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HEAVY-METAL ION UPTAKE BY PLANTS FROM NUTRIENT SOLUTIONS WITH METAL ION, PLANT SPECIES AND GROWTH PERIOD VARIATIONS

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SUMMARY

Rape, cucumber, wheat, oats and tomato were grown for one to two weeks in nutrient solutions with heavy metals added. Of the metal ions tested (Cr^{3+}, Cu^{2+}, Co^{2+}, CrO_4^{2-}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+} and Ag^{+}), manganese, nickel and lead exhibited the greatest mobility in cucumber plants, which resulted in the highest shoot/root concentration ratio. Silver was not translocated to the shoots of cucumber plants in measurable amounts.

When the plants were grown with 1.0, 10 and 100 μM cadmium or nickel in the solution, the shoot and root concentration increased 5–10 times if the metal ion concentration of the solution was increased 10 times.

The plants showed great differences in cadmium and nickel uptake. In the shoot, the cadmium concentration increased in the order: oats = wheat < cucumber = rape < tomato, and in the root in the order: oats < wheat < cucumber = rape < tomato. The great uptake of cadmium and nickel by tomato is notable and agrees with other reports.

The nickel, and especially the cadmium, concentration in roots and shoots increases with the age of the plant.

The results are discussed and related to other investigations. The need for research on the uptake mechanisms of non-essential heavy metals is emphasized.

INTRODUCTION

The flow of heavy metals through different pathways to foodstuffs and man is of fundamental interest for any discussion of heavy metals in relation to human health[22 28]. The contribution of heavy metals from air, water and food varies for people in different areas and for the metal studied. The atmospheric pollution may be most significant for lead, whereas mercury is probably most significant as a pollutant of aquatic food chains. The plant uptake may be important for the human intake of cadmium. For all heavy metals, the flow: soil → plant → animal → man is of great but varying interest.

In recent years, there have been many reports dealing with heavy metal uptake by plants. Especially cadmium has received much interest[1 3 5 6 9 10 11 15 16 20 21 22 24 26 28]. In most cases, the investigations are derived from actual pollution problems. The extensive studies of cadmium contamination of soils and rice plants caused by zinc mining in Japan is one example of this kind of work[22]. Many reports deal with possible risks of using contaminated sewage sludge for soil improvement. Uptake of heavy metals by agricultural plant species from sludge-manured areas may lead to an increased human intake[1 15 16 21 25 26]. The investigations are most often restricted to a limited number of soils and plant species in field and pot trials with the addition of contaminated sludge.

We still do not have the knowledge which enables us to make broad generalizations about the most common non-essential heavy metals and how these metals behave in plants and soils under varying environmental conditions. More extensive research is needed under varying field conditions and more studies of heavy metal uptake on a basic physiological level are required. Then the question of heavy metals in plants could perhaps in a natural way be linked to the extensive available knowledge about uptake, transport and function of 'trace elements' in plants[19 23].

In the present work, results are presented from experiments of heavy metal uptake from nutrient solutions. The experiments were performed with different metals, concentrations and plant species. The major part of this work concerns cadmium uptake and translocation with various plant species and growth periods.

MATERIALS AND METHODS

Plant material

The following plant species were used: Rape (*Brassica napus* L., variety oleifera, cultivar Svalöv's Gulle), cucumber (*Cucumis sativus* L., cultivar Weibull's Favör II), wheat (*Triticum sativum* Lam., cultivar Svalöv's Diamant II), oats (*Avena sativa* L., cultivar Svalöv's Sol II) and tomato (*Solanum lycopersicum* L., cultivar Weibull's Dansk Export).

Nutrient solution experiment

Seeds were germinated in the darkness at 25°C in petri dishes on moist filter paper four days for tomato and two days for the other plant species. Wheat and oats seeds were soaked in water 15 hours before transferring them to the petri dishes.

