Assessing Plant Phytoextraction Potential Through Mathematical Modeling

Cristina Gonnelli,[1]* Stefano Marsili-Libelli,[2] Alan Baker,[3] and Roberto Gabbrielli[1]

[1]Department of Plant Biology, University of Florence, Via Micheli 1, 50121, Florence, Italy; [2]Department of Systems and Computers, University of Florence, Via S. Marta 3, 50139, Florence, Italy; [3]School of Botany, The University of Melbourne, Parkville, IVC 3010, Australia

* **Author for correspondence:** Cristina Gonnelli, Department of Plant Biology, University of Florence, Via Micheli 1, 50121, Florence, Italy. Phone: 0039-055-2757392, Fax: 0039-055-282358, e-mail: fisveg@unifi.it.

ABSTRACT

One of the most serious and long-term consequences of environmental pollution is heavy metal contamination of soils. Elements such as zinc, cadmium, lead, nickel, and chromium are being released into the environment by many industrial processes and have now reached concentrations that are of concern. Phytoremediation is a new, low-cost, and environmentally friendly technique that relies on the natural properties of some plants to clean-up the ground through their ability to take up metals from the soil. Hyperaccumulator plants, capable of accumulating metals far in excess of any normal physiological requirement, represent a most promising tool for metal phytoextraction, but the *in field* establishment of their conditions for utilization needs a long period because of the plant life-cycle. The use of a mathematical model is proposed to process growth and uptake data from *in vitro* experiments for a rapid assessment of the time and concentration parameters for the deployment of hyperaccumulator plants for phytoextraction purposes. This preliminary research has been carried out using *Alyssum bertolonii* Desv., a nickel hyperaccumulator endemic to Italian serpentine soils.

KEY WORDS: phytoremediation, hyperaccumulation, nickel, *Alyssum bertolonii*.

I. INTRODUCTION

Most plants that are able to survive on metal-rich soils are known to exclude toxic elements from their shoots (Baker and Walker, 1990). However, a particular and interesting strategy of adaptation to metalliferous soils has been developed by heavy-metal hyperaccumulating plants. These plants are capable of accumulating metals far in excess of any normal physiological requirement; they can extract metals such as zinc, cadmium, manganese, nickel, cobalt, and copper from the soil and accumulate them in their shoots, frequently to percentage concentrations (Baker and Brooks, 1989a; Baker *et al.*, 1999). The ecological role of metal hyperaccumulation is still not entirely clear, but defense against herbivores and attack by fungal and bacterial pathogens could be possible explanations for this behavior (Pollard and Baker, 1997; Boyd, 1998).

Until a few years ago, hyperaccumulators were considered merely to be ecological and evolutionary curiosities. Only recently has their potential use for extracting toxic elements from soils been recognized as a subject for serious scientific investigation (Baker *et al.*, 1994). The fundamental problem of soil pollution has made hyperaccumulator plants prime candidates for phytoremediation work. Their ability to extract heavy metals from the soil can be exploited to reduce the metal content in polluted areas (Salt *et al.*, 1998).

Taxa so far discovered that have the ability to hyperaccumulate nickel are quite numerous (>320) and among them the genus *Alyssum* (Brassicaceae) contains 48 species in which the metal is present in exceptionally high concentrations in the shoots (>1000 mg k^{-1} in dried material) (Reeves, 1992; Brooks, 1998). This characteristic makes some members of the genus promising candidates for phytoextraction studies.

Direct *in field* trials suffer from serious limitations in the time needed to assess the proper conditions for plant utilisation. Therefore, it is necessary to find a fast and reliable alternative way to evaluate the time limits beyond which there is no further use in growing the plants on the contaminated soils. This limit needs to be assessed in relation to the time in which the maximum metal concentration in the plant is reached before plant development is impaired.

The purpose of this article is to propose an alternative method through *in vitro* experiments combined with the application of a specific mathematical model to evaluate growth and nickel uptake patterns by means of numerical simulation. In this way, a culture strategy can be devised to design a planting policy taking into account the physiological limits of plant use. This could, in principle, be applied to every kind of plant with phytoextraction capabilities.

The plant species studied is the nickel hyperaccumulator *Alyssum bertolonii* Desv. (Minguzzi and Vergnano, 1948), a species endemic to some Italian serpentine outcrops.

