AVAILABILITY OF Cd, Ni AND Zn TO RYEGRASS IN SEWAGE SLUDGE-TREATED SOILS AT DIFFERENT TEMPERATURES

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Abstract. A pot experiment to compare the availability of Cd, Ni and Zn to ryegrass (*Lolium perenne* L.) was conducted at 15 and 25 °C. For this purpose, three rates of sewage sludge (0, 10 and 50 t ha^{-1}) were applied in a loamy sand (LS) and a clay loam (CL). Heavy metal availability assessed by soil extractions with 0.05 M $CaCl_2$ and the organic matter content were monitored during a period of two years, while uptake by ryegrass was monitored over one year after addition of the sludge. The concentrations of Cd and Ni in both the ryegrass and the soil extracts increased significantly, during the first year, especially at 50 t ha^{-1}. However, in the second year metal availability reached a plateau. During the first year, in the ryegrass Zn concentrations did not show an increase, but in the soil $CaCl_2$-extracted Zn increased. During the same period, the organic matter content decreased rapidly, especially at 25 °C, in the first year and much more slowly in the second, giving a total decrease of 16%. Temperature had a marked effect on metal availability; both soil extracts and plant samples from the 25 °C treatment had greater concentrations of Cd, Ni and Zn than those at 15 °C. This may be attributed to the organic matter, which decomposed more rapidly at 25 °C. Moreover, soil-plant transfer coefficients (Tc) of the metals were significantly higher at 25 °C than at 15 °C, with Cd showing the greatest difference, followed in decreasing order by Zn and Ni.

Keywords: $CaCl_2$ extraction, heavy metals, metal bioavailability, organic matter, temperature, time

1. Introduction

Sewage sludge is the residue product of wastewater treatment and has been used in agriculture for many years as a fertiliser containing organic matter and macro-and micronutrients. However, it contains heavy metals, which may have adverse effects on crops and possibly humans (Solar-Rovira *et al.*, 1996). The ‘available’ fraction of heavy metals is that which can be readily mobilised in the soil environment and taken up by plant roots. Organic matter exerts a major control on the availability of metals to plants (Towers and Paterson, 1997; McBride *et al.*, 1997) and it is especially responsible for reducing the availability of Cd and Zn to plants. It has been reported that organic matter diminishes metal toxicity symptoms, because it adsorbs heavy metals and removes them from the soil solution (Alloway and Jackson, 1991; Bell *et al.*, 1991; El-Hassanin *et al.*, 1993). However, low molecular weight fulvic acids (forming part of the dissolved organic carbon, DOC) may increase metal mobility, as they can complex metals previously bound onto soil

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solid constituents and affect their speciation and solubility (Hamon *et al*., 1995). Organic matter is a dynamic entity in the soil and the amount and type of organic matter can vary with time. Temperature can have marked effects on the rate of decomposition of organic matter: increasing temperature accelerates the microbial decomposition process. Consequently, where sewage sludge has been applied, there is the possibility that high temperatures may lead to greater quantities of sludge-borne heavy metals being available to plants (Chang *et al*., 1997). This has important environmental implications, because crops on sludge-amended soils in warmer areas (such as the Mediterranean region) may take up proportionally more metals than those in cooler climates with similar concentrations of heavy metals applied in sewage sludges. However, the role of different ambient temperatures in the availability of heavy metals has not been widely recognised (Hooda and Alloway, 1993), and there is a need for more experimentation in order to be able to assess this risk.

The hypothesis, which this study was based on, is that higher temperature might affect the organic matter decomposition pattern, which in turn may increase the availability of heavy metals. The objective of the work was to investigate the effects of temperature on the organic matter status and the availability of heavy metals to plants in soil-sludge mixtures over a residual time of two years. The temperatures selected (15 and 25 °C) were intended to represent humid temperate conditions, such as in North Western Europe and Mediterranean climatic conditions, respectively.