The seedlings were placed on perforated plastic plates in test tubes containing about 150 ml of a solution containing 0.6 mM potassium nitrate, 1.5 mM calcium nitrate, 1.2 mM potassium dihydrogen phosphate, 0.2 mM disodium hydrogen phosphate, 1.0 mM magnesium sulphate, 0.05 mM iron (III) chloride and 0.05 mM sodium citrate. Heavy metals were added as nitrates with the exception of potassium chromate. ^{109}Cd, ^{63}Ni and ^{65}Zn were used for labelling corresponding heavy metal ions, usually 1.0 uc/200 ml. The pH of all solutions was 6.0.

The nutrient solution was changed after about two thirds of the growth period. Usually five replicates of each treatment were grown. The plants in test tubes were placed in boxes with five tubes each and grown in a controlled chamber for plant growth: 16 hours of light a day, 70 per cent relative humidity and 20°C. Radiation intensity was about 5 mW cm^{-2} from Sylvania 96 Fluorescent tubes, cool light/neutral, 50/50. Plants and solutions were not sterilized.

Nine rape plants were harvested after 12 days or earlier, 5 cucumber plants after 12 days, 14 oats or wheat plants after 7 days and 5 tomato plants after 14 days.

Harvest

After harvesting the plants were divided into shoots and roots. The parts were weighed. The roots were washed with 40 ml of 20 mM calcium nitrate in phosphate buffer, 1.0 mM, pH 7.0, three times – 30, 30 and 10 minutes.

Analysis

Plants grown with ^{109}Cd and ^{65}Zn were placed in plastic bottles and measured by Nuclear Data 130 with 4″ × 4 mm sodium iodide (Tl) crystal. ^{63}Ni was measured by means of a Beckman liquid scintillation system and the other metals with Varian AA5 atomic absorption spectrophotometer after the plant material was treated according to AOAC-method 6.062 for phosphorous.

RESULTS

Metal variations

The results obtained by cucumber plants are given in Fig. 1 and Table 1. Most of the metals have a considerably higher concentration in roots than in shoots. Manganese, lead and nickel show greater mobility than the other metals studied. Silver ions are probably reduced to metallic silver and appear as a black precipitate on the roots. Silver ions are not translocated to the shoots in measurable amounts.

