Ital. J. Agron. / Riv. Agron., 2006, 2:271-280

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

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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

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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 (Reevs 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 v*ar. 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 NH_4NO_3 extract (Blum et al., 1996, Prüeß 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 Na_2EDTA 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 varoius 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 NH_4NO_3 extract (Blum et al., 1996, Prüeß 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 H_2O_2 30%, 6 ml HNO_3 65%, 1 ml $HClO_4$ 70-72% according to Blum et al., 1996). The biomass dry weights (dryied 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, NH_4NO_3 extract, and water extract. Average ± SE, n = 2.

Metal	Total (mg kg^{-1})	EDTA 0,05M (mg kg^{-1})	NH_4NO_3 1M (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*

Table 2. EDTA applications schema. Numbers following treatment codes (second column) refer to the data sets used for calibrating and validating the models.

Codes	Data sets	EDTA dose (g kg^{-1} fine soil) I treatment (t = 0)	II treatment (t = 14 days)	*Total added*
Pot trials				
Pot/3	*1*		0.2	*0.2*
Pot/4	*2*	0.1	0.1	*0.2*
Pot/5	*1*		0.4	*0.4*
Pot/6	*2*	0.2	0.2	*0.4*
Pot/7	*1*		0.8	*0.8*
Pot/8	*2*	0.4	0.4	*0.8*
Pot/9	*1*		1.6	*1.6*
Pot/10	*2*	0.8	0.8	*1.6*
Lysimeter trials				
Lys/3	*3*		0.2	*0.2*
Lys/4	*4*	0.1	0.1	*0.2*
Lys/5	*3*		0.5	*0.5*
Lys/6	*4*	0.2	0.2	*0.5*
Lys/7	*3*		1.0	*1.0*
Lys/8	*4*	0.5	0.5	*1.0*
Lys/9	*3*		2.0	*2.0*

periments (Wenzel et al., 2002) clearly indicate that metal bioavailability in the soil increases as the EDTA application rate is increased and appears to be influenced by the time elapsed since the last application. This mobilisation appears to depend on the total initial metal concentration in the soil, the characteristics of the soil and the amount of chelate added. A functional, analytical model that is able to describe the effect of EDTA on the dynamics of metal mobilization in soil was developed assuming an asymptotic (saturation-type) metal lability response. We define M_i (*mg kg^{-1} fine soil* < 2 mm) as the bioavailable concentration in soil of metal *i-th*. Changes in M_i with respect to time t (*d*) can then be described as follow:

$$(Eq.\ 1) \qquad \frac{\partial M_i}{\partial t} = \lambda_i \cdot (M_{X,i} - M_i)$$

where $M_{X,i}$ is an asymptotic metal concentration value depending – on its hand – on the total amount of applied EDTA (X, *g kg^{-1} soil*):

$$(Eq.\ 2) \qquad \frac{\partial M_{X,i}}{\partial X} = k_i \cdot (M_{\max,i} - M_{X,i})$$

The natural metal bioavailability in soil was found very close to zero. Thus the hypothesis $M_i(t = 0)$ equal to $M_{0,i} \approx 0$ was assumed for the model. $M_{max,i}$ is the maximum theoretical bioavailable concentration in soil achievable for the metal *i-th* whith an EDTA dose $X \rightarrow \infty$. Parameter k_i (*(mg kg^{-1})$^{-1}$*) represents the "intensity" of response of M_i to increases in EDTA application rate; λ_i (*d^{-1}*) gives a measure of the mobilisation kinetics. Our experimental data suggest that k_i is independent of t, whereas λ_i is related to X according to the function:

$$(Eq.\ 3) \qquad \lambda_i = a_i \cdot X^{b_i}$$

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

$$(Eq.\ 4) \qquad M_i = M_{\max,i} \cdot \left[\ 1 - \exp(k_i X)\right] \cdot \left\{\ 1 - \exp\left[-(a_i \cdot X^{b_i}) \cdot t\right]\right\}$$

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 M_i (*mg kg^{-1}*) over the exposure time (*d*) gives the cumulative exposure E_i *(mg kg^{-1} d)*:

$$(Eq.\ 5) \qquad E_i = \int_0^t M_i\, dt$$

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_{pi} *(mg kg^{-1}* dw*)* at time t can firstly be expressed as a function of E_i ($R^2 > 0.60$):

