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Distribution of Metals in Grassland Soils Following Surface Applications of Sewage Sludge

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ABSTRACT

In order to decide on a suitable sampling depth for grassland soil treated with sewage sludge and to assess implications for grazing animals, a field trial on two soils was designed to estimate the distribution of metals in grassland soil profiles following surface applications of sludge. Thus the sites represented permanent grassland where no form of cultivation had taken place. Soil cores were taken using specialised equipment to 30 cm depth and divided into seven sections. Movement from the soil surface to a depth of 10 cm was observed for all of the seven metals, Cd, Cr, Cu, Mo, Ni, Pb and Zn, but most of the metal (60%–100%, mean 87%) remained in the upper 5 cm of soil. It was concluded that sampling to a depth of 5 or 7·5 cm would be most suitable for monitoring long-term grassland treated with surface applications of sludge.

INTRODUCTION

Grassland provides a valuable outlet for sewage sludge allowing application at times of the year when arable land is inaccessible because of wet soil conditions in the winter, or of cover by a standing crop in the summer. Also, grass grows well in response to surface dressings of sewage sludge and is not particularly sensitive to enhanced metal concentrations in the soil. These positive factors have led to a position in the UK where approximately 20% of the sewage sludge used in agriculture is applied to long-term pasture land, usually as a surface dressing. Negative factors include the problem of odour

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following surface applications to fields near houses, possible transmission of pathogens to grazing animals, and accumulation of sludge contaminants at the soil surface. Described below are the results of a study of the distribution of metals in grassland soil profiles following surface applications of sludge. The work was undertaken to assess the extent of metal accumulation at the soil surface and to indicate a suitable sampling depth for monitoring metal concentrations in grassland soils receiving surface dressings of sewage sludge.

METHODS

Field sites

The field study was carried out on a four-year old experiment on manurial value, with a known history of sludge application. In order to assess metal distributions in soil profiles, 18 experimental plots were sampled at each of two sites which had received surface applications of liquid digested sludge thickened in lagoons in the period 1978–1981. The sludge had been spread manually from calibrated buckets. Metal analyses of the sludges applied are given in Table 1. The sites were initially sown with perennial ryegrass, *Lolium perenne* L cv. S24, before liquid sludge was applied each autumn in an investigation of the manurial value of the sludge. One site was on a calcareous loam soil (Swaffham Prior Series), 30–40 cm deep over chalk, the land was gently sloping, but the infiltration rate of the soil was rapid and surface runoff did not occur. The other site was level and the soil was a sandy loam about 40 cm deep over sandstone and was very free draining (Cottenham Series). Background soil analyses for both sites are given in Table 2.

TABLE 1
Total Concentrations of Metals in Sludge

Application	*Metal* ($mg\ kg^{-1}$ *DS*)						
	Cd	*Cr*	*Cu*	*Mo*	*Ni*	*Pb*	*Zn*
Autumn 1978	63	700	1 020	32	320	650	2 050
Autumn 1979	64	810	1 120	35	340	710	2 350
Autumn 1980	61	773	1 107	32	370	687	2 350
Autumn 1981	59	770	1 060	35	350	670	2 200

TABLE 2
Background Soil Analyses

Site	*pH*	*Organic matter* (%)	*CEC*[a] (*meq 100 g*$^{-1}$)
Sandy loam (Cottenham series)	6·5	2·9	11·4
Calcareous loam (Swaffham Prior series)	8·0	3·7	17·4

[a] CEC = Cation Exchange Capacity.

Coring methods

In order to remove soil cores which were intact and had received no compaction, a specialised soil corer was developed at WRc. The soil corer comprised a stainless steel tube which was driven into the ground by a petrol-engined jack hammer (Fig. 1). The 50 mm diameter cutting edge of the cylinder was narrower than the tube, thus once the soil had been cut it could slide inside the tube with minimum contact with the sides, therefore avoiding compression of the soil core.

The cylinder with soil core was driven into the soil to a greater depth than required, and then rotated to shear the soil core from the subsoil. The cylinder was then raised by hand winch and laid on a flat surface with the top of the cylinder on a carriage. A plunger was used to push the deepest centimetre of soil through the cutting edge zone into the larger diameter cylinder, after which the whole core could be easily extruded from the cylinder onto the carriage and divided accurately into the appropriate sections for analysis.