Ag, Cu, Cd and Co markedly retarded root growth at the metal

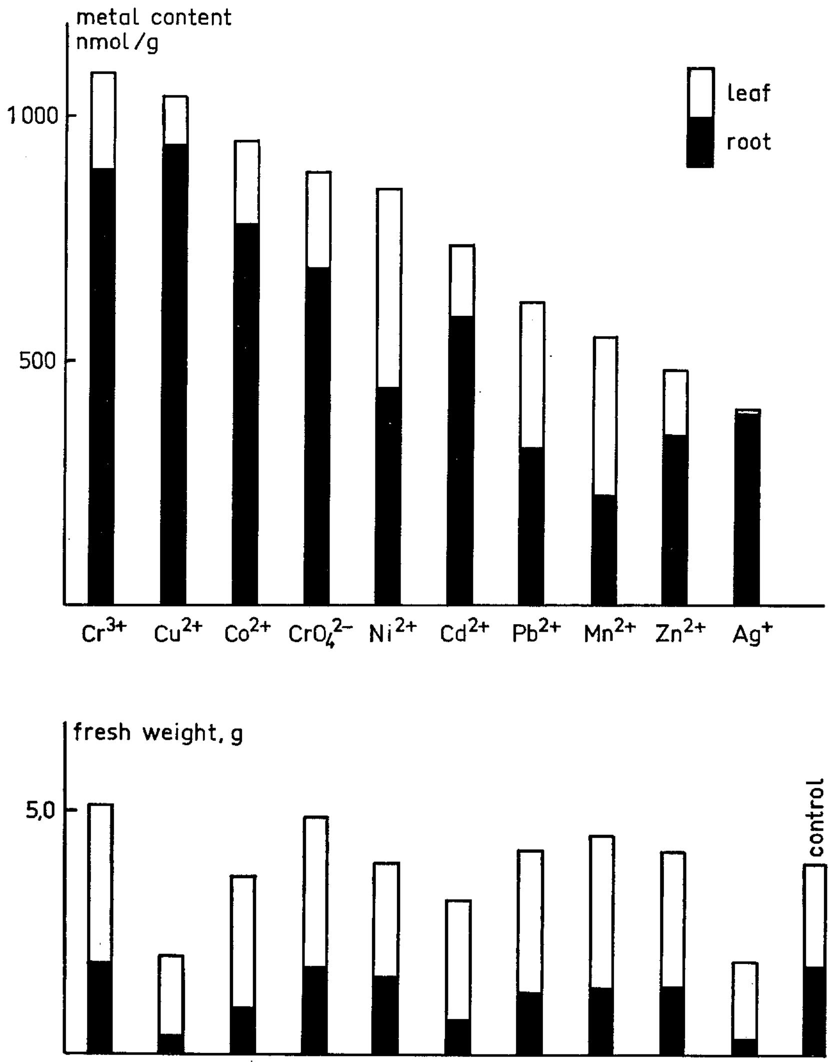

Fig. 1. Heavy metal concentration and fresh weights of cucumber plants grown for 12 days in nutrients solutions with a metal ion concentration of 10 μM (means of five replicates with five plants each).

concentration used. The other metals tested give no clear decrease in growth, in fact a stimulation of shoot growth may be noticed at least for chromium. In the nickel experiments, chlorotic spots appear on the leaves.

TABLE 1

Heavy metal concentration ratio shoot/root of cucumber plants grown for 12 days in nutrient solutions with a metal ion concentration of 10 μM, *cf* Fig. 1

Metal ion	Shoot/root	Metal ion	Shoot/root
Mn^{2+}	1.50	Cd^{2+}	0.25
Pb^{2+}	0.94	Cr^{3+}	0.23
Ni^{2+}	0.94	Co^{2+}	0.22
Zn^{2+}	0.41	Cu^{2+}	0.11
CrO_4^{2-}	0.29	Ag^+	< 0.05

TABLE 2

Nickel concentration of tops and roots from cucumber plants grown for 12 days in nutrient solutions with different nickel concentrations

Nickel conc., μM	Tops, nmoles/g fresh weight	Roots, nmoles/g fresh weight
1.0	22	22
10	195	213
100	1580	1710

Metal concentration variations

Rape, cucumber, wheat, oats and tomato were grown in nutrient solutions with 1.0, 10 and 100 μM of cadmium. An 10-fold increase in solution metal ion concentration results in a 5 to 10 times higher level of the metal in the plant (Fig. 2). Nickel affects the plants in a similar way, which can be seen from Table 2. Further more, there are no outstanding differences between plant species in this response to different cadmium concentrations. On the other hand, the plant species differ greatly in cadmium uptake at the same concentration.

Shoot cadmium increases in the order: oats = wheat < cucumber = rape < tomato and root cadmium concentration in the order: oats < wheat < cucumber = rape < tomato. For all plant species studied, there is noticed a slight decrease in growth at 10 μM and a strong retardation at 100 μM. Roots are most sensitive. At 1.0 μM, phytotoxic effects can be seen only in tomato plants which species is outstanding according to both uptake and sensitivity.

