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Orawan Siriratpiriya, Einar Vigerust and
Alf Reidar Selmer-Olsen

Effect of temperature and heavy metal application on metal content in lettuce

Virkning av temperatur og tungmetalltilførsel
på metallinnhold i salat

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   2. SKAARE, S. 1958. Forsøk med søtlupin. Forsk. Fors. Landbr. 9: 629–641.
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Effect of temperature and heavy metal application on metal content in lettuce

Virkning av temperatur og tungmetalltilførsel på metallinnhold i salat

BY

ORAWAN SIRIRATPIRIYA[1]), EINAR VIGERUST AND ALF REIDAR SELMER-OLSEN[2])

CONTENT

[1]) Present address: The Institute of Environmental Research, Chulalongkorn University, Phyathai Road, Bangkok, Thailand.
[2]) Chemical Analytical Laboratory, Agricultural University of Norway.

I. INTRODUCTION

The main purpose of this experiment has been to clearify the effect of temperature on the uptake of heavy metals in plants. The study was carried out in phytotrone with 6 different temperatures ranging from 9 to 24°C. The same amounts of heavy metals were applied as sewage sludge or inorganic salts to the soil and combined with different liming.

Some investigations concerning heavy metal uptake has been carried out elsewhere with different soil temperatures (e.g. soil heating). This, however, is not quite the same as changing the temperature in the growing cells.

The effect of soil temperature seems to be well documented as far as the effect of Zn is concerned. Less investigations seem to be done about the effect of temperature on uptake of other metals.

ELLIS et al. (1964), BAUER & LINDSAY (1965), MARTIN et al. (1965) and WALLACE et al. (1969) found increased availability for zinc at increasing soil temperatures.

HAGHIRI (1974) and CUTLER & RAINS (1974) reported increased Cd-concentration in plants at increasing soil temperature.

According SHEAFFER et al. (1979) increasing soil temperature did not consistently affect Cu, Ni, Cd or Pb concentration in maize tissue. Zn concentrations generally increased as soil temperature rose.

The concentration of Cd and Zn in several vegetables grown in sludge-ammended soil increased after soil heating (GIORDANO & MAYS 1977). GIORDANO et al. (1979) found in field experiment higher uptake of Cu and Zn in different crops as a result of soil heating.

RUSH & JARRELL (1981)found higher concentration in radish tops and roots of Ni, Zn, Mn, Cd and Cu at 30°C soil temperature than 15°C.

Cu content in maize and bush bean tended to increase with soil temperature while Mn and Al tended to decrease at the high temperature (WALLACE et al. 1969).

ARVIK and ZIMDAHL (1974)did not find any influence of soil temperature on lead uptake in plants.

LAL (1974) found small effects of soil temperatures upon heavy metal uptake by plants.

An important goal today is to be able to use soil analysis as a basis for predicting plant uptake of heavy metals. Soil pH has a thorough effect on the availiability of most metals. Therefore, neutral salts as 0.1 M $NaNO_3$ (HAENI & GUPTA 1983) and 0.05 M $CaCl_2$ (SAUERBECK & STYPEREK 1983) are recommended as soil extractants. Both methods are used in this study.

II. MATERIALS AND METHODS

Pot experiment was conducted in phytotrone with the following temperature levels: 9, 12, 15, 18, 21 and 24°C. The crops was lettuce (Wheelers Tom Tumb).

The humidity of the air blown through the cells was higher at higher temperatures in order to get approximately the same evaporation from the pots at different temperature levels. The soil material consisted of sand, with medium sand as the dominant fraction. To increase the buffer capasity of the soil 20 volume pst. loam and 10 volume pst. peat soils were added. The content of heavy metals in the soil and sewage sludge was, mg per kg dry matter:

	Cd	Zn	Ni	Cu	Mn	Fe	Mo
Soil	0.01	27.7	6.3	6.2	155		0.71
Sludge	3.41	1 050	68.3	572	34.1	10 200	6.35

The organic matter content of the mixtures was 3 pst. of the dry soil by weight. The clay content of the mixture was 4.5 pst. and with a silt fraction of 8 pst.

The following treatments were used:

A) Control.

B) Application of sewage sludge, 60 tons DM/hectare.

C) Heavy metals, amount per pot as B) applied only as inorganic chlorides.

The following heavy metals were applied: Cd, Zn, Cu, Ni, Mn and Mo. The application rate was based on the sludge analysis. The sewage sludge, from Bekkelaget purification plant, Oslo, was anaerobically digested, frozen and then air-dried. After freezing the sludge had a loose structure and this facilitated a thorough mixing with the soil.

The soil mixture had pH 5.4. A preliminary study showed that application of 1500 kg $CaCO_3$ per hectare should give a desired pH value of 5.6–5.8. This application rate was given to 3 replicates; to 1 pot per treatment $CaCO_3$ in amounts equivalent to 3000 kg/hectare, was applied.

To the treatments A) (Control), and C) (chemicals) compound fertilizer, type B, was applied in amounts equivalent to 1000 kg/hectare, approximately with the same nitrogen fertilizer effect as treatment B.

To treatment B, sludge, no N or P fertilizer was applied. The same amount of K as A and C was applied in sulphate form. $MgSO_4$, 1000 kg/hectare was applied to all pots.

5 l pots were filled with 6.6 kg dry soil. The pots were sown with lettuce and after germination the lettuce was thinned out to 4 plants per pot.

Soil samples were taken every second week, one mixed sample per replicate pots was taken after 2 weeks, later one sample per pot. The harvest followed this scheme:

Weeks after start:

T °C	24	21	18	15	12	9
1st harvest	6	6	7	8	10	12
2nd harvest	6	6	7	8		

An attempt was made to harvest at approximately the same stage of plant development and yield.

The yield, therefore, does not express the effect of temperature upon the growth. The time from sowing until harvesting does not reflect differences in botanical development. There was e.g. much difference in germination. At lower temperatures the lettuce also remained in the seedling stage for a longer period of time.

After the 1st harvest and soil sampling, the soil left in every pot was mixed, before sowing. Because lower pH than desired occurred during the first period, additional lime was mixed in for 2 of the replication pots. For each treatment the lime dosage was as follows:

	a	b	c	d
kg $CaCO_3$/hectare	1500	3000	4500	6000

with a) and b) unchanged from the first period.

The experiment was started at the end of February, and a change in the length and intensity of day-light occurred during the period. Artificial light, in addition to day-light, was used until the middle of April.

On the whole there has been little change in growth conditions during experiment period.

Because the lack of time we had to discontinue the experiment before the 2nd harvest at the temperatures 9 and 12°C.

After harvest the lettuce plants were thoroughly washed in destilled water. From each pot two samples were analysed.

After homogenization and drying the samples were ashed overnight at 400°C. This ash was treated with H_2SO_4 (1:1) in order to transform volatile salts to less volatile sulphates. After drying the temperature was raised to 700°C. All elements except Mo were determined by atomic absorption spectrophotometry in an acid solution of the ash. Mo was determined spectrophotometrically in a diisopropylether extract after treating an aliquote of the ash solution with Fe (III), SCN^- and Sn (II).

Ammonium and nitrate in soil were determined colorimetrically in a 2M KCl extract by respectively using phenol-hypochlorite (SELMER-OLSEN 1971) and naphtylethylenediamine and sulphanilamide after reduction to NO_2^- by a Cd-reductor (HENRIKSEN & SELMER-OLSEN 1970).

Soil samples taken from each pot after 2nd harvest and from the temperatures 24, 21 and 15°C were extracted with 0,05 M $CaCl_2$ and 0.1M $NaNO_3$ and the heavy metal content was determined.

III. RESULTS

A. SOIL PH

Soil pH is an important basis for the plant availiability of heavy metals.

In the preliminary study different amounts of lime were applied in order to get an optimal soil pH for the experiment (5.7 - 5.8).

The start pH was 5.8 (1500 kg $CaCO_3$/ha). The pH dropped, however, for all treatments, especially where sewage sludge was applied. This can be a result of the decomposition of organic matter. Fig. 1 and 2 show the development of pH during time and the influence of temperature (treatment B, sludge). At the highest temperatures there was a quick drop in pH and then a weak tendency to increase for the remaining time.

