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Original article

Effect of heavy metals contamination on root-derived and organic matter-derived CO_2 efflux from soil planted with *Zea mays*

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ABSTRACT

The CO_2 efflux from loamy Haplic Luvisol and heavy metal (HM) uptake by *Zea mays* L. were studied under increased HM contamination: Cd, Cu, and Ni up to 20, 1000, and 2500 mg kg^{-1} soil, respectively. Split-root system with contrasting HM concentrations in both soil halves was used to investigate root-mediated HM translocation in uncontaminated soil zones. To separate root-derived and soil organic matter (SOM)-derived CO_2 efflux from soil, $^{14}CO_2$ pulse labeling of 15-, 25-, and 35-days-old plants was applied. The CO_2 evolution from the bare soil was 10.6 μg C–CO_2 d^{-1} g^{-1} (32 kg C–CO_2 d^{-1} ha^{-1}) and was not affected by HM (except 2500 mg Ni kg^{-1}). The average CO_2 efflux from the soil with maize was about two times higher and amounted for about 22.0 μg C–CO_2 d^{-1} g^{-1}. Portion of assimilates respired in the rhizosphere decreased with plant development from 6.0 to 7.0% of assimilated C for 25-days-old *Zea mays* to 0.4–2.0% for 45-days-old maize. The effect of the HM on root-derived $^{14}CO_2$ efflux increased with rising HM content in the following order: Cd < Cu < Ni. In Cu and Ni contaminated soils, shoot and root dry matter decreased to 70% and to 50% of the uncontaminated control, respectively. Plants contained much more HM in the roots than in the shoots. A split-root system with contrasting HM concentrations allowed to trace transport of mobile forms of HM by roots from contaminated soil half into the uncontaminated soil half. The portion of mobile HM forms in the soil (1 M NH$_4$NO$_3$ extract) increased with contamination and amounted to 9–16%, 2–6% and 1.5–3.5% for Cd, Cu, and Ni, respectively. Corresponding values for the easily available HM (1 M NH$_4$OAc extract) were 22–52%, 1–20% and 5–8.5%. Heavy metal availability for plants decreased in the following order: Cd > Cu $\geq$ Ni. No increase of HM availability in the soil was found after maize cultivation.

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1. Introduction

Anthropogenic factors such as industrial activity, sewage disposal, traffic, etc. are mainly responsible for increasing heavy metals (HM) concentrations in soil. Heavy metals inhibit directly (altered catalytic function of enzymes, damage to cellular membranes, inhibition of root growth) and indirectly (dampened photosynthesis and mineral nutrient

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uptake, water stress, changes in photoassimilate accumulation, structural changes) all physiological processes in plants [2,7,26,29–31]. Another indirect effect of HM soil contamination is the decrease of microbiological activity, which is especially pronounced in the rhizosphere.

Close interactions between plant roots and microorganisms characterize the rhizosphere soil [5,17]. Organic substances released by roots play a key role in these interactions. Indirect effects include the solubility, sorption, and transport of both: nutrients and pollutants. Direct influences involve acidification, chelation, precipitation, and oxidation-reduction reactions [1,6,17,20–23,25,27,37]. Root exudates can mobilize or bind metal ions depending on substance composition, the solid phase and the metal involved [19,20,23,36]. Thus, root exudates could play a major role in the retention or mobilization of HM in the rhizosphere [9,23] and also affect their uptake by plants [36]. Most previous studies have approached this problem by investigating exudates collected from plants grown in nutrient solution, not under soil conditions. However, the composition and amount of root exudates can change depending on the HM and its concentration in the soil, thereby modifying binding of HM in the rhizosphere.

The distribution of contaminants in soil is frequently irregular and plants can stimulate root growth in the pollution-free zones. The split-root system with contrasting HM concentrations in the both soil halves provides an opportunity to investigate HM transport in roots and to verify a hypothesis about exudation of HM by plant roots. Such investigations have never been carried out before.

As a function of microbiological activity, the decomposition of soil organic matter (SOM) is reduced and less CO_2 will be evolved from HM-contaminated soil. In contrast to SOM-derived CO_2, the effect of HM on the emission of root-derived CO_2 is controversial. On one hand, the contaminant can decrease root growth and therefore impede the total underground carbon (C) translocation including root respiration and exudation. On the other hand, every type of pollution could increase the ineffective C losses such as root respiration and exudations. Therefore, the main effect of HM on the root-derived CO_2 efflux from soil is difficult to predict.

This study evaluated: (a) the root-derived and SOM-derived CO_2 efflux from the soil depending on the type and level of HM contamination; (b) effect of increasing level of HM soil contamination on HM distribution in maize plants; and (c) HM transport from contaminated to uncontaminated roots (HM re-translocation) by plant roots.

2. Materials and methods

The rhizodeposition, as well as root- and SOM-derived CO_2 effluxes of *Zea mays* L. grown on a loamy Haplic Luvisol were studied by $^{14}CO_2$ pulse labeling of shoots at three growth stages. Different levels of soil contamination by Cd, Cu, and Ni were investigated. A split-root system in two-compartment pots (Fig. 1) with contrasting HM concentrations in soil of left and right pot halves imitated the effect of irregular contaminant distribution. This allowed investigating the transport of HM from contaminated to uncontaminated soil part through the roots and also the release of HM together with exudates by roots into the uncontaminated soil half.