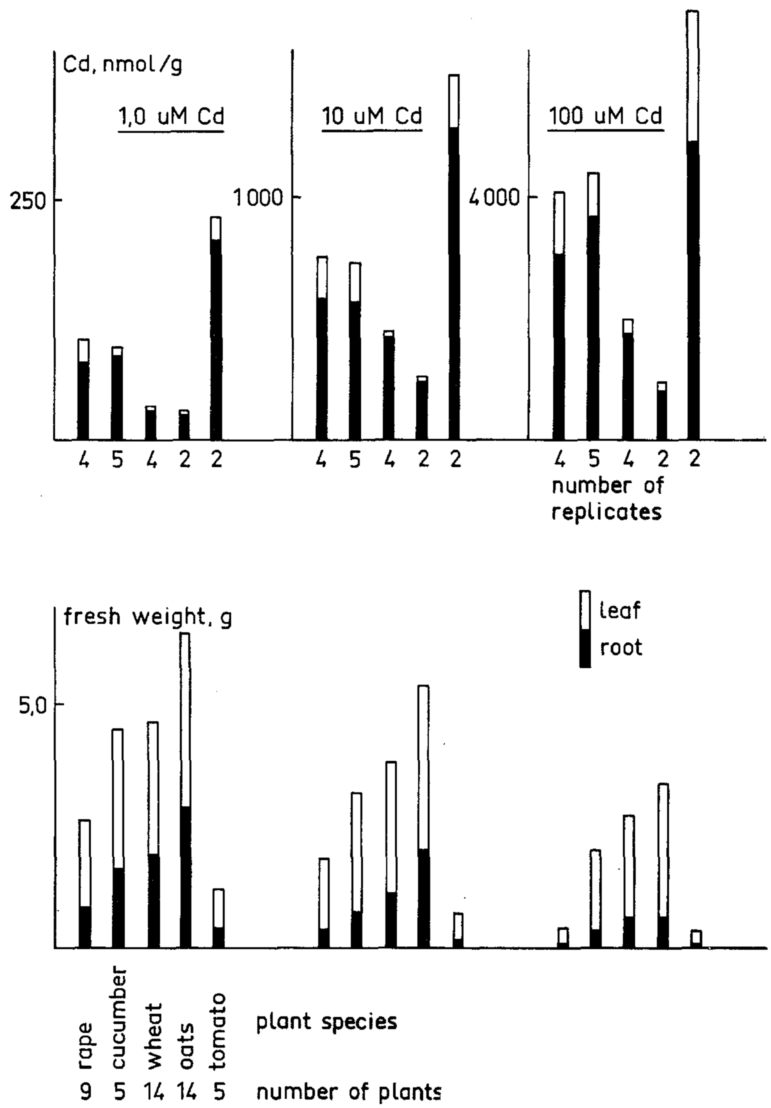

Fig. 2. Cadmium concentration of tops and roots from different plant species grown in nutrient solution with 1.0, 10 and 100 μM of cadmium.

Growth time variations

The plants were grown with 10 μM of cadmium or nickel and harvested at different ages. The results are given in Figures 3–7. Because of their high sensitivity, the experiment with tomato plants

