

Accumulation and Distribution of Ni⁶³ by Maize Seedlings Grown in Labeled Soils Amended with Sludge

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Abstract: A study describing the accumulation of Ni⁶³ in leaves of maize grown in various soil / sludge mixtures was conducted to evaluate the gross distribution and chemical forms of Ni⁶³ following absorption and translocation. The study demonstrated the presence of anionic nickel complexes Ni⁶³ in maize leaves indicating that nickel in plant may be species dependant. The electrophoresis results showed, Ni⁶³ associated with chlorophyll. High levels of nickel concentration occurring after sludge application could contribute to the destruction of this pigment. With an increased incubation time Ni-Spiked soil and soil / sludge mixtures decreased. The nickel concentration, thus the amount of sludge present influences the uptake of inorganic nickel by plants. Greater sludge application may cause phytotoxic effects on crops, so that the concentration of nickel and phytotoxic heavy metals in sewage sludge should be monitored. The amount of nickel in the soil plant system was found to change with time as did the soluble nickel complexes in plants, which are of interest and importance to animal and human nutritionists.

Key words: Accumulation, maize, Ni⁶³, electrophoresis, phytotoxicity, metal concentration

Introduction

Increasing application of sewage to agricultural land and continuous release of industrial wastes can be expected to increase soil levels of heavy metals such as nickel, resulting in a concomitant increase in the concentration of nickel in plants and possibly in the food chain (Cataldo *et al.*, 1978a). Plants readily take up nickel, and small amounts are found in most plants (Mishra and Kar, 1974 and Hooda *et al.*, 1997). Nickel is usually absorbed in ionic form, Ni²⁺, from the soil or the culture solution. There are a number of reports (Sims and Kline, 1991) that nickel is more easily absorbed by plants when supplied in the ionic form than when chelated. High nickel content of the soil accelerates the absorption of this element by the plants when other conditions, such as pH are favorable (Webber, 1972). This accelerated rate of nickel absorption does not actually depend on the net amount of nickel present, but on the chemical form of nickel present in the soil. Peterson (1971) showed nickel toxicity to plants growing in nickel amended soils, he indicated that the high concentrations of nickel added with sludge can cause yield reductions. Halstead *et al.* (1969) found that the addition of nickel chloride at 100 µg g⁻¹ resulted in a nickel concentration of 30 µg g⁻¹ in oat grain, while Cropper (1969) indicated that the addition of nickel as inorganic salts to a sandy soil decreased the yields of maize. However, Cunningham *et al.* (1975c) showed that yields were higher for organic (sludge) treatments than for inorganic treatments and the nickel concentration in plant tissue was higher following the organic treatments.

There is no general agreement on the forms in which major cations are translocated. Stability constants of trace element chelates of organic and amino acids have been calculated and the data show that these may be important as a factor governing the forms present in plants (Sillen and Martell, 1964 and Nouri, 1980). Tiffin (1966a) showed that the stabilities of organic acid chelates of iron are relatively high and soluble iron and organic acids are usually present in plants. He also indicated that iron is translocated in sunflower in the anionic form. The synthetic metal chelates being used in plant nutrition are of relatively large molecular weight

anions which are apparently easily absorbed by most plant roots and translocated to leaves (Sims and Kline, 1991). Timperley *et al.* (1973) showed that soluble nickel occurred in tree leaves as cationic complexes, whereas copper, zinc and iron occurred largely as anionic complexes.

The availability of highly specific radioactive ⁶³Ni²⁺ with a long half-life of 120 years has permitted the use of low non-toxic levels of the element in environmental and biological investigations.

The present study describes the accumulation of Ni⁶³ in the leaves of maize, grown in various soil/sludge treatments. Changes with time in the gross distribution and chemical forms of Ni⁶³, following absorption and translocation, were evaluated.

Materials and Methods

The soil and sludge samples were collected and prepared in London in 1990. Five sets of pots; (each comprising 3 pots containing 100 per cent soil, 90 per cent soil/ 10 per cent sludge and 50 per cent soil/50 per cent sludge), were prepared. Each pot was spiked with 30 µCi ⁶³Ni²⁺ (the isotope was present as nickel chloride in aqueous solution) and the sets of pots were left in a growth cabinet for periods of 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks at 21°C with periodic watering.