II. MATERIALS AND METHODS

A. Plant Material

Seeds of *A. bertolonii* were collected from plants living on an ultramafic outcrop near Pieve S. Stefano (Tuscany). They were germinated for 3 days in the dark on

floating trays held in 1 dm^3 vessels containing 400 mL of continuously aerated hydroponic solution with the following composition: KNO_3 0.06 mmol L^{-1}, $Ca(NO_3)_2 4H_2O$ 0.03 mmol L^{-1}, $NH_4H_2PO_4$ 0.01 mmol L^{-1}, $MgSO_4 7H_2O$ 0.02 mmol L^{-1}, $FeSO_4 7H_2O$ 0.18 μmol L^{-1}, tartaric acid 0.9 μmol L^{-1}, H_3BO_3 4.6 μmol L^{-1}, $MnCl_2 4H_2O$ 0.92 μmol L^{-1}, $CuSO_4 5H_2O$ 0.03 μmol L^{-1}, $ZnSO_4 7H_2O$ 0.077 μmol L^{-1}, and H_2MoO_4 0.06 μmol L^{-1}. The culture conditions were a 16-h (day) photoperiod, provided by fluorescent tubes (75 μE m^{-2}s^{-1}), at 23°±1°C and a relative humidity of 60 to 65%.

Data Collection During Growth at Different Nickel Concentrations

After germination, rafts with seedlings were placed in fresh hydroponic solutions amended with various $NiSO_4$ concentrations. From Day 6 of culture, the sampling was carried out every 2 days for 1 month.

After harvesting, plant roots were rinsed with distilled water and then carefully submerged in $CaCl_2$ 10 mM at 4°C for 10 min to remove the nickel adsorbed to the root cell wall. Root and shoot lengths of 20 plants were measured; the plantlets were then divided into shoots and roots and dried at 70°C for 1 d and subsequently weighed and decomposed by wet ashing with HNO_3 and $HClO_4$ (5:2 v/v). The concentrations of Ni in the digests were determined by atomic absorption spectrometry.

Total root length was chosen as a measure of nickel tolerance, because this is a growth parameter particularly sensitive to the presence of metal toxins (Baker and Walker, 1989b).

C. Selection of a Mathematical Model and its Calibration

It is well known that plant growth can be described in quantitative terms through mathematical models (France and Thornley, 1984). The most widely used of such models is the so-called Richards function (Causton and Venus, 1981), which is a modified logistic model. It has been used successfully to model vegetative growth on a very broad scale, from single leaf to full crop. Further, it can be applied not only to dry weight but to any morphologically meaningful growth parameters (e.g., length, volume, etc.). Like any logistic growth model, it assumes that the specific growth rate is a function of the current living biomass, multiplied by a limiting factor that has the ecological role of a carrying capacity. Compared with the basic logistic curve (for a thorough survey see, e.g., Begon and Mortimer, 1986; Renshaw, 1991), the Richards function has an additional parameter, in the form of a real number exponential, which is instrumental in tailoring the resulting sigmoidal growth curve to the characteristics of the particular plant being considered. Thus, the growth dynamics, considered as a continuous process, are described by the differential equation:

$$\frac{dX}{dt} = X\frac{r}{n}\left(1 - \frac{X^n}{K^n}\right) \qquad (1)$$

where in the present case X (cm) represents root length, K is the carrying capacity (cm), which in this case represents the final root length, $r \in \Re$ (d^{-1}) is the growth rate and $n \in \Re$ is the Richards exponent, adding flexibility to the dynamic response through modulation of the inflexion point. This basic growth equation, however,

should be complemented with a nickel absorption mechanism, for which the following dynamics was preferred to other candidate functions:

$$\frac{dC}{dt} = K_c(C_{sat} - C) \tag{2}$$

where C is the internal nickel concentration (μg g^{-1} dry weight), C_{sat} its saturation concentration, and K_c (d^{-1}) is a mass transport coefficient governing the rate of metal uptake. A conceptual justification of Eq. 2 can be found in regarding the uptake mechanism as a diffusion process. In this sense, Eq. 2 is a special case of Fick's law, where the mass transfer is governed by the concentration gradient. Similar equations are commonly used in modeling the diffusion of gas into the liquid phase (see Britton, 1986; Holland and Anthony, 1989).

It should be noted that the two equations, 1 and 2, are not directly linked. This is a consequence of the fact that to a large extent growth is not affected by nickel uptake, so the two processes can develop independently from one another. The interaction between these two equations is part of a very complex dynamics, which has been explored by Ågren and Bosatta (1996) and will be considered in order to make further progress in this field of research.

