Modelling Chelate-Induced Phytoextraction: Functional Models Predicting Bioavailability of Metals in Soil, Metal Uptake and Shoot Biomass

Pasqualina Sacco1, Fabrizio Mazzetto1, Luca Marchiol2

1Istituto di Ingegneria Agraria, Università di Milano
Via Celoria 2, 20133 Milano, Italy
2Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine
Via delle Scienze 208, 33100 Udine, Italy

Received: 9 December 2004. Accepted: 16 March 2006

Abstract
Chelate-induced phytoextraction of heavy metals from contaminated soils requires special care to determine, a priori, the best method of chelate application, in terms of both dose and timing. In fact, the chelate dose must assure the bioavailability of the metal to the plant without increasing leaching risk and giving toxic effects. Three mathematical models are here proposed for usefully interpreting the processes taking place: a) increased soil bioavailability of metals by chelants; b) metal uptake by plants; c) variation in plant biomass. The models are implemented and validated using data from pot and lysimeter trials. Both the chelate dose and the time elapsed since its application affected metal bioavailability and plant response. Contrariwise, the distribution strategy (single vs. split application) seems to produce significant differences both in plant growth and metal uptake, but not in soil metal bioavailability. The proposed models may help to understand and predict the chelate dose – effect relationship with less experimental work.

Key-words: phytoextraction, heavy metals, models.

1. Introduction
The efficiency of heavy metal removal by phytoextraction techniques depends mainly on: a) the bioavailability of the metals in the soil; b) the uptake and translocation capacity of the plants; and c) the plant biomass. Mobility and bioavailability of metals in the soil depend upon the metal, and are strongly influenced by environmental conditions (in particular pH, texture and organic matter content), but are generally always low. Induced phytoextraction aims at a better removal efficiency increasing soil metal bioavailability throughout rhizosphere processes (Wenzel et al., 2000), as well as the use of soil correctives (Ebbs et al., 1997), fertilisers (Bennet et al., 1998) or synthetic chelants. About chelate-assisted phytoextraction affects the chemical lability of metals in the soil and their accumulation in the shoots of phytoremediation crops see the overview of Wenzel et al. (1999) and McGrath et al. (2000). These results reveal some general trends in terms of a saturation-type effect of chelate application on metal mobilisation in the soil, whereas the type of relation between metal accumulation in shoots and the bioavailable metal pool is less consistent. Various studies (Blaylock et al., 1997; Hong et al., 1999) have found that EDTA tends to have a strong mobilising effect on metals when added to the soil in low concentrations, but that the mobilisation is progressively attenuated at higher doses, suggesting an asymptotic pattern. As was observed in our lysimeter experiments (Wenzel et al., 2002), adding greater amounts of EDTA, as the limit concentration is approached does not alter the bioavailable metal concentration and, considering the slow degradation of EDTA in the soil, the risk of leaching may persist even after the phytoextraction treatment (post harvest). On the other
hand, assuming an infinite quantity of bioavailable metal in the soil, phytoextraction efficiency will be limited by the physiological characteristics of the plants and the metal toxicity. In our case, Cu and Zn are essential micronutrients for plant growth, but can become toxic when present in high doses. Pb, instead, is always considered a toxic element even in trace quantities. Reference values in the literature for “normal”, “mean” and “toxic” metal concentrations in plant tissues (Reeves et al., 1995; Angeloni and Bini, 1992) are often dependent on plant species (or cultivar), and their adaptation characteristics, so determining a “limit” metal concentration is no simple matter. Likewise, the relation between metal concentration in soil and in shoots is not always clear. In some cases it is possible to hypothesise a non-asymptotic increase (Jackson and Alloway, 1992; Zao et al., 2000), while in other cases the data suggests a tendency toward an asymptotic value (Blaylock et al., 1997; Lombi et al., 2000; Wenzel et al., 2000; Wheeler and Power, 1995). In our experiments, a power-type relation emerged. There is no doubt that extraction efficiency depends first and foremost on the ability of plants to remove and accumulate the metals, however the usefulness of a crop for soil remediation also depends on its productivity in terms of plant tissue biomass (McGrath, 1998).