Both compartments of the split-root pot contained 312 g loamy Haplic Luvisol (C_{org} 1.2%, pH 6.8). The soil was taken from the top 10 cm (Ah horizon) at the experimental station Karlshof (South Germany, Stuttgart), air-dried, mixed, and passed through a 5-mm sieve. The following levels of HM contamination were tested: 0, 10, and 20 mg kg^{-1} for Cd; 0, 250, 500, and 1000 mg kg^{-1} for Cu and 0, 1000, and 2500 mg kg^{-1} for
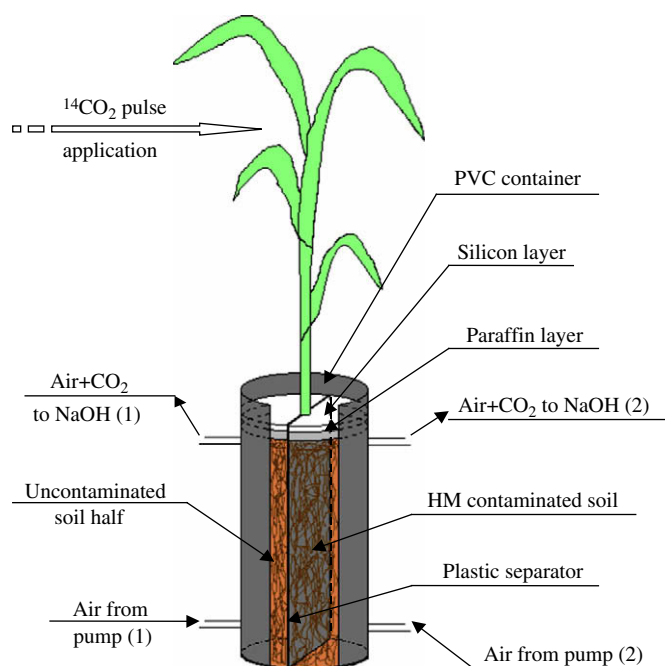

Fig. 1 – Two-compartment pot with split-root system for studying carbon rhizodeposition, CO_2 efflux and retranslocation of heavy metals.

Ni. Treatments with different combinations of HM levels in the soil of left and right pot halves were labeled as follows (left half: right half): for Cd – 0:0 (0), 0:10, 0:20, 20:20 (20); for Cu – 0:0 (0), 0:250, 0:500, 0:1000, 1000:1000 (1000); for Ni – 0:0 (0), 0:1000, 0:2500 mg kg^{-1}. Heavy metals were applied in the soil as water solutions of $CdCl_2$, $CuSO_4$, and $NiCl_2$ before filling the pots and the soil was carefully mixed.

Maize plants with four roots were transferred to the root-split pots by inserting two roots into each half of the split-root system. Plants were grown until shooting (45 days). One seedling of *Zea mays* L. (spec. Benicia) was grown in each pot at 26–28 °C day and 22–23 °C night temperature with a day-length of 14 h and light intensity of approximately 400 μmol m^{-2} s^{-1} at the top of the canopy. The soil water content of each pot was measured by weight and was adjusted daily to 60% of the available water holding capacity. One day before the first ^{14}C labeling, the root-soil compartments were separated from atmosphere by a layer of low melting point Paraffin (m.p. 42–44 °C; Merck Eurolab GmbH, Bruchsal) and overlaid with Silicon paste (NG 3170 of Fa. Thauer and Co. Dresden). To compare total unlabelled CO_2 evolution with and without *Zea mays*, unplanted soil was incubated under the same levels of Cd, Cu, and Ni contamination and the same experimental conditions.

The plants were labeled at 15, 25, and 35 days after sowing. Nineteen plants were ^{14}C labeled simultaneously during 1 h in a large Plexiglas chamber. Three labelings were done for replicate plants. Three ml of lactic acid were added to the Na^{14}CO$_3$ (460 kBq of ^{14}C pot^{-1}) solution to produce ^{14}CO$_2$. After 1 h, the remaining CO_2 was trapped in 1.0 M NaOH aqueous solution to remove the unassimilated ^{14}CO$_2$ from the Plexiglas chamber. Immediately after the labeling, the CO_2 efflux from soil was started and monitored during 10 days and the trap was changed every 2 days. Each left / right compartment of the pots had its own CO_2 trapping system with 10 ml of 1.0 M NaOH solution and was continuously pumped with a membrane pump. So, the total and ^{14}C labeled CO_2 efflux was monitored during maize growth separately from both soil-root halves.