Maize seeds (*Zea mays* L. cv. LG 11) were imbibed in distilled water, placed on a glass ballotini and allowed to germinate at 12°C. Seedlings of approximately 10 cm height were obtained seven days after germination. Nine uniform 10 cm seedlings were selected and planted in each pot and grown for a period of 10 days, the pots were watered daily and the draining was recycled. The leaves were harvested at the base of their stalk and 6 of the plants were weighed and then washed in distilled water before being separately dried overnight in an oven at 80°C and reweighed as dry matter. They were then digested with concentrated nitric acid and, after dilution with 1 N nitric acid, the total recovery was measured using a β scintillation counter. Another 3 fresh plants were ground in a pestle and mortar and extracted with deionized water. The residue was removed by centrifugation (10,000 g for 10 minutes) and re-

extracted twice more with the same volume of water. Aliquots of supernatant extract were transferred to glass vials and the radioactivity was assayed. The supernatant extract was then concentrated under vacuum in a rotary film evaporator at $\leq 40^{\circ}\text{C}$. Aliquots of concentrate were subjected to high-voltage paper electrophoresis (12 minutes at pH 5.3, using a Pyridine/acetic acid buffer, on Whatman No. 3 mm paper) to separate the soluble components into cationic, anionic and neutral forms. As standard markers, Ni⁶³Cl₂ and 5 amino acids, lysine, γ -amino butyric acid, asparagine, glutamic acid and aspartic acid were prepared. After electrophoresis the paper was cut into strips longitudinally, each strip was then cut again at 1 cm intervals across its length to be counted individually.

Results

The fate of Ni⁶³ in young maize leaves of plants grown in soil and the mixtures of soil/ sludge was determined. Tables 1 to 3 show the variation with time in the plant weights and distribution of water soluble Ni⁶³ in maize leaves grown in Ni⁶³-spiked soil and sludge.

The fresh and dry weights decreased significantly with incubation time for maize grown in 100 per cent soil, but increased irregularly for plants grown in 90 per cent soil/10 per cent sludge and 50 per cent soil. The majority of the activity was found in supernatant fractions, but this decreased with time. The mixture, 50 per cent soil/50 per cent sludge, had the greatest activity for most of the experimental period, the 90 per cent soil/10 per cent sludge the second highest activity, and the 100 per cent soil the lowest activity, but the specific activity, total activity, supernatant specific activity and supernatant total activity decreased significantly with time for all three different treatments. After 10 days of the growth, maize grown in 50 per cent soil/50 per cent sludge mixtures showed severe stunting and necrosis of leaf tips (Fig. 1), that grown in 100 per cent soil showed slight chlorosis and that in 90 per cent soil/10 per cent sludge mixtures showed remarkably good growth with little or no necrosis and no stunting. However, maize grown in 50 per cent soil/50 per cent sludge mixture, had a higher organic nickel concentration than with the lower proportion of sludge and the control soil growth medium. Table 4 compared this significant increase in nickel concentration in the maize leaves with the soluble and total nickel content of the growth media.

Electrophoretic distribution of soluble compounds: Radioactive scans of the high voltage electrophoretic paper used for the separation of aqueous extracts from maize seedlings supplied with Ni⁶³ in different soil/ sludge treatments with incubation time are shown in Fig. 2 to 4. At pH 5.3 anionic and cationic compounds migrate away from each other on the sheet and away from the sample application zone containing the neutral compounds. Electrophoresis is especially useful here since compounds could readily be classed as cationic, anionic or neutral forms. The water fractions from the maize leaves grown in 100 per cent soil, 90 per cent soil/10 per cent sludge and 50 per cent soil/50 per cent sludge were concentrated and an aliquot subjected to high voltage electrophoresis. The Ni⁶³ assay in leaves grown in 100 per cent soil showed two main areas of high Ni⁶³ concentration, one of which was electrophoretically neutral (b), while the other behaved as an anion for all the treatments (Figs. 5 and 6). Some small amounts of Ni⁶³ were present in cationic form in some of the extracts, especially those from earlier harvests. The standard Ni⁺² in all treatments behaved as a cationic form (a) as expected. After 1 day incubation the maize extracts of 100 per cent soil had two peaks, a large one at the neutral site and a smaller anionic one. After 1 week the anionic peak became bigger, after 2 weeks it became wider and after 4 weeks it had divided into two smaller peaks. By the end of 8 weeks there were 3 major peaks, one neutral and two anionic (Fig. 2).

