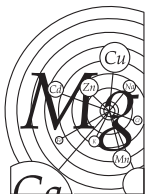


Heavy metal content of *Lactuca sativa* L. grown on soils from historic mining and smelting sites in Styria (Austria) described by the Electro-Ultrafiltration (EUF) method and kinetic models

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HEAVY METAL CONTENT OF *LACTUCA SATIVA* L. GROWN ON SOILS FROM HISTORIC MINING AND SMELTING SITES IN STYRIA (AUSTRIA), DETERMINED BY THE ELECTRO-ULTRAFILTRATION (EUF) METHOD AND KINETIC MODELS*

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ABSTRACT

Lettuce seedlings (*Lactuca sativa* L.) were grown in pot experiments on metalliferous soils sampled close to mining and smelting sites in the Province of Styria (Austria), in order to investigate effects of mineral fertilizer (NPK and PK). Additionally, soil samples were collected and extracted by modified electro-ultrafiltration (EUF) in five fractions after using diethylenetriaminepentaacetic acid (DTPA) to obtain the potentially available metals and to describe the release mechanisms with kinetic models. The NPK and PK fertilizer application decreased the Pb content in plants below the maximum threshold set up for human nutrition, but Cd increased slightly. Bioconcentration factors (BCF) for Cd were higher than for Pb and, according to the translocation factor (TSF), leaves contained more Cd but less Pb than corresponding roots. The largest heavy metal amounts of the metalliferous soils were released in the first two fractions of the EUF method. Closest fits in terms of the coefficient of determination (R^2) and recalculated results were obtained from the 2nd order polynomial model ($y = a + bt + ct^2$). In contrast to Cd, a statistically significant ($P \leq 0.05$) relationship between release rate b of the 2nd order polynomial model and the metal concentration in plants was achieved for Ni in both leaves and roots and for Pb only in leaves.

Keywords: lettuce, heavy metals, fertilizer, EUF method, kinetic model.

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INTRODUCTION

Heavy metals in soils pose a potential hazard to the environment as they seep into groundwater or are absorbed by plants when present in the soil as soluble ions in organic or inorganic complexes (SCHENK 2006, LIU 2018). Heavy metals show toxic effects in plants by blocking receptors, clogging pores or by interacting with the DNA. The uptake mechanisms, however, are often ion-selective and dependent on the properties of the membrane transporter (ALLOWAY 2013).

Plant species such as lettuce (*Lactuca sativa* L.) can accumulate increased amounts of heavy metals, like Mn, Pb, Zn or Cu, without showing any visible differences in growth (HODDA 1997, FERRI et al. 2012). SAGER et al. (2010) found that *Lactuca sativa* L. accumulated many trace metals at high concentrations per plant biomass, at maximum concentrations 5-10 days after planting the seedlings. The decreasing nutrient element content in lettuce leaves grown on mine tailings versus time, indicated increasing nutrient needs due to the toxic effects of metal stress. Universally accumulating plants like *Lactuca sativa* L. are therefore good metal hazard indicators.

In phytoremediation, the plants are harvested and incinerated, removing the heavy metals from biogeochemical cycles by landfilling the ashes (GHOSH, SINGH 2005). Use of the resulting ash to recover the metals has been termed as phytomining or agromining, carried out particularly for the recycling of nickel. Hyperaccumulator plants in particular with subsequent harvesting and biomass incineration generate a product that may be termed bio-ore, which is free of naturally occurring silicates, and may be leached with dilute acids (VAN DER ENT et al. 2015). On the other hand, the undesired uptake of heavy metals by agricultural plants can cause severe problems due to their enrichment in the food chain (KLOKE et al. 1984). Many authors report reductions of metal transfer to plants caused by compost, dung or manure additions (STERRETT et al. 1996, FRIESL-HANL et al. 2005) because of dilution in increasing biomass. A survey of trace elements in fertilizers sold in Austria revealed that most trace element contents in P-mineral-fertilizers (As, Ba, Be, Co, Cr, Cu, Li, Mn, Na, Ni, Pb) can be expected below levels encountered in soils, some occur within the concentration range in soils (Mo, Sr, V, Zn), and just Cd is expectable at higher levels (SAGER 1997, SAGER 2011). Addition of phosphate fertilizers was found to reduce the Cu, Mn, and Pb uptake of lettuce (STERRET et al. 1996). Addition of apatite or triple phosphate led to lower plant uptake of Pb from contaminated soils (LAPERCHÉ et al. 1997). Triple superphosphate is obtained by reaction of ground mineral phosphate with phosphoric acid and contains monocalcium phosphate as its essential ingredient. The immobilization of Pb by phosphate is due to the precipitation of pyromorphite, $\text{Pb}_5[\text{Cl}](\text{PO}_4)_3$ (MIRETZKI, FERNANDEZ-CIRELLI 2008).

Both desorption from soil as well as plant root uptake are kinetically driven processes, therefore modelling plant uptake via dissolution equilibria

is often inadequate. When the desorption from the soil matrix to the soil solution is slower than the uptake by plant roots, the kinetics of soil desorption directly reflects the soil–plant transfer (DANG et al. 1994, MOTLAGH 2012). Due to this complex behaviour, an experimental setup is needed to estimate potential hazards, which depends rather on the release rate and subsequent plant or gut uptake rate than on the total amount of occurrence. The actual release rate can be fitted by kinetic models, which consider possible interactions between metal ions, the soil solution and the soil matrix (ALGHANMI et al. 2015). If this information is obtained from dynamically operating analytical methods, predictions of environmental cycling of metals can be made more precisely than from simple snapshot methods.