Ten days after each labeling, all plants were cut, the root-soil column pulled out, and the roots carefully washed from the soil by hand. Shoots, roots, and soil were dried at 60 °C and pulverized in a ball mill (Fa Retch) prior to analysis of ^{14}C-activity. ^{14}C- activity of shoots, roots, and soil samples was measured with the scintillation cocktail Permafluor E+ (Canberra Packard) by a Liquid Scintillation Counter Tri-Carb 2000CA (Canberra Packard), after combustion of 1 g of sample within an oxidizer unit (Model 307, Canberra Packard).

^{14}C in the CO_2 collected in the NaOH solution was measured with the scintillation cocktail Rothisscint-22x (Roth Company) on 2-ml aliquots of NaOH after the decay of chemiluminescence. The ^{14}C counting efficiency was about 89% and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C-activity was standardized by addition of NaOH solution as a quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE).

The total CO_2 collected in the NaOH solution was measured by titration with 0.2 M HCl against phenolphthalein after addition of 0.5 M BaCl$_2$ solution [3]. The total C content in the shoots was considered to be 40% of dry mass and was

accepted as a constant. The total C content in the roots varied considerably because of different admixture of mineral soil particles. All calculations of the C content in the roots were based on C analyses.

Shoot and root samples were dissolved in H_2O_2 and HNO_3 in a microwave oven and analysed for Cd, Cu, and Ni contents by a Varian AA 400 and Varian AA 400Z atomic adsorption spectrometer (Varian, Mulgave, Australia). In the soil samples from each pot half, mobile forms of Cd, Cu, and Ni (1.0 M NH$_4$NO$_3$ extract) as well as easily available HM forms (1.0 M NH$_4$OAc extract) were determined [28,35].

The experiment was conducted with four replications. Analysis of variance and the LSD test at 5% error probability were applied to assess the significance of differences between treatments.

3. Results and discussion

3.1. Root-derived and SOM-derived CO_2 efflux from the soil depending on heavy metal contamination

The total unlabeled CO_2 evolution from the soil with *Zea mays* was compared with that from the bare soil incubated under the same conditions. In this study, the difference in CO_2 efflux

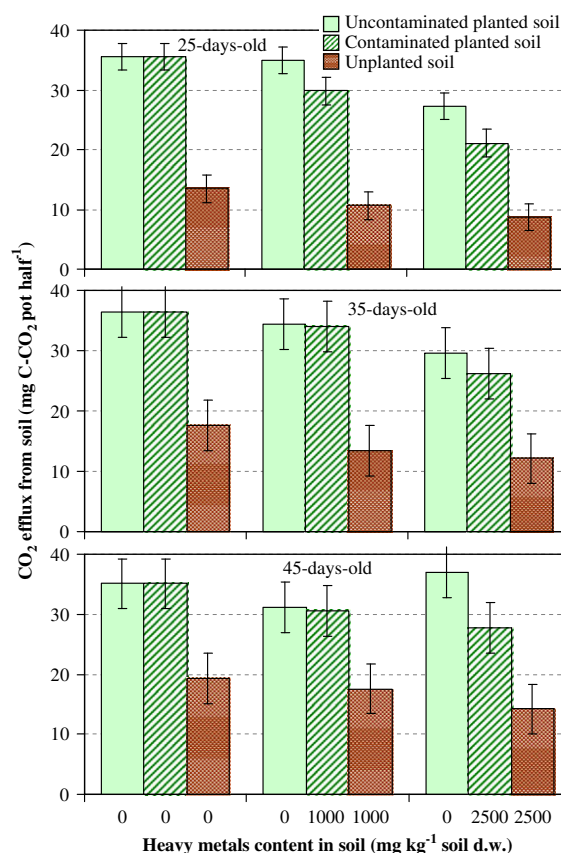Fig. 2 – Total CO_2 efflux (cumulative for 10 days, in mg C–CO_2 per each pot half; ±LSD, 5%) from unplanted soil and soil planted with maize depending on Ni content in soil and growth stage: 25 (top), 35 (middle), and 45 (bottom)-days-old maize.
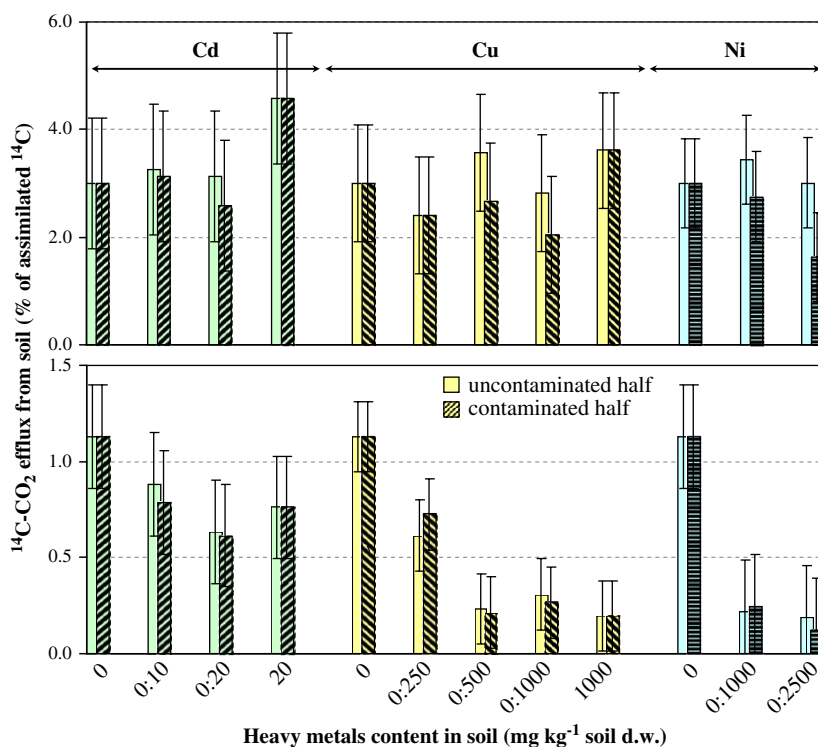
Fig. 3 – Cumulative root-derived ^{14}C–CO_2 efflux (% of assimilated C ± LSD, 5%) for 10 days of 25 (top) and 45-days-old (bottom) maize depending on the Cd, Cu and Ni contamination of soil (mg kg^{-1}). ^{14}C–CO_2 effluxes are presented for the planted soil from uncontaminated (left) and contaminated (right) pot halves. 0, 20, etc.: treatments without HM, with 20, etc. mg kg^{-1} HM in both pot halves; 0:10, etc.: treatments without HM in the left and with HM in the right pot halves.

