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Full Length Research Paper

Bioaccumulation of Cr, Hg, As, Pb, Cu and Ni with the ability for hyperaccumulation by *Amaranthus dubius*

John J. Mellem[1]*, Himansu Baijnath[2] and Bharti Odhav[1]

[1]Department of Biotechnology and Food Technology, ML Sultan Campus, Durban University of Technology, 41/43 Centenary Rd., Durban, 4001, South Africa.
[2]School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, P. Bag X54001, Durban 4000, South Africa.

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Amaranthus dubius **is a nutritious leafy vegetable that is widely distributed in Africa, Asia and South America. The rapid growth and biomass makes them one of the highest yielding leafy crops, which may be valuable for phytoremediation. Thus, this study investigates the potential of *A. dubius* for the hyperaccumulation and distribution of chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), copper (Cu) and nickel (Ni) in the different plant organs. The phytoremediation was calculated the by bioconcentration factor and translocation factor of plants grown under controlled conditions in a tunnel house. The metal accumulated was investigated using inductively coupled plasma mass spectroscopy (ICPMS). Following exposure to 25, 75 and 100 ppm of Cr, Hg, As, Pb, Cu and Ni respectively for four days, *A. dubius* accumulates Cr, Hg and Pb at the low concentration (25 ppm) and translocates it to the shoots, but at higher concentrations (75 and 100 ppm) the metal is only stored in the roots. In the case of As accumulation at all concentrations, the metal is translocated to the shoots; Cu and Ni indicate that the metal is stored in the roots mainly. This study indicates that *A. dubius* has limited potential for the bioaccumulation of Cr, Hg, Pb, Cu and Ni, but is capable of hyperaccumulating of As.**

Key words: *Amaranthus dubius*, bioconcentration factor, translocation factor, inductively coupled plasma mass spectroscopy.

INTRODUCTION

Concern has been expressed with regard to the accumulation of toxic heavy metals such as chromium (Cr), mercury (Hg) and lead (Pb) and their impact on both human health and the environment (Gardea-Torresdey et al., 2004). These metal contaminants are environmental pollutants from industrial and agricultural activities, sewage sludge leaching and mining waste, and are usually removed by physicochemical methods (Gratao et al., 2005). However for the remediation of large areas where the pollutants occur at low concentrations, use is made of bacteria, algae or plants (Rulkens et al., 1998). With current trends moving towards greener technologies, the focus is shifting to phytoremediation, where plants are used uptake metals or pollutants from the environment or transform them into harmless compounds (Berti and Cunningham, 2000; Salt et al., 1994). Phytoremediation presents a cheap, noninvasive, and safe alternative to conventional cleanup techniques and can be accomplished by phytoextraction, phyto-degradation, phytostabilization, phytovolatilization and rhizofiltration (Glick, 2003).

Numerous plant species have been identified for bioremediation, with certain plant species, known as hyper accumulators, being targeted, as they are able to accumulate potentially phytotoxic elements at concentrations 50 to 500 times higher than average plants. The high bioconcentration factor, which is the ability of the plant to extract metals from the soil and the efficient root to-shoot transport system endowed with enhanced metal tolerance provide hyper accumulators

*Corresponding author. E-mail: johnm@dut.ac.za.

with a high potential detoxification capacity. At present, there are nearly 400 known hyper accumulators, but most are not appropriate for phytoextraction because of their slow growth and small size (Salt and Kramer, 2000). Several researchers have screened fast-growing, high-biomass accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Kumar et al., 1995; Salt et al., 1995; Blaylock et al., 1997; Huang et al., 1997). In this study we chose *Amaranthus dubius* (marog or wild spinach) which is a popular nutritious leafy vegetable crop, rich in proteins, vitamins and minerals, and consumed in Africa, Asia and South America (Odhav et al., 2007).