The goal of this work was to investigate the effects of mineral fertilizers on yield and metal uptake of *Lactuca sativa* L. as an accumulator plant. For this purpose, lettuce was grown in pots containing especially selected soils from historic mining and smelting sites, sampled within the Province of Styria (Austria). To study the effects of amendments more precisely, the soil samples from the pot experiment were collected and extracted with a modified electro ultrafiltration (EUF) method and described with kinetic models.

MATERIALS AND METHODS

For the experiment, arable soils at or close to historic mining and smelting sites from Styria were selected (Figure 1). The samples were collected randomly from 3 to 4 points of each site (Arzwaldgraben, Kraubath,

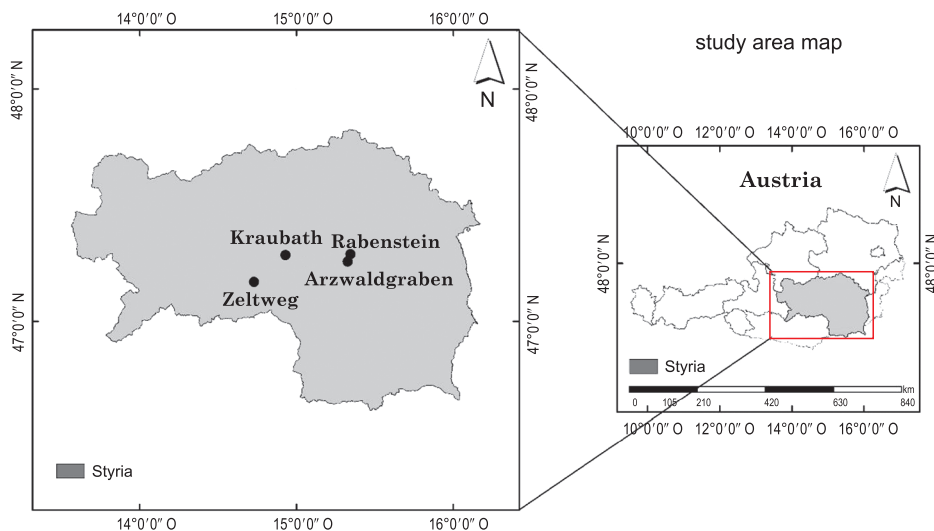


Fig. 1. Sampling map of the selected locations in Austria

Rabenstein and Zeltweg), from the 5-30 cm depth. Main characteristics of the metalliferous soils used were determined by standardized methods, i.e. soil pH in 0.01M CaCl_2 (ÖNORM EN 15933), electrical conductivity (ÖNORM L 1099), particle size distribution by the pipette method (ÖNORM L 1061-2), and total organic carbon after decarbonatization by combustion at 950°C (ÖNORM L 1080). Total N was obtained by the Kjeldahl's method (EN 16169), plant-available P and K in calcium-acetate-lactate extract (CAL; ÖNORM L 1087), Cd-Ni-Pb with the EUF method (by addition of 0.002 M DTPA) and Aqua regia by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Pot experiments were conducted transplanting one-week old seedlings in sets of three into the test substrate. The plants were well-watered every three days, and harvested after having reached the optimum marketable size ready. Lettuce seedlings were planted at Kick-Brauckmann pots containing 8 kg of dried soil (≤ 20 mm) from historic mining and smelting sites. Each pot was placed randomly in a foliar-covered greenhouse. Ten days after planting, urea (4.50 g), superphosphate (1.80 g) and potassium chloride (1.00 g) were added as a fertilizer in combinations as NPK and PK. After 40 days of growth, roots and shoots were harvested separately to obtain yields in terms of wet weight. The samples were dried at 65°C for 72 h, milled and analysed for total metal as well as C and N content. For determination of heavy metals, the samples were digested with nitric acid in closed pressure vessels by microwave-assisted heating, and the resulting digest was submitted to simultaneous multi-element analysis by ICP-OES (Perkin Elmer Optima 3000XL). Pure digests and 2 dilutions were read to target the optimum concentration ranges. The method had been approved by participation in respective ring tests.

To quantify the metal enrichment in various kinds of green plants, bioconcentration factors (BCF) and translocation factors (TSF) – CHANG et al. (2014), SINGH et al. (2017) were calculated. The BCF has been defined as a metal concentration in green plant divided by the concentration in soil (Aqua regia) and TSF represents a metal concentration in shoot divided by that in the roots. Both these accumulation factors reflect mobility and hazardous potential on the soil as well as phytoremediation capabilities of the green plant in question.

$$BCF = \frac{C_p}{C_s},$$

C_p – metal concentration in lettuce (mg kg^{-1} dw),

C_s – metal concentration in soil (mg kg^{-1}) obtained in Aqua regia.

$$TSF = \frac{C_L}{C_R},$$

C_L – metal concentration in leaves (mg kg^{-1} dw),

C_R – metal concentration in roots (mg kg^{-1} dw).