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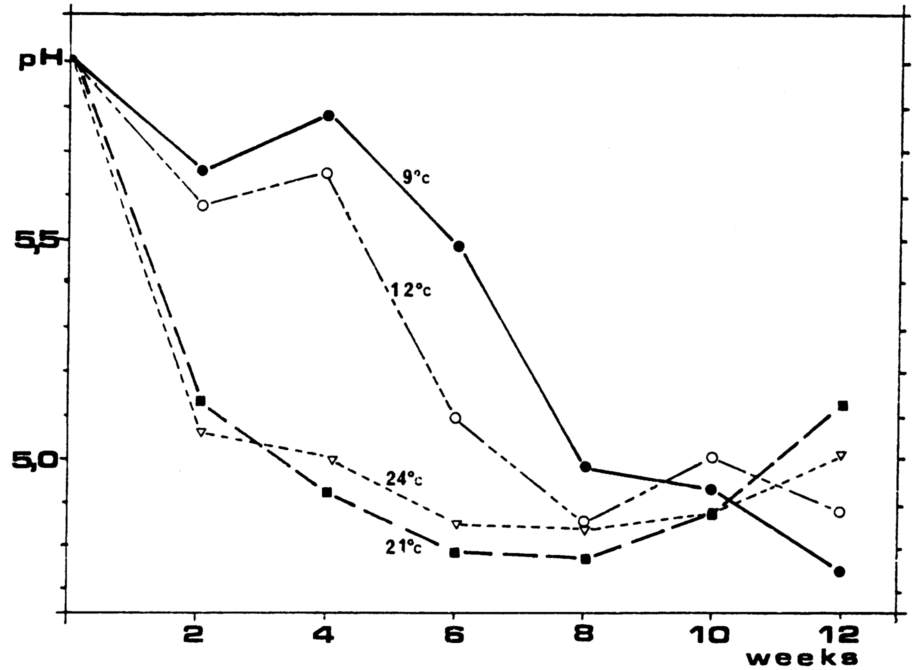

Fig. 1. Developments of soil pH at high and low temperatures for pots applied sewage sludge and 1500 kg $CaCO_3$ per hectare.

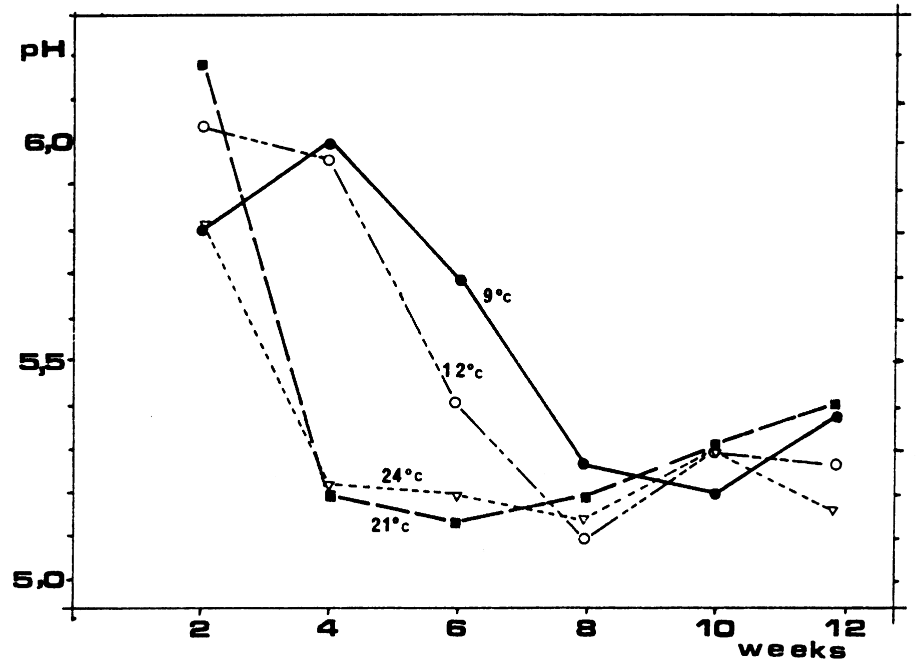

Fig. 2. Developments of soil pH at high and low temperatures for pots applied sludge and 3000 kg lime per hectare.

The pH development for the treatment Control (A) is shown in Fig. 3. After a drastic pH drop during the first two weeks the pH values increased a little.

Forsøksresultater:

1. Etter forsøksstart sank pH i jorda, sterkest for forsøksledd tilført kloakkslam. Nedgangen som hadde sammenheng med temperaturene, var rask ved høye og langsom ved lave temperaturer.
2. Tilføring av kloakkslam medførte høyere innhold av nitrat i jorda. Dette forklarer en del av pH-senkningen.
3. Tilføring av metaller i slam eller i uorganiske salter førte til tydelig høyere metallinnhold i salat i forhold til kontroll-leddet, unntatt for Mo. Innholdet av Cd og Ni var signifikant høyere etter tilføring av uorganiske salter (C) i forhold til slam (B). Det omvendte var tilfelle for Zn, Mn og Cu. Det høye metallinnholdet etter tilføring av slam i forhold til kjemikalier kan dels tilskrives senket pH.
4. Stigende temperatur har ført til betydelig høyere konsentrasjoner av Cd, Zn, Mn og Fe i salat ved begge høstinger. Denne temperatureffekten var uregelmessig for metallene Cu, Ni og Mo.
5. Stigende kalktilførsel ga tydelig lavere innhold av metaller i plantene, med unntak av Mo. Kalking har påvirket metallinnholdet i plantene i denne rekkefølgen: Mn, Zn > Ni, Cd > Cu > Fe og hvor virkningen overfor Cu og Fe var liten.
6. Innholdet av tungmetaller er bestemt i jord ved ekstraksjon med 0,05 M $CaCl_2$ og 0.1 M $NaNO_3$. Tilføring av metaller (ledd B og C) har i stor grad økt innholdet av ekstraherbart Cd, Zn og Ni. Mengden av ekstraherbart Cu var mindre påvirket av forsøksbehandlingen. Tilføring av kloakkslam har i særlig grad økt innholdet av Mn og Fe.

   Ulike temperaturer har i liten grad påvirket innholdet av ekstraherbare metaller fra jorda.
7. Stigende kalktilførsel reduserte mengden av ekstraherbart Cd, Zn, Ni og Mn meget sterkt, mens kalkingen bare hadde liten effekt på ekstraherbart Cu og Fe.
8. Korrelasjonene mellom innholdet av hvert metall i planter og jordekstrakter var dårligere enn ønskelig for en analysemetode ($r < 0.6$). En viktig årsak til dette var at både kalking og forsøksbehandlingen påvirket mengden av ekstraherbare metaller mer enn plantenes innhold. Plantene har tatt opp metallene gjennom hele vekstperioden, mens ekstraksjonen er foretatt i den tilstanden jorda hadde etter 2. høsting.
9. Presisjonen ved å bestemme innholdet i planter i denne undersøkelsen syntes å avta i denne rekkefølgen for de ulike metallene:
   Zn > Mn > Ni > Cd > Fe > Cu > Mo.

VII. ACKNOWLEDGEMENTS

The senior author is grateful to the Norwegian Agency for International Development (NORAD) for the fellowship financial assistance to carry out this research.