Research on the genus Amaranthus include *Amaranthus hybridus*, which was used to determine the effect of coal mine contamination on the uptake and distribution of lead, cadmium, mercury, nickel, manganese and iron; *Amaranthus tricolor* and *Amaranthus retroflexus* which were used for the uptake of cadmium, mercury, zinc and copper; and *Amaranthus spinosus* that was used for the accumulation of cadmium, zinc and iron (Jonnalagadda and Nenzou, 1997; Bigaliev et al., 2003; Prasad, 2003).

MATERIALS AND METHODS

Soil and plant culture

Wild source seeds of *A. dubius* were obtained from a cultivated area. Flower heads were collected, air dried and the seeds removed and stored in glass bottles in a refrigerator. The seeds were germinated for one week in potting soil at a pH of 7 to 8, with 140 ppm potassium, 77 ppm of phosphorous, 1850 ppm of calcium, 520 ppm magnesium, and 77 ppm sodium. Following this, specimens were put into seedling trays in friable loamy soil.

Six week old seedlings were transplanted into the pots, with 12 plants of uniform length (± 30 cm) placed a uniform distance apart in each pot. The pots were kept in a tunnel house at an ambient temperature. Salts of Cr, Hg, As, Pb, Cu and Ni [potassium dichromate ($K_2Cr_2O_7$), mercuric chloride ($HgCl_2$), arsenic trioxide (As_2O_3), lead nitrate ($Pb(NO_3)_2$), cupric nitrate ($Cu(NO_3)_2.3H_2O$) and nickel sulphate ($NiSO_4.6H_2O$)] were added to the potting soil as described by Gardea-Torresdey et al. (2004), Odjegba and Fasidi (2004) and Ahalya et al. (2005). A separate pot with untreated soil served as a control. The pots containing the plants were placed in drip trays so as to prevent any leachate from being lost (Giordani et al., 2005; Kos et al., 2003). The plants were watered daily with 500 ml of water per pot and all collected leachate was returned to the respective experimental pot.

Sampling

Three plants from each treatment were harvested after four days without damaging the roots and rinsed in distilled water to remove dust and soil mineral particles. The remaining plants were observed at four day intervals over a 16 day period for any phenological changes as a result of metal exposure. Plant samples were then separated into roots, stems and leaves and dried at 60°C in a convection oven for 48 h. Samples were milled to a fine powder using a Waring Commercial Laboratory Blender, placed in aluminum covered 500 ml Schott bottles and stored.

Extraction

Metal extraction using microwave digestion

The heavy metals were extracted using a microwave digestion procedure described by Zunk and Planck (Zunk and Planck, 1990). Plant samples (0.5 g) were weighed out in a digestion vessel and HNO_3 (5 ml) and H_2O_2 (2 ml) added (Roy et al., 2005). The parameters set for the microwave digestor were set as 5 steps, with samples exposed to different temperatures for different time periods. The power ranged from 0 to 600 Watts and time from 1 to 5 min. Upon completion of the 5 microwave digestion steps the samples were allowed to cool at room temperature for ± 45 min, decanted into a 25 ml volumetric flask and made up to the level with distilled water (Aksoy and Sahin, 1998; Minganti et al., 2004). This was then filtered with Whatman No 1 filter paper to remove any undigested particulates, and stored in a refrigerator for metal analysis using inductively coupled plasma – mass spectroscopy (ICPMS).

Heavy metal analysis

Inductively coupled plasma – mass spectroscopy (ICPMS): The metals were analyzed using ICPMS according to the procedure outlined by Zayed et al. (1998) and Wang et al.(1999). In this system samples are decomposed to neutral elements at high temperature argon plasma and analyzed based on their mass to charge ratios. There are four steps: (i) sample introduction; (ii) aerosol generation; (iii) ionization by an argon plasma source; and (iv) mass discrimination. Results obtained from ICPMS analysis were in mg/L and converted to parts per million (ppm).