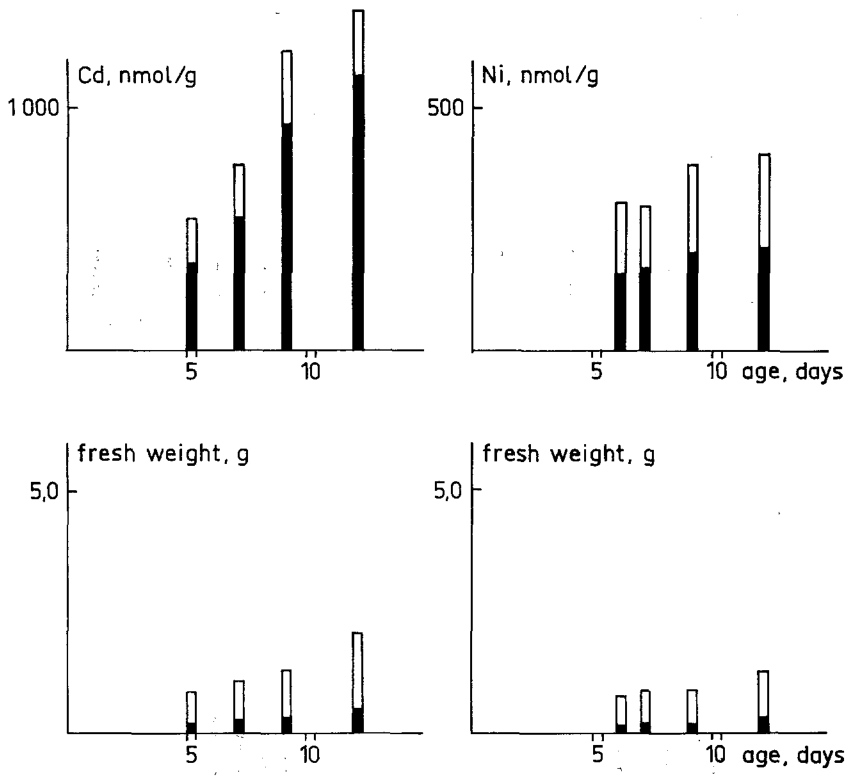

Fig. 3. Cadmium and nickel concentration and fresh weights of rape harvested at different ages and grown in nutrient solutions with a metal concentration of 10 μM (means of five replicates), metal content in nmoles/g fresh weight.

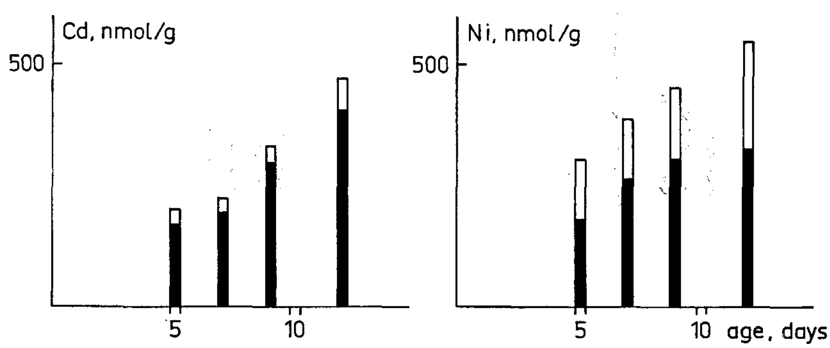

Fig. 4

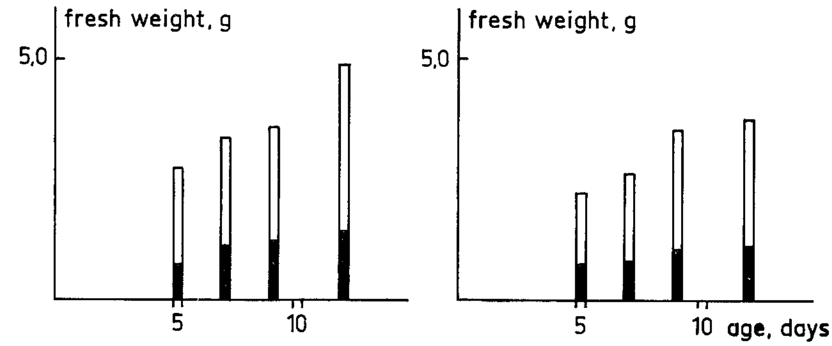

Fig. 4. Cucumber, see legend of Figure 3.

