# Modelling plant - metal uptake from contaminated soils

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## ABSTRACT

Heavy-metal contamination of land and waters used for production or potable supply is an ever-increasing concern. Transpiration by plants growing in contaminated sites reduces metal leaching and facilitates phytoaccumulation. In the case of phytoremediation, the plants can then be disposed of in an area where they pose no environmental risk. Results of pot trials and *in situ* analyses of plant-soil interactions were integrated to produce a generalised model that predicts plant metal-uptake. For some metals, plant uptake was found to be a function of transpiration, metal concentration in soil solution and a root absorption factor, $\phi$. The root absorption factor is a 'lumped parameter' describing the symplast/apoplast metal concentration quotient for a given plant species. The accumulation of B, Cd, Cu and Zn by poplars was determined using large lysimeters containing contaminated sawdust with three fertiliser treatments. Fertilisation had no effect on plant metal concentration. The root absorption factors calculated using this experiment were used to predict metal uptake by poplars in a lysimeter treated with sewage sludge, and a contaminated sawdust pile in the field. Leaf B concentrations could be accurately calculated using the model, while Cd, Cu and Zn concentrations were estimated at half their measured value. It was concluded that a consistent method of measuring the soluble fraction of metal in the soil solution is essential before an index of root absorption factors can be obtained for different plant species and metals.

## INTRODUCTION

All plants have the potential to remove metals from the soil of their root-zone. Some of these metals are essential for the plant functioning. Other metals may be taken up incidentally, and at high concentrations, may have a deleterious effect on plant growth.

Plant accumulation of heavy-metals can also facilitate their entry into the food-chain and directly or indirectly result in damage to ecosystems and human-health. Elevated heavy-metal concentrations in foodstuffs can also be used a non-tariff trade barrier.

Phytoremediation is technology that exploits plant-metal accumulation by using strategic planting and site management regimes to improve degraded environments. Studies by McGrath et al. (1993) and Baker et al. (1994) demonstrated that *Thlaspi caerulescens*, a plant that accumulates inordinate amounts of Zn, Cd and nickel, had potential use for the *in situ* remediation of Zn-contaminated soils. Since these initial reports, a whole suite of articles has appeared proposing the use of plants to remove heavy-metal contamination from soils. The technology, termed *phytoextraction*, involves the repeated cropping of plants on heavy-metal contaminated soils until the soils' metal concentrations have reached acceptable levels. After each cropping, the plant biomass can be removed from the area and may be burned to reduce its volume whereupon it can be stored in an appropriate area that does not pose a risk to the environment.

Nicks and Chambers (1994) reported a second potential use for hyperaccumulator plants: for economic gain in the mining industry. This operation, termed *phytomining* includes the generation of revenue by extracting saleable heavy-metals from otherwise sub-economic ore bodies.

Predicting plant metal uptake is therefore crucial in developing management strategies for sites with elevated heavy-metal loadings. In New Zealand, many agricultural lands contain elevated heavy-metal loadings due to proximity to a contaminated site or repeated applications of sewage-sludge, Cu-based fungicides and Cd-rich super phosphate.

The aim of this study was to develop a simple model that could be used to predict plant-metal accumulation, and we sought to test the model's ability to predict metal uptake by poplars in lysimeter and field studies. This study focused on plant uptake of boron, Cd, Cu and Zn from sawdust as well as from a sewage sludge amended soil. These metals were chosen because they are contaminants found in sawdust and sewage sludge, and could be measured easily in plant tissue, substrate and leachate.

## DERIVATION OF THE MODEL

### *Metal accumulation as a function of transpiration and bioavailable metal*

The accumulation of metals by plants relies on plants working as solar-driven 'biopumps'. The soil solution is drawn from the root zone through the plants' roots and stems to the leaves, where it is lost via transpiration. Any metal taken up in the soil solution that enters the roots will either accumulate in the subterranean realm, or more likely the aerial portions of the plant. Not surprisingly, the highest concentrations are often found in the leaves (Brooks *et al.*, 1998), as they are the major water sink. It is well documented that plant metal uptake is related to plant water uptake (Salt *et al.*, 1995; Hinchman *et al.*, 1996). For the purpose of modelling, we consider the amount of metal *M* (mg) removed by the plant is therefore proportional to the transpiration rate *T* (L $d^{-1}$) over a given period of time *t* (days).