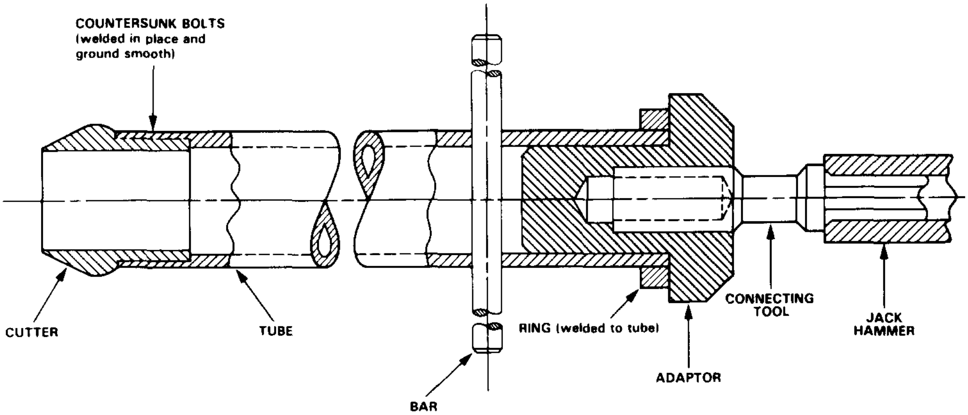


Fig. 1. Diagram of mechanical coring equipment.

Sampling and analysis

The established plots were 1·3 m wide and 8 m long; five core samples were taken from each of the plots, avoiding the edges. One core was taken randomly from each fifth of a plot, an area of 1 m by 1·5 m. In the first year, the winter of 1982/3, sampling depths were of 0–2·5, 2·5–5, 5–10, 10–20 and 20–30 cm. It was then decided that a more detailed assessment of metal movement down the soil profile could be obtained by subdividing two of the sections in each core (5–10 and 10–20 cm) in half. Division at 7·5 cm matched the sampling depth for permanent pasture recommended by the UK Ministry of Agriculture, Fisheries and Food (1982). These new divisions were used when sampling in the winters of 1983/4 and 1984/5, giving seven sections in all:

0–2·5, 2·5–5, 5–7·5, 7·5–10, 10–15, 15–20 and 20–30 cm.

All the samples were dried in an oven at a temperature not exceeding 30°C, ground to pass a 2 mm sieve, homogenised and further ground in an agate ball mill and subsequently analysed by X-ray fluorescence spectrophotometry (X-RFS), using a Philips PL1400 instrument. Concentrations of Cd, Cr, Cu, Mo, Ni, Pb and Zn were determined.

Experimental design and statistics

The experiment was designed around treatments from the original manurial value trial, which allowed comparison of unsludged and sludged (rate S1) treatments on eight plots. In addition, two replicates of higher rate (S2) sludged plot were sampled. Details of sludge applications are given in Tables 3 and 4. Thus, a total of 18 plots was sampled at both sites. Each core was divided into five, and later seven sections, as described above.

Effects were assessed initially on a factorial design with a sludge factor at two levels, no sludge and rate S1, and a depth factor at seven levels. This main factorial design incorporated eight replicates and therefore 2 × 7 × 8, that is, 112 observations. It should be noted that, in the previous manurial value experiment, two inorganic fertiliser treatments were also present in this main factorial design, calcium ammonium nitrate and sulphate of potash. Their effects, if any, were not relevant to this study and did not confound the sludge treatments. They were included to increase the power of the statistical tests.

Effects on the sludge treatments at rate S2 were studied in a smaller factorial design with the sludge factor at two levels, no sludge and rate S2, and a depth factor at seven levels. The two unsludged plots, without fertiliser treatment, were included from the main factorial design. Thus two

TABLE 3
Rates of Application of Sludge

	Treatment	
	S1	*S2*
	(*tds ha*$^{-1}$)[a]	
Sandy loam:		
1978	10·3	15·4
1979	11·3	16·9
1980	9·3	13·9
1981	11·1	16·6
Total	42·0	62·8
Calcareous loam:		
1979	11·1	16·7
1980	11·3	16·9
1981	12·3	18·5
Total	34·7	52·1

[a] tds = tonnes dry solids.

TABLE 4
Metal Additions in Sludge Added to Field Plots

S1 treatments	*Metal* (*kg ha*$^{-1}$)						
	Cd	*Cr*	*Cu*	*Mo*	*Ni*	*Pb*	*Zn*
Sandy loam:							
1978	0·65	7·2	10·5	0·33	3·3	6·7	21·0
1979	0·72	9·1	12·6	0·39	3·8	8·0	26·5
1980	0·57	7·1	10·0	0·31	3·4	6·4	21·8
1981	0·65	8·5	11·7	0·39	3·9	7·4	24·3
Total	2·59	32·0	44·8	1·42	14·4	28·5	93·6
Calcareous loam:							
1979	0·71	9·0	12·5	0·39	3·8	7·9	26·2
1980	0·69	8·7	12·5	0·37	4·2	7·8	26·5
1981	0·73	9·5	13·1	0·43	4·3	8·3	27·1
Total	2·13	27·2	38·0	1·19	12·3	24·0	79·8

replicates were available giving a total of 28 observations for each soil/metal combination.