2. Materials and methods

2.1 Experimental data

Soil taken from an area contaminated with heavy metals (principally Cu, Pb and Zn) was used to study induced phytoextraction by *Brassica napus* var. Petranova that was cultivated for 60 days both in pots and lysimeters. Details of the experiments are given elsewhere (Wenzel et al., 2002). The very low mobility of these metals (< 1% of the total under the original conditions) leads to a very low bioavailability that strongly limits metal uptake by plants. Heavy metals in the contaminated soil were investigated for (Table 1): a) the soluble fraction – available to plants and subject to leaching – by water extract 1:10 (Blum et al., 1996 – ÖNORM L 1092-93); b) the exchangeable fraction available to plants by 1M NH₄NO₃ extract (Blum et al., 1996, Prüeb et al., 1991 – DIN V 19730); c) the total amount of metals by extraction with strong acids (Blum et al., 1996), and d) a 0.05 M Na₂EDTA extraction (Blum et al., 1996 – ÖNORM L 1089-93), equivalent to an EDTA concentration of 186 g kg⁻¹ soil, performed in order to verify the effective increased metal mobility (41.7%, 29.3% and 25.6% of Cu, Pb and Zn of the total, respectively). EDTA was added to the contaminated soil at different concentrations, in single (12 days before harvest) and double (26 and 12 days before harvest) applications both on pots (as a solution) and lysimeters (as a powder). The various treatments performed and the relative data sets are given in Table 2. Five soil samples were taken from both the pots and lysimeters at different time and investigated for the bioavailable metal concentrations using a 1M NH₄NO₃ extract (Blum et al., 1996, Prüeb et al., 1991 – DIN V 19730) by ICP. Plant samples were taken five times from the pots and only once – at harvest – from the lysimeters. The total metal content in the shoots and roots was analysed by ICP following microwave digestion (0.5 g in 0.5 ml H₂O₂ 30%, 6 ml HNO₃ 65%, 1 ml HClO₄ 70-72% according to Blum et al., 1996). The biomass dry weights (dried 80 °C for 6 h) were also determined.

2.2 Metal Lability Model (MLM): Effect of EDTA on metal lability in soil

The data available in the literature (Blaylock et al., 1997; Wenzel et al., 2000; Hong et al., 1999; Li and Shuman, 1996) and the results of our ex-

### Table 1. Metals concentration in soil: total, EDTA extract, NH₄NO₃ extract, and water extract. Average ± SE, n = 2.

| Metal | Total (mg kg⁻¹) | EDTA 0.05 M (mg kg⁻¹) | NH₄NO₃ 1 M (mg kg⁻¹) | Water 1:10 (mg kg⁻¹) |
|-------|----------------|-----------------------|----------------------|---------------------|
| Cu    | 256.0 ± 3.2    | 94.3 ± 2.2            | 1.3 ± 0.0            | 0.3 ± 0.0           |
| % on total | 41.7         |                       | 0.6                  | 0.1                 |
| Pb    | 77.0 ± 5.8     | 22.6 ± 1.4            | -                    | 0.1 ± 0.0           |
| % on total | 29.3         |                       | -                    | 0.1                 |
| Zn    | 343.1 ± 18.4   | 88.1 ± 3.3            | -                    | 0.3 ± 0.1           |
| % on total | 25.6         |                       | -                    | 0.1                 |
The natural metal bioavailability in soil was found very close to zero. Thus the hypothesis $M_i(t = 0)$ equal to $M_{0,i} = 0$ was assumed for the model. $M_{\text{max},i}$ is the maximum theoretical bioavailable concentration in soil achievable for the metal $i$-th with an EDTA dose $X \to \infty$. Parameter $k_i$ ($\text{mg kg}^{-1}$) represents the “intensity” of response of $M_i$ to increases in EDTA application rate; $\lambda_i (d^{-1})$ gives a measure of the mobilisation kinetics. Our experimental data suggest that $k_i$ is independent of $t$, whereas $\lambda_i$ is related to $X$ according to the function:

(Eq. 3) \[ \lambda_i = a_i \cdot X^{b_i} \]

where the proportionality factor $a_i$ and the exponent $b_i$ are determined on experimental data. Note that for Zn it was found that $\lambda_i = \text{constant}$. In case of a single chelate dose $X$ applied when $t = 0$ we have:

(Eq. 4) \[ M_i = M_{\text{max},i} \cdot \left[1 - \exp(-k_i X^b) \right] \]

The combined use of Eq. 1 and Eq. 2 with their numerical integration is defined Metal Lability Model (MLM). The MLM concentration curves present typical rapid changes at each EDTA application (see curves A in Figure 1).

