JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
ISSN: 2339-076X, Volume 1, Number 1 (October 2013): 21-26

Research Article

The potential use of indigenous nickel hyperaccumulators for small-scale mining in The Philippines

E.S. Fernando[1], M.O. Quimado[1], L.C. Trinidad[2], A.I. Doronila[3,*]

[1] Department of Forest Biological Sciences, College of Forestry and Natural Resources, The University of the Philippines – Los Baños College, 4031 Laguna, Philippines
[2] Central Analytical Services Laboratory,National Institute of Molecular Biology and Biotechnology,The University of the Philippines – Los Baños College
[3] School of Chemistry, University of Melbourne, Victoria 3010, Australia

*corresponding author: adoro@unimelb.edu.au

Abstract : Uptake of nickel and three other heavy metals (copper, cobalt, and chromium) was examined in 33 species of the common and rare native vascular plants growing in an ultramafic area currently subjected to mining in Zambales Province, Luzon, Philippines. Leaf tissue samples were initially screened in the field using filter paper impregnated with dimethylglyoxime (1% solution in 70% ethyl alcohol) and later analyzed by atomic absorption spectroscopy. One species was found to be a hypernickelophore (>10,000 µg/g), eight species were nickel hyperaccumulators (>1,000 µg/g), nineteen species were hemi-accumulators (>100-1,000 µg/g), and five species were non-accumulators (<100 µg/g). This paper significantly adds to the list of hyperaccumulator species first reported for the Philippines in 1992. The findings will be discussed in context of using indigenous species for post mining ecological restoration and nickel phytoextraction in small-scale mining in the Philippines..

***Keywords**: hyperaccumulator species, nickel laterites, Zambales*

Introduction

Hyperaccumulators are a suite of plants with the rare ability to extract certain metals and metalloids, and to accumulate them in normally toxic shoot tissue concentrations without any evidence of physiological stress (Baker and Brooks, 1989; Reeves and Baker, 2000). The phenomenon has been observed in less than 0.2% of all angiosperms (ca 400 taxa), usually manifesting as extraordinarily high foliar concentrations (>1000 µg/g dry weight) of one of these elements in the leaf dry matter (Pollard et al., 2002). Hyperaccumulators are predominantly herbaceous and generally occur on substrates enriched in their hyperaccumulated elements. Host soils are most commonly serpentine, with elevated levels of heavy metals and magnesium, depleted in plant macronutrients, and supporting highly specialized floras (Brooks, 1998). Aside from understanding the unique ecophysiological adaptations of hyperaccumulators, there have also been studies on their application for metal prospecting, phytoremediation and mine restoration (Brooks, 1998; McGrath, 1998; Lombi et al., 2001; Macnair, 2003; Boyd, 2007). Thus far Ni, Zn, Cd, Cu, Pb, Co, Tl, Mn, As, Se are known to be hyperaccumulated, however over 70% of hyperaccumulators are specific to Ni (Brooks, 1998; Baker et al., 2000). Current understanding of hyperaccumulation is largely based on relatively easily accessible herbaceous species that hyperaccumulate Ni, Cd, and/or Zn (Minguzzi and Vergnano, 1948; Brooks et al., 1979; Gambi et al., 1979; Martens and Boyd, 1994; Krämer et al., 1996; 1997; Macnair et al., 1999; Pollard et al., 2000; Küpper et al., 2001; Robinson et al., 2003).