The soil samples from the pot experiment (after harvest) were examined at the Justus Liebig Laboratory of Südzucker AG Company in Rain am Lech (Germany) by using a modified electro ultrafiltration (EUF) method. The samples were dried at 40°C and sieved minor 2 mm and three replicates made. Only the sieve underflow was used for analyses. Each sample was divided into three replicates. The samples were then weighed (5.00 g) and introduced into the EUF chamber system. The principle of the EUF method is to suspend a soil sample in water (or solvent) and continuously remove the elements released into the solution without establishing equilibrium. For this purpose, the suspension is exposed to an electrical field, since electrical voltage is applied to two platinum electrodes, whereby the electrodes are separated from the suspension by electrical membranes. Due to the electric field, the cations dissolved in the aqueous phase migrate through the ultra-membrane to the cathode and the anions to the anode. In the original procedure, soils samples are extracted with desalinated water for 30 min (first fraction) and 5 min (second fraction) – NÉMETH (1982), HORN (2006). Heavy metals and trace elements can be obtained in the third fraction after 35 min by addition of a complexing agent like DTPA or EDTA (HORN 2006). Within this work, a modified method was performed by using 0.002 M DTPA ($C_{14}H_{23}N_3O_{10}$) aqueous solution. The soil samples were extracted in five separate fractions and collected every 5 min (in a total of 25 min). The extractions were carried out under the following conditions; 20°C, 200 V and max. 15 mA. After the extraction, the amount of the filtrate in the anode and cathode was weighed. The heavy metals were analyzed by using ICP-OES.

To describe the release of heavy metals from the EUF experiment, four kinetic models based on several works (MENGEL, UHLENBECKER 1993, FINZGAR et al. 2007, JELECEVIC et al. 2019) were applied. The cumulative curve of a modelled heavy metal released is shown both linearly (Elovich function, Linear function and Parabolic Diffusion Law) and nonlinearly (2nd order polynomial). To describe the release of heavy metals, the cumulated released amounts (y) were fitted versus time (t). Fitted constants b and c (2nd order polynomial) represent the slope whereas a represents the intercept of the fitted curves. The constant a represents the fitted initial concentration at the beginning of the extractions, when $t = 0$ (Table 1).

Table 1

Selected kinetic models

Kinetic model	$f(x)$	Release rate = dy/dt
Linear function	$y = a + bt$	$dy/dt = b$
Elovich function	$y = a + b \ln(i)$	$dy/dt = b/t$
Parabolic function	$y = a + b\sqrt{t}$	$dy/dt = b/\sqrt{t}$
2 nd order polynomial function	$y = a + bt + ct^2$	$dy/dt = b + 2ct$

y – measured concentration at time t

a, b, c – fitted constants

RESULTS AND DISCUSSION

Soil properties and heavy metal content

The soil pH ranges from slightly acid (pH 6.2) to slightly alkaline (pH 7.4). The content of humic substances has been termed as organic carbon times 1.72 (Table 2) and it is in the range of 2.8 to 5.4 wt%. The limit values

Table 2
Physical and chemical properties of the soils used

Parameter	Location			
	Rabenstein (Soil 1)	Arzwald- graben (Soil 2)	Zeltweg (Soil 3)	Kraubath (Control)
Geographic coordinates	47° 15.023' N 15° 18.450' E	47° 14.261' N 15° 16.798' E	47° 11.575' N 14° 43.325' E	47° 18.034' N 14° 55.955' E
Soil type	Eutric Cambisol	Eutric Gleysol	Dystic Cambisol	Eutric Cambisol
pH	6.9	7.4	6.2	7.3
EC ($\mu\text{S cm}^{-1}$)	225	301	213	151
Organic carbon*1.72	2.8	5.4	4.1	4.6
Sand (%)	46	26	30	44
Silt (%)	41	64	63	47
Clay (%)	13	10	8	9
N ($\text{g } 100 \text{ g}^{-1}$ dry weight)	0.18	36	0.21	0.24
P (mg kg^{-1} dry weight)	4	7	6	132
K (mg kg^{-1} dry weight)	39.1	39.1	53	179
DTPA (Cd mg kg^{-1} dry weight)	0.27	0.33	NQ*	NQ*
DTPA (Ni mg kg^{-1} dry weight)	2.83	1.63	1.24	0.98
DTPA (Pb mg kg^{-1} dry weight)	19.4	25.54	10.96	1.36
Aqua regia (Cd mg kg^{-1} dry weight)	1.8	2.5	0.24	0.20
Aqua regia (Ni mg kg^{-1} dry weight)	42	51	43	24
Aqua regia (Pb mg kg^{-1} dry weight)	340	800	130	21

* Not quantified

from the Austrian Landfill Ordinance for landfilling at excavated soil landfills (BMLFUW 2008) for soil excavation material have often been used for risk assessment of metals in soils. The reason is that excavated soil landfills are not equipped with a base liner so that materials which might harm the environment should not be landfilled there, but at a construction and demolition waste landfill or even higher class landfills which are equipped with more advanced barrier systems. When the limit values are exceeded,

the material must be deposited on landfills, although at many areas in Styria the average concentrations of some metals (without known anthropogenic activities) are above these values (JELECEVIC et al. 2018). Based on these values, the Pb total content reached high levels (130-800 mg kg⁻¹), even exceeding the limit values (150 mg kg⁻¹), in soil 1 and soil 2. The Cd content exceeded the limit values (2 mg kg⁻¹) in soil 2. In contrast, the Ni content was below the limit value in all samples. 13 to 15% of Cd, but only 3 to 8% of Ni and Pb are extractable by DTPA, which suggests that Cd is bound less tightly than Ni and Pb, and this is in agreement with results from sequential chemical extraction (VOLLPRECHT et al. 2020). Although the proportions of mobilizable Ni and Pb in DTPA over Aqua regia were within the same range, Ni was at about the average continental crust level (56 mg kg⁻¹) whereas Pb was strongly enriched versus the average continental crustal abundance (15 mg kg⁻¹) after WEDEPOHL (1995). The lowest mobility of Ni is found in the sample of Kraubath, where Ni is bound to olivine (Fe,Mg)₂SiO₄ (VOLLPRECHT et al. 2020).