Model (1-2) was calibrated with the experimental data using an optimized version of the flexible polyhedron search method. This algorithm, which is described in detail elsewhere (Marsili-Libelli, 1992) attempts to minimize the squared sum of errors between data and model response. The estimated parameters can then be obtained as $\hat{P} = arg\ \underset{P}{min}E(P)$, where:

$$E(P) = \frac{1}{N - n_p}\left[\sum_{i=1}^{N}\left(X_{exp}(i) - X_m(i)\right)^2 + \sum_{i=1}^{N}\left(C_{exp}(i) - C_m(i)\right)^2\right] \tag{3}$$

and the parameter vector $P \in \Re^{n_p}$ (in this case np = 5) is defined as:

$$P = \begin{bmatrix} r\ n\ K\ K_c\ C_{sat} \end{bmatrix}^T \tag{4}$$

It should be stressed that fitting model (1-2) to the experimental data does not reduce to a mere linear regression. In fact, in this case a nonlinear dynamic model is used, whereas linear regression can be applied only to algebraic linear models, which have no growth description capability.

III. RESULTS

A. Mathematical Model for Growth Under Heavy Metal Stress

Alyssum bertolonii root growth as a function of varying Ni concentrations in the culture medium is shown in Figure 1. The Richards model of Equations 1 and 2 was fitted to these growth data. During the 1-month experiment the roots grew until week 3 and then reached a characteristic maximum length related to Ni concentration in the medium. It can be seen that the model could fit adequately each growth assay with differing parameter values, as shown in Table 1.
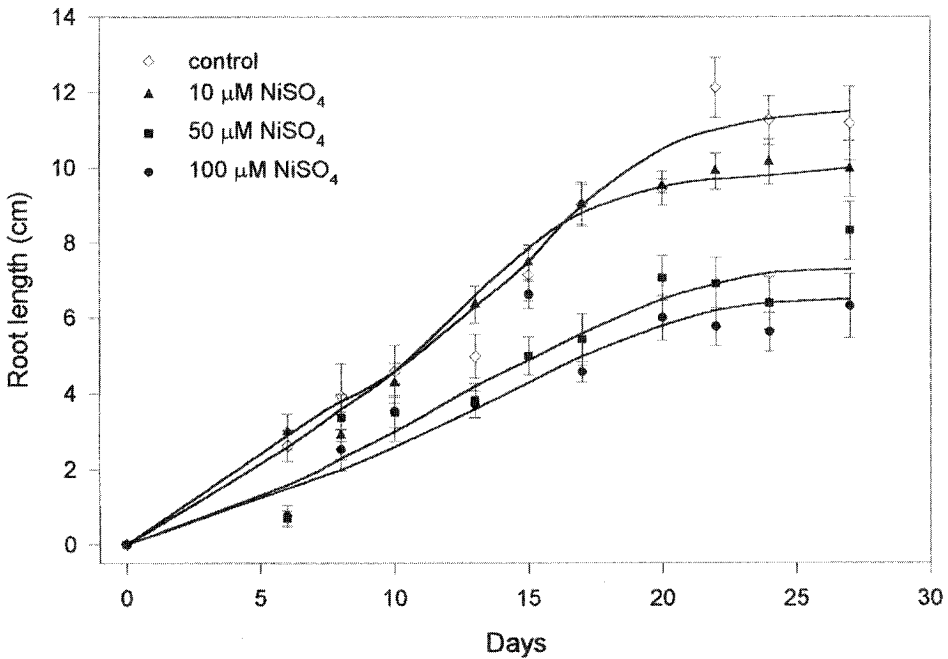
FIGURE 1. Root length of *Alyssum bertolonii* plantlets during growth in hydroponic culture with different $NiSO_4$ concentrations. Values are means of 20 replicates ± SE. The solid lines represent model response fitted to each data set.

TABLE 1. Fitted Parameters Values

	Parameter values				
$NiSO_4$ Concentration	$r\ (d^{-1})$	$n\ (-)$	$K\ (cm)$	K_c	$C_{sat}\ (\mu g\ g^{-1})$
control	0.1002	-0.1093	15.4012	---	---
10 μM	0.2544	0.6665	10.2388	0.2953	12635
50 μM	0.1959	0.4942	7.4790	0.2395	16487
100 μM	0.1613	0.2858	6.9051	0.2702	19604

The growth of control plants was similar to the 10 μM NiSO$_4$ treatment until about day 16. After this period, there was a statistically significant difference between the two curves and differing maximum values were reached. The other growth curves differed from the control during the entire growth period and reached lower maximum values.