Translocation factor

To evaluate the potential of *A. dubius* for phytoextraction, the translocation factor (TF) was calculated. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol et al., 2004). It is represented by the ratio:

$$\frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1, with values greater indicating translocation to the aerial part of the plant (Mellem et al., 2009).

Bioconcentration factor

The bioconcentration factor (BCF) was used to determine the quantity of heavy metals absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the formula (Ghosh and Singh, 2005):

$$\frac{\text{Metal concentration in tissue of whole plant}}{\text{Initial concentration of metal in substrate, that is, soil}}$$

The higher the BCF value the more suitable is the plant for phytoextraction (Blaylock et al., 1997). BCF Values > 2 were regarded as high values (Mellem et al., 2009).

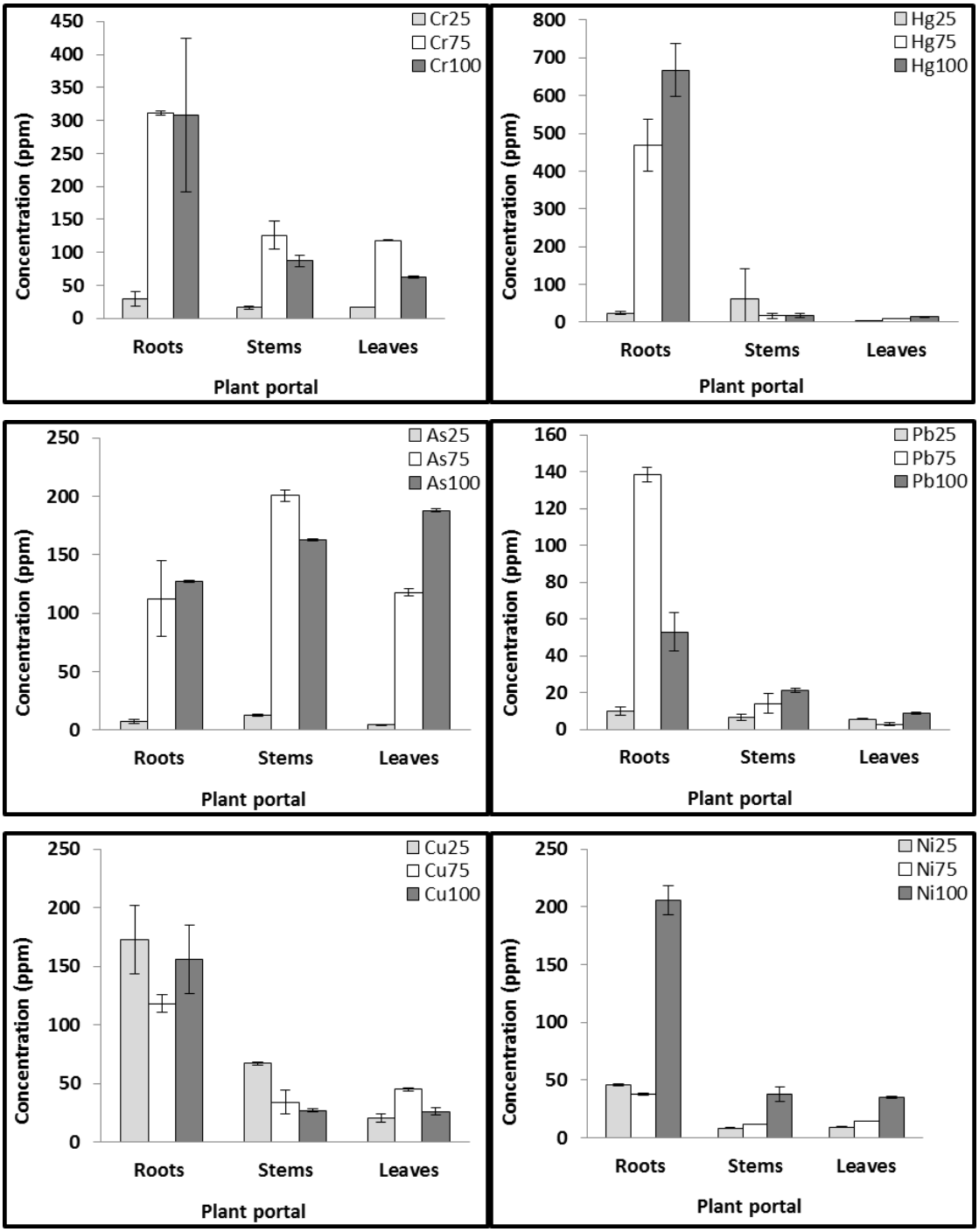

Figure 1. Individual metal distribution in the different portals of *A. dubius* from the three different concentrations (25 ppm, 75 ppm and 100 ppm) of each respective metal (Cr, Hg, As, Pb, Cu and Ni). [Bars denote mean ± standard deviation (n=3)].

RESULTS

Metal concentration in plant samples