$$M \propto \int_0^t T(t`) \quad dt \qquad [1]$$

Any water that is taken up by the plants' roots must first pass though the surrounding soil. During this time, only some fraction of the metal that is present in the bulk soil will be in solution. The remainder is likely to be bound to mineral particles and the organic matter in the soil. So bound it is therefore unavailable for uptake. Hence the total amount of metal that accumulates in the plant is related to the metal concentration that is in the soil solution (Robinson *et al.*, 1998; Robinson *et al.*, 2000), rather than the total metal concentration in the bulk soil. Thus we consider M to be proportional to the metal concentration in soil solution *C* (mg $L^{-1}$).

$$M \propto [C] \qquad [2]$$

### Root Absorption Factor

The total amount of metal that accumulates in the plant does not usually equal the accumulated product of the soil-solution concentration times the volume of water transpired by the plant. For a metal to be translocated to the aerial parts of a plant, it has to enter the root, either via the symplastic or apoplastic (Marschner, 1995) pathways where some active or passive filtering may occur.

Here we define the root absorption factor ($\phi$) as a dimensionless lumped parameter that represents the root xylem / soil solution metal concentration quotient,

$$\phi = \frac{[C]_r}{[C]} . \qquad [3]$$

where $[C]_r$ is the soluble metal concentration (mg $L^{-1}$) in the root xylem and $[C]$ is the soluble metal concentration (mg $L^{-1}$) in the soil solution.

The parameter $\phi$ is a simple lumped parameter encompassing the plethora of complex, and often poorly understood, biogeochemical factors that influence the passage of metals from the soil into the roots. Rhizobiological activity, root exudates, temperature, moisture, pH and the concentration of competing ions will affect $\phi$. The issue is further complicated by the fact that $\phi$ could change depending on the metal concentration in the soil solution. This would be particularly pronounced for essential elements such as Fe, Cu, Zn and Mn.

### Root Absorption Factor and plant responses to heavy metals

Baker (1981) divided plants species into three groups according to their responses to the heavy metal concentration in soil solution. For non-essential elements such as Cd, nickel and arsenic, plants having a very low $\phi$ are termed as being “excluders”. Most plants that occur naturally on metalliferous soils are recognised as being excluders.

Plants that have a relatively constant $\phi$ over a wide range of metal concentrations in soil solution are known as “indicators**”**. In this case, the concentration in the plant has a near linear relationship to the metal concentration in soil solution. Plants that do not occur naturally on metalliferous soils usually behave as ‘indicators’ when grown in the presence of the non-essential elements.

The third category of plants are those who tolerate very high concentrations of metal in their aerial parts, or have an active uptake mechanism for the metal. These plants would have a high $\phi$ and are known as “hyperaccumulators”.

For both excluders and hyperaccumulators, $\phi$ is constant over just a narrow concentration range. There can be a sudden increase in plant metal concentration at high soil-solution concentrations. At this point, the metal-uptake control mechanisms break down, and metal ‘floods’ into the plant in the transpiration stream. The actual phenomenon may be a saturation of the active uptake mechanism, or a break down of the plasma membrane at the apoplast/symplast interface. When this phenomenon occurs, the plants show toxicity symptoms and biomass production is reduced.

This change in $\phi$ with concentration can be modelled by adding a decay constant $K$

$$\phi(C) = \frac{\phi_1 C_1}{C_1 + K(C - C_1)} \qquad [4]$$

where $\phi(C)$ equals root adsorption factor at soil solution concentration $C$ (mg $L^{-1}$), $\phi_1$ equals the measured root adsorption factor at concentration $C_1$ (mg $L^{-1}$), and $K$ ($0 \leq K < 1$) is the decay constant. In the absence of information, here we assume $\phi$ a constant.