2. Materials and Methods

2.1. Experimental design

Two Brown Earth soils were collected in bulk from the University of Reading Sonning Farm. One soil was a clay loam (CL soil) of the Sonning series and the other was a loamy sand (LS soil) of the Rowland series. Sewage sludge was obtained from a sewage treatment plant and had 8% dry matter content. Some characteristic chemical and physical properties of soils and sludge are shown in Table I. These included: pH, organic matter by loss on ignition, $CaCO_3$, Al and Fe oxides, electrical conductivity and bulk density (Rowell, 1994), soil texture (Sheldrick and Wang, 1993), water-soluble cations (Rhoades, 1982), and total Cd and Zn concentrations (McGrath and Cuncliffe, 1985). Soils and sewage sludge were put in 4 L capacity pots and mixed thoroughly at sludge rates equivalent to 0, 10 and 50 t ha^{-1}. Half of the pots were placed in a greenhouse, maintained at 25 °C, and the other half in a constant temperature room at 15 °C. All pots were sown with twenty ryegrass (*Lolium perenne* L.) seedlings. The mixtures were maintained at field capacity moisture status, by regular irrigation from beneath.

TABLE I

Selected physical and chemical properties of the soils and sludge

	%	Dithionite extracted (%)		Water soluble (μg g^{-1})			
	$CaCO_3$	Al	Fe	Ca^{2+}	K^+	Na^+	Mg^{2+}
CL soil	3.00	0.13	1.32	265.00	12.90	37.15	7.65
LS soil	1.10	0.09	0.93	46.80	7.65	16.80	2.65
Sludge	–	–	–	–	–	–	–
	Sand	Silt	Clay	Texture	pH		LOI[a]
	(%)	(%)	(%)		H_2O	$CaCl_2$	(%)
CL soil	19.87	40.07	40.06	CL	7.65	7.19	10.41
LS soil	78.37	14.64	6.99	LS	7.21	6.68	2.37
Sludge	–	–	–	–	8.15	7.45	62.42
	EC[b]	CEC[c]	Bd[d]	*Aqua regia*-digested (μg g^{-1})			
	(dS m^{-1})	(cmol$_c$ kg^{-1})	(g cm^{-3})	Cd	Ni	Pb	Zn
CL soil	0.91	49.03	0.92	0.52	20.88	25.58	48.69
LS soil	0.17	8.47	1.21	0.34	13.93	17.82	34.69
Sludge	–	–	0.75	7.31	39.82	230.7	532.8

[a] Loss-On-Ignition.
[b] Electrical conductivity.
[c] Cation exchange capacity.
[d] Bulk density.

2.2. METHODOLOGY

Soil and plant samples were taken out of the pots at regular intervals (Weeks 0, 2, 4, 8, 16, 32, 48, 72 and 100 for the soil samples and Weeks 7, 14, 21, 28, 35, 44 and 51 for the ryegrass). It should be noted that in Week 14 there was a visible insect infestation, and in Week 28 a failure in the greenhouse heating. These adversely affected the metal concentrations in the plants. Soil samples were air-dried, lightly ground to pass through a 2 mm sieve and stored in plastic bags at room temperature until analysis. The samples were then analysed for organic matter by Loss-On-Ignition. 0.05 M $CaCl_2$ (Morgan and Alloway, 1984) was used to extract the 'available' fractions of Cd, Ni and Zn in the soil. This method has been reported as a useful index of biological availability for these metals (Andrewes *et al*., 1996). Total concentrations of Cd, Ni and Zn (Table IIa) were determined by *Aqua regia* digestion. For quality control reasons, the concentrations of Cd, Ni and Zn expected to be measured in the soil-sludge mixtures were also calculated for better comparison and they are presented in Table IIb, along with the quantities of sludge for each treatment in g pot^{-1} for easier comparison of the amounts applied. Metal availability was also assessed by calculating the Transfer coefficient (Tc) values of the metals. A Tc given by the formula $Tc = [M]_{plant}/[M]_{soil}$, where $[M]_{plant}$ is the concentration of a metal in the tissue of the test plant and $[M]_{soil}$ is the total concentration of the same metal in the soil where this plant was grown. The plant material was collected with stainless steel scissors and was washed with de-ionised water. After harvest the plants were dried in a forced draught oven at 75 °C until no further loss in their weight was observed, then ground and stored at room temperature in glass jars. The concentrations of Cd and Zn in the grass powder were determined by digestion with concentrated HNO_3. All soil extracts and digests and plant material digests were analysed for Zn and Ni concentrations with a Perkin Elmer Optima-3000 Inductively Coupled Plasma-Optical Emission Spectrometer. Cadmium concentrations in $CaCl_2$ soil extracts were analysed with a Graphite Furnace Atomic Absorption Spectrophotometer (Perkin Elmer 3030). For the extraction of DOC, 1:10 soil:water w/v ratio was shaken overnight, centrifuged and the supernatant was filtered through a 0.45 μm polysulfon membrane filter. The clear solution was stored in a freezer (–18 °C) until the analysis (Baham and Sposito, 1983) and it was then measured with a Shimadzu TOC-5000 Total Organic Carbon analyser. The statistical analysis was done with the SAS package, processing the data for Analysis of Variance (ANOVA). The LSD (Least Significant Difference) was then calculated for determining whether the treatments are significantly different. Analytical quality assurance was addressed by the systematic use of blanks, duplicates and analysis of a Certified Reference Soil (CRM 143R) and in-house sludged soil and barley leaf standards. Results of this quality control indicated that concentrations are within 10% error.