For extracts from maize grown in 90 per cent soil/10 per cent sludge (Fig. 3), the migration pattern was different. For all incubation times there were two larger single peaks, the anionic peak being much larger than the neutral one.

Electrophoresis using extracts from maize grown in 50 per cent soil/50 per cent sludge (Fig. 4) showed that 1 day incubation gave a neutral peak (b) which was bigger than the anionic peak. It seemed that 1 day's incubation caused the mixture to behave as soil, but after 1 week the anionic peak position changed and became bigger. After 4 and 8 weeks the neutral peak was smaller and was barely distinguishable from anionic peak.

To compare the concentration of different forms, the distinguishable peak areas for the neutral and anionic forms given in Fig. 2 to 4 were calculated and expressed as percentage of the total areas (Table 5). In 100 per cent soil the concentration of neutral forms exceeded that of anionic

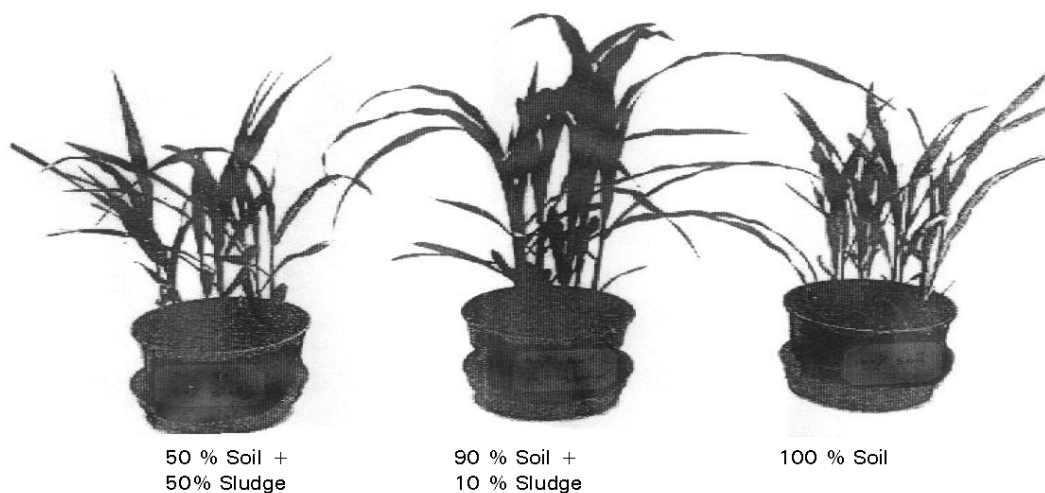


Fig. 1: 10-days-old maize grown in Ni⁶³-spiked soil/sludge mixtures (previously incubated for 8 weeks)

Fig. 2: High-voltage paper electrophoretic separations of the Ni⁶³ compounds present in the aqueous supernatant extract of fresh maize seedling leaves grown in 100 per cent soil after different incubation times. Marker substances (circles) corresponding to the electrophoretic distribution of Ni⁶³ compounds contained in the leaves extract (b) and (triangle) corresponding to ⁶³Ni⁺⁺ standard (a).

Fig. 3: High-voltage paper electrophoretic separations of the Ni⁶³ compounds present in the aqueous supernatant extract of fresh maize seedling leaves grown in 90 per cent soil, 10 per cent sludge after different incubation times. Marker substances (circles) corresponding to the electrophoretic distribution of Ni⁶³ compounds contained in the leaves extract (b) and (triangle) corresponding to ⁶³Ni⁺⁺ standard (a).