2. Application of sludge lead to increased nitrate content in the soil and this was partly responsible for the drop in pH.
3. Application of metals in sludge or as inorganic salts lead to a greater increase in heavy metal content in lettuce than for the control (except Mo).
   The content of Cd and Ni was significantly higher after application of metal salts (C) compared to sludge (B). The opposite was true for Zn, Mn and Cu. The high metal uptake from sludge was partly due to the lowered pH.
4. Increasing temperatures have resulted in considerably higher concentrations of Cd, Zn, Mn and Fe in lettuce for both harvests. The temperature effect on plant uptake of Cu, Ni and Mo was little and irregular.
5. Increased application of lime had clearly reduced the heavy metal uptake in plants, except for Mo. Liming affected the metal uptake in this order: Mn, Zn > Ni, Cd > Cu > Fe, with very weak effect for Cu and Fe.
6. Soil samples were extracted with 0.05 M $CaCl_2$ and 0.1 M $NaNO_3$. The amounts of extractable Cd, Zn and Ni were highly increased by both sludge and inorganic metal salts. The extractable Cu, however, was less affected by these treatments. The extractable amounts of Mn and Fe were particularly increased in sludge treated soils.
   The extractable amounts of metals from soil were only to a very little degree affected by the temperatures.
7. The amounts of extractable Cd, Zn, Ni and Mn were heavily reduced by liming, but only small effects upon extractable Cu and Fe were found.
8. The correlations between metal content in plants and soil extracts were rather weak ($r < 0.6$). One reason might be that both liming and treatments (B and C) affected the extractable metals far more than plant content. An explanation could be that the plants have taken up metals from the soil during the whole growing period while we have extracted the metals only after second harvest. The chemical and physical conditions in the soil could change quite a lot during the growing periods.
9. The ease at which precision is obtained, for heavy metals, in the investigation as a whole, seems to follow this sequence:
   Zn > Mn > Ni, Cd > Fe > Cu > Mo.

VI. SAMMENDRAG

Det er utført karforsøk i fytotron med følgende temperaturer: 9, 12, 15, 18, 21 og 24°C. For hver temperatur er følgende behandlinger sammenlignet: A) Kontroll, B) Tilført omsatt kloakkslam tilsvarende 60 tonn slamtørrstoff pr. hektar, C) Samme mengde av metallene Cd, Zn, Cu, Ni, Mn og Mo er tilført pr. kar i form av klorider, som tilført med slam i ledd B). I tillegg var disse behandlingene kombinert med ulike kalkmengder.

Salat var forsøksveksten, høstet 2 ganger, når plantene ved ulike temperaturer nådde samme størrelse. Veksttidens lengde tiltok med lavere temperaturer.

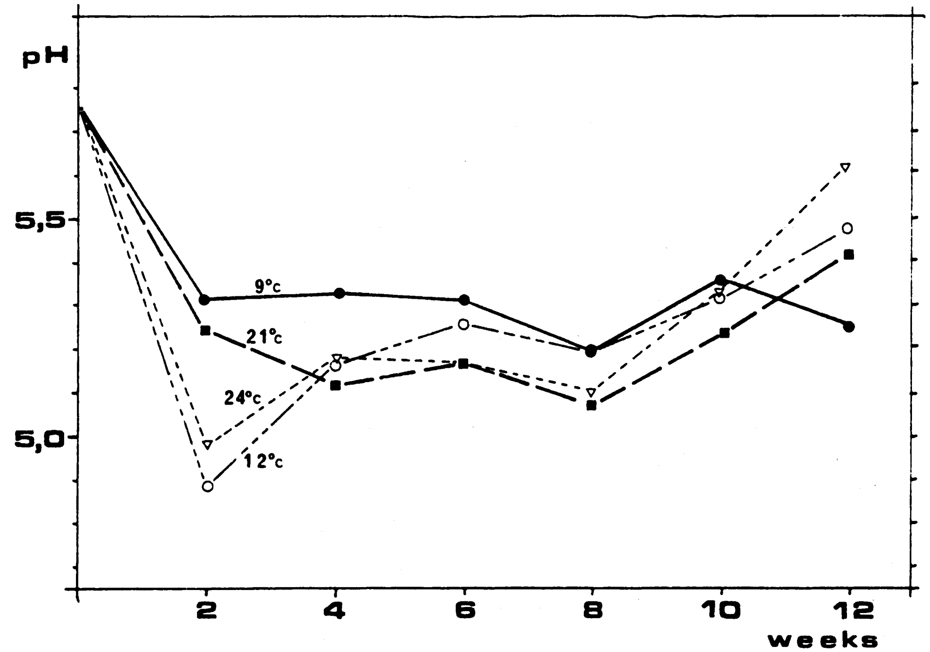

Fig. 3. Developments of soil pH at high and low temperatures for the control treatment and 1500 kg $CaCO_3$ per hectare.

From table 1 it can be seen that after 2 weeks pots with sludge (B) had higher pH than the other treatments (A and C), the reverse was, however, true and significant after 6 weeks.

Table 1. Effect of treatment on pH measured at different times. Average for different temperatures and lime applications.

Weeks after start	Treatments A	Treatments B	Treatments C	F-value
2	5.53	5.67	5.52	
4	5.28a	5.34a	5.28a	2.03
6	5.33a	5.02b	5.26a	30.7***

Numbers followed by the same or no letters are not significantly different at the 0.05 level according to Duncan Multiple Range Test.

The pH effect of different temperatures is shown in Table 2.

Table 2. Effect of temperatures on pH measured to different times, average for treatments and lime applications.

Weeks after start of expt.	Temperatures						F-value
	24	21	18	15	12	9	
2	5.40	5.73	5.50	5.62	5.53	5.63	
4	5.05a	5.14a	5.25b	5.34b	5.44c	5.57d	34.8***
6	5.14a	5.10a	5.20a	5.15a	5.22a	5.41b	7.21***

Numbers followed by different letters (horizontal lines) are not significantly different at the 0.05 level according to Duncan Multiple Range Test.

The calculations for pH show significant interaction between temperature and treatment (after 4 weeks: $F = 7.07$***, after 6 weeks, $F = 3.05$*). This is also indicated in Fig. 1 and 3.

To clearify the influence of pH on heavy metal uptake, the pH measurements at harvest time were used.

The time before harvest was particularly important for the size of yield, and at harvest time there were little differences in pH under different temperature levels, see Table 3.

Table 3. Soil pH at different temperatures. Average for treatments and lime applications.

	Temperatures						F-value
	24	21	18	15	12	9	
1st harvest	5.29a	5.25ab	5.18b	5.47c	5.44c	5.33a	9.87***
2nd harvest	6.19a	6.27ab	6.23ab	6.33b			2.46

At harvest there were very clear pH-differences between the treatments and liming levels, see Table 4.

Table 4. Soil pH at different treatments. Soil samples taken at the time of harvest. Average of different temperature.

		Treatment			F-value
		A	B	C	
1st harvest	1500 kg $CaCO_3$/ha	5.37a	4.83b	5.28c	116***
	3000 » »	6.20a	5.25b	6.08a	71.8***
2nd harvest	1500 » »	5.61	4.93	5.42	
	3000 » »	6.18	5.37	6.22	Lime ×
	4500 » »	6.98	6.10	6.90	treatm. n.s.
	6000 » »	7.37	6.37	7.32	
	Average	6.54a	5.76b	6.47a	161***

Metals applied as inorganic salts generally cause greater plant uptake and toxicity than when applied as sewage sludge (CUNNINGHAM et al. 1975a, 1975b, CHANEY 1982).

The total metal content in soil may give good correlation to plant uptake for one soil. This method gives, however, only poor estimation of the availability for plants grown in different soils. There is primarily a need for methods which in general can estimate the plant availability of metals.

Two neutral extractants are used ($CaCl_2$ and $NaNO_3$). The extracted amounts, as well as the plant availability are closely related to soil pH. Acid or strongly buffered extractants will, therefore, dissolve metals from soil in a quite different way than the plant roots do. This is the main reason why HAENI (1984) and SAUERBECK (1984) strongly recommend neutral extractants for description of plant availability. The extractability of metals using the $NaNO_3$ method is low. HAENI (1984) recommend this method especially for polluted soils. More metals are extracted by $CaCl_2$-solution and SAUERBECK (1984) recommend this as a better all-round method, especially for Cd.

In this experiment soil extractions with both methods gave a rather weak correlation to the plant concentration. The soil extractability seems to be more strongly connected to soil pH than the plant content. A possible reason for this might be that to some extent there are special properties round the roots e.g. another pH than generally in the soil. The root may extract from the nearest environment in another way than what appear by extractions in the laboratory. In addition the plants extract the metals during the whole period, while we extracted the soil samples after 2nd harvest.

Determination of the effect on metal content in plants is connected with uncertainties. These investigations indicate that the precision of the determinations of metals follows this order: Zn > Mn > Ni, Cd > Fe > Cu > Mo.

In an own study of this experiment SIRIRATPIRIYA (not published) found that size of roots decreased at increasing temperatures.

