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Natural and Induced Heavy-Metal Accumulation by *Arrhenatherum elatius*: Implications for Phytoremediation

Annabelle Deram,[a] Daniel Petit,[b] Brett Robinson,[c] Robert Brooks,[d,1] Paul Gregg,[d] and Chantal Van Halluwyn[a]

[a]*Laboratoire de Botanique et Cryptogamie, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Lille 2, P.O. Box 83, Lille F59006, France*
[b]*Laboratoire de Génétique et Evolution des Populations Végétales, UPRESA 8016, Université de Lille 1, Villeneuve d'Ascq F59655, France*
[c]*Horticultural and Food Research Institute of New Zealand Ltd., Palmerston North, New Zealand*
[d]*Soil and Earth Sciences, Institute of Natural Resources, Massey University, Palmerston North, New Zealand*

ABSTRACT

The uptake of cobalt, copper, lead, and nickel by the perennial metal-tolerant grass *Arrhenatherum elatius* was studied by growing the plants in two different substrates. These were a cobalt/copper/nickel ore (Experiment A) and base-metal tailings rich in lead (Experiment B). The enhancement of metal uptake by the plants following addition of EDTA was investigated in both experiments. In Experiment A, metal concentrations in dry plant matter were about equal to the acid-extractable (1 *M* hydrochloric acid) fractions in the

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[1] Corresponding author (e-mail address: r.brooks@massey.ac.nz).

supporting soils after addition of 4 g kg^{-1} EDTA. In Experiment B, the base-metal tailings contained 11,500 mg kg^{-1} (1.15%) lead. The EDTA addition induced 2.5% lead (dry matter) in the grass. This was reduced to ca. 1.0% upon addition of lime. The results indicate that *A. elatius* may have some potential in phytoremediation operations if EDTA is added to the substrate.

INTRODUCTION

Arrhenatherum elatius (L.), known commonly as 'tall oat grass' or 'French rye grass', is a perennial grass that has a worldwide distribution. It is found in virtually every environment, including fallow land, meadows, dunes, colliery spoil heaps and areas polluted with heavy metals such as near the base-metal smelter at Auby in Northern France (Ducousso et al., 1990). Soils from this site contain over 4% zinc, 0.8% lead, and 0.01% cadmium (Deram, unpublished data, 1999). Our field studies have shown that a single commercial crop of *A. elatius* has a dry biomass production of up to 10 t ha^{-1}. Under the most favorable conditions, it is possible to obtain two crops per year giving an annual biomass production about 70% of that of the high-biomass *Zea mays*. Genetic variation in populations of *A. elatius* has been studied by Cuguen et al. (1989) and Ducousso et al. (1990) who found that there was a higher selective diversity in plants growing over mine tips than over normal pastures.

Deram (unpublished data, 1999) showed that *A. elatius* accumulates significant concentrations of heavy metals when grown on a metal-rich substrate, such as the polluted soils around metal smelters near the town of Auby, Northern France. This plant is one of only three species found naturally on the most polluted soils, the other two being *Cardaminopsis halleri* and *Armeria maritima* subsp. *halleri*. The accumulation of heavy metals is important in pasture-production areas where soils may contain high levels of heavy metals, such as cadmium, arising from the application of cadmium-rich phosphate fertilizers (Loganathan and Hedley, 1997). High heavy-metal accumulation also has implications for the technology of phytoextraction, where plants are grown on metalliferous soils with the aim of removing the metals from the soils into the aerial portions of the plant. In the technique of phytoremediation (Salt et al., 1995), the plants are then harvested and burned to produce a "bio-ore" which can be buried; or sold if the metal is sufficiently valuable. The associated technology of phytomining (Chaney, 1983; Brooks et al., 1998; Robinson et al., 1997a, 1997b) is designed to sell the bio-ore for profit rather than purely for decontamination of polluted terrain.

Plants used in phytoextraction operations ideally should have the following properties: (1) a high biomass production, (2) a high bioaccumulation of the target metal, (3) be able to flourish on metalliferous soils, which, may also have nutrient deficiencies and other factors making plant growth unfavorable, (4) be easily propagated, and (5) tolerate a wide range of climatic conditions.