(plants versus bare soil) was accepted as equal to the contribution of plant roots to the whole CO_2 efflux. The CO_2 evolution from the bare soil was nearly constant during the incubation and amount to 10.6 µg C–CO_2 d^{-1} g^{-1} (32 kg C–CO_2 d^{-1} ha^{-1}, calculated based on a 25-cm-thick Ap horizon and a soil density of 1.2 g cm^{-3}). The average CO_2 efflux from the soil with *Zea mays* was about two times higher and amount to 22.0 µg C–CO_2 d^{-1} g^{-1} (66 kg C–CO_2 d^{-1} ha^{-1}). Thus, the contribution of plant roots to the total CO_2 efflux from the soil (calculated as the difference) was about 52%. Despite the low total C content of the soil used in the experiment, a high level of total CO_2 efflux was detected. A lower rate of total unlabelled CO_2 evolution from the soil with *Zea mays* (9.0 µg C–CO_2 d^{-1} g^{-1} ≈ 27.0 kg C–CO_2 d^{-1} ha^{-1}) has been reported by Liljeroth et al. [16]. The difference to our results can be explained by the smaller amount of soil (15 times) used in our experiment. Cd and Cu had no effect on the SOM-derived CO_2 efflux; only high concentrations of Ni diminished the values by about 30% (Fig. 2). However, total CO_2 efflux from planted soil diminished by application of 20 mg Cd kg^{-1}, 250–1000 mg Cu kg^{-1}, or 2500 mg Ni kg^{-1}.

$^{14}CO_2$ efflux derived from root respiration and rhizomicrobial respiration decreased with plant development on average from 3.0 to 3.5% (1st labeling) to 0.2–1% of the assimilated C (3rd> labeling) per pot half (Fig. 3). This decrease during plant development is explained by a higher belowground translocation of C by young plants, whereas old plants translocate it preferably into the shoots [4,13,14].

Labeling the plants in a $^{14}CO_2$ atmosphere allowed a distinction between SOM-derived and root-derived CO_2. So, the effect of HM contamination on the root-derived CO_2 was much stronger than that on the SOM-derived CO_2. Accumulation of HM in plant tissues during plant development results in an increasing negative effect on root respiration and exudation.

After the 1st and 2nd labeling, $^{14}CO_2$ evolution from the soil decreased with Ni contamination compared with that from uncontaminated soil. Cd and Cu did not affect $^{14}CO_2$ evolution.
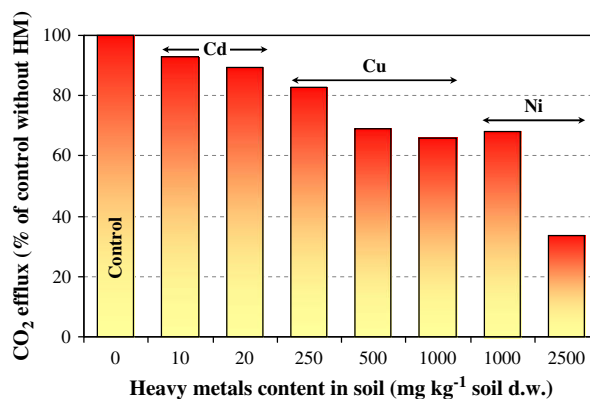