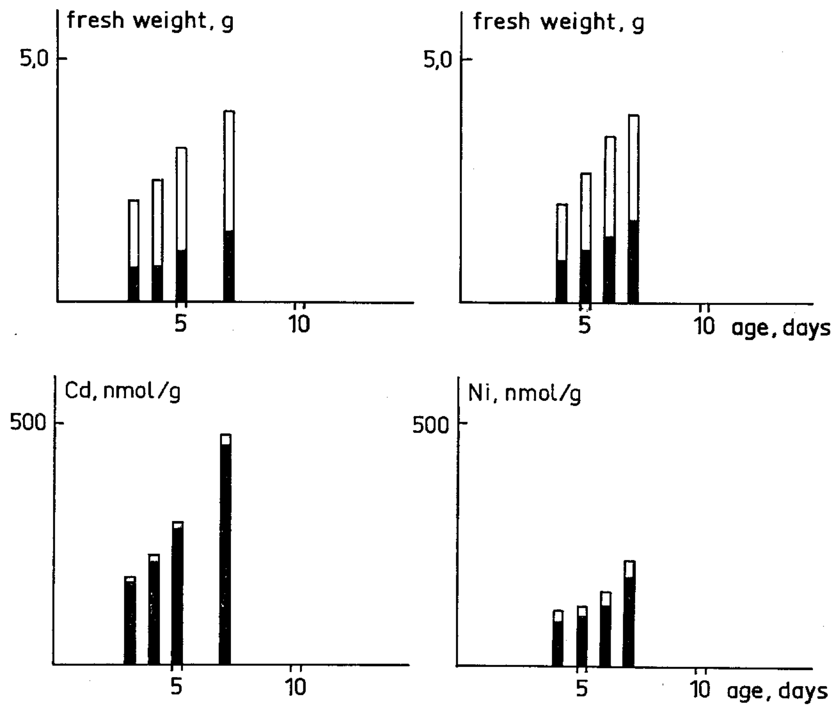

Fig. 5. Wheat, see legend of Figure 3.

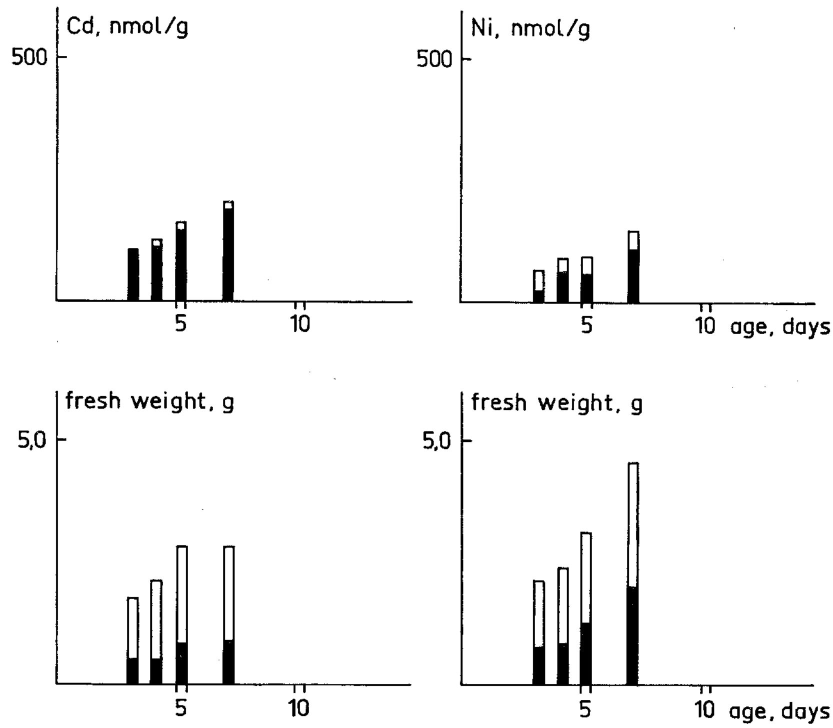

Fig. 6. Oats, see legend of Figure 3.