3. Results

3.1. Concentrations of Cd, Ni and Zn in ryegrass

The heavy metal concentrations in ryegrass were significantly higher at the higher sludge application rate (Figure 1) (please note units are in μg g^{-1}). Temperature also showed an effect on the plant concentrations of Cd and Zn. However, the concentrations of Ni in ryegrass did not show significant differences between temperature treatments. In both soils, the availability of Cd, Ni and Zn to plants was significantly higher at 25 °C than at 15 °C, and this increase was further enhanced at 50 t ha^{-1}. At 10 t ha^{-1} the differences between the two temperatures were only significant in the LS soil for Cd, and in the CL soil for Zn. The 50 t ha^{-1} sludge treatments are 5 times higher than would normally be used on agricultural and the increase in metal availability to plants between temperatures was much more substantial. For greater sludge applications the risk is much more apparent, even for soils with a high initial organic matter content (such as the CL soil in this study).

There was a trend of increasing Cd and Ni concentrations accumulated over time by ryegrass at 25 °C, except from the controls. Only the concentrations of Zn

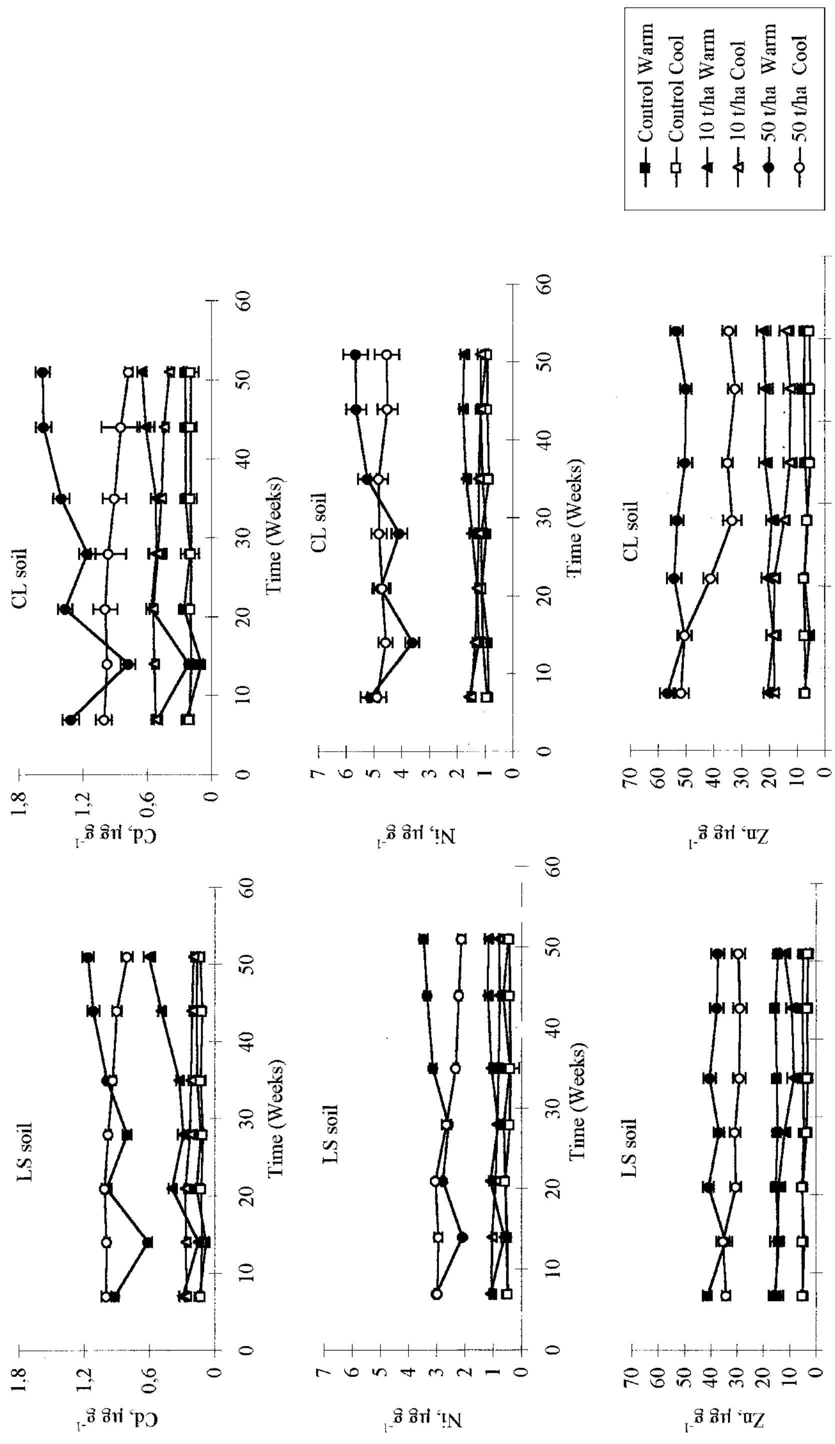