Fig. 4 – Total root-derived CO_2 efflux depending on HM content in the soil (average of 30 days for 45-days-old maize), % of uncontaminated control (C).
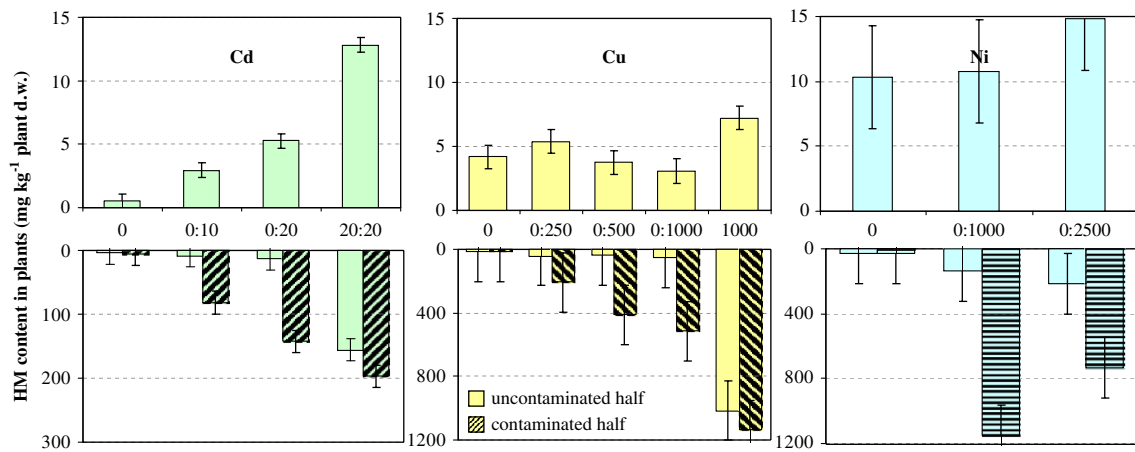
Fig. 5 – Cd, Cu, and Ni content in the 45-days-old maize shoots (top) and roots (bottom) depending on the level of HM contamination of soil. HM contents in the roots are presented for the roots from uncontaminated (left) and contaminated pot halves (right). 0, 20, etc.: treatments without HM, with 20, etc. mg kg^{-1} HM in both pot halves; 0:10, etc.: treatments without HM in the left and with HM in the right pot halves.

After the 3rd labeling, $^{14}CO_2$ efflux decreased more than after the previous two labeling periods and was equal in both pot halves of every treatment. Thus, a cumulative effect of HM contamination on the $^{14}CO_2$ evolution from the soil was observed during maize growth.

On average, the root-derived $^{14}CO_2$ efflux from the soil decreased in all treatments with HM contamination (Fig. 4). This effect increased in the following order: Cd > Cu > Ni, and the significance of the effect increased with raising HM content in the soil. As noted above, the HM did not affect SOM-derived CO_2 efflux, which varied from 42 to 55% of the total CO_2 evolution from planted soil.

3.2. Heavy metal uptake by maize plants

Soil contamination with all HM reduced the shoot and root biomass of maize. In soil with 1000 mg Cu kg^{-1} and 2500 mg Ni kg^{-1}, shoot dry matter (DM) decreased to 70 and 64% of the

control, respectively. In the case of Cd contamination, only shoot DM tended to decrease. Root DM decreased slightly in treatments with 10 and 20 mg Cd kg^{-1} and to about 50% in treatments with 1000 mg Cu kg^{-1}. These results are similar to those of Keltjens and van Beusichem [12] and Yang et al. [33,34], who clearly demonstrated Cu-induced root under-development by reduced total and specific root length. On the other hand, Yang et al. [32] found a much higher effect of Cd and Ni on maize growth: shoot DM decreased to about 50% that of the control when plants were grown in nutrient solution with Cd <14 μM and with Ni >60 μM. However, the results of nutrient solution studies are not directly comparable with the results obtained for plants grown on soil, because the most part of HM is absorbed on SOM and clay minerals and is not available for plant uptake.

Also in the uncontaminated soil halves, root mass decreased with Cd (10 and 20 mg kg^{-1}), Cu (1000 mg kg^{-1}), and Ni (1000 and 2500 mg kg^{-1}) contamination versus the control.
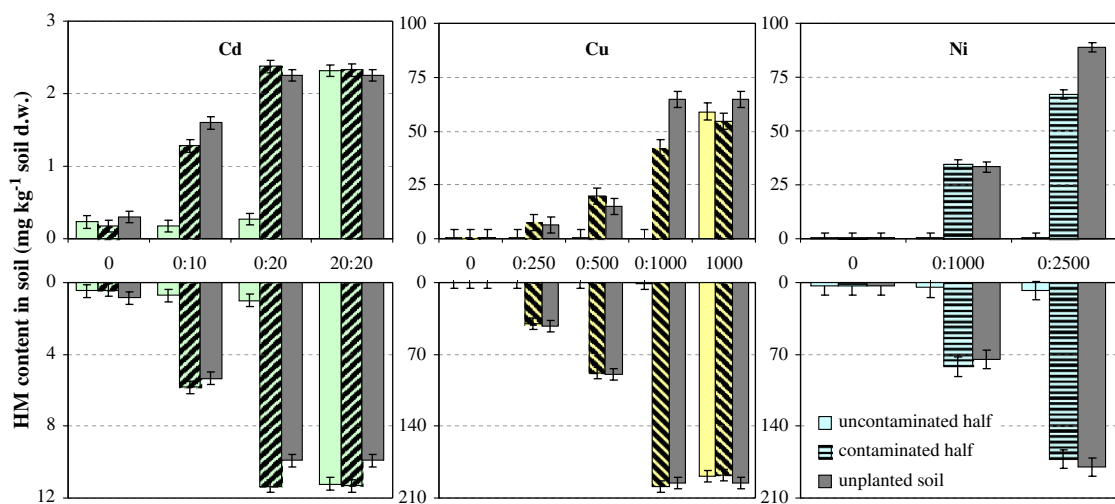