V. SUMMARY

Pot experiment was carried out in phytotrone with following temperatures: 9, 12, 15, 18 and 24°C. For each temperature the following treatments were compared: A) Control, B) Sewage sludge application, equivalent to 60 tons DM per hectare, C) The metals - Cd, Zn, Cu, Ni, Mn and Mo as chlorides, applied in the same amounts as in B) but without sludge. In addition the treatments were combined with different liming.

The crop was lettuce, harvested two times when the plants reached the same size. The length of the period increased by decreasing temperatures.

Results:

1. After start of the experiment, there was a greater drop in soil pH in the sludge treated soil. The drop in pH, which was related to the temperature, was faster at higher and slower at lower temperatures.

development. That means that the yield did not vary much. The calculations show a weak negative correlation between yield and metal concentration.

SHEAFFER et al. (1976) suggested that decreased yield at lower temperatures resulted in increased metal concentration in plants. If uptake of metals proceeds more slowly than formation of organic matter, the concentration in plants will decrease. This "dilution effect" is well discussed by JARRELL & BEVERLY (1981).

Increased pH have resulted in a very clear decrease in metal uptake in plants (except Fe) as shown in Fig. 11.

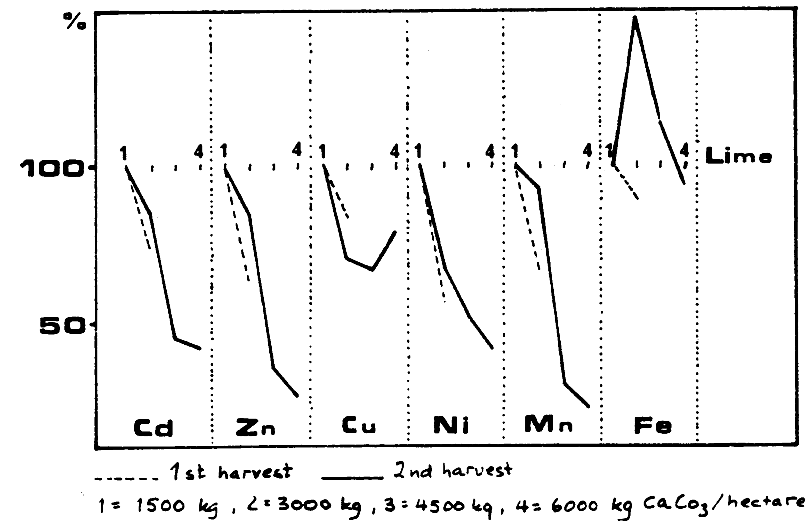

Fig. 11. The effect of increasing lime application (1500-6000 kg $CaCO_3$ per hectare) upon the heavy metal content in lettuce expressed relatively; concentration at 1500 kg $CaCO_3$ per hectare = 100.

The pH effect on the plant available heavy metal is briefly clearified (see e.g. DIJKSHOORN et al. 1983). Less attempts are, however, done in order to clearify the pH effect on different metals. In this experiment decreasing pH affected the availability of the metals in this order: Mn, Zn > Ni, Cd > Cu > Fe.

The pH effect on Cu concentration in lettuce was weak and not significant. On average there was no pH effect on Fe concentration. According DIJKSHOORN et al. (1983) the effect of soil pH on metal availiability followed this order:

Ni, Mn > Zn, Cd > Pb, Cr > Cu.

Application of heavy metals in inorganic salts resulted in significantly higher concentrations of Cd and Ni than from the sludge, despite the pH lowering effect of sludge. Concentrations of Mn, Fe, Zn and Cu in lettuce were higher from sludge than from inorganic metal source, the pH lowering effect of sludge being probably an important reason for Zn and Cu, while Mn and Fe also were affected by the conditions caused by the sludge application.

B. AMMONIUM AND NITRATE IN SOIL

Soil content of NH_4-N and NO_3-N is shown in Table 5.

Table 5. Ammonium and nitrate content, mg/kg dry soil, at different treatments. Average for different temperatures and lime applications.

	NH_4-N			F-value	NO_3-N			F-value
	A	B	C		A	B	C	
1st harvest	0.49a	5.1ab	8.2b	5.51**	25a	141b	38a	152***
2nd harvest	0.46a	0.68b	0.37a	8.44**	6.7a	136b	15a	251***

Sewage sludge (B) released nitrogen as NH_4-N which was converted to NO_3-N. Application of heavy metals as chemicals (C) affected the mineralization of N in a way which seems difficult to explain.

The content of inorganic N in soil at different temperature levels is shown in Table 6.

Table 6. Effect of temperatures on the content of N-compounds, mg N/kg dry soil. Average for different treatments and lime applications.

	Temperatures °C						F-value
	24	21	18	15	12	9	
NH_4-N							
1st harvest	7.97a	12.15ab	3.60b	1.27b	1.08b	1.52b	3.73**
2nd harvest	0.58a	0.60a	0.67a	0.18b			12.1***
NO_3-N							
1st harvest	70.6a	77.7a	87.1a	47.1b	45.3b	78.4a	5.75***
2nd harvest	60.2a	50.0ab	41.9b	57.3ab			2.39

The soil content of NH_4-N was lower at lower temperatures. This may be an effect of a lower decomposition rate and magnitude. The effect of lime application is shown in Table 7.

Table 7. Effect of lime on NH_4-N and NO_3-N content, mg/kg dry soil. Average for different treatments and temperatures.

	1st harvest kg $CaCO_3$/hectare		2nd harvest kg $CaCO_3$/hectare				F-value
	1500	3000	1500	3000	4500	6000	
NH_4-N	5.85	0.83	0.61a	0.36b	0.51ab	0.54ab	2.33
NO_3-N	67.7	67.7	68.8a	53.0b	44.4b	43.2b	4.95***

There is a significant interaction between treatment and temperature on the NH_4-N content (F = 42.6***). At temperatures from 9 to 18°C the NH_4-content was lower in soil treated with chemicals (C) than in the Control (A). At 21 and 24°C the NH_4-N content was several times higher in treatment C than A. There might have been a blockage of the nitrification in soil due to increased heavy metal influence at higher temperatures. This is, however, little reflected in the NO_3-N content in soil (interaction 2.92**).

C. Soil acidification

Decomposition of organic matter as well as nitrification may affect soil pH:

$NH_4^+ \rightarrow NO_3^- + 2H^+$

Formation of 1 kg NO_3-N will need 4 kg CaO for neutralization.

Regression between pH and NO_3-N content is calculated for soil samples taken at first harvest and for pots applied lime equivalent to 1500 kg $CaCO_3$/ha:

A, Control: pH = 5.65 - 0.011 NO_3-N, r = 0.80***

B, Sludge: pH = 5.21 - 0.0028 NO_3-N, r = 0.76***

C, Chemicals: pH = 5.39 - 0.0027 NO_3-N, r = 0.45

Application of sludge (B) gave the following drops in pH compared to treatment A, Control:

	After 1st harvest		After 2nd harvest			
Kg $CaCO_3$/ha	1500	3000	1500	3000	4500	6000
pH-units	0.50	0.89	0.58	0.87	0.84	0.71

From the relation between lime application and pH, the acid effect of 60 tons sludge DM is calculated to neutralize 1500–2000 kg $CaCO_3$.

The differences in the NO_3-N between treatments B and A, according to the regression equation between pH and NO_3-N, correspond to a pH-change of 0.33 pH-units for samples after 1st harvest and 0.36 for samples after 2nd harvest.

We can conclude that the formation of nitrate is partly responsible for the acid effect of sludge application. It is, however, very difficult to give an exact quantification of the very complicated effects on soil pH.

In soil samples taken 4 weeks after the start, electrical conductivity was determined in the same solution used for pH-measurements (soil: water = 1:2) and expressed in μmhos/cm:

Treatments			F-value
A	B	C	
266a	662b	335a	69.1

This increased salt content may be responsible, for a little part, for the measured acidification. Other acid effects may, however, be due to organic matter decomposition (Vigerust & Weng 1983).

Giordano & Mays (1977) reported an increase in Zn and Cd levels in leaves of maize due to soil heating. Haghiri (1974) suggested that the effect of soil heating on metal concentration in maize was greater when metal salts were applied instead of sewage sludge. Rush & Jarrell (1981) obtained increased metal concentration in radish with increased temperature. The treatments affected the plant concentration in this order: Al-sludge > Ca-sludge > Control.