Figure 1. Concentrations of Cd, Ni and Zn in the ryegrass (error bars represent the standard error of the four replicates each data point is the mean of).

TABLE IIa
Total concentrations of Cd, and Zn in the sludge-soil mixtures (μg g^{-1})

	LS soil			CL soil		
	Cd	Ni	Zn	Cd	Ni	Zn
Control	0.34 a	13.93 a	34.69 a	0.52 a	20.88 a	48.69 a
10 t ha^{-1}	0.38 ab	15.88 b	40.32 b	0.57 ab	21.51 b	53.27 b
50 t ha^{-1}	0.42 c	22.41 c	58.76 c	0.84 c	23.46 c	80.55 c

Different letters mean significantly different numbers within a column.

TABLE IIb
Added sewage sludge and expected concentrations of Cd, and Zn in the soil-sludge mixtures

	Sludge added (g sludge pot^{-1})	LS soil Concentrations of metals expected (μg g^{-1})			Sludge added (g sludge pot^{-1})	CL soil Concentrations of metals expected (μg g^{-1})		
		Cd	Ni	Zn		Cd	Ni	Zn
Control	–	0.34	13.93	34.69	–	0.52	20.88	48.69
10 t ha^{-1}	24.79	0.40	14.03	39.60	32.61	0.60	21.33	54.34
50 t ha^{-1}	123.96	0.64	15.35	57.20	163.05	0.92	23.05	77.30

in ryegrass stayed unchanged throughout the one-year period in the 7 consecutive harvests. However, at 15 °C in the CL soil Zn in ryegrass showed a decrease over time in the 50 t ha^{-1} treatment and this was also found for Cd in both soils, although less marked, while Ni showed a decrease with time in the LS soil.