Fig. 4: High-voltage paper electrophoretic separations of the Ni⁶³ compounds present in the aqueous supernatant extract of fresh maize seedling leaves grown in 50 per cent soil, 50 per cent sludge after different incubation times. Marker substances (circles) corresponding to the electrophoretic distribution of Ni⁶³ compounds contained in the leaves extract (b) and (triangle) corresponding to ⁶³Ni⁺⁺ standard (a).

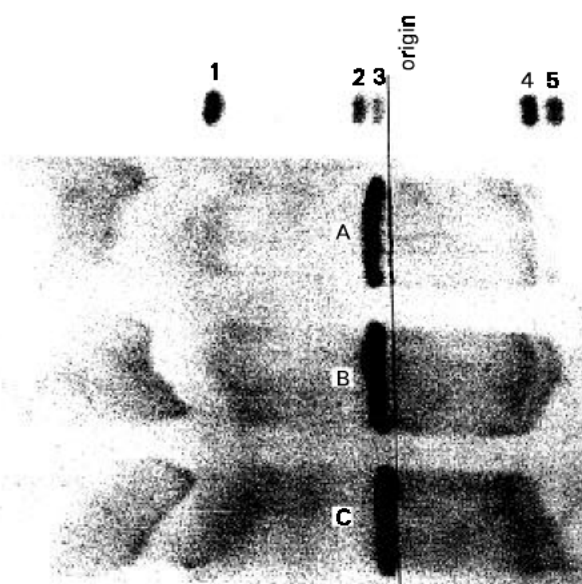


Fig. 5: Ninhydrin positive compounds in aqueous extracts of maize leaves from seedlings grown in various oil / sludge mixtures incubated for 1 day with Ni⁶³.
A (grown in soil), B (grown in 90/10 % soil/sludge) C (grown in 50/50% soil /sludge).
Amino acid standards:
1) Lysine 2)Y-amino butyric acid 3)Asparagine
4) Glutamic acid 5) Aspartic acid

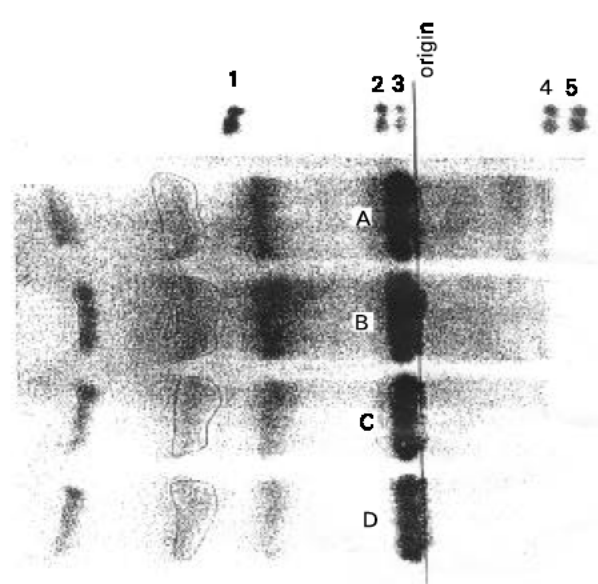


Fig 6: Ninhydrin positive compounds in aqueous extracts of maize leaves from seedlings grown in (90/10 % soil/sludge) mixtures incubated with Ni⁶³ for different periods of time.
A (1 week), B(2 weeks)
C(4 weeks) D(8 weeks)
Amino acid standards:
1) Lysine 2)Y-amino butyric acid 3)Asparagine
4) Glutamic acid 5) Aspartic acid.

Table 1: Distribution of water- soluble Ni⁶³ in maize leaves grown in Ni⁶³- spiked soil (100%) with time. (Fresh and dry weights are for 10-days-old plants growing in soil (100%) of various ages listed under the treatment).

