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## **Nickel and manganese accumulation and distribution in organs of nine crops**

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### ABSTRACT

Field experiments were carried out from 1999 to 2001 with nine crops (red beet, field pumpkin, chicory, common bean, barley, white cabbage, maize, alfalfa, common parsnip) to determine the nickel and manganese accumulation and distribution in the plants' organs. Based on the obtained results species suited for phytoremediation were selected. Within the red beet, field pumpkin, chicory, common bean and parsnip the maximum levels of nickel and manganese were found in the leaves. Within the barley and maize the highest amounts of investigated metals were found in the roots. The red beet, chicory, and common parsnip were characterized by the highest nickel concentration ratios (shoots/roots). The red beet and common parsnip were characterized by the highest manganese concentration ratios (shoots/roots).

## INTRODUCTION

In general, phytoremediation technologies include either the removing of the contaminants from the soil (phytoextraction) or reducing the risk posed by the contaminants by reducing exposure (phytostabilization). For phytoextraction to be successful, plants should possess sufficient accumulation of the metal intended to be extracted, fast growth with large biomass, suitable phenotype for easy harvest, treatment and disposal, and tolerance to site conditions (Vangrosveld and Cunningham 1998). The importance of biodiversity is increasingly considered for the cleanup of the metal contaminated ecosystems. Screening crop species for high metal uptake and tolerance is a general approach for further development of metal phytoextraction. Several researches have screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Prasad and Freitas 2003, Ciura et al. 2004).

To overcome the main biological bottlenecks limiting successful phytoextraction, a good knowledge about metal uptake, plant defence network against metal, as well as some possibilities to reduce chronic metal toxicity is fundamental. Poniedziałek et al. (1999) found differences between crops in the level of heavy metal accumulation in particular organs. They mention that metals' absorption and transport can be modified by many factors, i.e. cultivar, timing of production, and locality. It is necessary to try the phytoremediation efficiency of different crops in environmental conditions of Poland.

Ni and Mn in the soil originate mainly from natural sources, but high contents of these elements occur locally as a result of mining, fertilizer application, automobiles, industrial and manufacturing activities. Manganese and nickel availability and toxicity for plants is controlled by many factors, i.e. metal content in the soil, soil pH, redox potential, organic matter content, and plant genetics (Vega et al. 1992). A number of plants are known to enhance metals availability by releasing root exudates (Youssef and Chino 1991). In soil containing nonphytotoxic levels of metals (i.e. Ni) root-to-shoot transport rates are equal in hyperaccumulators and the other species (Kramer et al. 1997).

The aim of investigations was to determine nickel and manganese accumulation and distribution in organs of nine crops. Obtained results will let to select species for further investigations determining agronomy based optimisations as a tool for improvement of metal phytoextraction.

## MATERIAL AND METHODS

A field experiment was conducted from 1999 to 2001 at the Research Station of Agricultural University in Kraków, on soil classified as *Eutric Cambisols*, formed on loess.

The soil was characterized by  $\text{pH}_{\text{KCl}}$  4.8 and 1.2% organic carbon. The soil contained  $2.32 \text{ mg kg}^{-1}$  d.w. of exchangeable ( $15.1 \text{ mg kg}^{-1}$  d.w. of total) Ni and  $177.3 \text{ mg kg}^{-1}$  d.w. of exchangeable ( $333.2 \text{ mg kg}^{-1}$  d.w. of total) Mn. To determine exchangeable nickel and manganese content, soil samples (10 g) were treated with  $100 \text{ cm}^3$  0.01 M  $\text{CaCl}_2$  and shaken during 2 hours. After filtration of the solids, Mn and Ni were determined by ASA method, using Varian-SpectrAA 20 and air/acetylene flame under standard operating conditions. Total nickel and manganese content was determined by ASA method, after wet-mineralisation of soil samples (1.000 g) in a microwave oven MDS 2000 CEM, in a presence of 30%  $\text{HNO}_3$ . The organic carbon content was determined using Tiurin's method, based on the oxidising of C to  $\text{CO}_2$ , using bichromate of potassium as an oxidant (Ostrowska et al. 1991).