3.2. Cd AND Zn EXTRACTED WITH $CaCl_2$

In the 50 t ha^{-1} treatment, soil samples taken from the pots at 25 °C had greater $CaCl_2$-extractable metal concentrations than those from pots at 15 °C (Figure 2) (please note units are in μg kg^{-1}). This effect was particularly significant in the CL soil and the difference between temperatures was even greater in the higher sludge application rates. At 10 t ha^{-1}, however, in the CL soil, temperature did not have a very significant effect. The significant increase of metal concentrations with temperature in both the $CaCl_2$ extractions and ryegrass is also shown in Table III. In almost all cases, the levels of the metals were significantly higher at the end of the two-year residual period of the experiment than at the beginning (LSD 1.12 for Cd and 52.34 for Zn, $P < 0.05$). In the case of Ni, its concentrations increased in

TABLE III

Percentage increases in plant availability and soil extractability of Cd, Ni and Zn between 15 and 25 °C in the 50 t ha^{-1} treatment. The values are the means of the measurements taken between week 0 and week 100

	Plant concentration		$CaCl_2$ extractability	
	LS soil	CL soil	LS soil	CL soil
Cd	60	10	17	6
Ni	NS[a]	NS	10	NS
Zn	26	8	7	4

[a] Not significant.

the LS soil did not change significantly in the CL soil over time (LSD 20.23, $P < 0.05$).

3.3. Organic matter

The application of sewage sludge significantly increased the organic matter content of the soils in all treatments. After the beginning of the experiment the organic matter content of the soils was dependent on temperature. The decomposition of the organic matter was more rapid in the warm environment. This decomposition, however, was apparent even at 15 °C. The results in Figure 3 suggest that this decline occurred mostly in the first 4 months of the experiment; after that point the organic matter seemed to decline very slowly.

3.4. DOC concentrations

DOC increased over time in the CL soil, which initially (before sludge application) contained more organic matter (Table IV). DOC also increased in the control sludge treatment. The DOC status in the LS soil did not have the same pattern as that of the CL soil. In the LS soil, DOC showed a small decline in both the control and the 50 t ha^{-1} treatments.

3.5. Transfer coefficients

The Tc values were calculated taking into account the means of all measurements of Cd, Ni and Zn over time. The Tc value gives an indication of the mobility of the elements in the soil and this is particularly important for the heavy metals. High Tc values at high sludge application rates may indicate a risk of accumulation of this metal in the human food chain. The elevated sludge application rates led to greater Tc values for Cd, Ni and Zn in both soils, probably because the quantities