Fig. 6 – Mobile (top) and easily available (bottom) fractions of Cd, Cu, and Ni in the soil depending on the level of soil contamination after 45 days of maize growth. 0, 20, etc.: treatments without HM, with 20, etc. mg kg^{-1} HM in both pot halves; 0:10, etc.: treatments without HM in the left and with HM in the right pot halves.

In all treatments with Cu and Ni, root growth decreased more than shoot growth. It is explainable by much higher HM contents in roots versus the shoots. Concentrations in the shoots increased in both pot halves with rising Cd level and with 1000 mg Cu kg^{-1} (Fig. 5). When one pot half was contaminated, shoot concentrations slightly increased in all Ni treatments and remained unchanged in Cu treatments. According to many studies, low Ni and Cu contents in the shoot biomass of maize planted on strongly contaminated soils is associated with both: the significant negative effect of these HM on root growth [15,24] and with their extremely low root to shoot transport [8,12,32]. Low root to shoot transport was not affected by the level of HM contamination. Due to these effects, HM concentrations in maize roots were almost linearly correlated with soil contamination level (Fig. 5, bottom). It means that roots have no effective protection mechanism against HM uptake. Other studies report similar results [12,32].

3.3. Plant-mediated re-translocation of heavy metals

A linear increase of mobile and easily available HM contents in the soil was observed with their increasing total content in the soil (Fig. 6). For Cd, Cu, and Ni, the mobile HM forms (NH$_4$NO$_3$ extract) in the soil were 9–16%, 2–6% and 1.5–3.5% of total content, respectively, and the corresponding values for the easily available forms (NH$_4$Ac extract) were 22–52%, 1–20% and 5–8.5%. Moreover, in most treatments the HM contents in uncontaminated soil half increased with increasing soil contamination. The HM availability increased with increasing total HM contents in soil in the following order: Ni $\leq$ Cu $<$ Cd.

The amounts of mobile and easily available HM forms in the unplanted soil were the same or higher than those in the planted soil. This shows that the growing plants did not change the availability of HM in the soil, although several studies have described a HM mobilization by root exudates due to complexation and chelatization [10,18,37]. Plants can probably also decrease HM availability by exudation of various compounds, particularly mucilage: diffusion transport of any element in such viscous substances is lower than in the soil solution. Additionally, these compounds form metal-organic complexes that are unavailable for plants [23]. These authors found that maize root mucilage is able to bind HM in the following order: Pb $>$ Cu $\gg$ Cd. Thus, in the rhizosphere, a large retention of HM having a high affinity for the binding with organic molecules is expected. Moreover, many other components (clays, microbial products, etc.) in the root environment can combine with the root mucilage to form a mixed product termed mucigel [11] that can bind HM [23].

More mobile HM was found in the soil of uncontaminated pot halves than in the soil without HM (Fig. 7 top). As noted above, HM content increased in the roots of uncontaminated soil with increasing HM content in the contaminated soil. This fact is unknown and occurs by plant-mediated re-translocation of HM and by exudation of HM by plant roots together with organic substances. This merits further research into root-induced changes of HM mobility in soil by exudation or other mechanisms that control the transformation of HM in the rhizosphere of contaminated soils.