Data for shoot growth are not shown because they exhibit the same growth trend as for the roots, but are affected by larger relative errors caused by the smaller measured lengths.

B. Modeling the Metal Uptake Process

Figure 2 shows root Ni uptake during growth. Nickel concentration in the roots of plants treated with 100 μM NiSO$_4$ reached saturation after 2 weeks of growth at about 6800 $\mu g \ g^{-1}$ dry weight. When the concentration of 50 μM NiSO$_4$ was used to treat *A. bertolonii* plants, root Ni concentrations reached the same value, but only after 3 weeks of growth. In the 10 μM NiSO$_4$-treated plants root Ni concentrations increased during the growth period but not enough to reach the same saturation level as the other treatments. It was found that all growth data sets could be fitted to a sigmoid curve produced by the Richards model (1).

The Ni concentrations in shoots of plant exposed to different external Ni concentrations is shown in Figure 3. The uptake curve for 100 μM NiSO$_4$ plants achieved saturation levels in about 2 weeks of culturing and the maximum value reached was about 19000 $\mu g \ g^{-1}$ dry weight. The other two treatments showed similar trends but achieved lower values: 16000 $\mu g \ g^{-1}$ dry weight for 50 μM NiSO$_4$ and 12700 $\mu g \ g^{-1}$ for
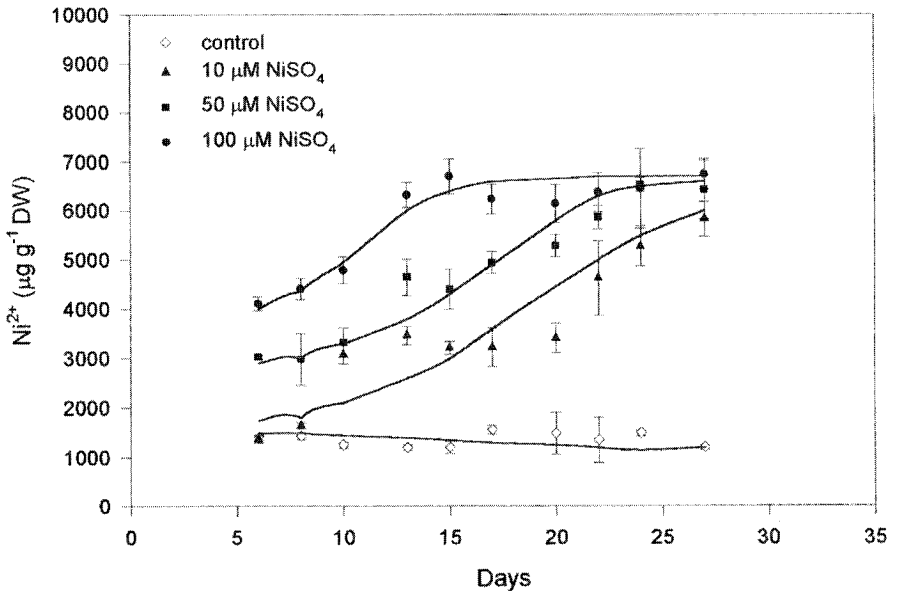

FIGURE 2. Nickel concentration in roots of *Alyssum bertolonii* grown in media with different NiSO$_4$ concentrations. Values are means of three replicates ± SE. The solid lines represent model response fitted to each data set.
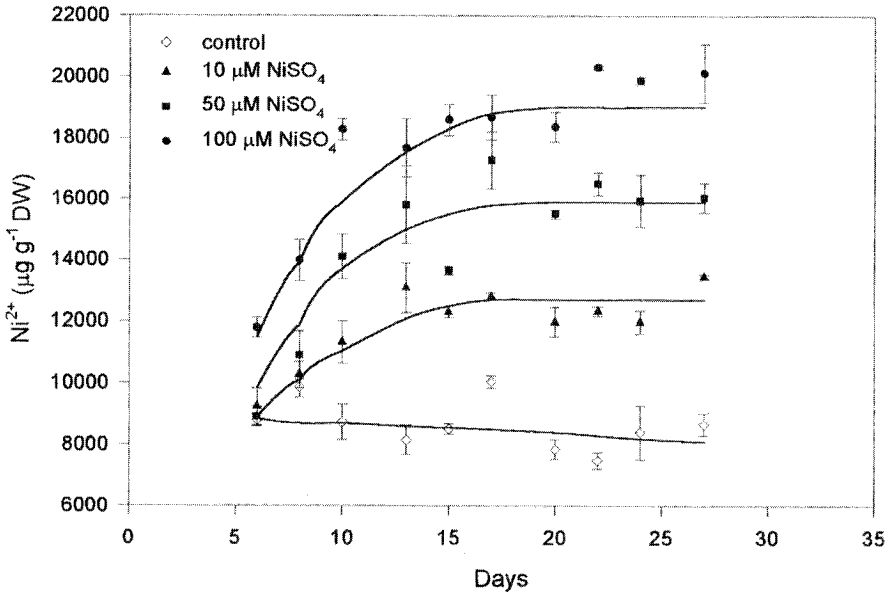
FIGURE 3. Nickel concentration in shoots of *Alyssum bertolonii* during growth on media with different NiSO$_4$ concentrations. Values are means of three replicates ± SE. The solid lines represent model response fitted to each data set.