High microbiological activity might decrease soil pH (Vigerust & Weng 1983). Increased CO_2-production in soil might lead to metal precipitation as carbonates, most pronounced, however, at higher pH.

A number of investigations show that plant availability of Zn is increased by increasing temperatures. (Sheaffer et al. 1979, Giordano et al. 1979, Ellis et al. 1964, Bauer & Lindsay 1965, Rush & Jarrell 1981.) Zn-deficiency is commonly reported to be most prevalent in cold wet soils during the early part of the season (Ellis et al. 1964). Wallace et al. (1969) reported increased Cu content in plants at higher soil temperature, with a variation among plant species.

Soil heating increased Cd concentration (as well as Zn) in broccoli and potato and in a weaker degree for several other vegetables (Giordano et al. 1979, Giordano & Mays 1976). Sheaffer et al. (1979) found higher uptake of Cd, Zn and Cu by maize and radish at increased soil temperature. Haghiri (1974) has reported higher Cd concentration of soybean shoots with increased soil temperature.

Rush & Jarrell (1981) found higher content of Zn, Mn, Fe, Cd, Ni and Cu in radish grown in soil at 30°C than at 15°C. On average the concentrations were increased 1,5 times. Ni was the metal which was most affected by temperature. This is contrary to our findings where Ni and Cu in plants were little affected by the temperature.

Increasing temperature resulted in lower selective binding ability to Vermiculite in this order: $Cu^{2+} > Zn^{2+} > Cd^{2+}$ (Bergseth 1982).

The increased plant uptake of Mn and partly Fe with higher temperatures might be a result of change in oxidation state caused by higher microbial activity.

Lagerwerff (1972) and Chang et al. (1979) reported that there appeared to be an effect on Cd concentration in plant tissue of field grown crops due to seasonal variations. Crops grown in soil which were warmer invariably contained higher Cd concentrations than those grown when temperatures were lower.

This experiment and the literature cited indicate that higher temperatures, generally, can lead to higher concentrations of heavy metals in plants. This might be an important statement especially for the total heavy metal strain from foodstuffs and whether the highest effects are obtained in colder or warmer areas.

Many experiments done in order to clearify the heavy metal uptake in plants are carried out in pot experiments. The temperature will here normally be higher than under field conditions. De Vries & Tiller (1978) found much lower effect on metal concentration in lettuce and onion in the field than in glasshouse. Sludge application brought about a sharp increase for most heavy metals in plant material from glasshouse experiments, where as the treatment caused very little increase in concentration in field grown plants. In the same way Kuntze et al. (1983) found higher uptake of Zn and Cu in plants from glasshouse experiment than under field condition. Comparison of element uptake in field and pot experiments is the next step of these investigations.

In this experiment the lettuce was harvested approximately at the same stage of

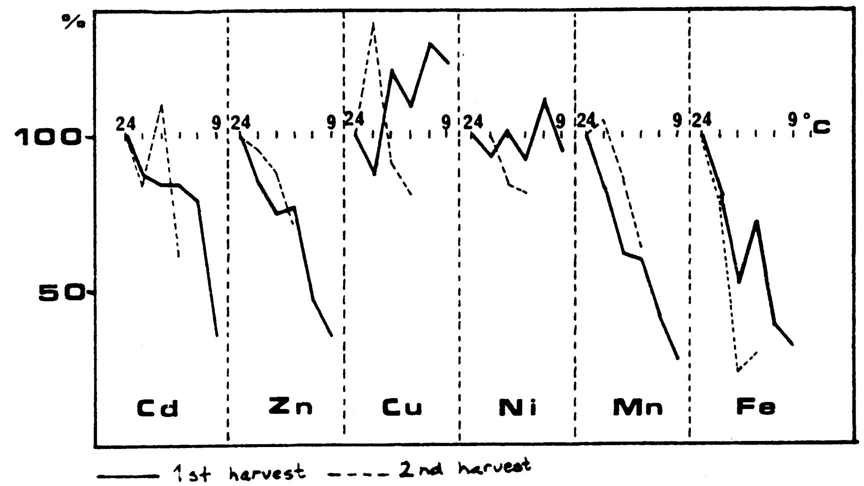

Fig. 10. The effect of declining temperatures (24–9°C) upon heavy metal content in lettuce expressed relatively; concentration at 24°C = 100.

(Barber 1974, Nielsen 1974) and also the viscosity of protoplasma (Nielsen 1974). Increased transpiration and also the lower viscosity of soil water increase the mass flow and diffusion to the plants (Barber 1974).

In this experiment in phytotrone the air blown through the cells had higher humidity at higher temperature. This system was used in order to keep the evaporation from plants and the water demand at approximately the same level for each temperature. No registration of water consumption was done. A rough estimation indicated a moderate increase in water consumption at higher temperatures. The growing period was, however, much longer at the lower temperatures.

As the metals in sewage sludge are mostly applied in organic form (Mitchell 1965) they could be released during the growing season by decomposition of organic matter. In this investigation elevation of temperatures seemed to increase the availability of metals from sewage sludge as well as from application in inorganic form and also from pots without applied metals.

A significant interaction between temperature and treatment upon the concentration of metals in lettuce was observed. Cd was, however, an exception.

Nielsen (1974) pointed out that the soil temperature influenced the rate of release of nutrients from organic as well as from inorganic compounds.

Miller (1974) observed a high correlation between soil temperature and CO_2 evolved from decomposition of an anaerobically digested sludge. A large fraction of the available Zn in soil was released from the decomposition of organic residues; thus higher temperatures inherent with the advance in season permit a greater biological activity which results in the release of available Zn.

Dowdy & Larson (1975) suggested that increased availability of Zn and Cu at higher temperature was due to metal mobilization by organic acids derived from decomposition of organic matter. Bauer & Lindsay (1965) reached the same conclusions for Zn.

D. Yield

The temperatures have highly influenced the growth of lettuce, and they were harvested after different times.

The effect of treatments and liming on the yield is given in Table 8.

Table 8. Effect of treatments on the yield of lettuce, dry matter, g/pot. Average for temperatures and lime applications.

kg $CaCO_3$ per ha	1st harvest			F-value	2nd harvest			F-value
	A	B	C		A	B	C	
1500	3.11a	2.16b	2.33b	11.8***	3.63	0.42	1.84	
3000	3.94a	4.43a	3.00a	0.96	4.14	2.76	4.46	
4500					6.71	7.54	7.22	
6000					6.57	7.86	8.27	
Average	3.32	2.73	2.51	3.29*	5.26	4.64	5.45	0.74

The fertilizing effect of sewage sludge has been compensated for by fertilizing of the other pots. This type of sludge has depressed the pH clearly with an expected decrease in growth.

At the lowest lime level the application of heavy metals decreased the yield of 1st and 2nd harvest by comparison with the control. This is probably a toxic effect of the heavy metals applied. At high lime application the treatment C resulted, however, in higher yield at 2nd harvest than obtained in the control. Therefore, we cannot exclude a possible positive effect of one of the minor nutrients.

E. Plant uptake of heavy metals

Concentration of heavy metals in lettuce according to the treatments is shown in Table 9.

Table 9. Concentration of heavy metals in plants at different treatments: A) Control, B) Sludge, C) Chemicals. Average of different temperatures and lime levels, mg/kg DM.

	Cd	Zn	Cu	Ni	Mn	Fe	Mo
1st harvest							
A. Control	1.57a	86a	14.2a	2.33a	334a	169a	64
B. Sludge	2.35b	409b	25.2b	4.28b	922b	373b	68
C. Chemicals	3.61c	370c	19.2c	5.64c	730c	167a	73
F-value	81.8***	266***	16.3***	180***	148***	66.7***	0.21
2nd harvest							
A. Control	1.08a	64c	14.9a	1.93a	125a	120	86
B. Sludge	1.95b	247b	23.7b	2.37b	635b	130	97
C. Chemicals	2.51c	194c	19.1ab	2.97c	248c	109	84
F-value	77.7***	79.7***	4.39*	17.2***	47.9***	2.17	0.60

The concentrations are significantly different for the treatments at both 1st and 2nd harvests for Cd, Zn, Cu, Ni and Mn. Cd and Ni were found in highest concentrations after metal application as chemicals, while Mn and Cu were taken up in largest amounts in sludge treated soils. This treatment gave very high Fe content in the lettuce at 1st harvest, but no significant differences at the second. There were no significant differences in the content of molybdenum.