TABLE 1. Composition (mg kg^{-1}) and pH of growth media used in the experiments.[a]

Growth medium	Experiment A[b]	Experiment B[c]
pH	5.5	3.5-7.0*
Lime	-	0-5000*
Cobalt	188	1
Copper	187	4500
Nickel	6300	1
Lead	-	6750

[a] NB: "total" metal concentrations were obtained by sample digestion with boiling 4 *M* hydrochloric acid.

[b] A: 'Champion' copper ore from Nelson, New Zealand mixed 1:3 (v/v) with pumice powder.

[c] B: Base-metal tailings from the 'Tui' Mine, New Zealand.

*Depending on amount of lime added.

Arrhenatherum elatius has all of the above properties except for a high accumulation of heavy metals. Huang and Cunningham (1996), and Blaylock et al. (1997) demonstrated that the addition of ethylenediaminetetraacetic acid (EDTA) to the soil where *Zea mays* was growing on a lead-contaminated site, induced the accumulation of this element to a concentration of 2% (20,000 mg kg^{-1}, dry weight) in the aerial portions of the plant. It may thus be possible to induce *A. elatius* to accumulate lead and other metals by the addition of chelating agents to metalliferous soils, making this plant potentially useful for phytoremediation.

The aims of the present study were to determine the effect on plant uptake of metals after addition of EDTA to metal-rich natural substrates, and hence assess the potential of *A. elatius* for the phytoremediation of soils polluted by these metals.

MATERIALS AND METHODS

Greenhouse Experiments

Plants were grown in natural and artificial media as follows. In the first set of experiments (Experiment A), *A. elatius* was grown in a modified copper ore (Table 1) consisting of a 1:3 mixture of ore:pumice. The pumice had negligible concentrations of the above heavy metals and was used to improve drainage of the finely divided ore. The copper ore (treated with 'Osmocote' slow release fertilizers) was from the abandoned Champion Mine near Nelson, New Zealand.

A second set of experiments (Experiment B) involved the use of fertilized ('Osmocote' slow release) mine tailings (undiluted) from the Tui Mine, Te Aroha, New Zealand. The composition of these tailings is also shown in Table 1.

For each set of experiments, two 500-mL pots were prepared for each liming regime and/or EDTA addition (total of 10 for Experiment A and 16 for Experiment B). In each pot, ca. 20 seeds of *A. elatius* were sown and the seedlings grown for a further eight weeks with weekly changing of pot positions to equalize light exposure. After four weeks, each pot was thinned to leave only four individual plants of similar size in each. At the same time, EDTA as the disodium salt (Na_2EDTA) was added as a 5% (w/v) solution to each series of pots at rates of 0 (control), 0.5, 1, 2, and 4 g kg^{-1}. When liming was required, the lime was added directly to the substrate before seed sowing.

The above-ground parts of plants (four from each pot and 104 in total) were harvested eight weeks after sowing, and placed individually in a drying oven at 80°C until a constant weight was achieved.

All pot trials were carried out in a greenhouse with temperatures set in the range of 15°C (night) to 25°C (day). There was no humidity control.

Chemical Analysis of Plants and Their Substrates

Plants to be analyzed for cobalt, copper, and nickel were prepared in the following manner. Subsamples (1 g) of each of four plants from each pot were weighed into 10-mL borosilicate test tubes. The tubes were heated in a muffle furnace overnight at 500°C. The next day, 5 mL of hot 2 *M* hydrochloric acid was added to each tube and the solutions mixed using a vortex mixer.

The remaining plants (for lead analysis) were weighed into 50-mL conical flasks. To each flask, 5 mL of a 3:1 mixture of concentrated nitric/perchloric acids was added and the mixture boiled until all the nitric acid had evaporated and fumes of perchloric acid began to appear. Then, 4 mL of hot (80°C) 2 *M* hydrochloric acid was added, together with a small volume of distilled water to give a final volume of 5 mL.

The acid-extractable (essentially total) metal concentrations in the substrates were determined by adding 20 mL of 4 *M* hydrochloric acid to 2 g subsamples weighed into 40-mL sealable centrifuge tubes. The mixtures were placed on an end-over-end agitator overnight, then centrifuged, filtered and the solutions stored for analysis. A simple acid extraction was sufficient to determine the total amount of metal added to the substrate as these metals were not bound to silicate matrices (where HF attack would have been needed). The untreated pumice contained negligible amounts of the elements being measured. Total metal concentrations are shown in Table 1.