There are still many parts of the tropics in which no plant collections have focused on metalliferous (including serpentine) soils, and in which little or no analytical work has been performed. Such areas include parts of the Philippines and Indonesia (Proctor, 2003, Reeves, 2003). Philippine ultramafics (geological formations which have high Mg/Fe ratios) comprise 5% of the nations land area, and most likely support large assemblages of extreme (>1% concentration) Ni hyperaccumulators, potentially equalling New Caledonia and Cuba where currently most of the known tropical species have been discovered to date (Reeves, 2003). It is expected that related taxa or even new plant species could be discovered in the area. The only thorough study to date was an expedition to Mt Bloomfield, Palawan in 1986, on which four woody species of Ni hyperaccumulators were discovered (Baker et al., 1992). That study strongly suggested that Ni hyperaccumulators surely remain to be found, but there was also the intriguing discovery of high Zn in some populations of a subspecies of the tree *Dichapetalumgelonioides*, for which the serpentine endemic sub species are Ni-hyperaccumulators; these need further investigation. The ultramafics of Zambales, the largest ultramafic formation in the Philippine archipelago may add to the known list of four species previously discovered and described in 1986. This preliminary study forms part of a comprehensive nationwide survey Ni hyperaccumulating species in the Philippines since 1986.

Materials and Methods

Site description

The Acoje mine site is located in the Zambales province, approximately 100 km (250 km by road) north of Manila, the Philippine's capital. The area is situated between the municipalities of Santa Cruz and Candelaria, and is geographically situated 15°42' north latitude and 120°03' west longitude with an altitude of 90 m above sea level (Figure 1). It is located on the Zambales Mountains, which are bordered to the west by a 6km coastal plain. Besides mining operations, land use within the mine area is mainly for forestry and subsistence farming. The climate of the area is classified as a type II, which is common in the western seaboard of the main island of Luzon. It is tropical with an average temperature of 27°C and 80% humidity. Average rainfall in the area is around 3,200 mm during the wet season (May to October) Minimum monthly rainfall (< 100 mm) occurs between the months of November to April (Pagasa, 2012). Zambales is underlain by the Zambales Ophiolite complex. This complex is composed of 3 major massifs namely Masinloc, Cabangan, and San Antonio with the Masinloc massif further divided into the Acoje Block and the Coto Block. The Acoje block is characterized by a complete ophiolite sequence with the presence of residual harzburgites, transition zone dunites, layered ultramafic cumulate rocks, layered gabbros, isotropic/massive gabbros, sheeted dike complex, and pillow lavas (Anon, 2008).

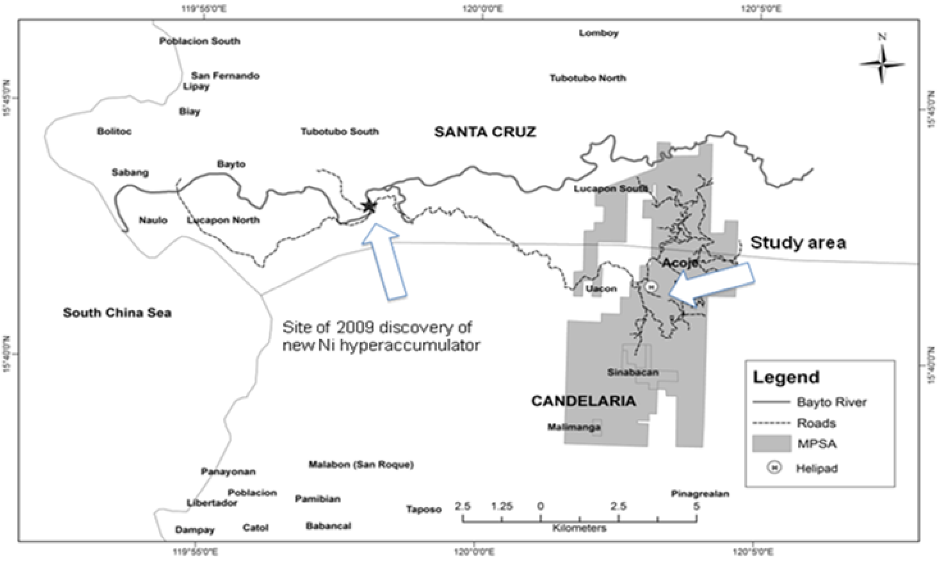

Figure 1. The study area in Acoje, Zambales Province.