	Time	Plant, fresh weight (g)	Plant, dry weight (g)	Plant, specific activity (dpm/g)	Plant, total activity (dpm)	Supernatant volume (ml)	Supernatant specific activity (dpm/ml)	Supernatant total activity ((dpm)	% soluble activity
Soil (100%)	1 day	1.7	0.21	14780	2752	1.78	1100	1940	71.2
	1 week	1.88	0.17	10630	2070	1.85	1 085	1790	86.5
	2 weeks	1.57	0.18	9390	1690	1.61	880	1420	84.0
	4 weeks	1.8	0.13	8 920	1 180	1.83	540	990	85.3
	8 weeks	1.29	0.12	8 780	1 085	1.42	640	910	83.9

Table 2: Distribution of water- soluble Ni⁶³ in maize leaves grown in Ni⁶³- spiked soil (90%) + sludge (10%), with time (Fresh and dry weights are for 10-day-old plants growing in soil (90%) + sludge (10%) of various ages listed under the treatment).

	Time	Plant, fresh weight (g)	Plant, dry weight (g)	Plant, specific activity (dpm/g)	Plant, total activity (dpm)	Supernatant volume (ml)	Supernatant specific activity (dpm/ml)	Supernatant total activity ((dpm)	% soluble activity
Soil (90%) + Sludge (10%)	1 day	2.29	0.2	14350	2870	2.93	775	2270	79.0
	1 week	2.2	0.22	13435	2620	2.28	910	2080	79.4
	2 weeks	2.4	0.18	13170	2370	2.60	770	2000	84.4
Soil (90%) + Sludge (10%)	4 weeks	2.6	0.19	10530	2000	2.89	588	1700	85.0
	8 weeks	2.5	0.23	7490	1780	2.89	570	1640	93.2

Table 3: Distribution of water- soluble Ni⁶³ in maize leaves grown in Ni⁶³- spiked soil (50%) + sludge (50%), with time (Fresh and dry weights are for 10-day-old plants growing in soil (50%) + sludge (50%) of various ages listed under the treatment)

	Time	Plant, fresh weight (g)	Plant, dry weight (g)	Plant, specific activity (dpm/g)	Plant, total activity (dpm)	Supernatant volume (ml)	Supernatant specific activity (dpm/ml)	Supernatant total activity ((dpm)	% soluble activity
Soil (50%) + Sludge (50%)	1 day	1.1	0.11	29400	3910	1.14	2395	2730	69.8
	1 week	1.25	0.13	23770	3090	1.4	1900	2680	86.0
	2 weeks	1.3	0.13	23120	2890	1.3	1740	2260	78.2
	4 weeks	1.5	0.13	14820	1900	1.83	950	1740	91.6
	8 weeks	1.48	0.15	10000	1600	1.56	790	1230	76.9

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Table 4: 10-day-old maize grown in soil/sludge mixture ($\mu\text{g g}^{-1}$ nickel in d. wt.)

Growth medium	Conc. in maize leaves (Total)	Conc. in growth water soluble	Medium total
Soil (100%)	2.5	0.1	14
Soil (90%) + Sludge (10%)	8.3	1.15	64
Soil (50%) + Sludge (50%)	18.3	11.2	286

Table 5: Neutral and anionic forms in electrophoretic separation of Ni⁶³ compounds in supernatant fraction of maize leaves (Expressed as percentages)

		1 day	1 week	2 weeks	4 weeks	8 weeks
Soil (100%)	Neutral	55	43	45	51	47
	Anionic	29	37	33	25	23
	(sum)	(84)	(80)	(78)	(76)	(70)
Soil (90%) + Sludge (10%)	Neutral	41	30	30	25	24
	Anionic	46	52	53	55	52
	(sum)	(87)	(82)	(83)	(80)	(76)
Soil (50%) + Sludge (50%)	Neutral	47	41	33	14	12
	Anionic	41	45	50	67	62
	(sum)	(88)	(86)	(83)	(81)	(74)

forms and increased over the first week before significantly decreasing. In soil/ sludge mixture the positions are different, the concentration of anionic forms generally exceeded that of neutral forms and increased significantly with time. The total Ni⁶³ activity was higher in maize grown in soil/sludge than in soil alone, and the activity always significantly decreased with time. Replicate electrophoretic papers were examined by radio- autography. Only extracts from maize grown in mixtures of soil/sludge left detectable traces on the film. The papers were than sprayed with ninhydrin so that the areas of radioactivity could be compared with the migration of amino acids and pigments (Figs. 5 and 6). Some Ni⁶³ was located in the neutral amino acid/pigment area but the remainder was not coincident with other standard amino acids.