10 µM NiSO$_4$-treated plants, respectively. After 16 days growth, when root and shoot development began to be significantly different for control and 10 µM NiSO$_4$-treated plants, the shoot Ni concentrations for these latter plants were about 12000 µg g^{-1} dry weight.

IV. DISCUSSION

A rapid *in vitro* experiment has been devised to model the growth of a metal-accumulating plant in order to identify time and concentration conditions for phytoremediation use.

The results obtained from the mathematical modeling of growth under nickel stress can allow estimation of the upper limit of Ni concentration that can be achieved by the plant without major growth inhibition. A concentration of 10 µM NiSO$_4$ can be tolerated by the plant without a severe reduction in biomass, so *A. bertolonii* has proven itself to be a very suitable candidate for phytoextraction of moderately Ni-polluted soils. For higher Ni concentrations, a major decrease in growth rate should be expected, although growth and metal uptake would still continue to a more limited extent.

At the critical time of about 16 days, when control and 10 µM NiSO$_4$ curves began to diverge, a shoot Ni concentration of about 12000 µg g^{-1} dry weight can be considered the toxicity threshold beyond which growth begins to be affected. In field applications, when this level is reached, harvesting should be done, replacing the crop with a new stand instead of maintaining the old plants with an impaired uptake

efficiency. This strategy is also supported by the fact that in the 10 μM $NiSO_4$-treated plants, once a Ni concentration of about 12000 μg g^{-1} dry weight has been reached, this should be considered the upper limit. Experimental evidence supporting this statement is that the Ni concentration in *A. bertolonii* plants growing in their natural habitat on serpentine soils (Minguzzi and Vergnano, 1948) is similar to that identified experimentally as the toxicity threshold. Therefore, an important conclusion can be drawn from these results: the metal content that plants reach in their natural habitats must be carefully taken into consideration during phytoextraction strategy design. Plants appear to take up metals not only in relation to soil content, but also in relation to their tolerance capabilities. When the maximum Ni concentration is reached, which still allows normal growth, it would be inappropriate to attempt to increase it further without anticipating a major growth reduction.

Root Ni uptake has shown a typical saturation trend for all concentrations: the Ni concentration to which every curve tends is 6800 μg g^{-1} dry weight. In shoots, however, different saturation levels are reached in relation to different Ni concentrations. In *A. bertolonii*, the following possible mechanism can be suggested for Ni uptake and translocation: roots may represent a 'sink' with a well-defined saturation level, which is reached more or less rapidly according to ambient Ni concentrations. To account for the existence of several saturation levels in the shoots the following explanation can be proposed: at between 15 to 20 days of development, control and treated plant growth rates began to decrease to zero. The cessation of growth and uptake seem to coincide with the timing and the attainment of saturation levels: it is possible that *A. bertolonii* takes up the metal only during its active growth phase. This hypothesis can explain why uptake stops after a fixed time, but regarding the different levels of saturation, a further explanation has to be proposed: the accumulation level may depend on both growth rate and external Ni concentration. Higher Ni concentrations induce higher accumulation levels because the metal influx is higher. At the same time, the lower growth rates caused by them affect the uptake reduction to a greater extent. Thus, there is a two-factor relationship between internal and external Ni concentrations due to different Ni flow and different growth rates: this could explain the nonlinear proportionality that is observed for nickel treatments and nickel saturation levels.

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