***Estimating the Root Absorption Factor***

The plant specific $\phi$ can be approximated using the plant's total water use, above-ground dry biomass, and the metal concentration in soil solution. We assume the following relationship holds

$$\phi = \frac{MB}{\sum T \quad C}, \qquad [5]$$

where $\phi$ equals the root absorption factor for the metal (dimensionless) $M$ equals the metal concentration in the above-ground dry biomass (mg $kg^{-1}$), $B$ equals above-ground dry biomass (kg), $\Sigma T$ equals the total water use (L) and $C$ equals the concentration of metal in soil solution (mg $L^{-1}$),

***Predicting plant metal uptake***

The local concentration of metal in soil solution and hence the potential amount of metal entering the plant roots, will be depth-dependent. The plant-root density also varies with depth. Most of the plant roots are near the surface and root density decreases with increasing depth. We assume potential metal uptake depends on root density. The amount of metal (mg) taken up by the plant can therefore be predicted by:

$$M\,(t) \quad = \quad \int_0^{z_R} \int_0^{t} \quad R(t`, z)\, T(t`)\, C(t`z)\, \phi\big(C\,(t`z)\big) \;\; dt \quad dz \qquad [6]$$

The average metal concentration in the plant is therefore:

$$[M] = M / B\,, \qquad [7]$$

where $M$ = the amount of metal taken up by the plant at time $t$, $[M]$ is the average plant metal concentration (mg $kg^{-1}$), $B$ equals above-ground dry biomass (kg), $z_R$ = the depth of the root-zone (m), $z$ = depth (m), $t$ = time (days), $R(t`,z)$ = root density fraction [(root mass at depth $z$) / (total root mass) at time $t$], $T$ = water use (L $day^{-1}$ at time $t$), $C_z$ = concentration of metal in soil solution (mg $L^{-1}$ at depth $z$ and time $t$), $\phi$= root absorption factor for the metal at depth $z$ and time $t$. Plant-metal uptake can be enhanced or retarded by changing the concentration of metal in the soil solution ($C$). This may be done by using soil amendments such as chelates and lime.

Alternatively, $\phi$, or the transpiration rate could be changed by selective breeding, or by gene manipulation.

## MATERIALS AND METHODS

### ***Lysimeter experiment with poplars in sawdust and sewage sludge amended soil***

Lysimeter experiments were performed in a shade-house at HortResearch, Palmerston North, New Zealand. Two sets of experiments were carried out, one with sawdust and the other with sewage-sludge amended soil. Sawdust was chosen because there are sites of contaminated sawdust world-wide, and from a practical perspective, sawdust provided a homogeneous plant-growth medium that would allow root density to be disregarded and the metal concentration in soil solution to be taken as constant over the time of the experiment. The sewage-sludge amended soil was used as a more realistic example to test the accuracy of the model using parameters gained from the sawdust experiment.

Seven thousand litres of sawdust was obtained from a sawdust pile at Kopu and homogenised using a digger. The bulk density of the sawdust was 0.23 and the water holding capacity was 420 mL kg$^{-1}$. In October 2000, eight 800 L weighing lysimeters were filled with the sawdust, and a 1 M long poplar pole (*Populus deltoides x P. yunnanensis*) planted in each. The lysimeters were fertilised with Hoagland's solution at three rates: low (2 lysimeters), medium (3 lysimeters) and high (3 lysimeters). These plant-nutrient treatments were used to determine effect of substrate fertility on the model's ability to predict metal uptake.

Table 1. Metal concentrations for sawdust (mg kg$^{-1}$ dry wt) and drainage (mg L$^{-1}$) value in brackets are the standard error of the mean.

| Element | Total | Drainage |
|---|---|---|
| B | 19.4 | 2.47 (0.09) |
| Cd | 8.1 | 0.21 (0.01) |
| Cu | 58.5 | 0.43 (0.07) |
| Zn | 13.9 | 0.61 (0.17) |

In a separate experiment, an 800 L lysimeter was partially filled with sieved soil (Manawatu fine sandy loam) and 9cm of dried sewage sludge from the Levin sewage treatment plant was placed on top. The lysimeter was irrigated and allowed to drain for one month before a poplar seedling was planted in it. This experiment was performed under different conditions to the sawdust experiment, so the biomass production and water use are not directly comparable. For the purposes of this study, however, this lysimeter experiment was used to compare predicted plant metal uptake against measured uptake.