2.3 Metal Uptake Model (MUM): Effect of EDTA on metal accumulation in shoots

A model of cumulative metal uptake for describing the effect of various chelate application rates and schedules must consider chelate-induced changes in metal bioavailability during the plant growth period. Here, we assume that metal accumulation in the phytoremediation crop depends both on: a) the concentration of the bioavailable metal in the soil, and b) the duration of exposure to any given concentration. During the growth period, $M_i$ changes as a result of chelate applications, while the exposure time depends on the duration of the cropping period and the number of individual applications. Integrating $E_i$ ($\text{mg kg}^{-1} \text{ d}$) over the exposure time $t$ gives the cumulative exposure $E_i$ ($\text{mg kg}^{-1} \text{ d}$):

(Eq. 5) \[ E_i = \int_0^t M_i \, dt \]

where $t = 0$ is the day of sowing and values for $M_i$ can be obtained performing MLM. The concentration of the $i$-th metal in the plant shoots $M_{p,i}$ ($\text{mg kg}^{-1} \text{ dw}$) at time $t$ can firstly be expressed as a function of $E_i$ ($R^2 > 0.60$):

| Codes | Data sets | I treatment ($t = 0$) | II treatment ($t = 14$ days) | Total added |
|-------|-----------|-----------------------|----------------------------|-------------|
| Pot/3 | 1         | 0.2                   | 0.2                        | 0.2         |
| Pot/4 | 2         | 0.1                   | 0.1                        | 0.2         |
| Pot/5 | 1         | 0.4                   | 0.4                        | 0.4         |
| Pot/6 | 2         | 0.2                   | 0.2                        | 0.4         |
| Pot/7 | 1         | 0.8                   | 0.8                        | 0.8         |
| Pot/8 | 2         | 0.4                   | 0.4                        | 0.8         |
| Pot/9 | 1         | 1.6                   | 1.6                        | 1.6         |
| Pot/10| 2         | 0.8                   | 0.8                        | 1.6         |

| Lysimeter trials | Codes | Data sets | I treatment ($t = 0$) | II treatment ($t = 14$ days) | Total added |
|-------------------|-------|-----------|-----------------------|----------------------------|-------------|
| Lys/3             | 3     | 0.2       | 0.2                   | 0.2                        |             |
| Lys/4             | 4     | 0.1       | 0.1                   | 0.2                        |             |
| Lys/5             | 3     | 0.5       | 0.5                   | 0.5                        |             |
| Lys/6             | 4     | 0.2       | 0.2                   | 0.5                        |             |
| Lys/7             | 3     | 1.0       | 1.0                   | 1.0                        |             |
| Lys/8             | 4     | 0.5       | 0.5                   | 1.0                        |             |
| Lys/9             | 3     | 2.0       | 2.0                   | 2.0                        |             |
where $M_{p0i}$ (mg kg$^{-1}$ dw) is the metal concentration in the shoots of a given phytoremediation crop grown on contaminated soil in the absence of EDTA applications, and $c$, $d$ and $e$ (adimensional) are experimental parameters. Eq. 6 describes a medium-long term effect which presumes an adaptation of the plants to high metal concentrations in the soil. However, such an equilibrium will be perturbed by each EDTA application, which causes a rapid increase in the current bioavailable metal concentration in the soil $M_i$ so that also this non-cumulative exposure term must be explicitly considered. In particular, in the present work the role of $M_i$ was interpreted as an inhibitory effect. Considering both non-cumulative ($M_i$) and cumulative ($E_i$) exposures $M_{pi}$ can be better expressed as ($R^2 > 0.70$):

$$M_{pi} = M_{p0i} + c \cdot E_i^d, \quad (c > 0 \text{ and } 0 < d < 1)$$

(Eq. 7)

where $M_{p0i}$ (mg kg$^{-1}$) is the metal concentration in the shoots of a given phytoremediation crop grown on contaminated soil in the absence of EDTA applications, and $c$, $d$ and $e$ (both adimensional) are calibrated simultaneously on experimental data. Note how $d < 1$ indicates that, as the value of $E_i$ increases, the corresponding incremental metal absorption by plants decreases. This can in practice be attributed to an asymptotic-like behaviour, by which when plants reach a certain limit they are no longer able to absorb any further metals. However, for the values found in our work, a “power” type relation between the absorbed metal and the cumulative exposure better matches the experimental data. The inhibitory effect of $M_i$ is expressed by exponent $e < 0$. Typical $M_{pi}$ behaviours produced by MUM are described by B-curves in Figure 1.