Iron was not applied to the soil in treatment C, the Fe content in this sludge used is naturally very high. The high uptake of Fe and Mn from sewage sludge might be caused by low pH and reducing effects from sludge decomposition. The microbiological activity has probably been higher before 1st harvest than later and this, possibly, could have an effect on the Fe uptake.

The relative change in the element concentration for treatments B and C is tabulated below, Control (A) = 100:

		Cd	Zn	Cu	Ni	Mn	Fe	Mo
1st harvest	B	150	476	177	184	276	221	106
	C	230	430	135	242	219	99	114
2nd harvest	B	181	386	159	123	508	108	113
	C	232	303	128	154	198	91	98

Zn was the element which was most affected by applied heavy metals.

The main question in this experiment has been to clearify the effects of different temperatures on the heavy metal uptake in plants, see Table 10.

Table 10. Effect of temperatures on the element concentrations, mg/kg DM. Average for different treatments and lime applications.

°C	Cd	Zn	Cu	Ni	Mn	Fe	Mo
1st harvest							
24	3.16a	407a	17.5ab	4.11a	1049a	375a	51a
21	2.74ab	347b	15.2a	3.82a	869b	306b	27a
18	2.67b	306b	21.1ab	4.21ab	650c	194c	56a
15	2.66b	314b	19.2ab	3.78a	633c	270b	99b
12	2.51b	190c	22.7b	4.55b	439d	148cd	50a
9	1.13c	146d	21.6b	3.91a	295e	121d	133c
F-value	18.2***	39.8***	2.16	2.75*	60.3***	21.8***	20.2***
2nd harvest							
24	2.07a	191a	18.9ab	2.64a	383a	207a	84a
21	1.75b	181a	25.7a	2.65a	405a	162b	148b
18	2.28a	168ab	17.2b	2.23b	328ab	49c	80a
15	1.27e	136b	15.4b	2.14b	245b	62c	44c
F-value	22.2***	3.90*	3.39*	5.17*	2.53	80.5***	15.33***

Increasing temperatures have, for 1st harvest of lettuce, lead to significantly higher concentrations of the metals Cd, Zn, Mn and Fe. The effect of temperatures on plant content of Cu and Ni was little and irregular. For molybdenum there were

Metals taken up by lettuce in relation to applied follow this succession:
1) Mn 2) Cd, Zn 3) Ni, Cu 4) Fe.

Metal uptake by plants was reduced far less by liming than the extractable amounts, compare e.g. Figs 4 and 7 (Zn) and Figs 5 and 8 (Ni). This fact reduces the regression between concentration in plants and the soil analysis.

The obtained correlation coefficients between concentration in plants and the extractable amounts of metal from soil with $CaCl_2$ and $NaNO_3$ were:

Methods	Cd	Zn	Cu	Ni	Mn	Fe
$CaCl_2$	0.47***	0.35*	0.32	0.38*	0.38*	0.04
$NaNO_3$	0.56***	0.37*	0.35*	0.48***	0.39**	0.17
Both methods	0.56***	0.37*	0.40*	0.60***	0.39**	0.25

This must be characterized as a rather weak correlation. The metal content in plants was better correlated to soil pH.

There was significant negative regression between Cd concentration in plants and Zn extracted by $CaCl_2$-solution.

G. Precision of the investigation

For 1st harvest there were 3 replication pots (except extra limed pots) and double analysis of yield samples were carried out for all metals. Standard deviation for 6 determinations with same treatment will show the precision of the method including plant uptake and chemical determinations. Standard deviation is calculated in pst. of average dry matter yield (CV pst.). These percentages were not much affected by temperature and treatment (A–C). The averages for all calculated CV pst. were in this order:

Zn 14.9, Mn 16.0, Ni 17.8, Cd 25.8, Fe 31.0, Cu 42.4, Mo 63.7.

There is a close correspondance between these values in the calculations of heavy metal concentration in plants (Tables 9, 10 and 11). The most significant effects were obtained for Zn and Mn. Ni, Cd and Fe come in an intermediate position while the least significant effects were obtained for Cu and Mo. This was, of course, a result of different responses to the metals in the experiment as well as analytical errors. The standard deviations do, however, give information about how precisely the various metals can be obtained in pot experiments like these.

IV. DISCUSSION

The effect of temperature on the concentration of different metals is shown as relative number in Fig. 10, concentration at 24°C = 100.

Temperature has a fundamental importance for plant growth. Dependent on plant species the rate of production and plant development is affected by temperature.

Evaporation of water from soil and transpiration from plants are highly dependent on the temperature. Increasing temperature decreases soil water viscosity

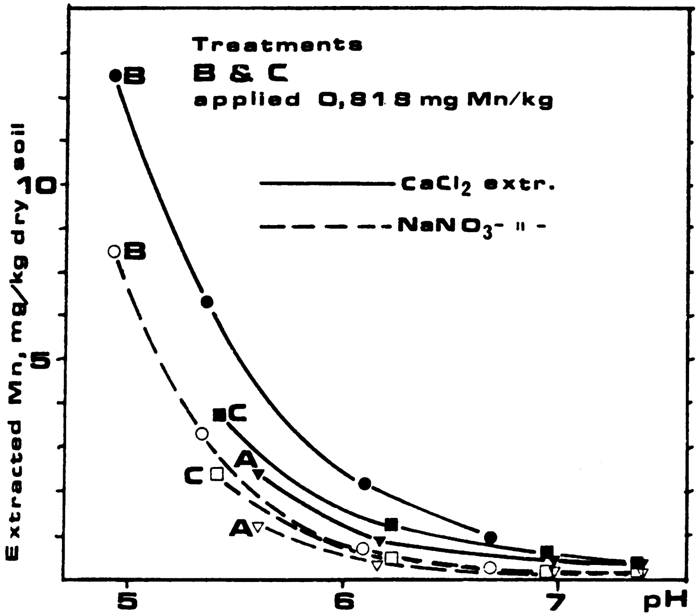

Fig. 9. The relation between extracted Mn and soil pH.

Soil treated with heavy metal salts have only released a part of the total metal content by extraction and soil pH seems to be important in these relations.

Table 15 shows the extractable amounts of metals in pst. of total content (including applied).

Table 15. Extracted metals in pst. of the total content in soil, including applied.

	$CaCl_2$			$NaNO_3$		
	A	B	C	A	B	C
Cd	(70)	22.9	28.3	3.0	4.3	3.8
Zn	0.4	5.3	4.5	0.2	2.4	1.8
Ni	0.7	3.2	3.7	0.3	1.4	1.4
Cu	1.8	0.9	0.7	1.5	0.6	0.7
Mn	0.6	3.4	1.0	0.3	1.9	0.5

The amounts extracted in % of the total were much higher for Cd than for other metals. The Cd contents in soil was, however, very low and because of that the percent values in table 15 are to some degree uncertain.

In relation to applied, the extracted amounts of metals fall in this order:
1) Mn 2) Cd, Zn, Ni 3) Cu 4) Fe.

significant differences but not regular at increasing temperatures and the tendency seemed to be different in 1st and 2nd harvests.

At the 2nd harvest a very marked effect of temperatures on the Fe-content in lettuce was found, but also for the other metals, there were clear tendencies of increased concentrations at increased temperature levels. For all 7 elements the concentrations in lettuce plants at 15°C were significantly lower than the concentrations at 21 or 24°C. For this investigation, can be concluded that higher temperatures lead to significantly higher concentration of Fe, Zn and Mn in lettuce. The response to temperature by Cd was clear but not quite regular at 2nd harvest. The nickel content was little affected. Of the metals determined Cu was least affected by different temperatures.

In the first period there were two rates of lime application, equivalent to 1500 and 3000 kg $CaCO_3$ per hectare, the first of these with 3 replications and the other with a single pot per treatment (A-C). In the statistical calculations all these were regarded as replications and the best expression for the effect of different liming is therefore given by the Duncan Multiple Test Range. After the 1st harvest all 4 pots within treatments received different lime applications. The effect of different liming on the element concentrations in plants is shown in Table 11.