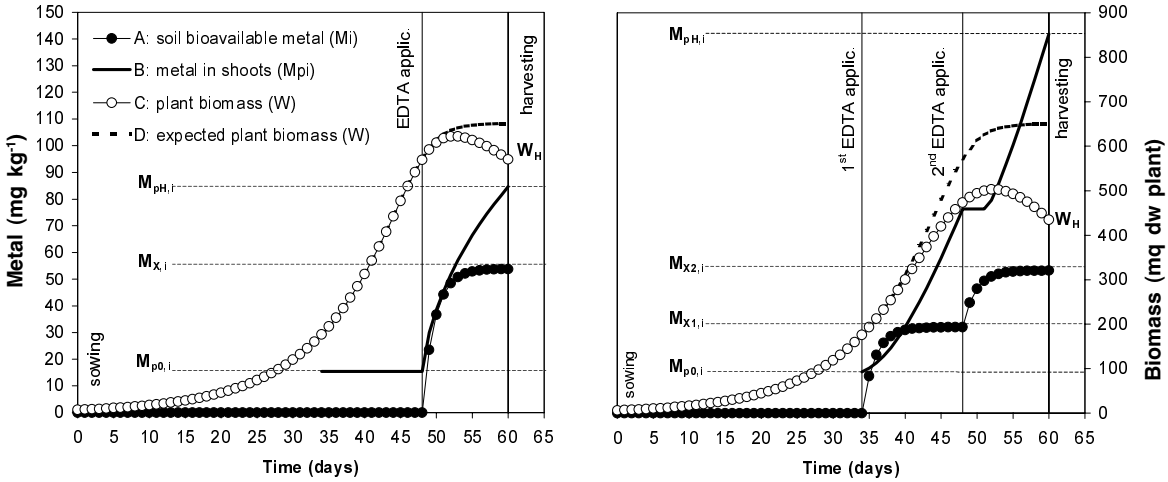

Figure 1. Simulation of induced phytoextraction processes with single (left) and double (right) applications of the same EDTA dose. A: bioavailable metal concentration in soil induced by EDTA applications (MLM); B: metal concentration in shoots (MUM); C and D: plant shoot biomass on ETDA treated and untreated soils, respectively (SBM). Sowing is at time = 0.

(*Eq. 6*) $M_{pi} = M_{p0i} + c \cdot E_i^{\,d}$, $(c > 0 \; and \; 0 < d < 1)$

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^{-1}) and d (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 ($R^2 > 0.70$):

(*Eq. 7*) $$M_{pi} = M_{p0i} + c \cdot E_i^{\,d} \cdot M_i^{\,e}\,, \quad (c > 0,\; 0 < d < 1,\; e < 0)$$

i.e., the Metal Uptake Model (MUM). The coefficients c (d^{-1}), 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:

(*Eq. 8*) $$\frac{dW}{dt} = \frac{\mu W (W_f^{\,n} - W^n)}{n W_f^{\,n}}$$

where W (g dw) represents the average dry weight of a plant at time t (d), Wf (g dw) is an asymptotic value of W for $t \rightarrow \infty$, μ (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-

tails in 21). Therefore, it was possible to analyse the variations in biomass in relation only to Zn concentrations, which had the lowest variability of all the metals considered. The effect of other metals, as well as of any EDTA dose was considered to be bundled into the parameters. Here we proposed a Shoot Biomass Model (SBM) that considers both the effect of: a) the daily amount of metal uptake (D = dMp/dt, mg kg-1 dw d-1); and b) the cumulated metal uptake since germination (M_p, *mg kg-1* dw). SBM can then be expressed as:

$$(Eq.\ 9)\ \frac{dW}{dt} = \frac{\mu' W (W_f^n - W^n)}{n W_f^n} - 10^{-3} \cdot \alpha\ M_p W (t - t_a)$$

with

$$\mu' = \mu \cdot \left(1 - \beta\ \cdot \frac{D}{D_{\max}}\right)$$

where t_a is the day of EDTA application, D_{max} *(mg kg-1* dw *d-1)* is the maximum daily variation of metal concentration in the biomass following the application, and α (d^{-2}) and β (*adimensional*) are experimental coefficients. The daily values of M_p are supplied by MUM. SBM assumes that the reduction in biomass caused by the EDTA treatments is proportional to the cumulative dose of metal in the biomass, to the biomass itself, and to the exposure time. In addition, a slowdown in growth, proportional to the daily metal uptake of the plants, is considered. Modifications in *W* shapes of plant growth on EDTA treated and untreated soils are respectively shown by curves C and D in Figure 1.