An analysis of variance was applied to estimate significant effects between the treatments for both factorial designs. The model used can be described as:

$$\text{metal}_{ijk} = \text{mean} + \text{sludge}_j + \text{depth}_k + \text{sludge} \times \text{depth}_{jk} + \text{e}_{ijk} \qquad (1)$$

where:

metal_{ijk} = the metal concentration of the *i*th sample from the *j*th sludge treatment at the *k*th depth
mean = the mean of all the metal concentrations
sludge_j = the effect of the *j*th sludge treatment
depth_k = the effect of the *k*th depth
$\text{sludge} \times \text{depth}_{jk}$ = the effect of interaction of the *j*th sludge treatment at the *k*th depth
e_{ijk} = the residual or error term of the *ijk*th sample

Data sets to which this type of analysis is applied should come from normally distributed populations. Tests of skew, performed on each set, indicated that Cu and Zn concentrations in the sandy loam, and Cd, Cr, Cu, Ni and Zn concentrations in the calcareous loam, were all positively skewed. Thus, a log transformation was applied to these data before the analysis of variance.

An additional model, similar to that above but with a year term instead of a sludge term, was used to determine significant effects between years on metal levels in S1 sludge-treated plots only. To balance the comparison between 1983 and 1984, where five depths only were sectioned in 1983, results for the two sections further divided in half in 1984 were averaged giving reduced data sets equivalent to those from 1983.

RESULTS

General

The most comprehensive set of results was that for 1984, covering both soil types. Mean concentrations of each metal throughout the soil profile, on both sites in 1984, are illustrated in Fig. 2. Results are from the main factorial design, where sludge was added at rate S1; increases are of metal due to added sludge above that found in background soil. Results for the S2 sludge treatment, and for years 1983 and 1985, are not shown because they