Heavy metal concentration in lettuce

After treatment with fertilizers, different yields from lettuce plants (edible parts) with respect to zero addition samples were obtained. Higher yields were achieved after the application of NPK and even more when the PK fertilizer was added (Figure 2).

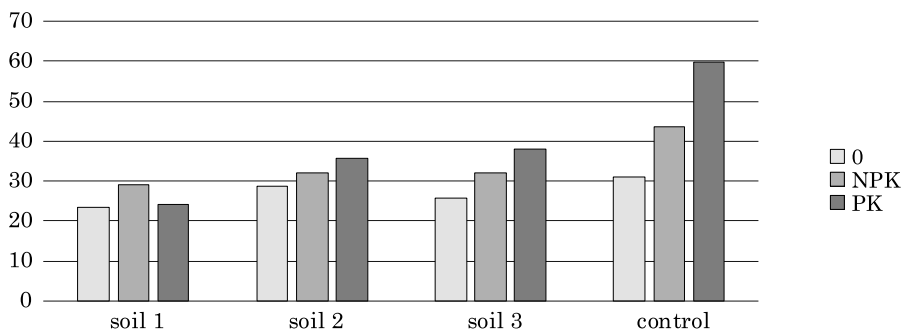


Fig. 2. Yield of an individual lettuce plant (g wet weight) under different fertilization regimes

In 2006, the EU set thresholds for maximum permissible levels of hazardous substances in food, e.g. in leafy vegetables at 0.2 mg kg⁻¹ for Cd and 0.3 mg kg⁻¹ for Pb per wet weight (EC 2006). Using dry weight and water content, recalculated concentrations in lettuce leaves grown in this experiment exceed thresholds just in one case concerning soil 1 (for Cd, after adding the PK fertilizer) – Tables 3, 5. Mineral phosphate fertilization reduced the uptake of Pb significantly, which has been observed by other authors as well (STERRET et al. 1996, LAPERCHE et al. 1997). The nitrogen component (as NPK) caused no significant differences on heavy metal uptake in relation

Table 3
Metal concentration in leaves and roots (mg kg⁻¹) in dry weight and total carbon (%), nitrogen (%) and C/N ratio in lettuce leaves

Amendments	Cd (± 0.1)		Ni (± 0.2)		Pb (± 0.3)		C/N ratio		
	leaves	roots	leaves	roots	leaves	roots	C	N	C/N
Soil 1 (none)	2.29±0.08	1.36±0.05	1.53±0.37	2.40±0.50	2.22±0.06	7.07±0.84	43.32	2.89	14.98:1
NPK	2.86±0.35	1.20±0.05	1.70±0.58	1.91±0.48	1.61±1.98	7.57±0.39	42.10	3.96	10.63:1
PK	2.99±0.18	0.93±0.05	2.17±0.34	1.93±0.37	1.91±0.05	9.03±0.21	40.60	2.01	19.90:1
Soil 2 (none)	1.62±0.07	0.90±0.07	1.01±0.33	1.27±0.44	4.00±2.51	12.2±1.27	42.36	3.21	13.20:1
NPK	2.43±0.09	1.44±0.05	1.09±0.37	1.30±0.31	3.52±0.89	14.23±1.30	41.96	3.92	10.70:1
PK	1.46±0.05	0.88±0.04	1.90±0.31	1.32±0.35	2.50±0.18	10.93±0.82	41.76	3.55	11.26:1
Soil 3 (none)	1.42±0.04	0.63±0.04	1.70±0.29	1.53±0.19	1.77±0.19	1.99±0.53	42.69	3.11	13.72:1
NPK	1.10±0.05	0.38±0.04	1.63±0.23	1.47±0.26	1.01±0.25	2.01±0.34	42.28	3.45	12.26:1
PK	1.31±0.03	0.52±0.055	1.18±0.27	1.53±0.31	0.30±0.09	2.63±0.42	41.76	3.55	11.76:1
Control (None)	0.50±0.03	0.93±0.01	1.41±0.37	1.82±0.43	0.50±0.19	5.08±0.15	43.78	4.26	10.28:1
NPK	0.58±0.03	0.33±0.04	1.03±0.23	1.10±0.25	0.31±0.32	0.98±0.11	41.96	4.77	8.64:1
PK	0.60±0.04	0.20±0.02	1.11±0.38	1.03±0.12	0.49±0.28	2.14±0.30	41.76	3.90	10.66:1

Table 4

Results of the unpaired *t*-test parameter of two groups to show significant differences

Heavy metal	Leaves			Roots		
	none/NPK	none/PK	NPK/PK	none/NPK	none/PK	NPK/PK
Cd	4.36*	10.85*	1.48	11.21*	21.95*	11.44*
	20.98*	4.45*	26.81*	18.99*	1.72	19.91*
	13.76*	3.88*	11.12*	6.25*	2.99*	2.12
	8.19*	5.46*	2.27	0.84	5.09	2.54
Ni	0.48	3.02*	1.92	1.87	2.40	0.32
	0.25	4.81*	4.77*	0.34	0.85	0.45
	0.66	3.28*	2.97	0.08	0.07	0.12
	1.24	0.55	0.55	3.44*	5.01*	1.15
Pb	1.39	2.62	1.99	2.14	5.17	5.64*
	2.43	3.79*	3.39*	3.17*	1.89	5.19*
	5.73*	15.22*	5.27*	0.54	1.86	1.70
	0.59	0.65	1.03	3.63*	1.18	6.70