The objects of investigations were nine crops selected according to their different taxonomy position and information cited in literature about their remediation efficiency: red beet (*Beta vulgaris* var. *cicla* L.) – ‘Wodan F<sub>1</sub>’, field pumpkin (*Cucurbita pepo* L. convar. *giromontiana* Greb.) – ‘Astra F<sub>1</sub>’, chicory (*Cichorium intybus* var. *foliosum* Hegi) – ‘Rubello F<sub>1</sub>’, common bean (*Phaseolus vulgaris* L.) – ‘Tara’, barley (*Hordeum vulgare* L.) – ‘Stat’, white cabbage (*Brassica oleracea* var. *capitata* L.) – ‘Krautman F<sub>1</sub>’, maize (*Zea mays* L. convar. *saccharata* Koern.) – ‘Trophy F<sub>1</sub>’, alfalfa (*Medicago sativa* L.) – ‘Vela’, common parsnip (*Pastinaca sativa* L.) – ‘Półdługi Biały’.

Crops were grown under standard agronomic conditions on experimental plots (plot area -  $9 \text{ m}^2$ ) in four replications, with random blocks method. Crops were harvested at the stage of harvest maturity. The fresh weight and the heavy metal content were determined in the morphological organs of the investigated crops: red beet – roots and leaves, chicory – roots, rosette leaves, and head, field pumpkin – roots, stem, leaves, and fruits, common bean – roots, stem, leaves, and pods, barley – roots, straw, and grain, white cabbage – roots, stem, rosette leaves, and head, maize – roots, stem, leaves, husks, shank, and grain, alfalfa – roots and shoots, common parsnip – roots and leaves. Plant samples were rinsed in demineralised water, dried in  $105^\circ\text{C}$ , and ground using a colloidal grinder (Retsch). Analyses of Ni and Mn content were carried out using ASA method, following prior dry-mineralisation of 5.000 g plant samples at  $500^\circ\text{C}$ , dissolved in 20%  $\text{HNO}_3$  (Ostrowska et al. 1991). The mean concentrations of heavy metals in aerial parts of investigated plants were calculated according to formula: mean metal conc. = sum of (metal conc. in each plant part x fraction each part contributes to total shoot biomass).

The results were statistically evaluated using analyses of variance, significant differences between means were calculated by the Student t-test at  $p = 0.05$ . Coefficients of simple correlation were calculated between the amount of metals in particular plants organs ( $N = 12$ ) at  $p = 0.001$ .

## RESULTS AND DISCUSSION

There were significant differences in the nickel and manganese levels in the morphological organs of the investigated crops. Within the red beet, field pumpkin, chicory, common bean and parsnip the maximum levels of both elements were found in the leaves (Figs 1 and 2).

The leaves of the red beet contained 4.1 times more of nickel and 3.7 times more of manganese than the storage roots (Figs 1 and 2). A significant correlation coefficient was found between the amount of nickel and manganese in the leaves and the roots of the red beet, i.e.  $r = 0.942$  and  $r = 0.922$ , respectively (Table 1a). It confirmed the theses that plants accumulate both nickel and manganese, and distribute it in tissues proportionally to its level in the soil. Gambuś (1991) compared the capacity of taking up heavy metals by seven species of vegetables. He found higher accumulation of nickel in leaves of red beet in comparison with storage roots, irrespective of the level of metal in the soil.

Within the field pumpkin the lowest level of nickel ( $3.12 \text{ mg kg}^{-1} \text{ d.w.}$ ) was found in the fruits (Fig. 1). Significantly higher level of this element was found in the stem ( $3.99 \text{ mg kg}^{-1} \text{ d.w.}$ ), roots ( $10.34 \text{ mg kg}^{-1} \text{ d.w.}$ ) and leaves ( $11.41 \text{ mg kg}^{-1} \text{ d.w.}$ ). The gradient of manganese concentration declined in the order: leaves ( $66.5 \text{ mg kg}^{-1} \text{ d.w.}$ ), roots ( $18.3 \text{ mg kg}^{-1} \text{ d.w.}$ ), fruits ( $17.7 \text{ mg kg}^{-1} \text{ d.w.}$ ), and stem ( $14.2 \text{ mg kg}^{-1} \text{ d.w.}$ ) (Fig. 2).