The uptake of Cr ranged from 16 to 310 ppm, with the highest concentration of Cr being accumulated in the root system (Figure 1). In the samples exposed to Cr the highest concentration of Cr was accumulated by the plants grown in the soil containing 75 ppm. The plants exposed to Hg showed no visible phenotypical changes, with the samples exposed to each of the different Hg concentrations showing a uniform growth rate. The

Table 1. Comparison of metal (Cr, Hg, As, Pb, Cu and Ni) accumulation in the roots, stems and leaves of *A. dubius* grown in metal treated soil after 4 days.

Metal	Concentration (ppm)	Roots	Stems	Leaves	TF†	BCF‡
	25	30 ± 11	17 ± 2	17 ± 1	1.1	2.6
Cr	75	311 ± 3	126 ± 22	118 ± 1	0.8	7.4
	100	308 ± 117	87 ± 9	63 ± 2	0.5	4.6
	25	24 ± 4	61 ± 80	3 ± 0	2.6	3.5
Hg	75	468 ± 69	16 ± 6	9 ± 0	0.1	6.6
	100	667 ± 70	17 ± 5	14 ± 1	0.1	7.0
	25	7 ± 2	13 ± 1	4 ± 0	2.4	1.0
As	75	112 ± 32	201 ± 5	118 ± 3	2.8	5.7
	100	127 ± 32	163 ± 17	188 ± 4	2.8	4.8
	25	10 ± 2	6 ± 2	6 ± 0	1.2	0.9
Pb	75	138 ± 4	14 ± 5	3 ± 1	0.1	2.1
	100	53 ± 10	21 ± 1	9 ± 1	0.6	0.8
	25	173 ± 28	67 ± 18	20 ± 3	0.5	10.4
Cu	75	118 ± 8	34 ± 10	45 ± 1	0.7	2.6
	100	156 ± 29	27 ± 1	26 ± 3	0.3	2.1
	25	46 ± 1	9 ± 0	9 ± 1	0.4	2.6
Ni	75	38 ± 1	12 ± 0	15 ± 0	0.7	0.9
	100	206 ± 13	38 ± 7	36 ± 1	0.4	2.8

Units in ppm ± standard deviation associated with the mean value (n = 3); †translocation factor (ratio of metal concentration in aerial portals to those in roots) - values > 1 are regarded as high ; ‡ bioconcentration factor (ratio of metal concentrations in plant tissue to those in soil) - values > 2 are regarded as high.