Measurements of pH were performed by shaking 2 g of substrate with 10 mL of distilled water in sealed vials placed for 24 hours in an end-over-end mechanical shaker. After allowing the material to settle, pH was measured in the supernatant

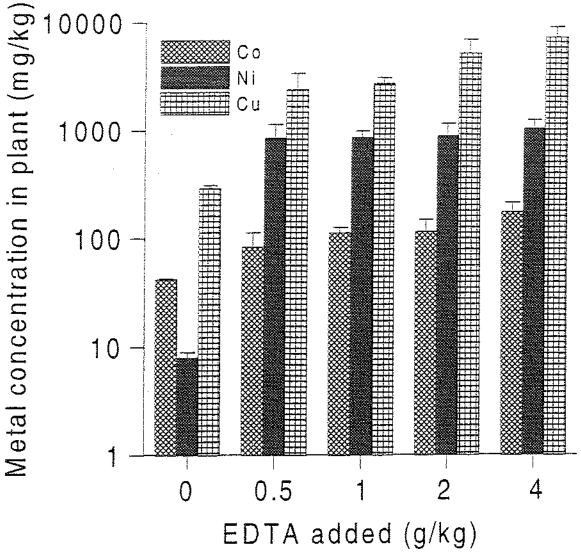

FIGURE 1. Effect of EDTA amendment (N=8 for each element and amendment) on uptake of copper, cobalt, and nickel by *Arrhenatherum elatius* (mg kg^{-1} dry mass) from Champion Mine copper ore diluted 1:3 with pumice (Experiment A). Error bars are standard errors of the mean.

All samples were stored in 35-mL polythene containers and analyzed using flame atomic absorption spectroscopy (FAAS). The standards for the workings curves were prepared in 2 *M* hydrochloric acid to match the bulk composition of the sample solutions.

RESULTS AND DISCUSSION

Accumulation of Heavy Metals by *Arrhenatherum elatius* from a Cobalt/Copper/Nickel Ore After EDTA Amendment

Addition of EDTA to a growth medium of copper ore (Experiment A) amended with pumice to improve drainage, resulted in a dramatic increase in cobalt, copper and nickel concentrations in *A. elatius*. The increases shown in Figure 1 were from 200 to 7,500 mg kg^{-1} for copper, 40 to 175 for cobalt and 8 to 1,276 for nickel. Values in this figure are shown on a logarithmic scale in order to include three plots in the same figure. A linear scale would have shown greater differences for individual elements. For all three elements there was a statistically significant increase of concentration for increasing dosages of EDTA, albeit with some evidence of leveling off in the case of nickel for EDTA additions above 0.5 g kg^{-1}. Addition of EDTA (even at the lowest treatment level) immediately caused necrosis in the plants, no doubt because of the very high phytotoxicity of copper that thereby became available to the plant. This necrosis would not be a disadvantage in a phytoremediation project because the vegetation would be harvested in any case. It is envisaged that plants under field conditions would be allowed to grow to full size before addition of EDTA, as is done for lead phytoremediation (Blaylock et al., 1997).

Relative uptakes of the three metals by the grass were 37.5 for copper, 4.4 for cobalt, and 160 for nickel. The differences may be in part because of different phytoavailability among the three elements. In terms of total metal plant/ore concentration quotients, the respective values were 40 for copper, 1.1 for cobalt, and 0.17 for nickel. The very high relative accumulation of copper in the plant is very noteworthy because here we have a case of a metal-tolerant grass that is easy to grow and has a fairly high biomass. If a similar behavior were to occur under field conditions, a crop of *A. elatius* grown over a hectare of copper-rich soil (assuming a biomass yield of 10 t ha^{-1}) might be expected to remove 75 kg of copper after addition of EDTA.

Accumulation of Lead by *Arrhenatherum elatius* from Tui Base Mine Tailings After EDTA Amendment

Addition of EDTA to Tui Mine base metal tailings (Experiment B) resulted in a dramatic increase in the lead concentration in *A. elatius* from <100 mg kg^{-1} (dry weight) to about 25,000 mg kg^{-1} (Figure 2). This is comparable, and indeed superior, to the 2% lead recorded by Huang and Cunningham (1996) when HEDTA was added to lead-contaminated soil (2,500 mg kg^{-1}) in which *Zea mays* was grown. Before addition of EDTA, the lead concentration in this plant had only been about 100 mg kg^{-1} (dry weight).