The rosette leaves of chicory accumulated 10.2 times more of nickel and 2.2 times more of manganese in comparison with the leaves formed in the head (Figs 1 and 2). There were no significant differences in the level of nickel in the roots and leaves formed in the head of chicory. A significant correlation ( $r = -0.945$ ) was found between the level of Mn in the rosette leaves and the head of the investigated species (Table 1a).

The leaves of the common bean were the most contaminated with Ni and Mn, i.e.  $16.73$  and  $43.13 \text{ mg kg}^{-1} \text{ d.w.}$ , respectively (Figs 1 and 2). The pods were characterized by low levels of both elements in spite of high mobility of investigated metals in plants. A significant correlation coefficient ( $r = 0.849$ ) was found between the level of manganese in the common bean pods and leaves (Table 1a). Kuboi et al. (1986) proved that plants of *Fabaceae* family are generally characterized by low trace element absorption. In the case of Ni, Mallan and Farrant (1998) reported the following distribution in soybean plant: roots > leaves > mature seeds > pods.

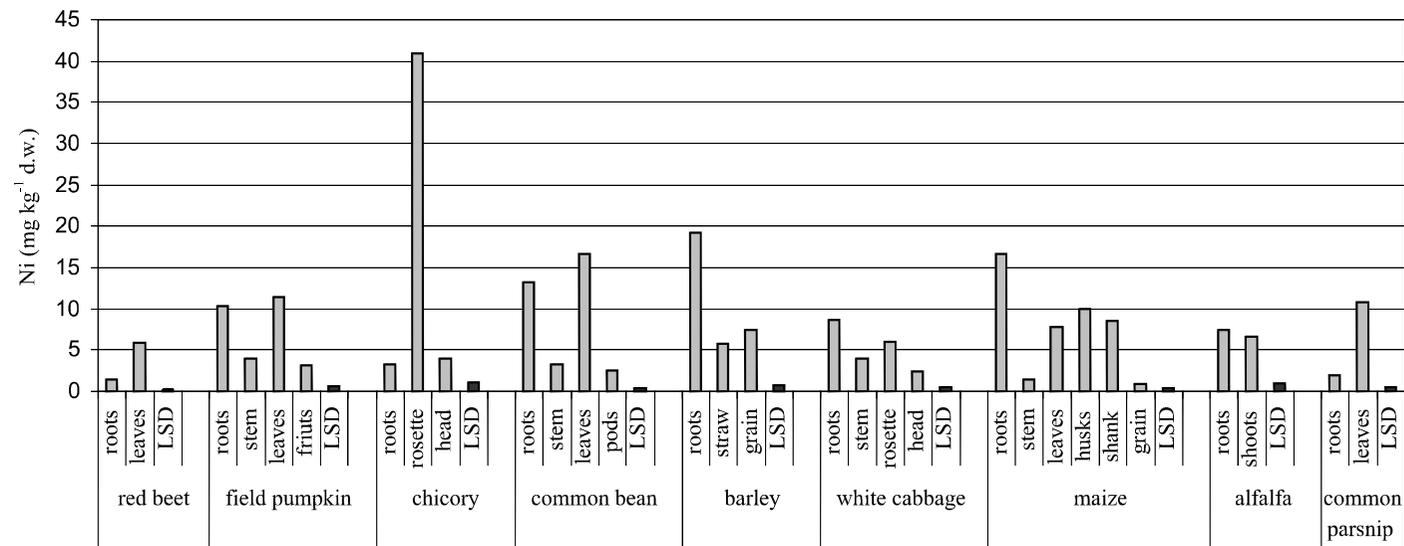


Figure 1. Nickel distribution among organs of investigated crops, average for 1999 – 2001 (mg kg<sup>-1</sup> d.w.)