Table 11. Concentrations in plants at different lime levels, mg/kg DM. Average for different treatments and temperatures.

Kg $CaCO_3$/ha	Cd	Zn	Cu	Ni	Mn	Fe	Mo
1st harvest							
1500	2.67a	316a	20.3	4.53a	721a	244	71
3000	1.98b	203b	17.1	2.66b	486b	219	60
F-value	8.07***	39.8***	3.61*	44.0***	17.7***	3.27*	1.76
2nd harvest							
1500	2.71a	274a	24.4a	3.70a	560a	105a	101
3000	2.30b	234b	17.0b	2.50b	515a	156a	73
4500	1.22c	96c	16.4b	1.94c	161b	120b	87
6000	1.13c	72d	19.2ab	1.51d	123b	98a	96
F-value	70.9***	67.0***	2.24	65.1***	26.3***	9.09***	1.27

Increasing applications of lime have very clearly depressed the concentration of Cd, Zn, Ni and Mn in the lettuce plants. There is a weak tendency in the same direction for Cu and partly Fe. Liming had no significant influence on the concentration of Mo in plants.

pH is logarithmic. For a limited pH interval a nearly linear relation between pH and some biological data as e.g. growth is shown. In this investigation pH, therefore, is calculated as an ordinary number.

We also have calculated the correlation between element concentration in plants and pH as an ordinary number. These calculations are done for the whole material for 1st and 2nd harvests and also for each of the treatments. The regression and correlation coefficients including significance levels are given in Table 12.

Table 12. Coefficients of regression, b, and correlation, r, between metal concentration in plants and soil pH. (The calculations include all temperature levels and lime applications.)

	Cd		Zn		Cu		Ni		Mn		Fe	
	b	r	b	r	b	r	b	r	b	r	b	r
All 1st harv. 72 obs	-0.48	0.17	-234	0.53***	-7.4	0.35	-1.81	0.45***	-510	0.56***	-164	0.44***
A » 24 »	-0.69	0.42*	-45.8	0.61**			-1.1	0.45*	-261	0.57**	- 74	0.39
B » 24 »			-276	0.33			-2.8	0.70***				
C » 24 »	-1.03	0.38	-238	0.66***			-3.58	0.82***	-442	0.62***		
All 2nd harv. 54 obs	-0.92	0.58***	-138	0.71***	-3.4	0.22	-0.98	0.56***	-350	0.65***		
A » 18 »	-0.31	0.62**	-37.3	0.87***					- 68	0.72***		
B » 18 »	-1.35	0.71***	-172	0.67**			-1.03	0.68**	-558	0.66**		
C » 18 »	-1.54	0.84***	-186	0.93***			-2.4	0.93***	-255	0.72***		

No value for b and r means correlation not sign. at 15% level.

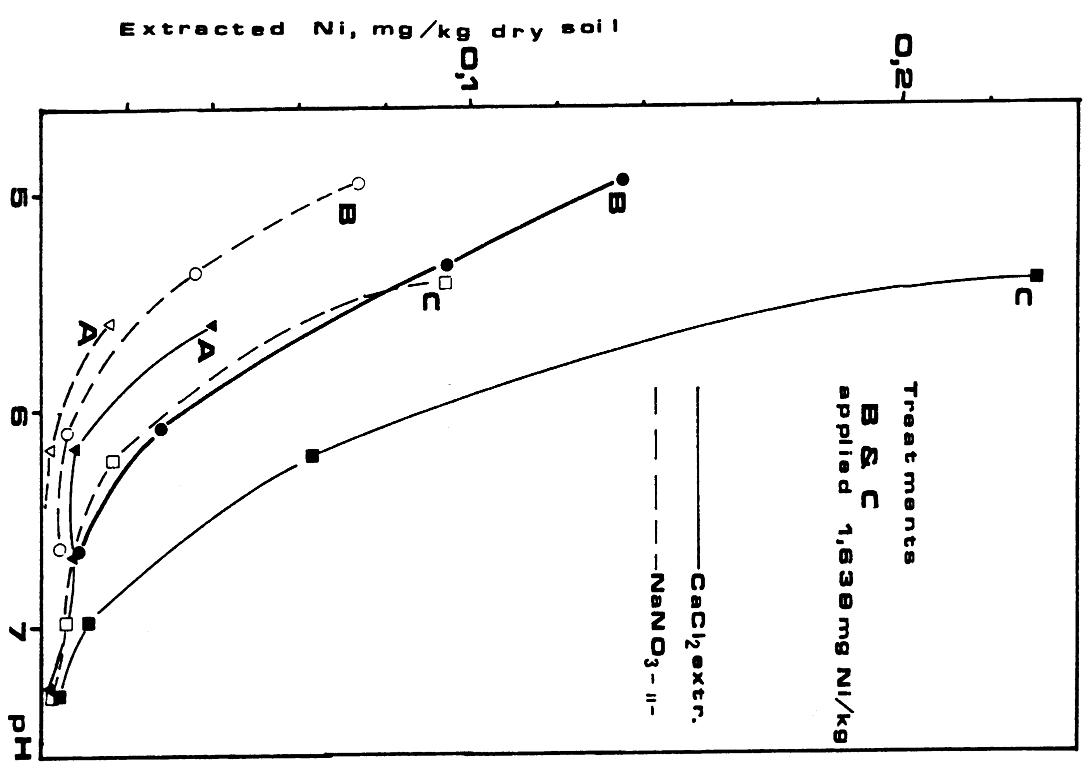

Fig. 8. The relation between extracted Ni and soil pH.

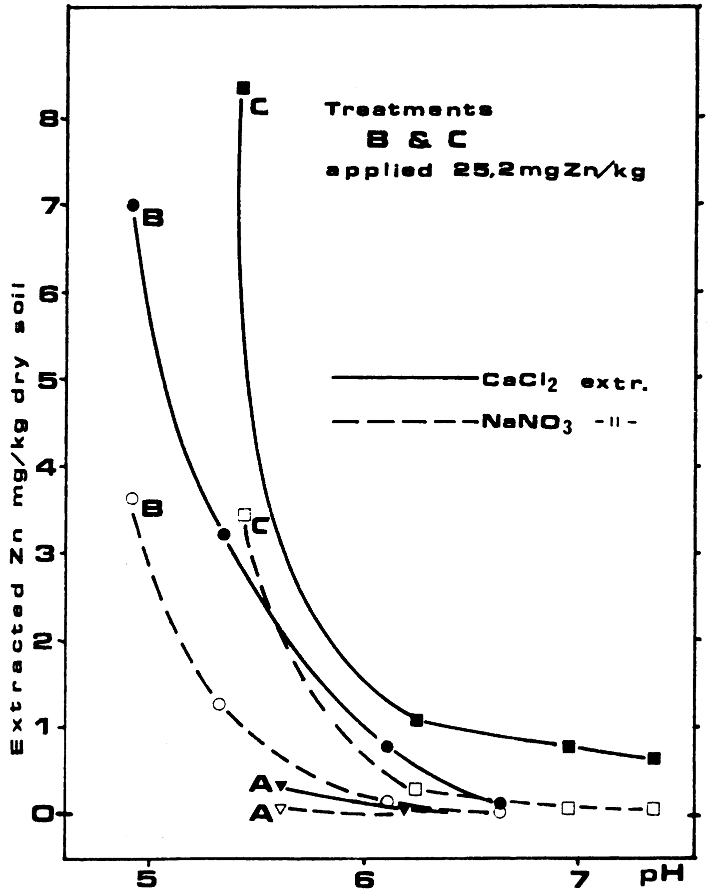

Fig. 7. The relation between extracted Zn and soil pH.

The concentrations of Cu and Mo were not significantly correlated to soil pH while this correlation was slight for Fe. The other metals were negatively correlated to pH. In most cases the highest correlation coefficients were found for treatment C, (chemicals).

As typical examples the linear regressions for Zn and Ni are shown in Figs 4 and 5.