2.4 Shoot Biomass Model (SBM): Effect of EDTA on crop biomass

Plant growth on contaminated soil without EDTA applications was simulated by a Richards (1969) function:

$$\frac{dW}{dt} = \mu W(W_f^n - W^n)$$

(Eq. 8)

where $W$ (g dw) represents the average dry weight of a plant at time $t$ (d), $W_f$ (g dw) is an asymptotic value of $W$ for $t \to \infty$, $\mu$ (d$^{-1}$) is the specific growth coefficient and $n$ (adimensional) is an experimental coefficient. A decrease in plant biomass has been experimentally observed to correspond to an increase in EDTA concentrations and bioavailable metals in the soil. From the available data it is not possible to establish whether this effect is due to metal or chelate toxicity (Vassil et al., 1998). It has also been observed that the relative ratio between the metals Pb, Zn and Cu in the soil and in plant tissues remains constant over time ($r > 0.90$, de-
cluded data from set 2, as well as data sets 3 and 4. In a second step, to closely simulate lysimeter conditions, the model parameters were re-calibrated using data sets 3 and 4, but excluding the final sampling data of set 4, after used for validation. Both MUM and SBM were calibrated on the complete pot data sets and tested against lysimeter data sets (harvest). For the numerical integration of the three models a simple Euler method with time step = 1 day was used. No interactions between metals were considered, because the amounts of EDTA applied were sufficient to rule out competition between metal cations for chelation. For validation, we verified that the models estimated values fell within the respective confidence intervals calculated on observed data for the last sample both in pots and lysimeters (MLM), and for samples harvested from the lysimeters (MUM and SBM) by means of the Student’s t test at 95 and 99% (df = 3).

3. Results and discussion

3.1 Metal Lability Model (MLM)

Model calibration on pots (Table 3) shows that the metals responded differently to the application of EDTA: in particular, the induced bioavailability was Cu > Zn > Pb. In fact, for Cu both the intensity of response to EDTA and the mobilisation rate were high, whereas Pb, as we know, is not easily mobilised. MLM calibrated to pot data sets, provided the daily dynamics of bioavailable metal concentrations in the soil for the different EDTA treatments with a good fit to experimental data (Figure 2), also accepted in most cases by the validation test.
MLM applied to the lysimeters provided worst correspondence with experimental observations: the simulation data tended to undershoot the experimental values for Pb and Zn. Anyhow, no case was rejected by validation, thanks to the high variability of trials data (Figure 3). To closer reproduce metals dynamic in lysimeters, MLM parameters were re-calibrated to lysimeter data sets (Table 3), in the manner described previously, considering that: a) EDTA was applied as a powder in lysimeters and as a liquid solution in pots, b) uncontrolled field conditions, and other factors which are not accounted for by the model could have occurred, c) growth of plants was irregular for any given treatment. The values of $k$ are higher for lysimeters than for pots, which indicates a greater intensity of response to EDTA applications. The re-calibrated MLM provided good fitting results with simulated values (Figure 4) that were always accepted by the validation test (Figure 3). The ratio $p_i = M_{X,i}/M_{\text{max},i}$ (in %) is an estimation index of the induced metal mobilisation in the soil. Considering pot trials, an EDTA application of $X = 0.825 \text{ g kg}^{-1}$ to soil induced $p_{Cu} = 57\%$ and $p_{Zn} = 54\%$ showing a similar dynamic behaviour for the two metals. On the other hand, a value of $p_{Pb} = 27\%$ confirmed the low mobilisation for Pb. Increasing the amount of chelant that is applied increases the mobilisation of the metals, albeit in ever diminishing increments: doubling the applied EDTA dose ($X = 1.650 \text{ g kg}^{-1}$) a $p_i$ increase of 44% was achieved by Cu and Zn, against an increase of 74% for Pb (47% of $M_{\text{max},Pb}$). As far as the time is concerned, the highest mobilisation rate was observed for Cu: with a single application $X = 0.825 \text{ g kg}^{-1}$ a $M_{0.825,Cu} = 54 \text{ mg kg}^{-1}$ was reached in 12 days time. For Zn and Pb the analogous times were 20 and 26 days, respectively. The 50% of $M_{0.825,i}$ is reached after only 1, 2 and 3 days for Cu, Zn and Pb, respectively. The same behaviour – in terms of both $p_i$ and time – was observed in lysimeter trials. Moreover, no significant differences were found between single or split chelant distributions. What is more, depending on the metal, there is substantial mobilisation already at low chelate doses: for $X = 0.05 \text{ mg kg}^{-1}$ the metal availability exceeded the threshold values associated with phytotoxicity (2 and 10 mg kg$^{-1}$ for Cu and Zn respectively, Prüeß, 1994) and water quality risks (1,3,5 mg kg$^{-1}$ for Cu, Pb, Zn respectively, Prüeß, 1994).