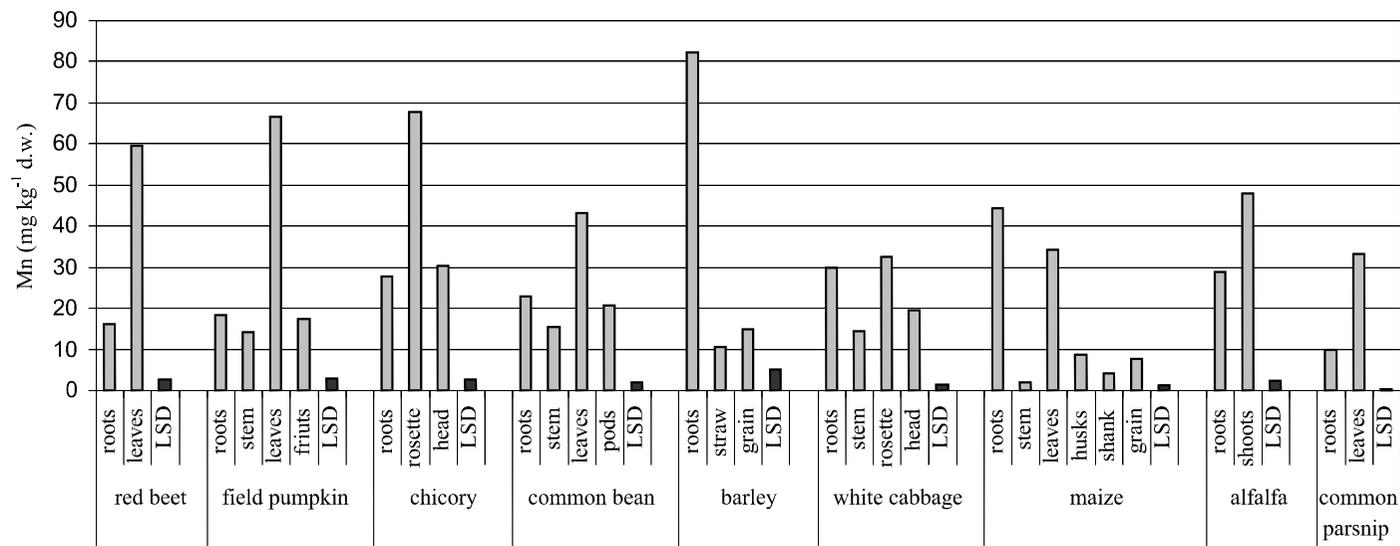


Figure 2. Manganese distribution among organs of investigated crops, average for 1999 – 2001 (mg kg<sup>-1</sup> d.w.)

Table 1a. Coefficients of simple correlation (r) between the amount of metals in tested plant organs (\* – r significant at p = 0.001)

	Nickel	Manganese
Red beet		
Roots – leaves	0.942*	0.922*
Field pumpkin		
Roots – leaves	-0.439	-0.352
Roots – stem	-0.307	0.006
Roots – fruits	0.325	-0.498
Leaves – stem	0.221	-0.767
Leaves – fruits	0.224	-0.279
Stem – fruits	-0.794	0.609
Chicory		
Roots – rosette	0.108	0.582
Roots – head	-0.303	-0.448
Rosette – head	0.315	-0.945*
Common bean		
Roots – leaves	-0.501	-0.384
Roots – stem	0.637	0.219
Roots – pods	-0.081	-0.564
Leaves – stem	-0.567	-0.584
Leaves – pods	0.175	0.849*
Stem – pods	0.428	-0.717
Barley		
Roots – straw	-0.013	0.932*
Roots – grain	0.236	-0.040
Straw – grain	0.409	-0.040
White cabbage		
Roots – stem	0.906*	0.930*
Roots – rosette	0.155	0.460
Roots – head	-0.479	0.571
Rosette – stem	0.363	0.615
Rosette – head	0.646	0.939*
Stem – head	-0.309	0.736

Table 1b. Coefficients of simple correlation ( $r$ ) between the amount of metals in mentioned plants organs (\* –  $r$  significant at  $p = 0.001$ ).