2.5 Statistics, numerical integration, model calibration and validation

The parameters of the models here proposed were estimated performing a conventional regression approach (Least Square Method via simplex convergence; StatSoft, 1995). For MLM, the maximum mobilisation potential $M_{max,i}$ was assumed to be equal to the concentration achieved with a 0.05 M EDTA extraction (i.e., X = 186 g kg^{-1}) that largely exceeds the chelate doses typically employed in induced phytoextraction. Soil data were divided into 4 data sets (Table 2) for the purposes of calibrating and subsequently validating the models. MLM was calibrated using sets 1 and 2, but excluding the final sampling data from the latter set. Validation was performed using the previously excluded 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 ther 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).

Table 3. Values of the parameters *k*, *a* and *b* of MLM, estimated through *Eq. 5* in pots and lysimeters.

MLM parameters	Cu	Pb	Zn
Pots trials			
k	1.0	0.4	0.9
a	0.5	0.3	0.3
b	0.8	1.2	0.0
R^2	0.9	0.9	0.9
Lysimeters trials			
k	3.0	0.9	1.7
a	0.4	0.4	0.3
b	1.3	1.3	0.4
R^2	0.7	0.8	0.9

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

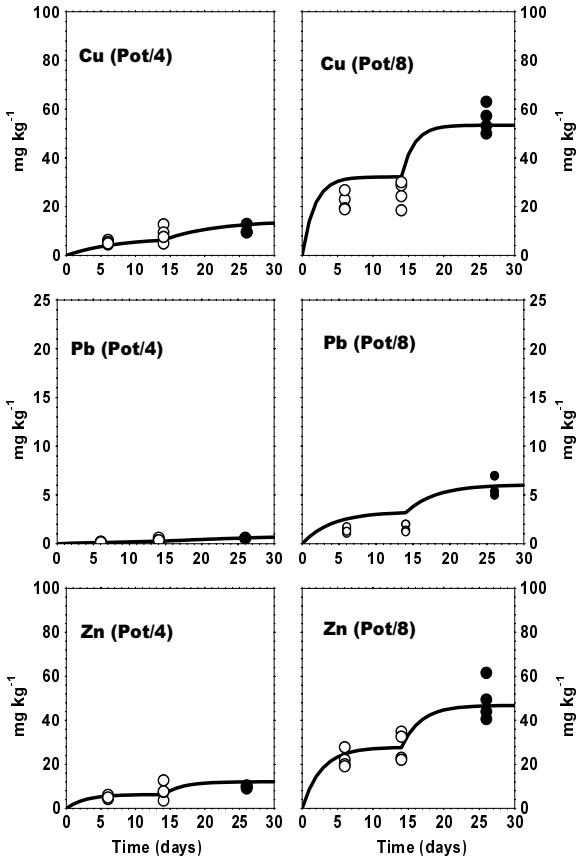

Figure 2. Bioavailable metals simulations for application of 0.17 g EDTA kg $^{-1}$ soil (left; Pot/4) and 0.83 g EDTA kg^{-1} soil (right; Pot/8), performed by MLM (ϒ: calibration set data; λ: validation set data). 1st EDTA application: t = 0; 2nd EDTA application t = 14 days).

(Figure 3). 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 $p_i = M_{X,i} / M_{max,i}$ (in %) is an estimation index of the induced metal mobilisation in the soil. Considering pot trials, an EDTA application of X = 0.825 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 g kg^{-1}) a p_i increase of 44% was achieved by Cu and Zn, against an increase of 74% for Pb (47% of $M_{max,Pb}$). As far as the time is concerned, the highest mobilisation rate was observed for Cu: with a single application X = 0.825 g kg^{-1} a $M_{0.825,Cu}$ = 54 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 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 experi-

Table 4. MUM model: values of coefficients *c*, *d* and *e* (*Eq. 7*) estimated for pot trials.