* significant at $P \leq 0.05\%$ probability

Table 5

Cd- and Pb-concentrations in lettuce leaves (mg kg^{-1}) recalculated for wet weight

Amendments	Cd	Ni*	Pb
Soil 1 (none)	0.16±0.01	0.10±0.03	0.15±0.01
NPK	0.16±0.02	0.09±0.03	0.09±0.11
PK	0.20±0.02	0.14±0.02	0.13±0.02
Soil 2 (none)	0.09±0.01	0.05±0.02	0.22±0.14
NPK	0.13±0.01	0.06±0.02	0.18±0.01
PK	0.10±0.01	0.12±0.02	0.16±0.01
Soil 3 (none)	0.09±0.01	0.10±0.02	0.11±0.01
NPK	0.05±0.01	0.07±0.01	0.05±0.01
PK	0.07±0.00	0.06±0.01	0.02±0.01
Control (none)	0.02±0.00	0.06±0.02	0.02±0.01
NPK	0.03±0.01	0.04±0.01	0.01±0.01
PK	0.03±0.00	0.05±0.02	0.02±0.01

* The European Commission (EC 2006) did not define the permissible values for nickel.

to the PK fertilizer. Presumably, phosphate application lead to the formation of hardly soluble Pb-phosphates, which remain stable in soils and which are hardly plant-available (MELAMED et al. 2003).

The mineral fertilizer treatments occasionally resulted in increased Cd uptake, due to increased Cd input to the soils from the mineral phos-

phate component, versus dilution by increase of biomass. In naturally occurring apatite ($\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$), Cd can substitute some Ca in the crystal lattice (McCONNELL 1973), which should scavenge the additional input of Cd. However, as this was not observed in the experiments, it is suggested that plants can take up the Cd after dissolution of the primary Cd-bearing phases of the fertilizer before re-precipitation as Cd-bearing phosphate. Screening of elemental contents of fertilizers sold in Austria revealed sample ranges of trace metals. Median concentrations (= expectable values) for Cd in triple phosphates were 13.5 mg kg^{-1} , which is about 50 times as much as the Cd present in the investigated soils, leading to a load of $6.5 \text{ g ha}^{-1} \text{ ha}$ for a P-fertilization of $100 \text{ kg ha}^{-1} \text{ ha}$, and in compost 0.043 mg kg^{-1} , leading to a respective load of $7.9 \text{ g ha}^{-1} \text{ ha}$. In contrast, in soil 3 and 4, the addition of NPK and PK did not increase Cd uptake by lettuce. It is suggested that the small amounts of Cd released from the fertilizer are below the equilibrium concentration controlled via solubility or adsorption processes. Ammonium, nitrate, lime and K-salts contained just 0.09 resp. 0.05 mg kg^{-1} Cd. In most cases, Pb and Ni concentrations in fertilizers were below soil levels (SAGER 2009). The Ni content ranged within background values (KABATA PENDIAS, PENDIAS 2001). Contrary to Cd and Pb, traces of Ni are essential for green plants as a component of urease (ALLOWAY 2013), but current data show that there is no malnutrition of Ni. Data about the N-content of lettuce leaves prove sufficient N-supply. NPK fertilization increased the N levels as expected.

When individual concentration measurements follow a Gaussian distribution, a *t*-test can be performed to prove if two values are significantly different. The *t*-coefficient is calculated from the number of measurements and the individual variances (Table 4). If the *t*-coefficient is higher than 2.45, the two values are significantly different.

Bioconcentration (BCF) and translocation (TSF) factors

Whereas the BCF for Pb ranged within 0.002-0.026, it was markedly higher in the case of Cd (0.58-5.83), which can be explained by the competition of Cd^{2+} with Ca^{2+} . Although most non-essential metal ions enter plant tissues just by passive transport (no energy required; the metals enter with water), Cd may use active transport paths for Ca (HART et al., 2002). Highest BCFs were obtained for the slightly acid soil 3 (pH 6.2) and control soil. This might be due to the fact that the mobility of Cd increases with decreasing pH, as surface charge of soil minerals becomes more positive, thereby yielding repelling forces between ions and surfaces. INTAWONGSE, DEAN (2008) found that $\text{BCFs} \leq 0.9$ for Pb and ≤ 2.7 for Cd are marginal and do not pose a concern to the environment, which is the case for all investigated soils concerning Pb and in many cases also regarding Cd. On the other hand, high metal BCFs of the plant species signify their capacity for decontamination, thus reducing the pollution load of aquatic and terrestrial ecosystems (SINGH et al. 2017). These high BCFs were not observed for the soils investigated within this study. For Ni, the BCFs were below 1.