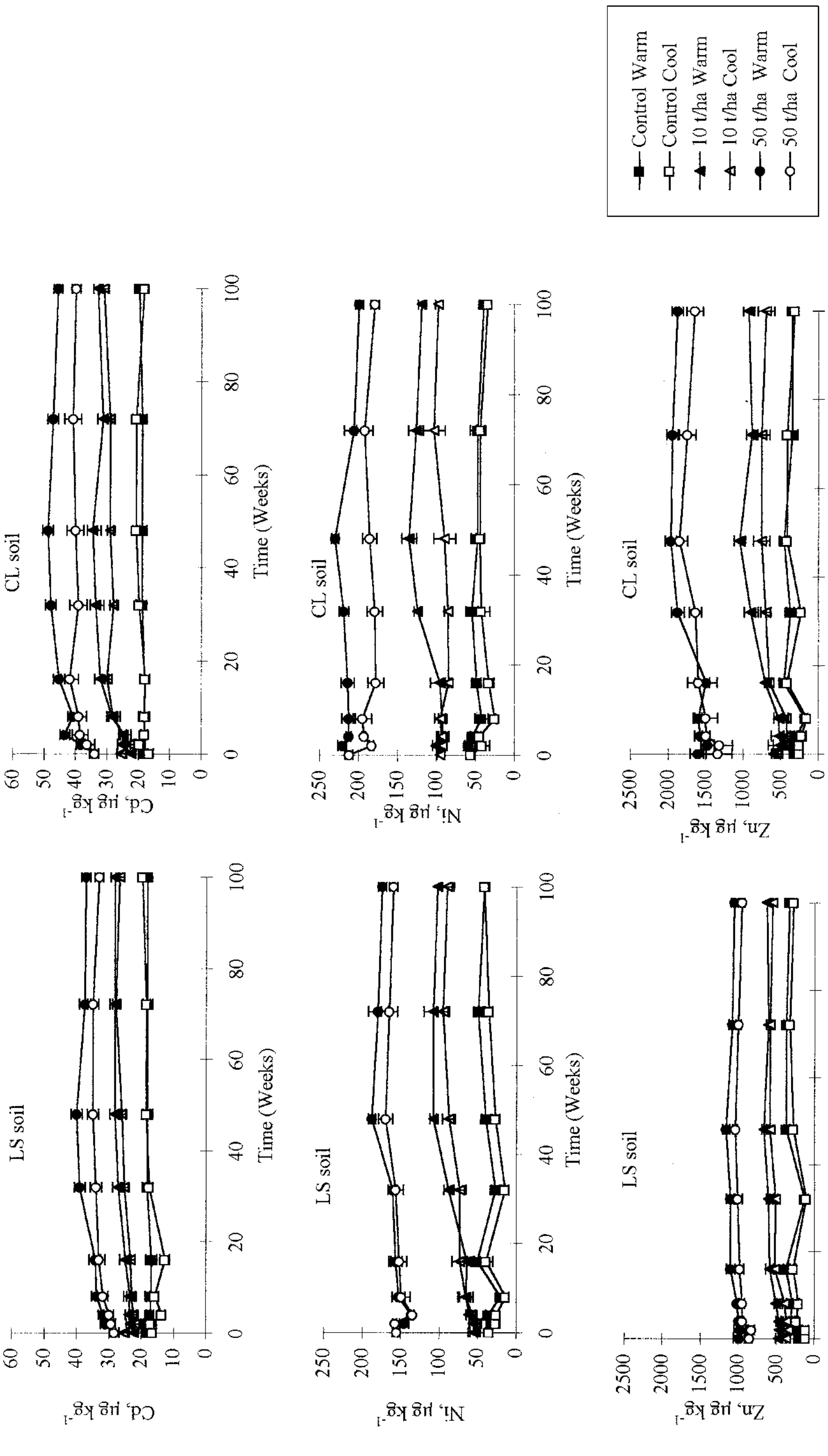

Figure 2. Concentrations of Cd, Ni and Zn extracted with $CaCl_2$ (error bars represent the standard error of the four replicates each data point is the mean of).