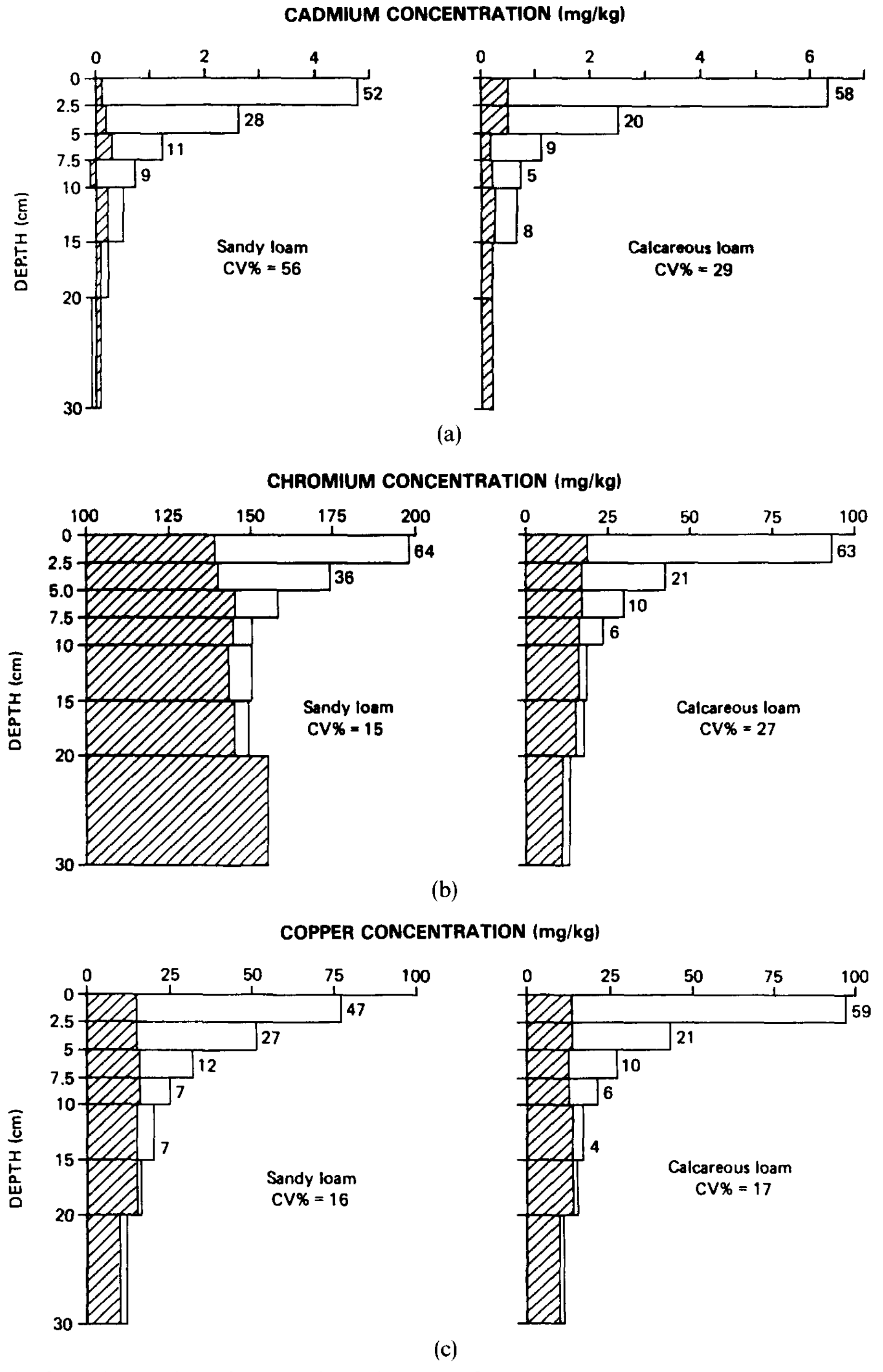
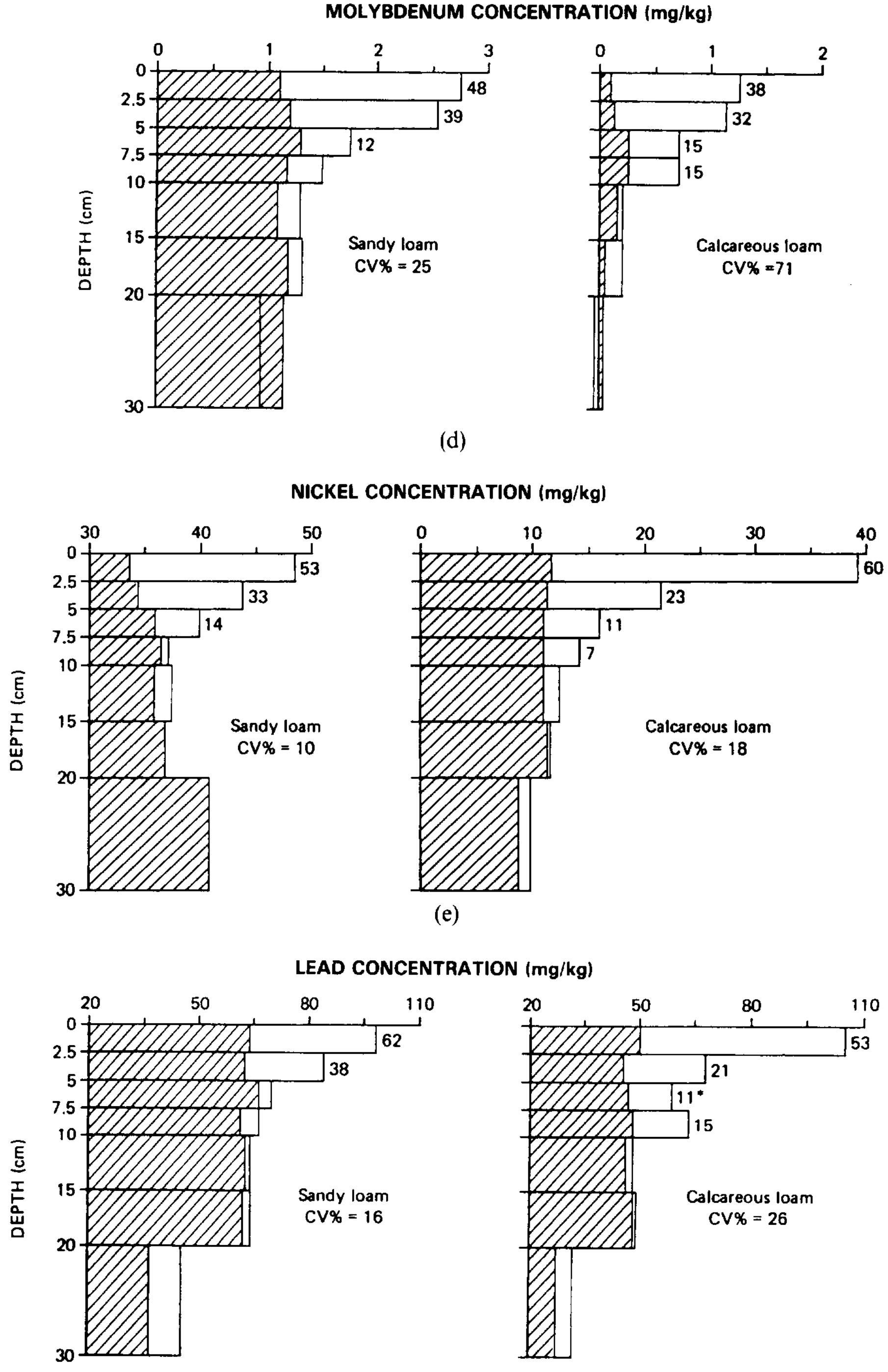