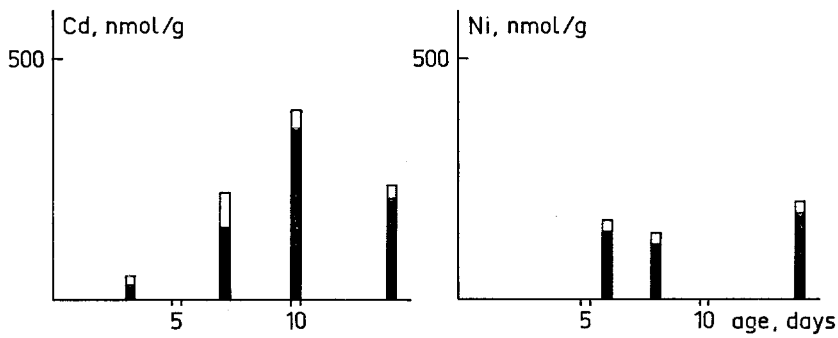

Fig. 7

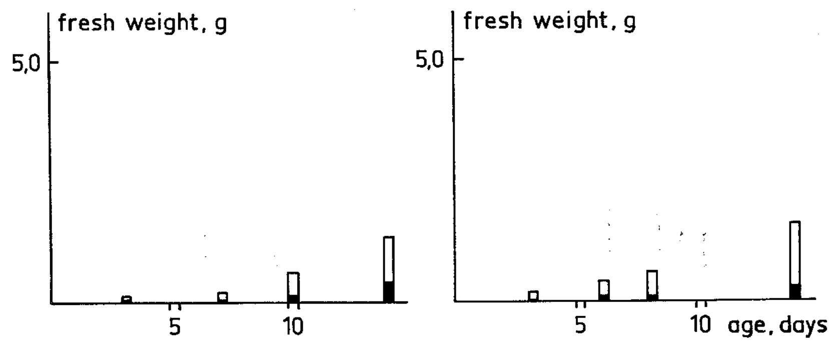

Fig. 7. Tomato, metal concentration 1.0 μM, see legend of Figure 3.

TABLE 3

Heavy metal concentration ratio shoot/root of different plant species grown in nutrient solutions with cadmium or nickel concentrations of 10 μM, *cf* Figures 3–7

Plant	Age, days	Cd	Ni
Rape	12	0.24	0.92
Cucumber	12	0.17	0.67
Wheat	7	0.06	0.16
Oats	7	0.08	0.38
Tomato (1.0 μM)	14	0.14	0.12

TABLE 4

Concentration ratio cadmium/nickel for plants grown in nutrient solutions with a metal concentration of 10 μM, *cf* Figures 3–7

Plant species	Shoot	Root
Rape	1.5	5.3
Cucumber	0.3	1.2
Wheat	0.6	2.5
Oats	0.4	1.7
Tomato (1.0 μM)	1.2	1.2

were performed with a metal ion concentration of 1.0 μM. See Fig. 7.

The cadmium and nickel concentration increases with the age of the plant, with the exception of tomato. There are outstanding differ ences in the mobility of the metals in different plant species. Thus, rape and cucumber have a higher shoot/root ratio for both cadmium and nickel concentration than the other plant species (Table 3).

As can be seen from Table 4, plant specificity also exists regarding cadmium and nickel uptake. Rape has a higher level of cadmium than of nickel in the shoot but cucumber has much lower.

DISCUSSION

Heavy metals usually accumulate more in the root than in the shoot. This result is obtained from experiments with nutrient solutions as well as soil tests. Huffman and Allaway[7] studied chromium uptake by mature bean and wheat plants in nutrient solution and found more than 90 per cent of the total plant Cr in the roots. Uptake and translocation of Cr differed only slightly between Cr(III) and Cr(VI) sources which agrees with the results presented here. Similar results about chromium uptake and translocation are also reported by Verfaillie[27].

A greater amount of the cadmium than of the nickel which is taken up by plants is found in the root; the cadmium concentration is most often higher than that of nickel (Table 3, 4). The root cadmium is probably mainly absorbed on the root surface and not available for translocation. Thus, in an investigation of cadmium uptake from contaminated soil[10], it was found that the roots had a several times higher level of cadmium than the shoots, but only in the root parts which were directly exposed to the contaminated soil. In root parts not exposed to the soil, cadmium content was only slightly higher than in the shoots. Therefore, in most reported experiments, a higher concentration of cadmium is found in the root than in the shoot[9 10]. Radish may be an exception[5].

Also mercury is found in a similar distribution between plant parts, with the highest level in the roots[8].

Of the metals studied here, lead, manganese and nickel show the greatest mobility in cucumber plants (Table 1, Fig. 1). Minami, Yasuda and Araki[18] found a high lead mobility in soil culture corn experiments. Of the metals tested, they noticed great mobility of lead and cadmium but less of cobalt, copper and nickel. Nothing can be concluded from the differences of my results because of different experimental conditions.