Table 2. Metal concentrations for sludge & soil (mg kg$^{-1}$ dry wt) and drainage (mg L$^{-1}$) value in brackets are the standard error of the mean.

| Element | Sludge total | Soil total | Drainage |
|---|---|---|---|
| B | 19.2 | 24.2 | 0.40 (0.14) |
| Cd | 6.7 | 0.6 | 0.31 (0.01) |
| Cu | 645.5 | 50.7 | 0.47 (0.06) |
| Zn | 974.4 | 79.3 | 0.34 (0.02) |

Lysimeters were given a measured amount of water daily to maintain their volumetric water content between 50 and 60% for the sawdust experiment, and between 40 and 50% for the sewage-sludge experiment. Drainage was collected weekly, weighed, and stored for analysis. An estimation of plant water use was made by subtracting the drainage from the daily weight change. It was assumed that evaporation from the surface of the lysimeters was negligible. Every fortnight 10% of the leaves were removed. The total leaf area and biomass of the tree was estimated by determining the weight and area of the excised leaves. The leaves were then dried, ground and stored for analysis. In early April, 2001, the above-ground portions of the trees were removed and the dry biomass determined.

### *Field trial with poplars*

On the 26th July, 2000, five hectares of a contaminated sawdust pile near Kopu, New Zealand were planted in poplars, willows and *Eucalyptus*. The site was fertilised according to plant requirements. In April, 2001, nine poplars were removed along with sawdust from the root-zone. Leaves and stems were separated and the samples dried and weighed then stored for analysis.

Table 3. Metal concentrations in sawdust (mg kg$^{-1}$ dry wt) from sawdust pile near Kopu. Values in brackets are the standard error of the mean.

| Element | Total |
|---|---|
| B | 4.1 (1.1) |
| Cd | 6.2 (0.6) |
| Cu | 123 (13.4) |
| Zn | 28 (4.1) |

### *Sample preparation and elemental determination*

Approximately 0.2g portions of ground sawdust, soil, leaf and stem samples were accurately weighed into 50 mL Erlenmeyer flasks. Concentrated nitric acid (10 mL) was added to each tube and the mixtures heated on a heating block until a final volume of ca. 3 mL was reached. The samples were then diluted to 10 mL using deionised water and stored in polythene containers. Elemental determinations were made using Inductively Coupled Plasma Emission Spectroscopy (ICPES).

### *Modelling plant metal uptake*

The changing plant metal concentration was predicted using equations [6] and [7]. It was assumed that there was no variation in concentration with depth. The average metal concentrations in the drainage were used as an approximation of metal concentration in soil solution. The low standard errors (Tables 1 & 2) indicate that the concentration of metals in soil solution was reasonably constant for the duration of the experiment. The soluble metal concentration from the contaminated sawdust field trial was estimated by multiplying the metal concentrations in the drainage from the sawdust lysimeter experiment by the quotient of the respective total concentrations. This calculation assumes that the isotherm for metal solubility in sawdust is constant over the measured range.

Equation [5] was used to estimate the root absorption factors ($\phi$) from the sawdust lysimeter experiment. These $\phi$ values were used to calculate metal uptake for both the sludge-treated lysimeter and the field trial.

## RESULTS AND DISCUSSION

### *Lysimeter experiment with sawdust*

Fig. 1(A) shows the cumulative biomass production of the poplar leaves for the three N treatments. The ‘low’ treatment showed signs of N deficiency with small chlorotic leaves, while plants from the ‘medium’ and ‘high’ treatments appeared normal. The large difference in biomass production between the ‘medium’ and ‘high’ N treatments implies that N levels were sub-optimal in this high C:N ratio medium, even in the ‘high’ treatment. The water use of the trees in the three treatments (Fig 1(B)) was proportional to biomass production. The water use efficiencies at the end of the experiment were 1.4, 1.8 and 2.0 kg m$^{-3}$ for the low, medium and high treatments respectively.

At the end of the experiment, an analyses of variance revealed that there were no significant differences ($p$>0.05) between N treatments for the metal concentrations in the poplar leaves (Table 4). These results indicate that the plant-nutrient concentrations of the substrate had little effect on the metal concentration in poplar leaves and consequently will not affect the uptake calculations. Therefore only the three trees in the 'high' N treatment were used in the modelling exercise.