uptake of Hg ranged from 3 to 666 ppm with the root system accumulating the highest concentration of Hg. In the soil containing As the pattern was similar to that of the plants grown in the soil containing Cr. The plant samples in the soil containing 25 ppm of As showed no phenotypical, changes however the samples exposed to 75 and 100 ppm showed both chlorosis and severe wilt. The concentration of As accumulated ranged from 4 to 200 ppm. The accumulation of As was different in comparison to the other modes of accumulation thus far with the lowest accumulation in the roots (Figure 1). The plant samples exposed to Pb showed chlorosis among all three concentrations (25, 75 and 100 ppm) with a uniform growth rate between the 25 and 75 ppm Pb concentrations, while the 100 ppm showed a slightly slower growth rate. Leaves in this treatment were larger in comparison to plants exposed to other metals. The concentration of Pb accumulated ranged from 2 to 138 ppm with the highest concentration accumulated by the roots (Figure 1). In the plants grown in the soil containing Cu there were no visible phenotypical changes between the three concentrations, however the plant exposed to 25 ppm showed a slower rate of growth in comparison to those grown in 75 and 100 ppm. The concentration of Cu accumulated ranged from 20 to 172 ppm with the highest concentration being accumulated by the roots (Figure 1). The plants grown in the soil containing Ni showed no phenotypical changes apart from the leaves appearing darker green in color when compared to the plants exposed to the other metals. Concentrations of Ni accumulated ranged from 8 to 205 ppm with highest concentration being stored in the roots. There was a difference in the growth rates between the different concentrations with the plants exposed to 100 ppm being the highest followed by 25 and 75 ppm.

Translocation of metal from the soil to the aerial parts of the plant (TF Values)

The Translocation Factor (TF) of each metal under investigation (Cr, Hg, As, Pb, Cu and Ni) and each of the 3 different concentrations (25, 75 and 100 ppm) for each metal is recorded in Table 1. From the TF results the closer to 0, the higher the concentration of the metal stored in the roots instead of being translocated to the shoots/aerial parts of the plant. From the results, *A. dubius* accumulated Cr, Hg and Pb at 25 ppm and

translocates it to the shoots, but at higher concentrations (75 and 100 ppm) the metal were stored in the roots. In the case of As accumulation at all the concentrations, the metal was translocated to the shoots. The Cu and Ni results indicated that the metal was stored mainly in the roots.

Index of plant to accumulate metal with respect to soil concentration (BCF values)

The bioconcentration factor (BCF) is used to indicate the ability of the plant to absorb metals from soil. In this study BCF values > 2 were regarded as high (Table 1). For plants exposed to Cr, Hg and Cu the BCF values were > 2 for all three soil types (25, 75 and 100 ppm). In the case of As the BCF values were only > 2 for the plants exposed to the soils containing 75 and 100 ppm. Plants exposed to Pb had BCF values > 2 for plants grown in soil containing 75 ppm Pb. For the plants exposed to Ni those grown in the soil containing 25 and 100 ppm had BCF values > 2.

DISCUSSION

A. dubius clearly demonstrates that it can tolerate difficult soil conditions. Furthermore plant samples collected had well-developed rooting structures at lower concentrations (25 and 75 ppm). The implication of metal contamination and the role it plays in the physiology of plants depends on the metal speciation, which is responsible for its mobilization, uptake and resultant toxicity in the plant system (Shanker et al., 2005).

The study showed that for Cr at 25 ppm there was no toxic effect but at higher concentrations such as 75 and 100 ppm the plants showed signs that affect the growth and development. The observation of the growth of the plant over a sixteen day period indicated that *A. dubius* tolerated high Cr concentrations as indicated by the high BCF index (Table 1). However, the ability of *A. dubius* to be considered for phytoextraction has to be viewed with caution, as the TF index indicates that only when the Cr concentration is 25 ppm is Cr being translocated to the aerial parts of the plant. Findings by Gardea-Torresdey et al. (2004) support Cr being concentrated in the roots and not translocated to the aerial parts of the plant by determining the uptake and accumulation of Cr by *Convolvulus arvensis* L.