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

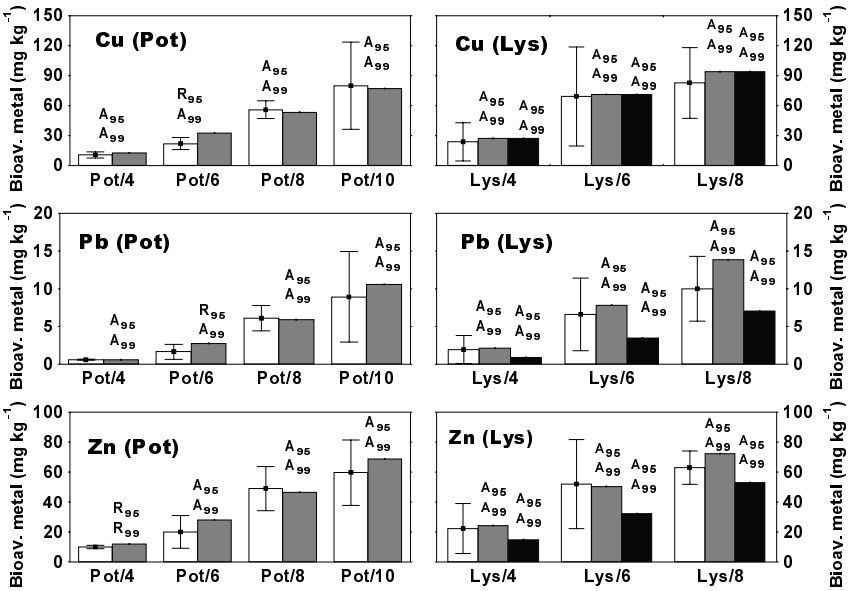

Figure 3. Validation confidence range (0.95) for pots (left) and lysimeters (right). The blank column represents experimental data (mean ± confidence 0.95 range). For pots the dashed column represents the estimated values. For lysimeters the last two columns show the values estimated through the MLM calibrated on lysimeters data (dashed) and pot data (black) respectively. Labels indicate if the validation test accept (A) or reject (R) the model estimation at 95% and 99%.

ments 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 ($R^2 = 0.98$): μ (d^{-1}) = 0.390, n = 3.770, W_0 (g dw) = 0.006, W_f (g dw) = 0.650. The additional parameters used by SBM to reproduce effects of EDTA treatments on plant biomass in pots – α and β – were found to be $1.49 \cdot 10^{-5}$ (d^{-2}) and $1.43 \cdot 10^{-2}$, 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

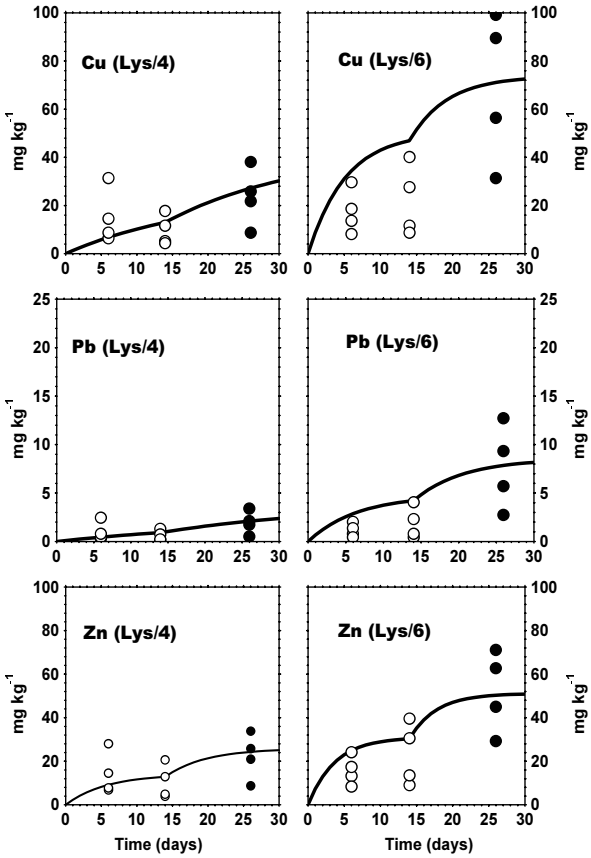

Figure 4. Bioavailable metals simulations for application of 0.2 g EDTA kg^{-1} soil (left; Lys/4) and 0.5 g EDTA kg^{-1} soil (right; Lys/6), performed by MLM calibrated on lysimeters (Y: calibration set data; λ: validation set data). 1st EDTA application: t = 0; 2nd EDTA application t = 14 days.