In the pots with different concentrations in both halves, HM content in the roots from uncontaminated soil increased with increasing HM in the contaminated soil, particularly at the highest concentrations (Fig. 7 bottom). High contents in the roots from the uncontaminated soil half probably reflect increased HM bioavailability in the uncontaminated soil half (Fig. 7 top) and / or HM transport by the roots from the
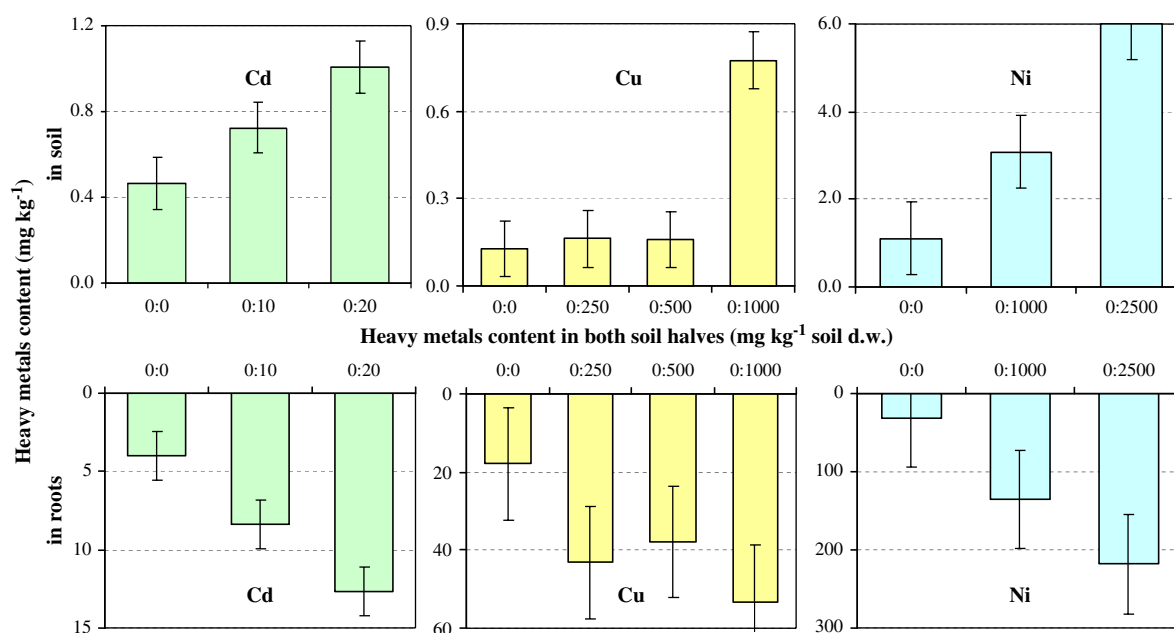

Fig. 7 – Translocation of HM by roots from contaminated half to uncontaminated half. Top: the mobile HM (mg kg^{-1}) in the uncontaminated soil half after 45 days of maize growth. Bottom: Cd, Cu, and Ni contents (mg kg^{-1}) in maize roots from uncontaminated soil half. 0, 20, etc.: treatments without HM, with 20, etc. mg kg^{-1} HM in both pot halves; 0:10, etc.: variants without HM in the left and with HM in the right pot halves.

contaminated soil half. To our knowledge, no other studies were published showing plant- mediated transport of HM in uncontaminated soil parts.

4. Conclusions

- High HM contamination of the soil decreased mainly the root-derived CO_2 efflux in the following order: $Cd > Cu > Ni$. Consequently, the contribution of maize plants to the total CO_2 evolution from the soil decreased with raising HM concentration in soil. Only extremely high Ni contamination decreased the CO_2 efflux originated from SOM.
- Cd and Cu concentrations in the shoots increased with increasing Cd level and with 1000 mg kg^{-1} Cu. The content of all HM in the roots was linearly correlated with the level of soil contamination.
- Bioavailability of HM increased with increasing their total content in soil in the following order: $Ni \leq Cu < Cd$
- Using a root-split system, root-mediated transport from contaminated soil parts and exudation of HM into uncontaminated soil parts was observed.