Hyperaccumulating plants are defined by accumulating 100 times more than non-accumulator plants growing in the same substrate. About 300 species accumulate nickel, 26 cobalt, 24 copper, 19 selenium, 16 zinc, and 11 manganese (BROOKS et al. 1998). Target plants must tolerate, absorb and translocate contaminant metals and produce sufficient biomass, and also allow repeated planting and harvesting. The success of phytoremediation depends on adequate plant yield and high metal concentrations in plant shoots (SHEN et al. 2002). The TSFs for almost all variants are > 1 for Cd and < 1 for Ni and Pb, i.e. lettuce is a hyperaccumulating plant for Cd, but not for Pb. A similar result was found by SAGER et al. (2010) after adding soluble metal salts to soils on lettuce plants. They noted that Cd also preferably accumulated in the shoots and Pb in the roots. Considering that the content of Pb in the roots was much higher than that of Cd, it seems reasonable that the retention capacity regarding Pb is reached earlier. Contrary to Pb, phosphate fertilizer increases TSFs for Cd (Table 6). It is suggested that

Table 6

BCF and TSF for Cd, Ni and Pb

Amendments	BCF			TSF		
	Cd	Ni	Pb	Cd	Ni	Pb
Soil 1 (none)	1.28±0.06	0.06±0.01	0.006±0.001	1.58±0.09	0.63±0.05	0.31±0.08
NPK	1.61±0.21	0.05±0.02	0.005±0.006	2.43±0.25	0.89±0.15	0.21±0.10
PK	1.67±0.11	0.05±0.01	0.006±0.001	3.34±0.10	1.16±0.05	0.21±0.20
Soil 2 (none)	0.61±0.03	0.03±0.01	0.006±0.008	0.57±0.18	0.71±0.20	0.08±0.02
NPK	0.92±0.04	0.03±0.01	0.004±0.004	2.12±0.10	0.85±0.30	0.33±0.05
PK	0.58±0.03	0.03±0.01	0.003±0.001	2.35±0.17	1.46±0.17	0.26±0.15
Soil 3 (none)	5.83±0.17	0.03±0.01	0.013±0.001	1.82±0.15	1.13±0.10	0.42±0.10
NPK	4.58±0.22	0.03±0.01	0.008±0.001	1.69±0.16	1.07±0.45	0.24±0.17
PK	5.42±0.14	0.03±0.01	0.002±0.001	1.54±0.34	0.80±0.09	0.23±0.05
Control (none)	3.33±0.24	0.08±0.01	0.026±0.001	0.88±0.08	0.78±0.04	0.39±0.05
NPK	4.00±0.31	0.05±0.01	0.016±0.002	1.29±0.19	0.91±0.24	0.16±0.21
PK	4.00±0.35	0.04±0.01	0.026±0.001	1.32±0.45	1.10±0.50	0.28±0.02

the increased growth due to fertilizer addition is associated with improved transport of dissolved species from the roots to the leaves.

Modified electro-ultrafiltration method (EUF) and kinetic models

Contrary to selective leaching methods, which approach mobilizations like a snapshot, the EUF simulates desorption from the solid soil particles, accelerated by an electric field. In order to avoid alkaline precipitations at the cathode as an effect of H^+ consumption, a complexant has to be added, like DTPA. In our experiment, the released concentrations should remain about constant during the dissolution of a proper solid phase or the saturation of the extractant, and then follow a desorption curve until exhaustion.

The results of the modified electro ultrafiltration method (EUF) are presented in Table 7. The largest amounts of heavy metals were released in the first two fractions. For all samples the highest Cd concentrations, which

Table 7

Heavy metal concentrations in 5 fractions of the EUF method and the *t*-test values

Heavy metal	Amend-ments	Fraction (mg kg ⁻¹)					<i>t</i> -test
		1	2	3	4	5	
Cd	Soil 1 (none)	0.311±0.500	0.203±0.013	0.120±0.007	0.085±0.003	0.061±0.004	0.606
	PK	0.222±0.041	0.168±0.010	0.112±0.002	0.076±0.0.04	0.053±0.024	
	Soil 2 (none)	0.386±0.010	0.297±0.008	0.183±0.004	0.134±0.003	0.090±0.004	0.290
	PK	0.343±0.051	0.263±0.011	0.175±0.002	0.124±0.004	0.093±0.006	
	Soil 3 (none)	0.075±0.018	0.020±0.005	0.007±0.003	0.001±0.001	0.000±0.000	0.140
	PK	0.058±0.003	0.022±0.004	0.009±0.003	0.003±0.001	0.000±0.000	
	Con-trol (none)	0.050±0.019	0.014±0.003	0.007±0.005	0.029±0.039	0.030±0.036	-
Ni	Soil 1 (none)	1.340±0.261	1.287±0.227	0.752±0.180	0.691±0.176	0.533±0.207	2.174
	PK	0.887±0.213	0.638±0.152	0.497±0.101	0.400±0.140	0.301±0.157	
	Soil 2 (none)	0.729±0.367	0.946±0.273	0.529±0.160	0.424±0.118	0.322±0.074	0.037
	PK	0.658±0.229	0.739±0.262	0.592±0.192	0.523±0.152	0.458±0.144	
	Soil 3 (none)	0.479±0.178	0.388±0.461	0.282±0.401	0.199±0.391	0.153±0.463	4.833*
	PK	0.482±0.099	0.814±0.056	0.685±0.033	0.618±0.032	0.640±0.026	
	Con-trol (none)	1.045±0.599	0.386±0.136	0.316±0.078	0.351±0.117	0.396±0.225	-
Pb	Soil 1 (none)	21.11±1.818	23.59±1.046	18.41±1.234	16.72±0.426	13.71±0.554	0.425
	PK	18.69±1.872	21.46±1.247	19.10±1.222	16.49±0.440	13.69±0.664	
	Soil 2 (none)	64.21±3.232	74.43±2.974	61.28±1.298	51.20±0.977	40.80±0.516	0.674
	PK	61.41±6.488	68.02±5.943	56.00±2.422	45.30±0.798	37.14±0.538	
	Soil 3 (none)	8.382±0.362	7.945±0.121	5.868±0.042	4.458±0.080	3.348±0.058	0.404
	PK	9.338±2.693	8.405±0.355	6.426±0.200	4.832±0.223	3.617±0.218	
	Con-trol (none)	2.210±0.727	1.329±0.069	1.028±0.043	0.892±0.140	0.828±0.224	-