Fig. 2. Distribution of metals through soil profiles, 1984. Key: ▨, mean concentration of metal in unsludged soil (background), $n = 8$; ☐ ×, mean concentration of metal in sludged soil, × = percentage of total significant increase above background ($P < 0{\cdot}05$, or $P < 0{\cdot}1$ if marked*); CV%, coefficient of variation per cent (the standard error of individual experimental units expressed as a percentage of the grand mean).

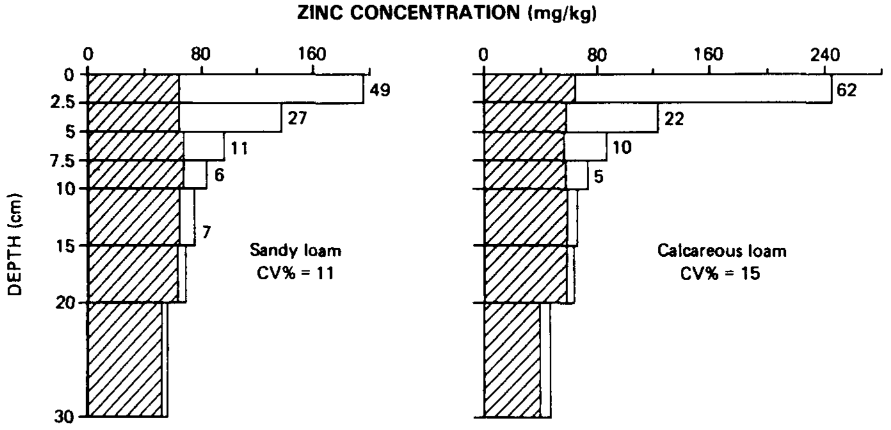


(d)

(e)

(f)

Fig. 2.—*contd.*



(g)

Fig. 2.—*contd.*

were broadly similar to treatment S1 in 1984. Increases, which were significant by an analysis of variance ($P < 0{\cdot}1$), have been calculated as percentages of the total significant increase measured at all depths. These percentages are included in Fig. 2. Also, the standard error of individual metal determinations is included, expressed as a percentage of the grand mean of each metal/soil combination (CV%).

Cadmium

The distribution of Cd down the soil profile was similar on both soil types. Figure 2 illustrating results for 1984, shows significant increases down to the 10 to 15 cm section on the calcareous loam. Of the total significant increase in Cd concentration, the average proportion which penetrated to 5 to 7·5 cm was 89% (both soils). Approximately four-fifths remained in the upper 5 cm.

Chromium

On the sandy loam, where the Cr concentration in the background soil exceeded the increase in concentration resulting from sludge addition, significant increases were confined to the upper 5 cm. Increases were significant to the 7·5 to 10 cm section on the calcareous loam, 84% of which was in the upper 5 cm.

Copper

Increases, illustrated in Fig. 2, were significant to the 10 to 15 cm section on both soils. Eighty-six to 90% of the total was found above 7·5 cm, and 74 to 80% in the upper 5 cm.

Molybdenum

Both background concentrations and increases for Mo were low, being 0 to 3·4 mg kg^{-1}, including the S2 treatments; consequently, standard errors were high, as indicated by high CV% (Fig. 2). Increases were still found to be significant to the 7·5 to 10 cm section on the calcareous loam, 70% of which was in the upper 5 cm. On the sandy loam, increases were significant only above 7·5 cm, with 87% in the upper 5 cm.