Exposure of *A. dubius* to doses of Hg up to 100 ppm showed no visible phenotypical changes, and uniform growth rate. The lowest uptake of Hg, 3 ppm was found to be in the leaves whilst the roots were found to have 666 ppm. There are several other studies that also show that plant roots accumulate Hg when they were exposed to Hg-contaminated soils (Kalac and Svoboda, 2000). Laboratory studies showed that plant roots absorbed Hg from solution and roots accumulated much greater amount of Hg than shoots (Godbold and Hütterman, 1988). Our studies did not investigate the absorption of the gaseous Hg via the stomata of leaves, however studies of Cavallini et al. (1999) reported that uptake of mercury oxide by the leaf increased with increasing Hg vapour concentration, temperature, and illumination. Nonetheless, as plant roots were able to efficiently take up Hg from the available Hg pool in the soil and to accumulate Hg in roots, phytostabilization might be a promising approach to remediate aged Hg-contaminated soils (Figure 1). In this process, the massive plant root system traps the bioavailable Hg and reduces the leakage of Hg from contaminated soil.

Plant samples in the soil containing 25 ppm of As showed no phenotypical changes, however, the samples exposed to 75 and 100 ppm showed changes. The accumulation of As was different in comparison to Cr and Hg in that the lowest level was found in the roots and the highest levels were found in the leaves (Figure 1). The root length was found to increase for 25 ppm over a sixteen day period, but at 75 ppm this increase was only observed to day 12, thereafter root growth stopped. The absorption of As from the soil by the roots was lower than that of leaves and shoots. Further evidence indicating that As was translocated in the leaves and shoots was indicated by the TF and BCF values respectively (Table 1). As the objective was to evaluate *A. dubius* for the phytoremediation of As, this study showed that it can tolerate As levels of 75 ppm, and can also translocate most of the As to the aerial parts of the plant up to 100 ppm. Thus the conclusion from this study was that *A. dubius* is a hyperaccumulator of As.

In the study the effect of exposing *A. dubius* to Pb showed uniform growth rate at 25 and 75 ppm of Pb, and slightly lower growth rate at 100 ppm. A similar trend was observed with root length over a sixteen day period. Analysis of the Pb concentrations in the different plant portals showed that most of the Pb was in the roots (Figure 1). The ability of the plant to take up Pb from the soil was only evident at 75 ppm which had a BCF value > 2. However, ability of the plants to move the lead to the aerial parts of the plant was limited, and only the plants exposed to 25 ppm showed a TF > 1. *Thlaspi rotundifolium* and *Thlaspi caerulescens* show similar results (Reeves and Brooks, 1983). Results of the study were not consistent, and therefore it was difficult to conclude that *A. dubius* is a hyperaccumulator of Pb.

It is well known that elements such as Cu, Mo, Ni, Cr, and Zn, among others, are essential for plant growth in low concentrations (Taiz and Zeiger, 1998). However, beyond certain concentrations, these same elements become toxic for most plant species (Blaylock and Huang, 2000). Thus the effect of Cu and Ni was studied. The results for both metals showed that Cu and Ni did not show any visible damage to *A. dubius* and higher doses gave higher growth rates. The study also showed that

most of the Cu and Ni is stored in roots and very little is translocated to the aerial parts (Table 1).

All higher plants are capable of accumulating heavy metals in different concentrations. However a significant difference in metal accumulation exists between and within plant populations. *A. dubius* is a cosmopolitan plant with a rapid growth rate, yields a high biomass and is easy to harvest and the metal is sequestered from the soil and transported to the aerial parts. The translocation Factor (TF) of each metal under investigation (Cr, Hg, As, Pb, Cu and Ni) and each of the 3 different concentrations (25, 75 and 100 ppm) for each metal showed that *A. dubius* accumulates Cr, Hg and Pb at 25 ppm and translocates it to the shoots, but at higher concentrations (75 and 100 ppm) the metal was only stored in the roots. In the case of As accumulation at all the concentrations, the metal is translocated to the shoots. The Cu and Ni results indicated that the metal is stored in the roots mainly. Therefore this study shows that *A. dubius* can be defined as a hyperaccumulator of As since it has the ability to extract, translocate As to the aerial parts of the plant and tolerate high levels of this metal.

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