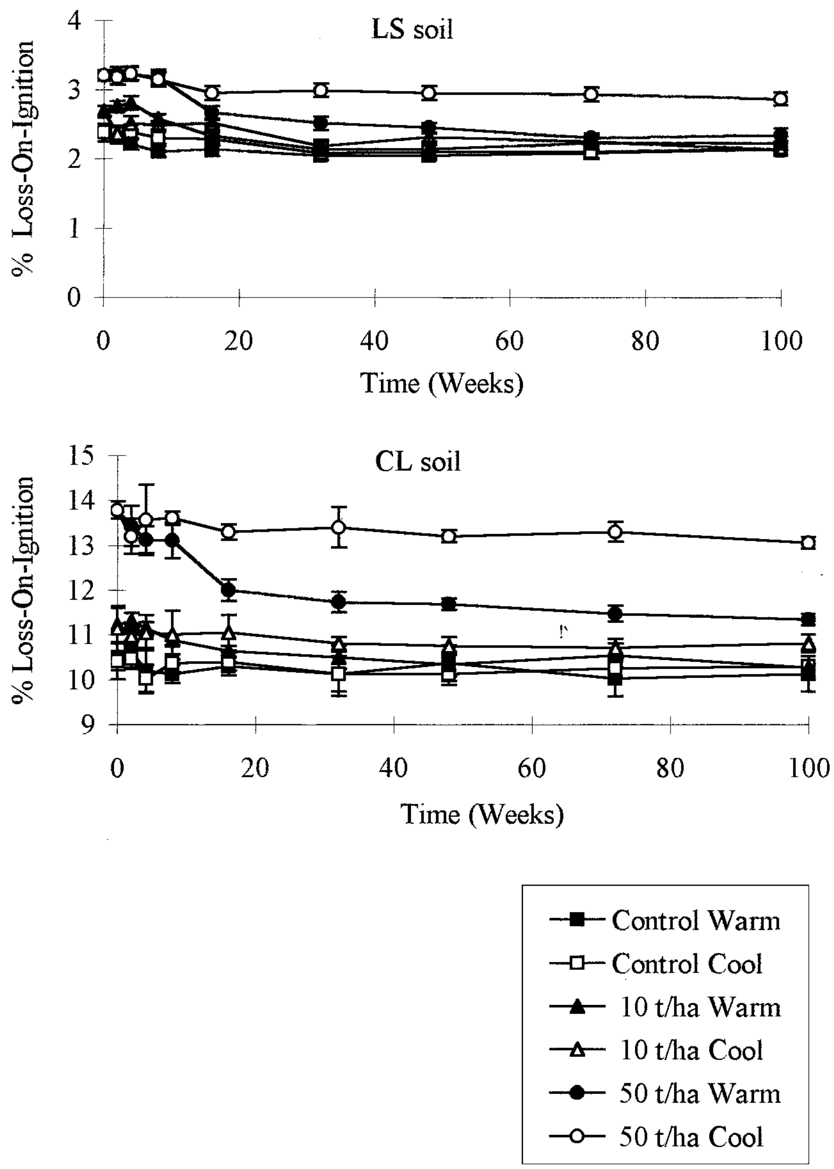

Figure 3. Percentage Loss-On-Ignition (error bars represent the standard error of the four replicates each data point is the mean of).

of the heavy metals introduced with the sludge were substantially higher at 50 t ha^{-1}. Besides, the Tc values were higher at 25 °C than at 15 °C.

TABLE IV

DOC status over time (mg L^{-1})

	LS soil		CL soil	
	Week 4	Week 100	Week 4	Week 100
Control	26.4	19.0	54.8	58.1
50 t ha^{-1}	36.9	33.5	60.2	66.9
LSD test:	Time × sludge = 2.1		Time × sludge = 3.8	

TABLE V

Transfer coefficients of Cd, Ni and Zn (where Tc = $[M]_{plant}/[M]_{soil}$. For the calculation of Tc the means of all measurements of Cd, Ni and Zn over time in plant and soil samples were taken into account)

	LS soil					
	25 °C			15 °C		
	Cd	Ni	Zn	Cd	Ni	Zn
Control	0.56 a	0.04 a	0.16 a	0.43 a	0.03 a	0.08 a
10 t ha^{-1}	1.56 b	0.09 a	0.36 b	0.48 a	0.05 a	0.29 b
50 t ha^{-1}	2.74 c	0.21 b	0.65 c	1.75 b	0.12 b	0.48 c
	CL soil					
	25 °C			15 °C		
	Cd	Ni	Zn	Cd	Ni	Zn
Control	0.45 a	0.04 a	0.15 a	0.40 a	0.04 a	0.13 a
10 t ha^{-1}	1.16 b	0.08 a	0.45 b	0.65 a	0.04 a	0.24 b
50 t ha^{-1}	1.72 c	0.21 b	0.68 c	1.00 b	0.20 b	0.42 c

Different letters mean significantly different numbers within a column.

4. Discussion

The extractability and plant availability of Cd, Ni and Zn were affected by the additions of sewage sludge to the soils as in the 50 t ha^{-1} treatment they were significantly higher than the control. Even at 10 t ha^{-1} metal extractability and availability were higher than the control (for Cd in the LS soil and for Zn in the CL soil). This is important, because it shows that even in sludge applications similar to the rates applied in common agricultural practice (8–10 t ha^{-1}) there is a risk of enhancement of heavy metal availability under warmer conditions, as far as potentially mobile elements such as Cd and Zn are concerned. The higher metal extractability and availability with the higher sludge application was also shown by the Tc values, which were higher at 50 t ha^{-1} than at 10 t ha^{-1} and the control. This may also indicate that Cd, Ni and Zn were less strongly bound onto the soil surfaces than the indigenous metals before adding the sludge. Besides, the trends of increase over the two-year period in the amounts of Cd and Ni accumulated in the foliage of the ryegrass and the changes in the amounts of metals extracted by $CaCl_2$ at 25 °C were significant. However, at 15 °C the metal concentrations in ryegrass declined over time. This decreasing trend was also reported by Villaroel *et al*. (1993) and Chang *et al*. (1983), and may have been caused by the weaker growth of the plants at 15 °C, compared with those plants grown at 25 °C.