Nickel

As for Cr, background concentrations of Ni exceeded increases resulting from sludge addition in the sandy loam soil. Significant increases were above 7·5 cm on this soil, compared to 10 cm on the calcareous loam. Eighty-six and 83% of the total increase remained in the upper 5 cm of the sandy loam and calcareous loam, respectively.

Lead

On the sandy loam, Pb increases were not significant below 5 cm, as found for Cr on this soil. Again similar to Cr, background concentrations exceeded the increases due to sludge. Increases were significant to 7·5 to 10 cm on the calcareous loam, with 74% in the upper 5 cm.

Zinc

The distribution of zinc down the soil profiles was similar to Cu; significant increases in concentration were found to 10 to 15 cm on the sandy loam, and to 7·5 to 10 cm on the calcareous loam. Seventy-six to 84% of these increases remained in the upper 5 cm.

DISCUSSION

Mass balance

Metal concentrations in sludge and quantities of sludge applied were given earlier in Tables 1 and 3. The metal additions arising from the applications were given in Table 4. If the metal added had mixed evenly to a depth of 10 cm in the soil, and the density of the sludged soil is assumed to be 1 g cm^{-3}, then the expected increase in metal concentration in mg kg^{-1} is equivalent to the addition figure in kg ha^{-1}. Actual significant increases measured throughout any profile can be expressed as an equivalent uniform increase in a 10 cm column of soil. Hence amounts of sludge metal added to the plots can be compared with significant increases in the 30 cm depth sampled.

Table 5 compares the theoretical additions with the total significant increase found in the earliest set of soil samples taken in 1983, approximately one year after the final sludge application. Standard errors associated with the mean increases from which the effective increases to 10 cm were calculated are given (SEM). Errors associated with the theoretical additions could not be estimated from the available data, but are expected to be of the same order as the mean increase standard errors. Although there are no statistically significant losses or gains in Table 5, it is of interest that for all elements on the sandy loam soil and for four of the seven elements on the calcareous soil, there was an apparent net loss of metal. Other researchers have reported this effect on cultivated land (for example Chang *et al.*, 1984; Hinesly *et al.*, 1984). The results reported in Table 5 refer just to soil samples taken in 1983. This apparent initial loss of metal, over a period of four years since the sludge applications begun, might be explained by differences in

TABLE 5

Increases in Soil Metal Compared to Theoretical Additions

	Cd	*Cr*	*Cu*	*Mo* (*mg kg*$^{-1}$)	*Ni*	*Pb*	*Zn*
Sandy loam:							
Increase[a] (0–10 cm)	1·95	29·4	41·9	0·85	9·9	18·8	83·5
Addition[b]	2·59	32·0	44·8	1·42	14·4	28·5	93·6
Difference	0·64	2·6	2·9	0·57	4·5	9·7	10·1
SEM[c]	0·29	12·2	3·0	0·26	2·0	6·2	7·4
Calcareous loam:							
Increase[a] (0–10 cm)	2·96	29·8	42·5	0·95	11·2	23·1	71·3
Addition[b]	2·13	27·2	38·0	1·19	12·3	23·9	79·8
Difference	0·83	2·6	4·5	0·24	1·1	0·8	8·5
SEM[c]	0·28	2·9	3·0	0·29	1·3	4·5	6·3

[a] Increase = total significant ($P < 0{\cdot}1$) increase in metal concentration in 1983 (mg kg^{-1}) at any depth expressed as an effective increase in a 10 cm section of soil, density 1 g cm^{-3}.

[b] Addition = theoretical addition of total metal in sludge applications in kg ha^{-1}, which, if mixed evenly to a depth of 10 cm of soil density 1 g cm^{-3}, is directly equivalent to an expected increase in metal concentration in the soil profile (mg kg^{-1}).

[c] SEM = standard error of the mean increases in soil metal concentration measured (mg kg^{-1}).

actual soil density to that assumed of 1 g cm^{-3}. Expected increases in soil metal concentration from known additions would be lower for soil densities greater than 1 g cm^{-3}. In contrast, the effect of sludge dry solids added to the soil might be expected to reduce soil density by the addition of organic matter. The combination of these effects would be expected to act equally on all metal concentrations; however, apparent losses varied widely between different metals. Laboratory density measurements on the <2 mm air dry samples indicated a mean density for unsludged treatments of 0·99 and 0·87 g cm^{-3}, for the sandy loam and calcareous loam respectively. There was no significant variation with depth, or between unsludged and sludged treatments. Thus, allowing for density suggests that apparent losses may have been even greater, particularly on the calcareous loam. Also, subsequent losses occurred particularly for Cr, Ni, Pb and Zn on the sandy loam in later years (see Table 6).