* significant at *P* ≤ 0.05% probability

is also the most mobile heavy metal, were extracted in the first fraction while the highest Ni and Pb concentrations vary between the first two fractions. These easily extracted fractions are the most important as they readily move to the soil solution and contaminate the food chains (FANGUEIRO et al. 2005). In opposite to the accumulated Pb concentrations in leaves, the EUF method extracted slightly higher Pb concentrations from soil 3 in the PK variant in contrast to the untreatable variant. The Pb root concentration, however, showed a higher concentration in the PK variant. The differences between abiotic EUF experiments and plant uptake experiments are in agreement with the explanation of the behaviour of Pb within a plant by the retention capacity of the root, as this intra-plant barrier is not relevant for EUF. According to the *t*-test, however, a significant relationship was achieved only for Ni.

As the criteria for the goodness of fit of the cumulative curves with regression lines, the coefficient of determination (R^2) – MENGEL, UHLENBECKER (1993), JELECEVIC et al. (2019) and the mean ratio between recalculated and measured values were selected (JELECEVIC et al. 2019). The high coefficients of determination and the mean ratio between recalculated and measured values of about 100% show that the selected mathematical functions describe the calculated cumulative curves very well. However, the 2nd order polynomial provides a better description of the release of heavy metals than the other mathematical fittings (Table 8). Several authors (FINZGAR et al. 2007, JELECEVIC et al. 2019) also reported a successful description of the release curves using the 2nd order polynomial function. Such functions show a decreasing slope which corresponds to a decreasing release rate with increasing time (Figure 3). Considering that heavy metals are present

Table 8
Coefficient of determination (R^2) and the mean values of the 4 kinetic models

Heavy metal	Treatment	Statistics	Mathematical function			
			Linear	Parabolic	Elovich	2 nd order polyn.
Cd	none	R^2	0.900	0.927	0.932	0.993
		mean	100.7	95.22	99.55	107.1
	PK	R^2	0.895	0.947	0.977	0.973
		mean	101.9	97.22	100.2	103.9
Ni	none	R^2	0.980	0.992	0.978	0.999
		mean	101.8	101.1	97.61	100.4
	PK	R^2	0.979	0.997	0.989	0.999
		mean	101.4	99.72	99.27	99.85
Pb	none	R^2	0.979	0.998	0.986	0.999
		mean	101.5	99.24	99.20	100.0
	PK	R^2	0.974	0.998	0.986	0.999
		mean	102.7	98.94	98.98	100.0

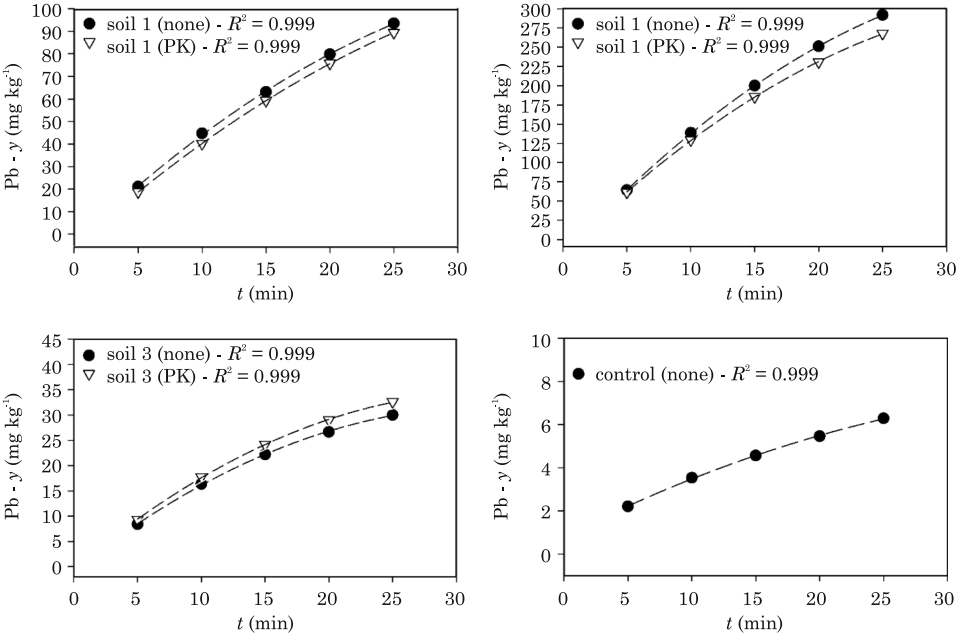


Fig. 3. Cumulative Pb release described with the best fitted model - 2nd order polynomial model

in different mineralogical bonds and the destruction of stronger bonds requires more time, it seems reasonable that after a certain time the fraction of a heavy metal that is released from a stronger bonding form increases. Noticeable differentiations were only detected for Cd, which indicates that there were somewhat higher variations in the release between fractions. This can be explained by the increasing relative measurement uncertainty with decreasing concentrations.

The intercept a is related to the thickness of the boundary layer, and the slope b is the intra- particle diffusion rate constant (Table 9). There is immediate utilization of the most readily available desorbing sites (“surface diffusion”) until the desorbed amount reaches a constant value, which is due to slow diffusion from surface sites into the inner pores (“pore diffusion”).