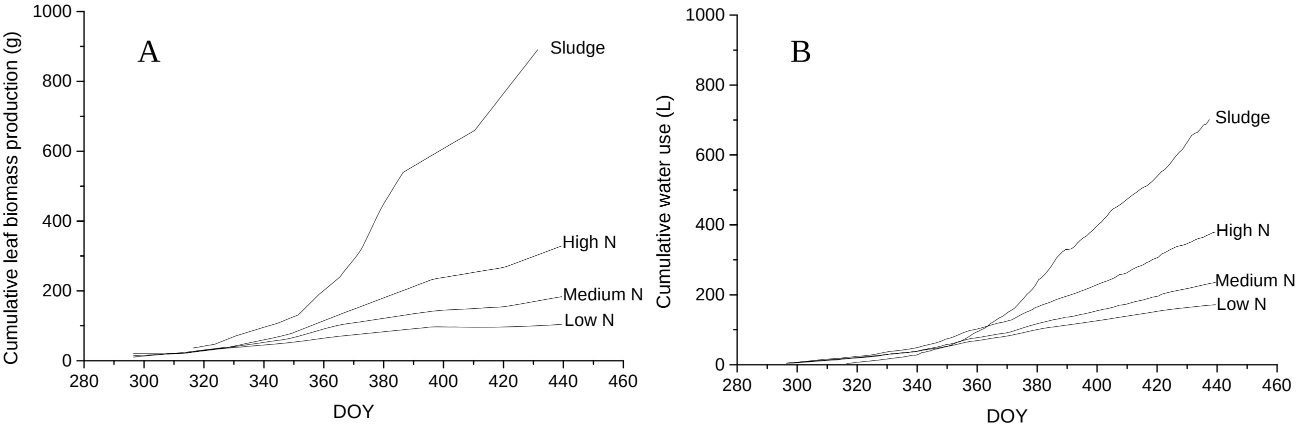

Figure 1 (A and B). Cumulative leaf biomass and total water use of the poplars. DOY = day of year. It should be noted that the sludge treatment is not directly comparable to the three N treatments as it was conducted under different conditions

Fig. 2(A-D) shows the measured and calculated leaf-metal concentrations for the sawdust lysimeter experiment. Average leaf metal concentrations increase over time as the total amount of water transpired per unit leaf area increases. This increase is consistent with seasonal reallocation of photosynthetic products from leaf production early in the season to stem and root development as the season progresses.

Table 4. Measured and calculated metal concentrations (mg kg$^{-1}$ dry weight) from the lysimeter experiments and field-trial. Values in brackets are the standard error of the mean. Root absorption factors ($\phi$) were calculated from Eqn. [5] using data from the sawdust lysimeter experiment.

| | B | Cd | Cu | Zn |
|---|---|---|---|---|
| High N | 437 (49) | 2.3 (0.2) | 11.4 (3.0) | 473 (50) |
| Medium N | 459 (15) | 2.4 (0.3) | 2.1 (1.1) | 497 (111) |
| Low N | 396 (65) | 2.6 (0.2) | 1.5 (0.2) | 403 (61) |
| | | | | |
| Measured sludge | 45 | 4.5 | 15.9 | 212 |
| Calculated sludge | 43 | 2.1 | 6.9 | 89 |
| | | | | |
| Measured field conc. | 94 (45) | 0.4 (0.0) | 9.1 (0.7) | 405 (56) |
| Calculated field conc. | 92 | 1.8 | 24.0 | 952 |
| | | | | |
| Calculated $\phi$ | 0.13 | 0.009 | 0.02 | 0.36 |

Using the average $\phi$ calculated over the duration of this experiment (Table 4), the predicted leaf metal concentrations were reasonably close to the measured values all the elements. (Fig. 2(A-D)). The irregular behaviour of copper compared to the other three metals may be due to plant regulation of Cu deposition in leaves, or redistribution of Cu by phloem transport.