There are two distinct habitat types found within the study area, the forested areas and grassland areas. The distribution of these habitat types is dispersed. The forested areas are mostly characterized as secondary mature forests with the primary forest observed in the vicinity of Mt. Lanat. Grasslands are found on areas previously disturbed by mining operations. In addition, some of the disturbed areas are being regularly utilized for swiden/shifting agriculture.

Sampling and Analyses

Plant specimens were collected, tagged then placed in plant presses from the study site, the valley of the Baytoriver,Sta Cruz which leads up the Acoje mine site. This was not far from the original discovery of a new Ni hyperaccumulator in the ultramafic outcrop in the Bayto river valley (Doronila et al., 2010) On return to the field camp semi quantitative screening for Ni accumulation was performed on washed plant material. Leaf, stem and root fragments were crushed on prepared filter paper, which were previously impregnated with 1% dimethylglyoxime dissolved in 95% ethanol. Formation of pink to red colour indicates a Ni concentration > 1000 µg/g of dry plant matter (Reeves, 2003). Further elemental analyses were performed on the plant material.Elemental analyses of plant material was followed the method developed at Massey University (Reeves, 2003). The procedure is briefly described. The plant material was air-dried for a week then selected material for analysis was then oven-dried overnight at 60 ^{o}C. About 25 mg was weighed to 0.1 mg into borosilicate tubes for dry ashing in a muffle furnace. The temperature was raised to 200 ^{o}C over 1 hour and then to 500 ^{o}C for 4 hours. After cooling overnight the ash was dissolved in 5.00 mL of 2M HCl. The solutions were then analyzed for the metals Cu, Co, Cr and Ni with the aid of an atomic absorption spectrophotometer. Surface soil samples were also collected from 10 different sites in the plant study area. These were air dried in the laboratory then the following standard analyses were performed on the 2mm fraction obtained after sieving: soil pH and electrical conductivity in H_2O, organic matter (%) and cation exchange capacity (me/100g soil). Soil samples were also digested with aqua regia (3:1 conc Hydrochloric acid: Nitric acid) then diluted appropriately for metals analyses of Cu, Co, Cr and Ni with the aid of an atomic absorption spectrophotometer.

Results and Discussion

Soil sampling

The mean and standard deviation of metal concentration (% n = 10) for Ni, Cu, Co, Cr and Fe in the soil samples from the Acoje, Zambales field survey are respectively: 0.77 ± 0.15; 0.01 ± 0.001; 0.05 ± 0.02; 1.01 ± 0.05 and 25.34 ± 7.22. These are typical values from ultramafic outcrops (Brooks 1998). Moreover, physicochemical values obtained from the soil samples (n = 10) were pH 5.9 ± 0.3, % organic matter 3.6 ± 1.6, Electrical conductivity 102.6 ±52.3 µs/cm and cation exchange capacity 42.1 ± 19.9 meq/100g soil. These values are similar to other tropical serpentine tropical regions (Brooks, 1987).

Metal uptake by Plants

A collection of 33 species from 21 plant families was obtained from a field survey and these were analyzed for their metal content. The metal uptake of the species is presented in tables 1 - 3 which summarizes them according to the operational classification of metal uptake used by Reeves (2003). Aside from nickel Cu, Co and Cr were also analysed in the plant tissues. The levels obtained were considered to be typical values in most plants. The main aim of the study was to investigate the nickel uptake. Five species from 5 plant families were non-accumulators (<100 µg/g of Ni in their dry tissue) Table 1.

Table 1. Non-nickel accumulating species from Acoje, Zambales (< 100 µg/g Ni in dry matter). Tissue levels of copper, cobalt and chromium are also included.Bdl = below detection limit.

