	461.7	4.63	5.56	188.7
	0.98	5.64	55.57	553.2	497.4	3.68	5.64	143.7
	0.75	3.14	50.42	1282.0	418.2	2.01	8.29	145.7
	0.79	4.09	43.08	995.1	354.7	2.52	6.13	132.1

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