3.2 Metal Uptake Model (MUM)

MUM was firstly calibrated on pot data sets (Table 4) and then tested by comparing simulation results with lysimeter observations (Figure 5). Only few cases were rejected by the validation test (Figure 6). It is important to note the high variability of the data from pot experiments.

| MUM parameters | Cu     | Pb     | Zn     |
|----------------|--------|--------|--------|
| $c$            | 0.594  | 0.339  | 1.320  |
| $d$            | 1.337  | 1.024  | 0.919  |
| $e$            | -0.981 | -0.627 | -0.350 |
| $R^2$          | 0.729  | 0.761  | 0.801  |

Figure 2. Bioavailable metals simulations for application of 0.17 g EDTA kg$^{-1}$ soil (left; Pot/4) and 0.83 g EDTA kg$^{-1}$ soil (right; Pot/8), performed by MLM ($\gamma$: calibration set data; $\lambda$: validation set data). 1st EDTA application: $t = 0$; 2nd EDTA application $t = 14$ days.

Table 4. MUM model: values of coefficients $c$, $d$ and $e$ (Eq. 7) estimated for pot trials.
mments used for calibration; only for Zn a value of $R^2 > 0.80$ was achieved. In fact, the simulations for Zn were also closer to the observations. Simulations clearly show that a given chelate dose, when split into two successive applications, produces higher metal accumulation in plants, compared with a single application. For example, with the experimental protocol used here, an EDTA treatment $X = 1$ g kg$^{-1}$ in two successive applications of 0.5 g each (26 and 12 days before harvest) produces, at harvest, a concentration in plant tissues of 200 mg Cu kg$^{-1}$ dw, 20 for Pb and 355 for Zn, compared with 86, 11 and 246 mg kg$^{-1}$ dw for a single application (12 days before harvest). Based on these results, it would appear advantageous to divide the total chelate dose into several applications during the plant growth period, as this gives plants time to adapt to the new, increased availability of metals, attenuating the concentration spikes that follow each individual application. It should also be noted that single application treatments with chelate doses of 1 and 2 g kg$^{-1}$ do not produce significant increases in the metal absorbed in the shoots, compared with a dose of 0.5 g kg$^{-1}$, whereas the superior efficiency of split applications appears to be confirmed (see Sacco, 2000).

3.3 Shoot Biomass Model (SBM)

The parameters of the Richards growth function (Eq. 8) were firstly fitted to the pot data sets, referred to contaminated soil without any additions of EDTA. The following values were obtained ($R^2 = 0.98$): $\mu (d^t) = 0.390, n = 3.770, W_0 (g \text{ dw}) = 0.006, W_f (g \text{ dw}) = 0.650$. The additional parameters used by SBM to reproduce effects of EDTA treatments on plant biomass in pots – $\alpha$ and $\beta$ – were found to be $1.49 \times 10^{-5}$ ($d^{-2}$) and $1.43 \times 10^{-2}$, respectively. SBM validation was then carried out on lysimeters data sampled at harvest. This required a new calibration of Richards parameters due to the different growth conditions observed with respect to the pot trials. A new $W_f$ value was then obtained (0.50 g dw) indicating a lower biomass yield (on untreated soil an average of 0.15 g dw plant$^{-1}$ lower than in pots) probably even due to the bad weather occurred in the final phase of the experiment (large quantities of dead leaves were found in the lysimeters at the harvest day). Despite of those unfavourable test conditions, SBM provided biomass growth curves (lower diagrams in Figure 5) able to simulate the actual plant behaviour on different EDTA treated soils with acceptable results in most cases (Figure 6). Anyhow the model still needs to be validated on other experiments, with more frequent biomass observations at different growth stages. SBM systematically provides simulated data indicating a more pronounced biomass decrease for double application treatments with respect to single EDTA applications. This why