In soil culture and nutrient solution experiments with ryegrass[12 13], the same pattern of distribution in root and top has been found for lead as for other metals with a several times higher content in the root than in the top. My results, on the other hand, show great mobility of lead in cucumber. These results, however, cannot really be compared until the experiments are performed with the same plant species and/or growth medium. Plant specificity, lead concentration in nutrient solution or other medium differences may explain the divergence of mobility of lead.

Furthermore, when ryegrass was starved of sulphur, lead had a greater mobility with a higher level in tops than in roots[14]. Malone, Koeppe and Miller[17] studied accumulation of lead by corn roots and showed that lead could precipitate on root surface and cell walls. If lead precipitates on root surfaces, it is possible that a measured difference in mobility is due to a different root treatment after the uptake period. Thus, there are several possible reasons that lead has a different mobility in the experiments referred to here.

In the experiments with plants of varying ages, the cadmium and nickel concentration increases with age for rape, wheat, cucumber and oats. Tomato results in a more irregular pattern. This could be explained by the fact that the real metal concentration of the solution varies more because plant uptake influences solution level to a higher degree at 1.0 than 10 μM.

In experiments with oats, Cutler and Rains[3] obtained similar distribution between roots and tops as in my results. Moreover, the cadmium concentration and the top/root ratio increased with time during the 28 days of growth. In field trials with rice, Takijima and Katsumi[22] noticed an increase in cadmium concentration for the entire growth period up to maturity, while zinc increased only slightly and copper and lead decreased. This suggests that cadmium has special characteristics for plant uptake.

Uptake of nickel and cadmium is greatly dependent on the nutrient solution concentration in my experiment. A 10-fold increase of solution concentration results in an increase of between 5 to 10 times in the plant. This response is rather similar for all the plants tested with cadmium. Possibly, there is a more linear relationship in the lower part of the concentration range. Turner[24] studied cadmium uptake for 5 weeks at approximately 10 times lower solution concentrations. Lettuce, radish and swiss chard showed similar responses as in my experiments but beet root and carrot increased their uptake only slightly at the high concentrations.

The results obtained by Turner and those of Page, Bingham and Nelson[20], from experiments lasting 3 weeks, usually show a lower plant/solution cadmium ratio compared to my experiment, which suggests that this ratio decreases with time.

The experiments on cadmium and nickel uptake confirm that great species diversity exists. These differences in uptake are of an amplitude which could be of interest for choosing crops to grow on contaminated soils. This is also pointed out by Turner[24].

The outstandingly high cadmium uptake by tomato agrees with several other reports performed under varying experimental conditions[20 24]. Tomato has also shown particular attributes regarding mercury uptake. Van Loon[25] analysed mercury from different plant parts of six plant species in a field trial with mercury-contaminated sewage sludge. Only in the tomato plants there was a significant increase in mercury concentration.

The slight differences in cadmium mobility in Tables 1 and 3 and a similar disagreement of nickel concentration in Table 4 and Fig. 4 may be due to varying light intensities in the chamber between the series of experiments.

The research done on heavy metal uptake by plants up to the present day is most often carried out on a practical agronomic basis[1 4 5 6 7 8 9 10 12 15 16 18 21 22 25 26 28]. Of a more basic physiological character are the studies by Malone, Koeppe and Miller[17] on lead accumulation in corn roots and the work by Cutler and Rains[3] on cadmium uptake by barley plants and roots. They found three mechanisms for cadmium uptake: exchange adsorption, irreversible binding and diffusion. It is called diffusion because the temperature response does not indicate metabolic uptake.

It has recently been concluded that cobalt, on the other hand, is actively absorbed by barley roots[2]. Otherwise not very much is known about the mechanisms for the uptake of non-essential heavy metals. The same is true for translocation, with the exception of nickel[23]. More research is needed before it is possible to understand the uptake and translocation of non-essential heavy metals and their appearance compared to the metals which are essential for plant growth.

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