Statistical analyses were carried out to relate the fitting parameter b of the 2nd order polynomial order, which characterizes the dissolution rate, with the plant uptake concentrations (leaves and roots), yields of the edible part and C/N ratio. Table 10 shows that the most significant relationships ($P \leq 0.05$) were found for Pb (leaves and roots) and slightly worse for Cd (leaves). There was no significant relationship with Cd as well as with yields and C/N ratio.

Processes of trace element plant uptake include the supply of trace elements from the solid to the soil solution, the absorption by plants from the solution, as well interactions between soil and plants. Most plants can probably obtain their required nutrients from low concentrations, whereas

Table 9

Values of the constants of the kinetic models

Heavy metal	Amendments	Mathematical function								
		Elovich		Linear		Parabolic		2 nd order polyn.		
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>
Cd	Soil 1 (none)	-0.159	0.292	0.245	0.023	-0.044	0.169	0.089	0.050	-0.001
	PK	-0.194	0.257	0.161	0.020	-0.094	0.149	0.029	0.043	-0.001
	Soil 2 (none)	-0.326	0.440	0.290	0.034	-0.153	0.256	0.056	0.074	-0.001
	PK	-0.324	0.409	0.242	0.032	-0.166	0.238	0.048	0.066	-0.001
	Soil 3 (none)	0.059	0.008	0.059	0.002	0.037	0.012	0.033	0.006	0.000
	PK	0.008	0.020	0.036	0.002	0.017	0.011	0.014	0.005	0.000
	Control (none)	-0.033	0.045	0.024	0.004	-0.021	0.028	0.051	-0.001	0.000
Ni	Soil 1 (none)	-1.952	2.009	0.816	0.159	-1.193	1.173	0.029	0.294	-0.005
	PK	-1.018	1.143	0.551	0.091	-0.597	0.670	0.160	0.158	-0.002
	Soil 2 (none)	-1.490	1.374	0.416	0.108	-0.955	0.798	-0.254	0.223	-0.004
	PK	1.740	1.420	0.187	0.115	-1.233	0.837	-0.132	0.169	-0.002
	Soil 3 (none)	-2.373	1.672	-0.126	0.136	-1.793	0.990	-0.333	0.172	-0.001
	PK	-0.569	0.638	0.314	0.050	-0.337	0.372	0.035	0.098	-0.002
	Control (none)	-0.450	0.862	0.691	0.071	-0.164	0.514	0.721	0.067	0.000
Pb	Soil 1 (none)	-54.10	44.62	6.461	3.600	-38.08	26.29	-4.264	5.438	-0.061
	PK	-55.59	43.73	3.526	3.542	-40.11	25.81	-5.549	5.098	-0.052
	Soil 2 (none)	-173.2	141.2	18.79	11.36	-122.2	83.05	-19.88	17.99	-0.221
	PK	-153.5	128.0	20.71	10.28	-107.1	75.23	-15.51	16.49	-0.207
	Soil 3 (none)	-13.89	13.48	4.644	1.071	-8.831	7.881	-0.659	1.980	-0.030
	PK	-14.73	14.53	5.228	1.156	-9.298	8.502	-0.356	2.114	-0.032
	Control (none)	-1.996	2.497	1.390	0.202	-1.101	1.471	0.821	0.299	-0.003

Table 10

Spearman correlation coefficient for the constant *b* of the polynomial 2nd order in relation to several plant parameters

Heavy metal	Unit	Leaves	Roots	Yield	C/N
Cd	<i>r</i>	0.679	0.162	-0.250	0.286
	<i>P</i> _{2 tailed}	0.094	0.728	0.589	0.535
Ni	<i>r</i>	-0.036	-0.054	-0.601	0.536
	<i>P</i> _{2 tailed}	0.939	-0.901	0.148	0.215
Pb	<i>r</i>	0.893*	0.857*	-0.107	0.214
	<i>P</i> _{2 tailed}	0.001	0.014	0.819	0.645

* significant at $P \leq 0.05\%$ probability

at high levels, a 5-10-fold reduction in the absorption rate has been observed. The plant root modifies the soil environment in its immediate vicinity both by excreting chemically active substances, e.g. to establish mycorrhiza, and by absorbing water and ions (LONERAGAN 1975).

CONCLUSION

Prediction of soil to plant transfer is important to minimize food and feed contamination, or to optimize phytoremediation. Although N-supply was already sufficient, effects of the application of various fertilizers were investigated because this can be easily changed by actual farming practices. In spite of high pseudo-total metal levels, accumulation of Cd and Pb in lettuce grown thereof was for the most samples marginal and below hazardous ranges. In particular, soil 2 contained Pb at 800 mg kg⁻¹, but the respective lettuce did not exceed thresholds in edible parts. In the case of exceeding the limit values for foodstuff, P-fertilizers used in common farming practice can play a significant role by decreasing the mobility of heavy metals like Pb. The addition of P- fertilizer lowered Pb uptake, presumably due to Pb-phosphate formation in the soil. Cd inputs from mineral phosphates did not necessarily increase Cd concentrations in lettuce leaves because of dilution from increased plant growth. Monitoring release rates from soil might be closer to natural conditions than single step mobilizable fractions or sequential leaching. In the EUF-method, release from soil suspensions is accelerated by an electric field so that it can be measured within a short time span. Although the model calculations resulted in different release rates, the correlation coefficients between constant b and plant uptakes were statistically significant for Ni and Pb but not for Cd. As an outlook, variations of the EUF parameters (electrolyte -complexant and pH, voltage) might optimize correlations with plant uptake.

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