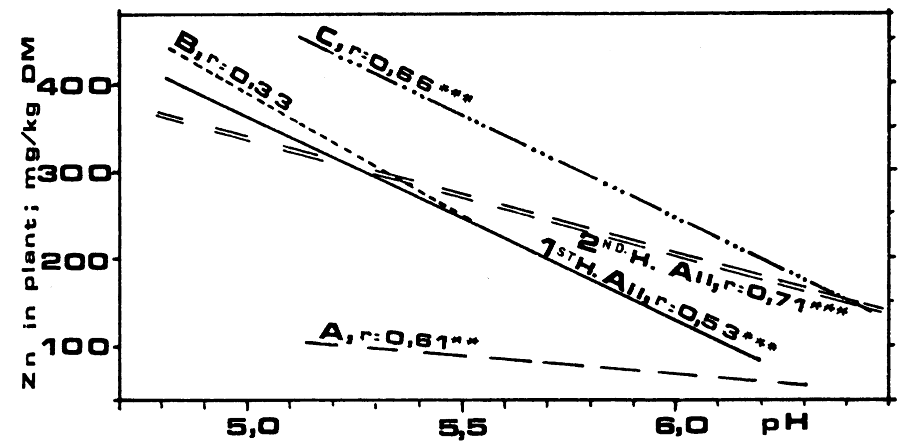

Fig. 4. Zn content in lettuce as a function of soil pH. For 1st harvest treatment A, B and C and for all pots. In addition regression line for 2nd harvest, all pots.

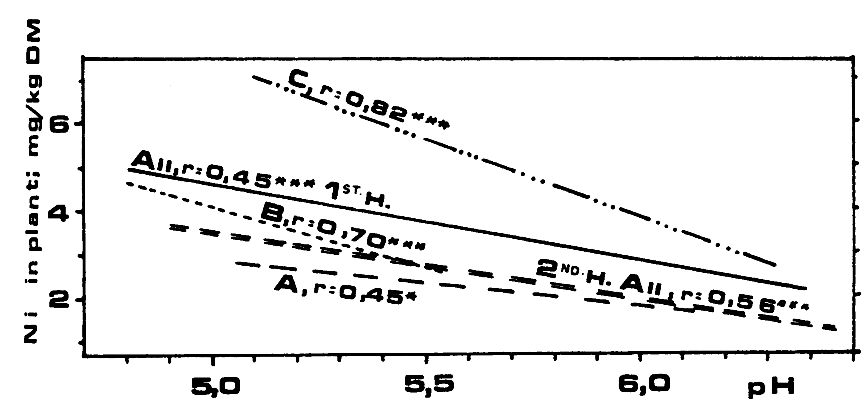

Fig. 5. Ni content in lettuce as a function of soil pH. For 1st harvest treatment A, B and C and for all pots. In addition regression line for 2nd harvest, all pots.

The lines for control (A) are nearly horizontal, indicating little correspondence of pH to metal concentration in plants.

Table 9 showed on average significantly higher Zn concentration in plants for treatments B, sludge, than for treatment C, chemicals. Fig. 4 shows that if the soil pH was the same for all treatments, the Zn content in plants treated with metals in chloride form should have been considerably higher than in plants grown in sludge-treated soil.

It can be concluded that the heavy metal content in plants grown in sludge-treated soil is affected by the applied metals and also by the decreased pH.

Fig. 5 shows that, at the same pH levels, the Ni concentrations in lettuce grown on sludge treated soils only slightly exceed the concentrations in the same plant species grown on control pots.

The concentrations of heavy metals were slightly negatively correlated with the yield. This effect was significant for Cd and Zn at 2nd harvest and for Ni and Mn at both harvests.

F. EXTRACTABLE HEAVY METALS IN SOIL

After second harvest the soil samples from 24, 21 and 15°C were extracted and the heavy metal content is presented in Table 13.

Table 13. Content of heavy metal in soil extracted by $CaCl_2$ (1) and $NaNO_3$ (2), mg/kg dry soil, according to treatments. Average for different temperatures and lime applications.

	Cd	Zn	Cu	Ni	Mn	Fe
1. ($CaCl_2$)						
A. Control	0.007a	0.11a	0.109a	0.045a	0.98a	0.052a
B. Sludge	0.021b	2.78b	0.170b	0.221b	5.23b	0.129b
C. Chemicals	0.026c	2.37c	0.147c	0.257c	1.48a	0.056a
F-value	56.1***	237***	74.1***	148***	33.6***	6.02**
2. ($NaNO_3$)						
A. Control	0.0003a	0.05a	0.096a	0.022a	0.39a	0.068
B. Sludge	0.0039b	1.29b	0.121b	0.099b	2.97b	0.076
C. Chemicals	0.0035b	0.94c	0.135c	0.100c	0.75a	0.063
F-value	6.26**	167***	20.4***	52.7***	44.3***	1.66

Applications of metals to soil by sludge or chemicals have significantly increased the extracted amounts from soil compared to the control.

For Zn, Mn and Fe the extracted amounts were higher in B than C, and the reverse was true for Ni and partly Cd.

The amounts of extractable metal were little affected by the temperature. For all metals, however, there was a tendency to less extractability from soil kept at the lowest temperature, significant for Cu, Ni and Mn for both methods and for $NaNO_3$-extractable Fe.

The extractable amounts of metals are to a very high degree affected by the liming, Table 14.

Table 14. Content of heavy metals in soil extracts, mg/kg dry soil, at different lime applications. Average for different treatments and temperatures.

	Cd	Zn	Cu	Ni	Mn	Fe
1. $CaCl_2$						
1500 kg $CaCO_3$/ha	0.034a	5.21a	0.230a	0.450a	5.87a	0.165a
3000 -»-	0.020b	1.45b	0.135b	0.183b	2.83b	0.067b
4500 -»-	0.012c	0.28c	0.108c	0.051c	1.08c	0.061b
6000 -»-	0.005d	0.06c	0.095d	0.013d	0.47c	0.024b
F-value	62.2***	489***	214***	336***	27.3***	9.03***
2. $NaNO_3$						
1500 kg $CaCO_3$/ha	0.0072a	2.43a	0.117	0.204a	3.73a	0.087a
3000 -»-	0.0017b	0.52b	0.111	0.062b	1.33b	0.058b
4500 -»-	0.0008c	0.08c	0.118	0.017c	0.31c	0.058b
6000 -»-	0.0007d	0.01c	0.123	0.012c	0.10c	0.073ab
F-value	12.0***	395***	0.96	160***	47.0***	5.26**

Liming has drastically decreased the extractable amounts of metals, especially Zn. The influence of soil pH on the extractable amounts of heavy metals is shown in Figs 6–9.

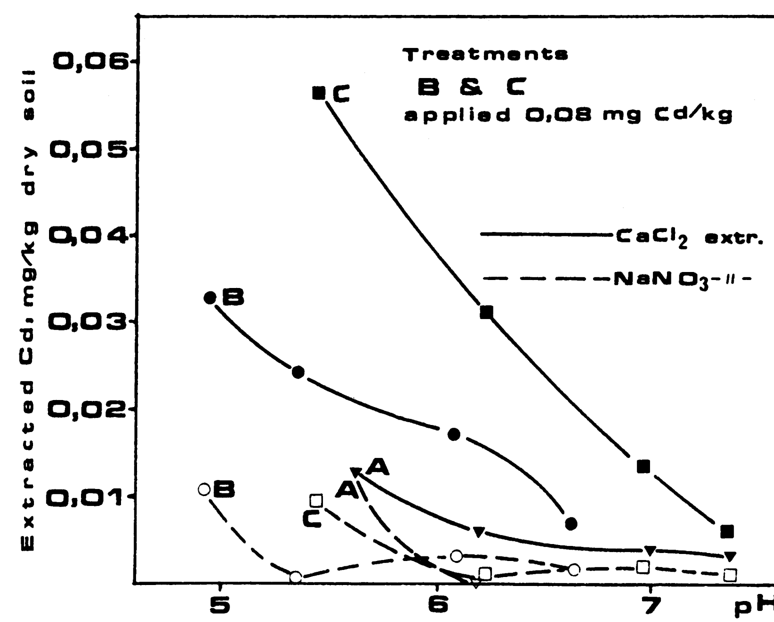

Fig. 6. The relation between extracted Cd and soil pH.