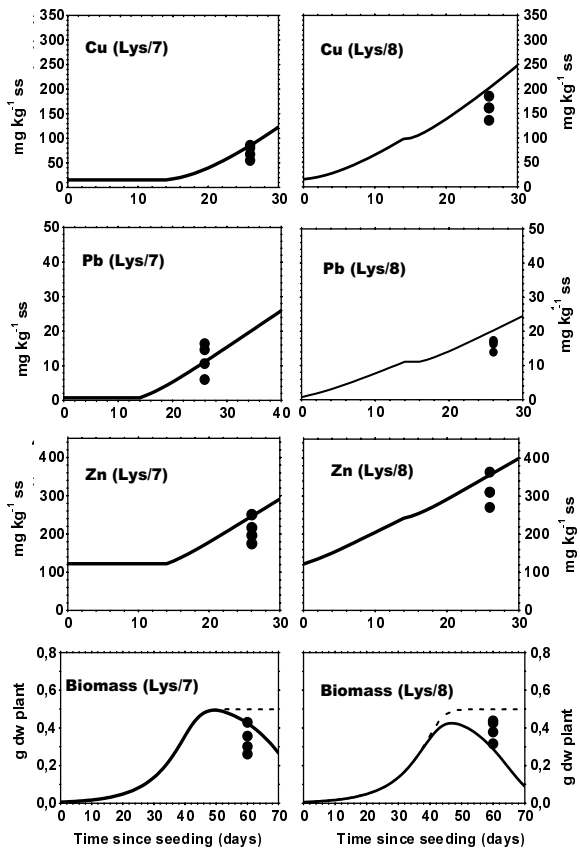

Figure 5. Metals uptake (by MUM) and biomass production (by SBM) simulations for single (left; Lys/7) and double (right; Lys/8) application of 1 g EDTA kg^{-1} soil in lysimeters (•: validation set data). 1st EDTA application: t = 0; 2nd EDTA application t = 14 days).

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:

$$(Eq.\ 10) \qquad I_{p,i} = 10^{-3} \cdot d_p \cdot W \cdot M_{p,i}$$

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 $I_{pH,i}$ 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 $I_{pH,i}$ 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

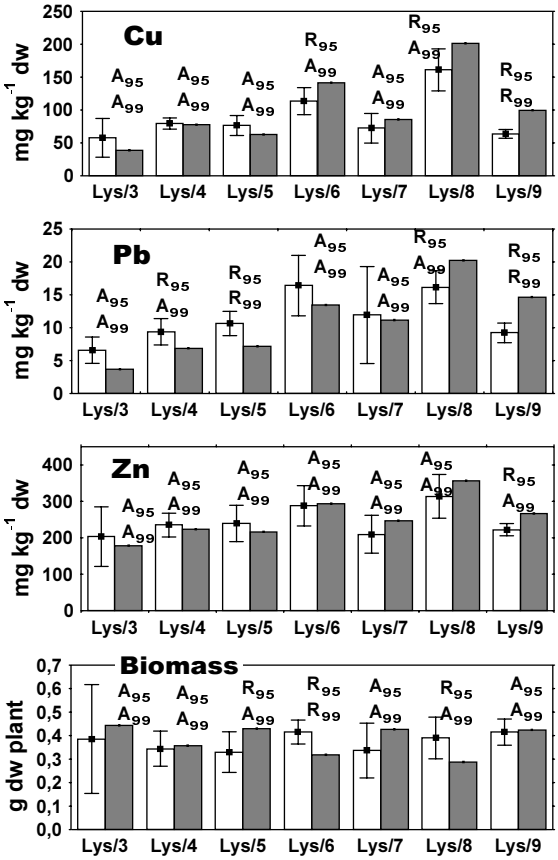

Figure 6. Validation confidence range (0.95) for lysimeters based upon a t-Student test (df = 3). The blank column represents experimental data (mean ± confidence 0.95 range). The dashed column represents the values estimated by MUM and SBM. Labels indicate if the validation test accept (A) or reject (R) the model estimation at 95% and 99%.

into account the phytotoxicity effects that can strongly reduce biomass yields throughout: a) a lower growth rate response (say, lower μ and W_f), and b) a direct plant tissue lost (usually leaves with an 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

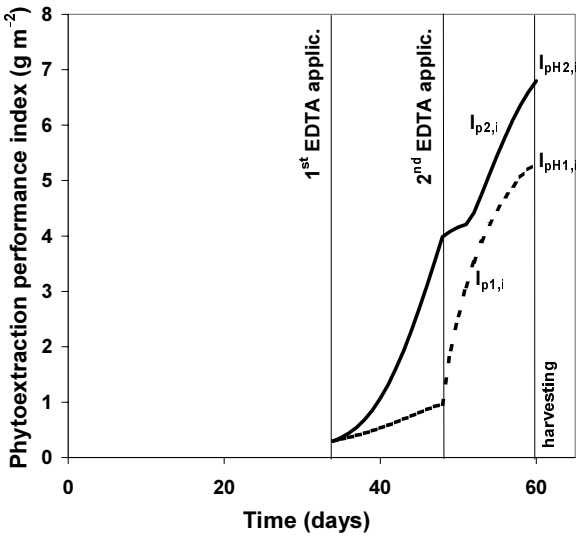

Figure 7. Simulation of the phytoextraction performance index ($I_{p,i}$), for the single (dotted line) and double (solid line) EDTA applications described in Figure 1. For the single application no EDTA was applied corresponding to 1st EDTA application, and the whole EDTA dose was applied corresponding to the 2nd EDTA application. For the double application the same dose was distributed into the two applications.

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.

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