![Graphs of metal concentrations in plant tissues](image-url)
SBM was calibrated on pot data sets where this behaviour was always observed. For example, considering an EDTA dose \( X = 1 \) g kg\(^{-1} \) soil, SBM provides, at harvest, a 16% biomass decrease for the single application against 40% for the double application. In general it was more difficult to observe this trend on lysimeters owing to the high variability of biomass data at harvest, featured by no statistically significant differences among the various treatments here considered (Sacco, 2000).

### 3.4 Global phytoextraction performances

The combined use of the above three models could be useful for an a priori evaluation of the overall performance of any induced phytoextraction process. In practical terms, such a performance depends on the total quantity of the \( i \)-th metal that plants remove from the soil, i.e. the product of the metal concentration in the shoots and their dry biomass. We define the phytoextraction performance index \( (I_{p,i} \) g m\(^{-2} \)) as following:

\[
I_{p,i} = 10^{-2} \cdot d_p \cdot W \cdot M_{p,i}
\]

where \( d_p \) (m\(^2\)) is the number of plant per m\(^2\). A qualitative example of \( I_{p,i} \) curves (for single and double EDTA applications) is shown by Figure 7, where \( I_{p,H,i} \) indicates the performance index at harvest. Considering again an EDTA dose \( X = 1.00 \) g kg\(^{-1} \) soil and a \( d_p = 110 \) plant m\(^{-2} \) (measured in lysimeter trials), in double application \( I_{p,H,i} \) is increased by about 72%, 33% and only 2% for Cu, Pb and Zn, respectively, as compared with single application. In general terms, the chelate distribution must be assessed taking
into account the phytotoxicity effects that can strongly reduce biomass yields throughout: a) a lower growth rate response (say, lower $\mu$ and $W_f$), and b) a direct plant tissue lost (usually leaves with a higher metal concentration as shoots). The latter effect causes part of the metal taken up by the plants to return to the soil via leaf loss. A similar problem exists for roots. It was not possible to implement an uptake model based on the available root data, however analyses showed a higher metal concentration in roots than in shoots. The metal stored in roots is only temporarily separated from the soil, because it can normally be assumed that plants are harvested without roots. This is why one of the preferred proprieties of a hyperaccumulator should be good translocation efficiency. Running the models here proposed, for all the treatments considered the best $I_{pH,i}$ performances were observed at lower chelate doses (0.17 and 0.20 g kg$^{-1}$ soil for pots and lysimeters, respectively) in double applications. However, our results showed that only less than 1% of the bioavailable metals were removed by the plants. Contrariwise, the quantity of metal leached by rain was 74 times greater than that removed by plants in the case of Cu, 86 times for Pb and 36 times for Zn (Sacco, 2000). It is clear, therefore, that under the climate conditions in which these trials were performed, *B. napus* (L.) is not suitable for phytoremediation of heavy metal contaminated soils. Because the proposed models appear to satisfactorily reproduce the dynamics of the processes involved in phytoextraction, it would be interesting to verify them in future with additional experiments on other potentially suitable plants and surely even including the role of leaching. In any case, although additional data on plant behaviour and leaching is needed to determine the optimal chelant application rate, we can already state that the use of high doses of strong chelants such as EDTA (> 1 g kg$^{-1}$ soil) does not appear to be justified.
References

Angeloni M., Bini C. 1992. Trace elements concentrations in soils and plants of western Europe. In: Adriano D.C. (ed.): Biogeochemistry of trace metals, 19-60.

Bennet F.A., Tyler E.K., Brooks R.R., Gregg P.E.H., Steward R.B. 1998. Fertilisation of hyperaccumulators to enhance their potential for phytoremediation and phytomining. In: Brooks R.R. (ed.): Plants that hyperaccumulate heavy metals. CAB International, 249-259.

Blaylock M.J., Salt D.E., Dushenkov S., Zakharova O., Gussman C., Kapulnik Y., Ensley B.D., Raskin I. 1997. Enhanced Accumulation of Pb in Indian Mustard by Soil-Applied Chelating Agents. Environmental Science and Technology, 31:860-865.