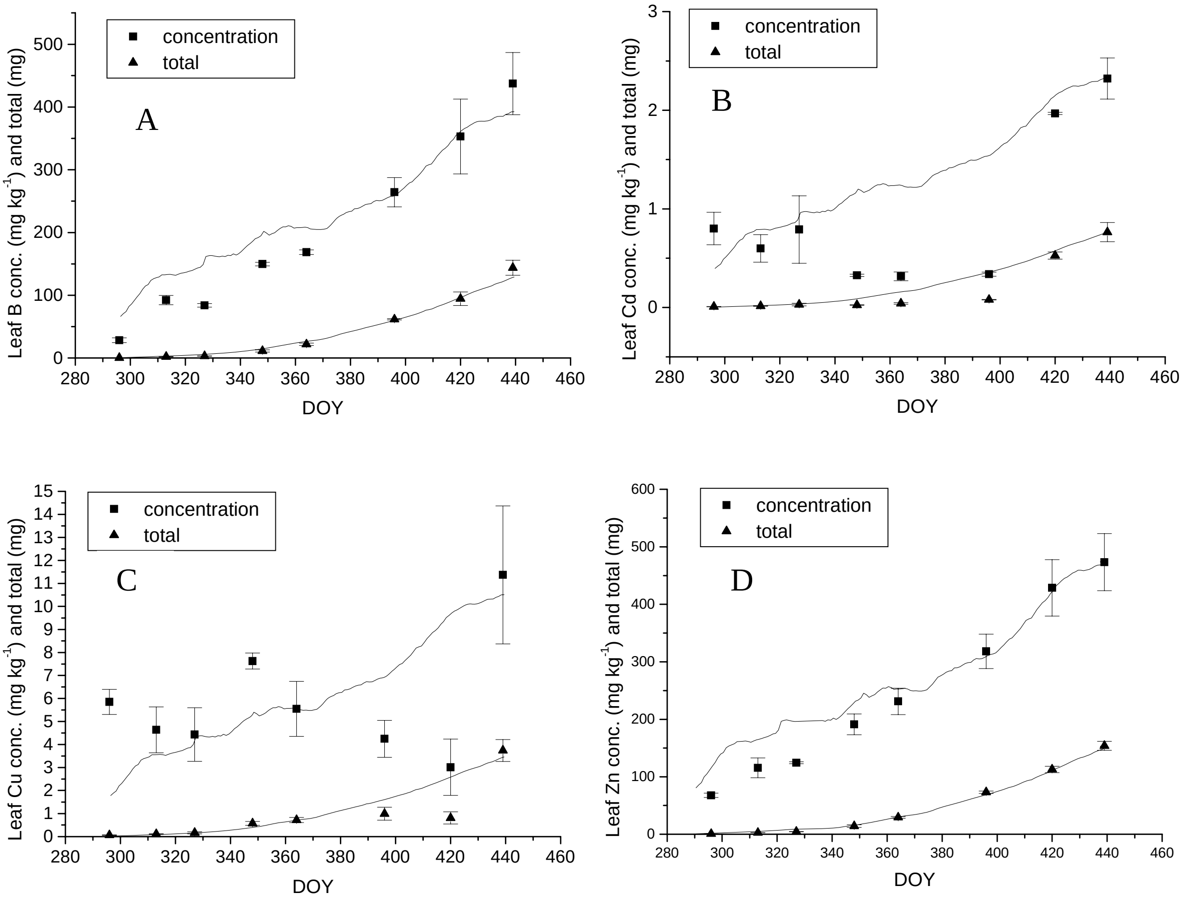

Figure 2 (A – D). Measured (points) and calculated (lines) plant metal uptake by poplars growing in lysimeters filled with sawdust. DOY = day of year. Note the changed scale on the ordinates.

### *Lysimeter experiment with sewage sludge*

Measured and calculated leaf - metal concentrations from the sludge lysimeter experiment are shown in Fig 3(A-D). As with the sawdust lysimeter experiment, leaf metal concentrations increased over time. Using $\phi$ values derived from the sawdust lysimeter experiment, the calculated leaf-metal concentrations were accurate for B but only half of the measured value for Cd, Cu and Zn. Our calculations could not take into account the depth-wise variation of soluble metal in this experiment. With the exception of B, the concentrations of the other metals were much higher in the sewage sludge than the underlying soil (Table 2). Therefore, the soluble metal in the sludge at the top of the lysimeter may have been re-adsorbed as it moved down the soil profile. This would result in an underestimation of soluble metal from the drainage and a consequent underestimation of calculated plant-metal concentration using Eqns. [6] and [7]. Nevertheless, a 50% error is well within the variation in metal concentration for poplars growing on a metalliferous soil (Robinson *et al.,* 2000).