	Nickel	Manganese
	Maize	
Roots – stem	-0.413	-0.262
Roots – leaves	0.708	-0.452
Roots – husks	-0.618	0.490
Roots – shank	-0.713	-0.486
Roots – grain	-0.005	0.051
Leaves – stem	-0.527	0.605
Leaves – husks	0.304	0.725
Leaves – shank	0.885*	0.584
Leaves – grain	-0.427	-0.583
Stem – husks	-0.402	0.601
Stem – shank	-0.498	0.758
Stem – grain	0.364	-0.369
Husks – shank	0.093	0.566
Husks – grain	-0.311	-0.395
Shank – grain	-0.505	-0.334
	Alfalfa	
Roots – shoots	0.990*	0.950*
	Common parsnip	
Roots – leaves	-0.708	0.217

The monocotyledons (barley and maize) accumulated high amounts of nickel and manganese in the roots (Figs 1 and 2). Nickel is a mobile microelement, easily absorbed by plants and transported in xylem and floem and accumulated in high amounts even in the seeds (Zeller and Feller 1999). The seeds of examined species were not contaminated with nickel in high amounts. The level of Ni in the shoots of barley was about 2 times lower (the level of Mn – 5 times lower) in comparison with the roots. The organs of maize, characterized by the lowest level of nickel, were the grain ( $0.79 \text{ mg kg}^{-1} \text{ d.w.}$ ) and stem ( $1.45 \text{ mg kg}^{-1} \text{ d.w.}$ ), in the case of manganese: stem ( $2.00 \text{ mg kg}^{-1} \text{ d.w.}$ ) and shank ( $4.13 \text{ mg kg}^{-1} \text{ d.w.}$ ). The significant correlations were found between the level of manganese in the roots and straw of barley ( $r = 0.932$ ), and the level of nickel in the leaves and shank of maize ( $r = 0.885$ ) (Tables 1a and 1b). Jasiewicz and Sendor (1995) investigated the toxicity and accumulation of Ni in maize depending on its content in aquacultures. The authors found the highest level of this element in maize roots, then in stalks, and the lowest in leaves and inflorescences.

Table 2. Nickel and manganese accumulation in roots and shoots of investigated crops, average for 1999 – 2001 (mg kg<sup>-1</sup> d.w.)

	Red beet	Field pumpkin	Chicory	Common bean	Barley	White cabbage	Maize	Alfalfa	Common parsnip
Nickel									
Roots	1.43	10.34	3.26	13.16	19.19	8.60	16.71	7.42	1.87
Shoots	5.86	6.91	18.78	4.07	6.02	3.14	4.02	6.55	10.84
Concentration ratios (shoots/roots)	4.09	0.66	5.76	0.30	0.31	0.36	0.24	0.88	5.79
LSD <sub>0.05</sub> for:									
species 2.43									
organs 1.14									
interaction 3.43									
Manganese									
Roots	16.27	18.32	27.63	22.97	82.32	29.96	44.38	29.06	9.87
Shoots	59.65	39.37	45.35	26.69	11.55	22.19	10.28	48.00	33.18
Concentration ratios (shoots/roots)	3.66	2.14	1.64	1.16	0.14	0.74	0.23	1.65	3.36
LSD <sub>0.05</sub> for:									
species 6.02									
organs n.s.									
interaction 9.65									

In the case of white cabbage, organs the most contaminated were the roots (8.60 mg kg<sup>-1</sup> d.w. of Ni and 29.96 mg kg<sup>-1</sup> d.w. of Mn), and the leaves of rosette (5.94 mg kg<sup>-1</sup> d.w. of Ni and 32.54 mg kg<sup>-1</sup> d.w. of Mn) (Figs 1 and 2). Baker et al. (1991) compared remediation efficiency of six species from *Brassicaceae* family (two crops and four wild growing hyperaccumulators). In this experiment the white cabbage accumulated in aboveground tissues amounts of Ni comparable with received in the present work. Authors suggested that crops were much less effective in remediation than hyperaccumulators in spite of high biomass production.