Blum W.E.H., Spiegel H., Wenzel W.W. 1996. Bodenzustandsinventur. Konzeption, Durchführung und Bewertung. Wien.

Ebbs S.D., Lasat M.M., Brady D.J., Cornish J., Gordon R., Kochian L.V. 1997. Phytoextraction of cadmium and zinc from contaminated soil. Journal of Environmental Quality, 26:1424-1430.

Hong P.K.A., Li C., Banerji S.K., Regmi T. 1999. Extraction, recovery, and biostability of EDTA for remediation of heavy metal-contaminated soil. Journal of Soil Contamination, 8:81-103.

Jackson A.P., Alloway B.J. 1992. The transfer of cadmium from agricultural soils to the human food chain. In: Adriano D.C. (ed.): Biogeochemistry of trace metals, 109-158.

Li Z., Shuman L.M. 1996. Extractability of zinc, cadmium, and nickel in soils amended with EDTA. Soil Science, 161:226-232.

Lombi E., Zhao F.J., Dunham S.J., McGrath S.P. 2000. Cadmium accumulation in populations of Thlaspi caerulescens and Thlaspi Goesingense. New Phytophystologist, 145:11-20.

McGrath S.P. 1998. Phytoextraction for soil remediation. In: Brooks R.R. (ed.): Plants that hyperaccumulate heavy metals. CAB International, 261-287.

McGrath S.P., Dunham S.J., Carrell R.L. 2000. Potential for phytoextraction of zinc and cadmium from soil using hyperaccumulator plants. In: Terry N., Banuelos G., Vangronsveld J. (eds.): Phytoremediation, 111-130. Ann Arbor, Michigan.

Prüß A. 1994. Einstufung mobiler Spurenelemente in Böden. In: Rosenkranz D., Einele C., Harres M. (eds.): Ergänzbares Handbuch der Maßnahmen und Empfehlungen für Schutz, Pflege und Sanierung von Böden, Landschaft und Grundwasser, 15: I/94, Erich Schmidt Verlag, Berlin.

Prüß A., Turian G., Schweikele V. 1991. Ableitung kritischer Gehalte an NH4NO3-extrahierbaren ökotoxikologisch relevanten Spurenelementen in Böden SW-Deutschlands. Mittlg. Dtsch. Bodenk. Ges., 66:385-388.

Reeves R.D., Baker A.J.M., Brooks R.R. 1995. Abnormal accumulation of trace metals by plants. Environmental Management, September 1995:4-8.

Richards F.J. 1969. The quantitative analysis of growth. In: Steward F.C. (ed.): Plant Physiology, 3-76. Academic Press, New York.

Sacco P. 2000. La fitoestrazione assistita su suoli contaminati da metalli pesanti. Prove in vasi e lisimetri e valutazione di applicabilità come tecnica di bonifica. PhD thesis. Università degli Studi di Udine, 313.

StatSoft, Inc. (1995). STATISTICA for Windows [Computer program manual]. Tulsa, OK.

Vassil A.D., Kapulnik Y., Raskin I., Salt D.E. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol., 117:447-453.

Wenzel W.W., Puschenreiter M., Horak O. 2000 Role and manipulation of the rhizosphere in soil remediation/rev egetation. In: Proceedings of SoilRem 2000 (International Conference of Soil Remediation), October 15-19, 2000, Hangzhou, China, 166-168.

Wenzel W.W., Adriano D.C., Salt D., Smith R. 1999. Phytoremediation: a plant-microbe-based remediation system. Bioremediation of contaminated soils, Agronomy Monograph, 37:457-508.

Wenzel W.W., Unterbrunner R., Sommer P., Sacco P. 2002. Chelate-assisted phytoextraction using canola (Brassica napus L.) in outdoors pot and lysimeter experiments. Plant & Soil, 249:83-96.

Wheeler D.M., Power I.L. 1995. Comparison of plant uptake and plant toxicity of various ions in wheat. Plant and Soil, 172:167-173.

Zhao F.J., Lombi E., Breedon T., McGrath S.P. 2000. Zinc hyperaccumulation and cellular distribution in Arabidopsis halleri. Plant, Cell and Environment, 23:507-514.