### *Field trial with poplars*

The leaf metal concentrations of the poplars from the field trial are shown in Table 4. Calculated B concentrations were accurate, however, Cd and Cu concentrations were underestimated and Zn concentrations were overestimated.

Leaf-to-stem concentration quotients ratios for all the poplars at the end of the experiment averaged 4.2, 1.9, 0.7 and 2.1 for B, Cd, Cu and Zn respectively. Using these ratios, and a leaf-stem biomass quotient for poplars in the first year of 1 (Robinson *et al.*, 2000), the total metal uptake of the plants can be calculated.

The sludge lysimeter experiment and the field trial demonstrate the difficulty of relating the easily measurable total-metal concentration of the substrate to the soluble or 'bioavailable' fraction. There are a plethora of techniques for measuring metal bioavailability using chemical extractions (Ernst, 1996). Each method removes a different fraction of the total metal. Therefore $\phi$ values obtained using one method should only be applied in future studies that same method.

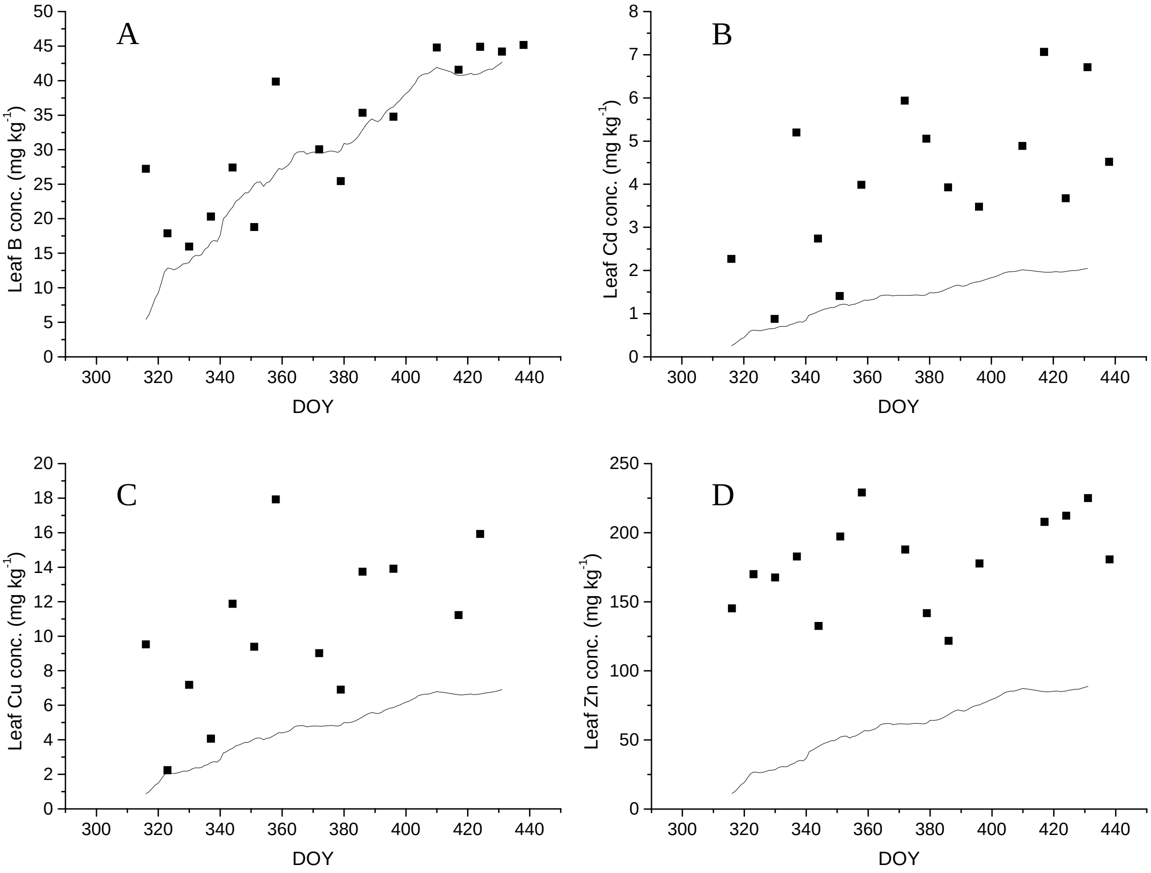

Figure 3 (A – D). Measured (points) and calculated (lines) plant metal uptake by a poplar grown in a lysimeter filled with sewage sludge amended soil. DOY = day of year.

## *Conclusions*

The model [6] is analytically simple, and is a rationally convenient description of the whole plant-metal uptake processes. Root absorption factors for metal-uptake by a given species could well be different when the plant is grown in different soils. This is because at a single solution concentration, the ease with which a metal enters the plant's symplast will be affected by the cocktail of other ions that are present in soil solution, as well as other factors such as soil pH, moisture and temperature. The model can be modified, on the basis of more information, to include a more mechanistic approach of root uptake. Such complexity could increase utility, but require additional parameters.

Upon entering the root, the model assumes that all the metal will be translocated to the above-ground portions of the plant. Analysis of the root material invariably shows that this assumption is not correct due to the presence of the metal in the root tissue. Stephan and Scholz (1993) demonstrated that metals in the aerial plant parts could be further translocated within in the phloem. Any relocation of metals back to the below-ground portions via the phloem will result in an over estimation of the amount of metal that is extracted.