The effect of lime addition in our experiments was to reduce the lead uptake by a factor of two as the percentage of lime exceeded 0.125%. This is no doubt due to the effect of raising the pH of the growth medium whereby lead, like most heavy metals, is rendered less mobile (Brooks, 1998). Despite the high uptake of lead, our plants survived the experiments, unlike studies with *Zea mays* (Huang et al., 1997) that only survived for 10 days after addition of EDTA.

The Potential of *Arrhenatherum elatius* for Phytoextraction

The low natural bioaccumulation factors (plant/soil metal concentration quotients) of *A. elatius* for the heavy metals studied, do not favor the use of this plant for phytoextraction without some sort of soil amendment to enhance metal solubility. The addition of EDTA, enhanced the accumulation of cobalt, copper, lead and nickel to levels where the plant may be of some use for phytoremediation soils weakly contaminated with any or all of these metals.

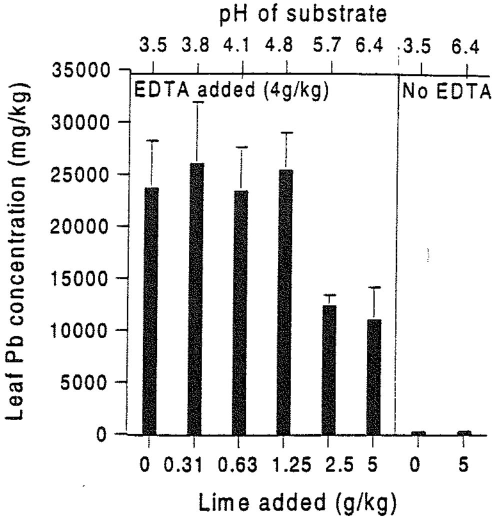

FIGURE 2. Uptake of lead by *Arrhenatherum elatius* (N=8 for each amendment) from Tui Base Mine tailings (Experiment B) with and without added lime and with and without a constant EDTA application of 4 g kg^{-1}.

Even though EDTA is non-toxic to humans (it is even used as a clinical treatment for lead poisoning), it must be recognized that addition of EDTA to the substrate could have unwanted environment ramifications if heavy metals other than lead are solubilized. However, the half life of EDTA in the environment has been studied by Means et al. (1980) who have shown that it is of the order of 6 months under normal conditions. Care should therefore be taken that sites are chosen to avoid any immediate runoff into local waterways. In assessing any environmental problems it will be necessary to weigh the use of EDTA against the other more serious consideration of just leaving the toxic lead *in situ*.

Radioactive ^{60}Co could conceivably be removed from sites contaminated by nuclear waste such as around Chernobyl in the Ukraine. Unpublished studies (K.W. Brown, personal communication) in the late 1970s were centered around the use of the cobalt hyperaccumulator *Haumaniastrum katangense* to remove radioactive cobalt from a contaminated site. The main problem with this plant species is its relatively low biomass production and sensitivity to cold conditions. Given the rapid biomass production, and tolerance to a wide range of climates, *A. elatius* may be effective for cobalt phytoremediation. EDTA is relatively inexpensive and its use can be justified on economic grounds when phytoremediation is compared with other far more expensive alternatives.

Copper, particularly near smelters, is a common industrial pollutant. It does not lend itself to phytoremediation without chelate amendment because although several hyperaccumulators of copper are known (Brooks and Malaisse, 1985) they are all from the Democratic Republic of Congo (formerly Zaïre), have a small biomass, and are not tolerant of cold conditions.

The results obtained in this study indicate the potential of *A. elatius* for phytoremediation of contaminated soils. These data should not however, be extrapolated unreservedly to the field situation because the forms of the metals *in situ* vary considerably and cannot be modeled by simple pot trials. The microflora of the soil may affect the uptake of heavy-metals by plants and our pot-trials were conducted using sterilized material. Furthermore, the effect of the heavy-metals and local climatic conditions on biomass production is unknown. Field trials investigating the *in situ* decontamination of heavy-metal contaminated soils using *A. elatius,* particularly in the vicinity of smelters in Northern France where it is already growing, should be a logical sequel to this present study.

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