Metal uptake by ryegrass growing on the plots during the period of sludge application has been estimated from metal concentrations in ryegrass during 1980 and yield figures covering all years. Results are summarised in Table 7. The crop uptake of each element is also expressed as a percentage of the total sludge addition, and these figures illustrate the negligible amounts that have been lost by this route from the total metal applied to soil.

Differences between years

To provide a summary of increases in soil metal concentrations due to sludge added, the total significant increases in the soil profile for each year,

TABLE 6
Total Significant Increase in Metal Concentration at all Depths Expressed as an Equivalent Increase in a 10-cm Soil Column (S1 Treatment mg kg^{-1})

	Sandy loam				*Calcareous loam*			
	1983	*1984*	*1985*[a]	$\bar{x}$[b]	*1983*	*1984*	*1985*	$\bar{x}$
Cadmium	1·95	2·22	1·95	2·09	2·96	2·52	2·60	2·70
Chromium	30	23	18	27	30	30	39	33
Copper	42	33	36	37	43	35	46	41
Molybdenum	0·85	0·86	1·15	0·86	0·95	0·77	1·44	1·05
Nickel	9·9	7·1	5·3	8·5	11·2	11·4	14·9	12·5
Lead	19	14	11	16	23	26	32	27
Zinc	84	67	66	76	71	73	102	82

[a] Figures extracted from smaller data set; three plots/treatment instead of eight used for all other sets.
[b] Mean of 1983 and 1984 only.

TABLE 7
Estimates of Metal Uptake by Ryegrass on Sludged Treatments

	Cd	*Cu*	*Ni*	*Pb*	*Zn*
			(S1-g ha^{-1})		
Sandy loam—four seasons, 1979–1982:					
Crop uptake	4·5	260	160	84	1 640
Total sludge addition (%)	0·17	0·58	1·1	0·29	1·8
Calcareous loam—three seasons, 1980–1982:					
Crop uptake	4·5	130	33	<30	720
Total sludge addition (%)	0·21	0·34	0·27	<0·13	0·90

expressed as an increase in 10 cm, are given in Table 6. On the sandy loam, decreases greater than 20% were seen between 1983 and 1984 for Cr, Cu, Ni, Pb and Zn. A comparison of mean metal concentrations in the S1 treatments between the same period indicated no significant loss of metals, considering concentrations throughout the 30 cm as a whole, but did show significant loss of Cu, Ni, Pb and Zn from the top 0 to 2·5 cm section specifically. The increases in metal concentrations for the calcareous loam fluctuated between years, but showed no overall trend.

Mechanisms of mobility

Metal movement from the surface down the soil profile could have occurred by physical infiltration of sludge particles following rainfall or by the action of the soil fauna, especially earthworms. Solubilisation and leaching of metals could also occur, and if important, this should be more evident on the sandy loam soil of lower pH. Judging from the distribution within the sampled profile (Fig. 2), Cd, Cr, Cu, Ni and Zn showed equal penetration on the two soil types. This suggests mobility was largely independent of soil type and this agrees with work by Brown *et al.* (1983) for Cd, Cu, Ni and Zn. When, however, the overall amount of sludge metal found in the sampled profile was compared with theoretical additions, there was some evidence of Cr, Ni and Pb losses from the 30 cm profile in 1983 on the sandy loam. Increases of these metals continued to drop in the following two years. There was further evidence of Cu and Zn loss from the 30 cm profile in 1983 and 1984, but not in 1985 on the same soil. The significance of these apparent losses could not be measured, because of uncertainty in errors associated with the theoretical additions of sludge metal added to soil. In contrast, there was no evidence of loss of any metals on the calcareous loam from the sampled profile in 1983, or in subsequent years. Formation of comparatively soluble anionic molybdate might explain greater movement down the profile of Mo on the calcareous soil. Estimated rainfall totals at each site were as follows (mm):

	During sludge application	During soil analysis	
		1983/84	1984/85
Sandy loam	2425	536	545
Calcareous loam	2099	596	653

The sludge applications began a year earlier on the sandy loam (Table 4) resulting in a higher total rainfall during sludge application compared to the calcareous loam. The yearly rainfall was higher on the calcareous loam and there is no evidence from this study that rainfall was associated with greater movement of metals down the profile of the sandy loam soil.