Acknowledgments

REFERENCES

[1] F. Awad, V. Römheld, H. Marschner, Mobilization of ferric iron from a calcareous soil by plant-borne chelators (phytosiderophores), J. Plant Nutr. 11 (1988) 701–713.
[2] J. Barcelo, C. Cabot, Ch. Poschenrieder, Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv Contender). II. Effects of Cd on endogenous abscisic acid levels, J. Plant Physiol. 125 (1986) 27–34.
[3] C.A. Black (Ed.), Methods of Soil Analysis, Part 2, American Society of Agronomy, Inc. Publisher, Madison, 1965, pp. 1562–1565.
[4] R. Brouwer, Functional equilibrium: sense or nonsense? Neth. J. Agric. Sci. 31 (1983) 335–348.
[5] E.A. Curl, B. Truelove, The Rhizosphere, Springer, Berlin, 1986.
[6] B. Dinkelaker, V. Römheld, H. Marschner, Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupine (*Lupinus albus* L.), Plant Cell Environ. 12 (1989) 285–292.
[7] M. Greger, S. Lindberg, Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris* L.) Cd^{2+} uptake and sugar accumulation, Plant Physiol. 66 (1986) 69–74.
[8] Y. Guo, H. Marschner, Uptake, distribution and binding of cadmium and nickel in different plant species, J. Plant Nutr. 18 (1995) 2691–2706.
[9] W.J. Horst, A. Wagner, H. Marschner, Mucilage protects meristems from aluminium injury, Z. Pflanzenphysiol. 105 (1982) 435–444.
[10] W.P. Inskeep, S.D. Comfort, Thermodynamic predictions for the effects of root exudates on metal speciation in the rhizosphere, J. Plant Nutr. 9 (1986) 567–586.
[11] H. Jenny, K. Grossenbacher, Root soil boundary zone as seen by electron microscope, Soil Sci. Soc. Am. Proc. 27 (1963) 273–277.
[12] W.G. Keltjens, M.L. Van Beusichem, Phytochelatins as biomarkers for heavy metal toxicity in maize: single metal effects of copper and cadmium, J. Plant Nutr. 21 (1998) 635–648.
[13] Y. Kuzyakov, G. Domanski, Carbon input by plants into the soil, Review. J. Plant Nutr. Soil Sci. 163 (2000) 421–431.
[14] Y. Kuzyakov, A. Kretzschmar, K. Stahr, Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil, Plant and Soil. 213 (1999) 127–136.
[15] T.M. Lexmond, P.D.J. Vorm, The effect of pH on copper toxicity to hydroponically grown maize, Neth. J. Agric. Sci. 29 (1981) 209–230.
[16] E. Liljeroth, P. Kuikman, J.A. Van Veen, Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels, Plant Soil 161 (1994) 233–240.
[17] H. Marschner, Mineral Nutrition of Higher Plants, 2nd ed. Academic Press. Harcourt Brace & Comp. Publ., London, 1995.
[18] M. Mench, E. Martin, Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L, Plant and Soil 132 (1991) 187–196.
[19] M. Mench, J.L. Morel, A. Guckert, Liaison du cadmium avec la fraction macromoleculaire soluble des exudats racinaires de mais (*Zea mays* L.), C.R. Acad. Sci. Paris 301 (1985) 379–382.
[20] M. Mench, J.L. Morel, A. Guckert, Metal binding properties of high molecular weight soluble exudates from maize (*Zea mays* L.) roots, Biol. Fertil. Soils 3 (1987) 165–169.
[21] M. Mench, J.L. Morel, A. Guckert, B. Guillet, Metal binding with root exudates of low molecular weight, J. Soil Sci. 39 (1988) 521–527.
[22] W. Merbach, E. Mirus, G. Knof, R. Remus, S. Ruppel, R. Russow, A. Gransee, J. Schulze, Release of carbon and nitrogen compounds by plant roots and their possible ecological importance, J. Plant Nutr. Soil Sci. 162 (1999) 373–383.
[23] J.L. Morel, M. Mench, A. Guckert, Measurement of Pb^{2+}, Cu^{2+}, Cd^{2+} binding with mucilage exudates from maize (*Zea mays* L.) roots, Biol. Fertil. Soils 2 (1986) 29–34.
[24] W. Savage, W.L. Berry, C.A. Reed, Effects of trace element stress on the morphology of developing seedlings of lettuce (*Lactuca sativa* L. Grand Rapids) as shown by scanning electron microscopy, J. Plant Nutr. 3 (1981) 129–138.
[25] G. Schilling, A. Gransee, A. Deubel, G. Lezovic, S. Ruppel, Phosphorus availability, root exudates, and microbial activity in the rhizosphere, J. Plant Nutr. Soil Sci. 161 (1998) 465–478.
[26] S. Tu, J.N. Brouillette, Metal ion inhibition of cotton root plasma membrane ATFase, Phytochemistry 26 (1987) 65–69.
[27] N.C. Uren, H.M. Reisenauer, The role of root exudates in nutrient acquisition, Adv. Plant Nutr. 3 (1988) 79–114.
[28] A.R.A. Usman, Y. Kuzyakov, K. Lorenz, K. Stahr, Remediation of a soil contaminated with heavy metals by immobilizing compounds, J. Plant Nutr. Soil Sci. 169 (2006) 205–212.
[29] F. Van Assche, H. Clijsters, Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relation *Phaseolus vulgaris* L. treated with zinc and cadmium, Environ. Pollut. 52 (1988) 103–115.
[30] M.D. Vazquez, Ch. Poschenrieder, J. Barceld, Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.), Ann. Bot. 59 (1987) 427–438.
[31] H.W. Woolhouse, Toxicity and tolerance in the responses of plants to metals, in: O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler (Eds.), Physiologica Plant Ecology. Encyclopedia of

Plant Physiology III, New Series, Vol. 12C, Springer-Verlag, New York, 1983, pp. 245–300.

[32] X. Yang, V.C. Baligar, D.C. Martens, R.B. Clark, Plant tolerance to nickel toxicity: I. Influx, transport, and accumulation of nickel in four species, J. Plant Nutr. 19 (1996) 73–85.

[33] X. Yang, V.C. Baligar, D.C. Martens, R.B. Clark, Plant tolerance to nickel toxicity: II. Effects on influx and transport of mineral nutrients in four species, J. Plant Nutr. 19 (1996) 265–279.

[34] X. Yang, V.C. Baligar, D.C. Martens, R.B. Clark, Cadmium effects on influx and transport of mineral nutrients in plant species, J. Plant Nutr. 19 (1996) 643–656.

[35] H. Zeien, G.W. Bruemmer, Chemische Extraction zur Bestimmung von Schwermetallbindungsformen in Böden, Mitt. Dtsch. Bodenkd. Ges. 59 (1989) 505–510.

[36] F. Zhang, V. Römheld, H. Marschner, Effect of zinc deficiency in wheat on the release of zinc and iron mobilisating root exudates, Z. Pflanzenernaehr. Bodenkd 152 (1989) 205–210.

[37] F. Zhang, V. Römheld, H. Marschner, Release of zinc mobilizing root exudates in different plant species as affected by zinc nutritional status, J. Plant Nutr. 14 (1991) 675–686.