Discussion

The results from the water extractions of maize leaves indicated that the approximate percentages of Ni⁶³ from the leaf tissue which occurred in the supernatant fraction were : 71 to 87 per cent for leaf tissues grown in 100 per cent soils, 79 to 93 per cent for 90 per cent soil/10 per cent sludge, and 70 to 92 per cent for 50 per cent soil/50 per cent sludge. Timperley *et al.* (1973) and Cataldo *et al.* (1978b) indicated 90 per cent of the total nickel in the leaf tissues of the species they investigated occurred in the supernatant fraction. The fresh and dry weight yields have been significantly depressed by high sludge application, but with lower sludge application, yield and Ni⁶³ activity were higher. Mishra and Kar (1974) and Hooda and Alloway (1994) reported that nickel has significant effects on yields of crops when used at non-phytotoxic levels. At higher concentrations the decrease in the yield is probably due to nickel's toxic effects, causing severe chlorosis and necrosis of the leaves. The present study demonstrated the presence of anionic nickel complexes in maize leaves, this contrasts with the work of Timperley *et al.* (1973) and Kelley *et al.* (1975), who indicated that Ni²⁺ existed in cationic complexes in nickel accumulating species. This suggests that the chemical fate of Ni²⁺ in plants may be species dependent. Tiffin (1971) has demonstrated the presence of a single anionic nickel complex in the exudates of maize, carrot, tomato, cucumber and peanut when Ni²⁺ concentrations are within physiological limits for the plant. When Ni²⁺ concentrations exceeded 1 to 3 μM for maize and tomato, a minor cationic complex began to appear in addition to the anionic form, suggesting a 'saturation' concentration for the anionic carrier. The cationic complex was not present in carrot, cucumber, of peanut when

nickel concentrations were increased. In the present work, it seemed that, on the basis of electrophoresis results, the Ni⁶³ was associated with chlorophyll, the high levels of nickel occurring after sludge application could contribute to the destruction of this pigment; indeed, Tiffin (1971) has noted chlorosis of younger leaves in plants given high level of nickel. Sauerbeck (1991) reported that excess nickel induced chlorotic symptoms similar to the iron deficient chlorosis in some plants, which suggests that nickel treatment decreased the iron content in leaves. It has been shown that nickel additions to soils reduced that total iron content of barley (Moreno, 1996).

Varying the incubation time for the nickel spiked soil and soil/sludge mixtures prior to planting affected the nickel concentration in the maize seedlings significantly, there was a decrease with increasing incubation time. This trend might be expected since increasing incubation time would have permitted more decomposition of the sludge and allowed the release of greater amount of organic chelating material. Increasing the incubation time have reduced the availability of inorganic nickel by the following mechanism:

- 1 Immobilization of nickel through microbial action.
- 2 Reversion of weakly adsorbed nickel to strongly adsorbed and more stable forms
- 3 Transformation of freshly precipitated nickel minerals to greater amounts of crystalline and less soluble forms.

In general, during the first four weeks, plants grown at the higher level of sludge application had higher Ni⁶³ activity than plants grown with a lower level of sludge application, or in the control soil treatment. However, the Ni⁶³ activity of the plants from the low level sludge application increased during the following four weeks to a level beyond that of plants from the higher level. Thus, over the experimental period, the amount of sludge present influenced the uptake of inorganic nickel (as Ni⁶³) by plants and the levels of organic nickel in the plant were significantly increased by the greater sludge application. Therefore, increasing the uptake of nickel by plants by increasing sludge application may cause phytotoxic effects on crops. This makes it advisable to monitor the forms and concentration of nickel or other phytotoxic heavy metals in sewage sludge at the time of their application as a fertilizer. The chemical form of nickel is normally more important than the net metal concentration in the soil-plant system; indeed; the amount of available nickel in the soil plant system was found to change with time as it came to equilibrium, as did the proportions of soluble nickel complexes in the plants, a

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phenomenon of interest and importance to animal and human nutritionists.

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