Significance of metal concentrations in the soil surface

Metal accumulation at the soil surface is important because of direct ingestion of soil by grazing animals, which represents a significant source of metals in the diet of ruminants (Field & Purves, 1964; Healy, 1968, 1970; Thornton, 1974; McGrath *et al.*, 1982). Animal health problems following ingestion of fluoride-rich sludge from the surface of grassland have been

TABLE 8
Metal Recovery and Concentration Factors at Different Sampling Depths (1984 Data)

Sampling depth (cm)	*Metal recovery (%)*[a]	*Concentration factor*[b]
2·5	55	2·20 ×
5·0	83	1·66 ×
7·5	92	1·23 ×
10·0	98	0·98 ×
15·0	100	0·67 ×
20·0	100	0·50 ×
30·0	100	0·33 ×

[a] Mean value for all elements on both soils.

[b] If sludge dressings to the soil surface applied × kg ha^{-1} of metal then the concentration which will result in a soil sample to the depth shown is indicated by the concentration factor assuming the soil has a density of 1·0 g cm^{-3} and other variables are as in the experimental work reported.

reported (Davis, 1980), but effects on animals of other contaminants ingested by this route need to be evaluated. An informative account of the problem in relation to sewage sludge and grazing animals is given by Fleming (1986). It is emphasised that surface accumulation can be avoided by occasional ploughing to mix sludge metals through the profile, or by using subsurface injection to apply sludge.

Depth of soil sampling for surface-dressed grassland has to be chosen to provide a practical estimate of the extent of accumulation in the upper few centimetres of soil and of the total addition of metal to the soil. Table 8 reports the estimated recovery of sludge-borne metal (based on total significant increase over background levels) at successive depths, together with a concentration factor indicating increases in metal concentration which sludge metal would produce in each sample. A sampling depth of 2·5 cm is impractical for operational purposes. Sampling to either 5·0 cm or 7·5 cm would recover most of the metal and show up metal accumulation at the surface. Sample depths of beyond 7·5 cm effectively collect the sludge metal, but cannot realistically indicate metal accumulation in the soil profile, because the effect of sludge is diluted by background soil from further down the profile. The results suggest that a sampling depth of 5·0 cm or 7·5 cm is appropriate for grassland, following surface applications of sludge.

TABLE 9

Percentage of Total Significant Increase in Metal Concentration Remaining in the Upper 5 cm

	Sandy loam				*Calcareous loam*			
	1983	*1984*	*1985*	$\bar{x}$	*1983*	*1984*	*1985*	$\bar{x}$
Cadmium	100	80	69	83	61	78	73	71
Chromium	100	100	99	100	87	84	67	79
Copper	77	74	62	71	67	80	64	70
Molybdenum	100	87	53	80	61	70	32	54
Nickel	79	86	100	88	88	83	64	78
Lead	100	100	100	100	82	74	61	72
Zinc	79	76	71	75	89	84	62	78

Table 9 presents details for each element of the increases found in different years at the 5·0 cm sampling depth on both soils. It shows how most of the metal is recovered by sampling to this depth and how the precise extent varies according to the element in question and the time after application of the sludge.

CONCLUSIONS

Metal increases above background values were observed to a depth of 30 cm on both soil types. Significant increases were found only to a depth of 10 cm on average for all seven metals, differences between metals being small. The order of mobility judged from increases to 30 cm was:

(i) sandy loam $Cu > Zn > Mo > Cd > Ni > Pb = Cr$
(ii) calcareous loam $Mo > Cd = Cu = Pb > Cr = Ni = Zn$

The principal mechanism of metal movement, independent of the soil types, was thought to be infiltration of sludge particles in soil water and movement by earthworms. There was also some evidence of Cr, Cu, Ni, Pb and Zn mobility beyond the sampled depth on the sandy loam soil of pH 6·5, but this was not conclusive. In contrast, on the calcareous loam soil Mo was the most mobile of the metals studied.

Of the metal added to the land through successive surface applications over 3–4 years, 60–100% (mean 87) could be accounted for in the top 30 cm. Of the total significant increase in metal concentration, on average 83% was found in the upper 5·0 cm of soil and 92% in the upper 7·5 cm. Sampling to either of these depths would be suitable for monitoring grassland surface-dressed with sludge, especially to assess hazard in relation to grazing cattle. These findings provide a basis to specify a sampling depth for grassland in guidelines for sludge utilisation on land. The UK Ministry of Agriculture booklet on sludge (MAFF, 1982) already recommends a sampling depth of 7·5 cm for grassland.

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