According to Gardea-Torresdey et al. (1996) the silica-immobilized alfalfa has the potential to be used as a biofilter for removal and recovery of nickel ions from contaminated waters. In the present experiment there were no statistical differences in the level of Ni in the roots and shoots of this species (Fig. 1). In the case of Mn the shoots contained 1.6 times more of this element in comparison with the roots (Fig. 2). Significant correlations were found between levels of both nickel and manganese in roots and shoots of alfalfa, i.e.  $r = 0.990$  and  $r = 0.950$ , respectively (Table 1b).

The level of Ni and Mn in common parsnip leaves was slightly higher than in roots. Leaves of mentioned species contained 6.0 times more of nickel and 3.4 times more of manganese than the storage roots (Figs 1 and 2).

Baker (1981) suggested three types of plant-soil relationship: accumulators, excluders, and indicators. The operation of these mechanisms can be seen by comparing metal levels in the roots and aerial parts of plants. Heavy metal concentration ratios (shoot/root)  $>1$  are characteristic for accumulators;  $<1$  for excluders. The data for the nickel distribution suggest the consistent differences between species: concentration ratios for the red beet, chicory and common parsnip are characteristic for accumulators, the other species – for excluders (Table 2). In the case of manganese – the most efficient accumulators are red beet and common parsnip. It should be stressed that species may act both as accumulators and as excluders over different ranges of soil metal concentration. Accumulators and excluders are the extremes of physiological response of plants to heavy metals. ‘Indicator’ behaviour is seen as intermediate type of response which may or may not reflect a direct link between uptake and metal tolerance (Baker 1981).

According to Ciura et al. (2004), among investigated species, the most effective in removing nickel and manganese from the soil was the field pumpkin ( $13.2 \text{ mg m}^{-2} \text{ year}^{-1}$  of Ni and  $66.5 \text{ mg m}^{-2} \text{ year}^{-1}$  of Mn), because of the high fresh matter yield. The cited and reported results show that the efficiency of cleaning soils polluted with nickel and manganese depends both on the biomass production and possibilities of particular species for metal accumulation in harvestable organs.

## CONCLUSIONS

1. Within the red beet, field pumpkin, chicory, common bean and parsnip the maximum levels of nickel and manganese were found in the leaves.
2. Within the barley and maize the highest amounts of investigated metals were found in the roots.
3. The red beet, chicory, and common parsnip were characterized by the highest nickel concentration ratio (shoots/roots).
4. The red beet and common parsnip were characterized by the highest manganese concentration ratios (shoots/roots).
5. For red beet and alfalfa a significant correlation between the level of Ni and Mn in the roots and above ground organs was found.

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#### DYSTRYBUCJA NIKLU I MANGANU W ORGANACH DZIEWIĘCIU GATUNKÓW ROŚLIN UPRAWNYCH

**Streszczenie:** Doświadczenia polowe przeprowadzono w latach 1999 – 2001 w celu zbadania poziomu i dystrybucji niklu i manganu w organach morfologicznych 9. gatunków roślin uprawnych (burak ćwikłowy, dynia zwyczajna, cykoria, fasola szparagowa, jęczmień, kapusta głowiasta biała, kukurydza cukrowa, lucerna i pasternak). Na podstawie uzyskanych wyników określono możliwość wykorzystania roślin uprawnych w technikach fitoremediacyjnych. Najwyższy poziom niklu i manganu stwierdzono w liściach buraka ćwikłowego, dyni zwyczajnej, cykorii, fasoli szparagowej i pasternaku. W przypadku jęczmienia i kukurydzy największą ilość badanych metali zawierały w korzenie. Najwyższy wskaźnik akumulacji niklu (pędy/korzenie) charakteryzował następujące gatunki: burak ćwikłowy, cykorię i pasternak, a manganu: burak ćwikłowy i pasternak.

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