On the other hand, Zn levels did not significantly increase in the ryegrass over time in each of the sludge application rates. This is difficult to explain, because Zn is generally regarded as being relatively mobile and similar in behaviour to Cd (Evans, 1989). It is interesting to note that the increase over time in Cd and Ni concentrations happened concurrently with the decomposition of the organic matter in the soil-sludge mixtures. The decomposition of the organic matter leads to the breaking of the humic macro-molecules and this process could release metals formerly bound onto the organic matter into more labile forms, thus increasing metal availability to plants (Alloway, 1997). This process may have facilitated the increase over time of the plant available fractions of Cd and Ni in the soil system. McBride (1995) also stated that sewage sludge-borne organic matter will not possess the same ability to sorb metals over time, as this ability is likely to decrease with maturation, probably due to decomposition. This increase in Cd and Ni extractability and plant availability over time shown in this study was also found by White *et al*. (1997). They found (under similar ambient temperature conditions and sludge application rates) that over a nine-year period Cd, Pb and Zn extractability was significantly higher at the end of the experiment than at the beginning. Their work provided evidence that heavy metal extractability may be significantly affected by the decomposition of organic matter, in the residual period after the application of sewage sludge.

The decomposition of organic matter can have another effect; it may lead to the formation of low molecular weight organic ligands (DOC), which are believed to increase metal availability. In this experiment DOC levels increased in the CL soils, but decreased in the LS soil at both control and 50 t ha^{-1}. This suggests that the decomposition of the organic matter in the CL soil did not significantly contribute to organic compounds of the molecular weight range that characterises the DOC compounds (less than 1000 g mol^{-1}) (Harter and Naidu, 1995). This could also be an indication that the low molecular weight organic compounds were not so strongly adsorbed and thus more prone to leaching than they were in the CL soil. However, this behaviour of DOC over time showed that the effect of DOC on the rate of increase that took place over time in the $CaCl_2$ extractions in the two soils was not very clear. This suggests that the predominant mechanism which led to the increase in metal availability was not the formation of soluble organo-metallic complexes, but the release of the metals into forms which are easier for the plants to absorb (into the soil solution or loosely bound onto soil surfaces). The same mechanism was also suggested by Hani *et al.* (1996).

Cadmium, Ni and Zn concentrations in the ryegrass and their soil extractability were higher at 25 °C than at 15 °C during the two-year period. This increase in metal availability was shown in the Tc values, which were consistently higher at 25 °C than at 15 °C for all metals and all sludge application rates. Hooda and Alloway (1993) found that increasing temperature may increase the plant uptake of heavy metals. They argued that this was probably caused by reduced plant growth at lower temperatures. However, the results of this study indicate that the behaviour in plant uptake was also caused by the chemical reactions in the soil system. Temperature significantly affected heavy metal extractability, especially in the CL soil and at the higher sludge application rates. It should be noted that plant concentrations were affected by temperature more significantly in the same soil at the 50 t ha^{-1} than at 10 t ha^{-1}. Since $CaCl_2$ extractions showed the same trends in metal availability as shown in Table III the increase in metal uptake cannot only have happened due to higher evapotranspiration rates at 25 °C. The reason is probably the decomposition of the organic matter which was more rapid at 25 °C probably due to more intensive microbial activity. This decomposition process is likely to have released metals into more bioavailable forms.

In this study, Cd was the most mobile element, as it had greater Tc values than Zn by a factor of 3–5. Cadmium also had greater Tc values than Ni by a factor of approximately 10. The same was indicated in Table III, where Cd has by far the most substantial increase between 15 and 25 °C, followed by Zn and Ni. The same was also reported by Li and Shuman (1996), Sloan *et al.* (1997) and Moreno *et al.* (1996).

5. Conclusions

Organic matter played a key role in heavy metal availability and plant uptake. Fast decomposition of organic matter (encouraged by higher temperature regime) might have led to greater quantities of heavy metals being taken up by plants as it was evident from the 25 °C treatments. This gave rise to greater Tc values, which may suggest greater mobility of heavy metals and possible risks of food chain accumulation in cases of heavy sewage sludge applications. Temperature also had a marked effect on heavy metals, enhancing their availability to plants especially in the higher sludge application rates. Cadmium was more mobile than Ni and Zn in this study; the order of mobility according to Tc values was Cd > Zn > Ni.

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