The depthwise distribution of fine roots does not always equate with total root uptake, even for water. Roots tend to be more active when the surrounding soil is moist (Clothier and Green, 1997). The model for simplicity and utility considers a uniform moisture distribution in the soil.

Future work will focus on validating the model for other species using a variety of methods for estimating metal solubility in the substrate. Hopefully, an index of $\phi$ values for a suite of species can be obtained to permit predictions of phytoextraction capacity and allow assessments of the efficacy of phytoremediation and land treatment.

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## REFERENCES

Baker A J M 1981 Accumulators and excluders – strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654.

Baker A J M, McGrath S P, Sidoli C M D and Reeves R D 1994 The possibility of in situ metal decontamination of polluted soils using crops of metal-accumulating plants – a feasibility study. Res. Conser. Recyc. 11, 41-49

Clothier B E and Green S R 1997 Roots: the big movers of water and chemical in soil. Soil Sci. 162(8), 534–543.

Ernst W H O 1996 Bioavailability of heavy metals and decontamination of soils by plants. Appl. Geochem. 11: 163 – 167.

Hinchman R R, Negri C M and Gatliff E G 1996 Phytoremediation: using green plants to clean up contaminated soil, groundwater and wastewater. Proc. International Topical Meeting on Nuclear and Hazardous Waste Management, Spectrum 96. Seattle WA.

Marschner H 1995 Mineral nutrition of higher plants. Academic press. London, UK. 65 p.

McGrath S P, Sidoli C M D, Baker A J M and Reeves R D 1993 The potential for the use of metal-accumulating plants for the *in situ* decontamination of metal-polluted soils. *In* Integrated Soil and Sediment Research: A Basis for Proper Protection. Eds. H.J.P Eijsackers and T.Hamers. pp.673-676. Kluwer Academic Publishers, Dordrecht.

Nicks L and Chambers M F 1994 Nickel farm. Discover. September. 19p.

Robinson B H, Leblanc M, Petit D, Brooks R R, Kirkman J H, Gregg P E H 1998 The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant and Soil. 203, 47-56.

Robinson B H, Mills T M, Petit D, Fung L E, Green S R and Clothier B E 2000 Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. Plant Soil. 227, 301-306.

Stephan U W and Scholz G 1993 Nicotianamine: Mediator of Transport of Iron and Heavy Metals in the Phloem? Physiol. Plant. 88, 522–529.

Salt D E, Prince R C Pickering I J, & Raskin I 1995 Mechanisms for cadmium mobility and accumulation in Indian mustard. Plant Physiol. 109, 1427-1433.