Family	Species	Tissue	Nickel	Copper	Cobalt	Chromium
Calophyllaceae	*Calophyllum pentapetalum*	Leaves	99	4	8	1
		Stems	47	7	2	bdl
Goodeniaceae	*Scaevola micrantha*	Leaves	99	6	13	-
		Stems	88	5	24	-
Myrsinaceae	*Ardisia sp.*	Leaves	79	2	1	4
		Stems	39	4	1	bdl
Myrtaceae	*Leptospermum flavescens*	Leaves	69	4	6	-
		Stems	69	4	10	-
Podocarpaceae	*Podocarpus sp.*	Leaves	58	2	1	1
		Stems	33	4	2	1

The second most frequent group were the nickel hyperaccumulators (>1000 µg/g of Ni in their dry tissue) Table 2 wherein 9 species from 7 plant families had elevated Ni content. Of these *Phyllanthus erythrotichus* (>10,000 µg/g)was found to be a hypernickelophore. This is a discovery of the 2nd extreme species of the genera in the Philippines. The previous one, *Phyllanthus balgooyi* was recorded together with *Dichapetalum gelanoidess* sp. *Tuberculatum* (Dichapetalaceae) in 1986 from Mt Bloomfield in Palawan (Baker et al., 1992). Hemiaccumulator species (100 - 999 µg/gof Ni in their dry tissue) Table 3 and 4 were the most abundant group with 19 species belonging to 15 plant families.

The current research project is surveying the major ultramafic regions of the Philippines and to date has increased the number of hyperaccumulating species to 14. To put this in context, approximately 50 taxa are known to hyperaccumulate Ni in New Caledonia (Reeves et al., 1996). This number is second only to Cuba, where approximately 130 hyperaccumulators have been identified (Reeves et al., 1996). The potential use of hyperaccumulators for harvesting metals requires a concerted effort firstly through discovering these species.

Harnessingthe capacity then of the fast growing indigenous hypernickelophores would be the optimal strategy. Many are shrubs of 1–5 m in stature, including species of *Buxus, Ariadne, Rinorea, Psychotria, Euphorbia* and *Phyllanthus* from Cuba, New Caledonia, or parts of the Philippines and Indonesia; these appear to have potential for use in rehabilitation, remediation or phytomining of ultramafic soils in tropical countries (Reeves, 2003). This technology can be accessible to third world agrarian communities, which rely on small-scale mining methods to enhance their livelihoods. They can propagate hyperaccumulators which they can farm the spent Ni laterite tailings followed by harvesting, burning and ashing the biomass to produce a nickel concentrate.

Table 2. Nickel hyperaccumulator species from Acoje, Zambales (> 1000 µg/g in dry matter). Tissue levels of copper, cobalt and chromium are also included.Bdl = below detection limit.

Family	Species	Tissue	Nickel	Copper	Cobalt	Chromium
Chrysobalanaceae	*Licania splendens*	Leaves	2,728	7	15	2
		Stems	681	7	4	4
Flacortaceae	*Xylosmalu zonense*	Leaves	1,795	7	4	bdl
		Stems	141	3	1	bdl
Myrtaceae	*Syzgium sp.*	Leaves	1,006	6	3	10
		Stems	107	5	2	8
	Decaspermum blancoi	Leaves	1,996	-	-	-
		Stems	1,845	-	-	-
Phyllanthaceae	*Breyniasp 1.*	Leaves	3,593	17	16	9
		Stems	602	9	5	6
	Phyllanthus erythrotichus	Leaves	17,519	7	35	13
	*New hypernickelophore	Stems	973	5	7	44
Rubiaceae	*Psychotria sp.*	Leaves	795	4	1	8
		Stems	1,402	4	3	5
Sapotaceae	*Planchonella obovata*	Leaves	1,005	2	bdl	bdl
		Stems	640	2	bdl	bdl
Verbenceae	*Callicarpa sp.*	Leaves	1,052	17	5	36
		Stems	331	-	-	-

Table 3. Nickel hemiaccumulator species from Acoje, Zambales (100 – 199 µg/g Ni in dry matter). Tissue levels of copper, cobalt and chromium are also included.Bdl = below detection limit.

Family	Species	Tissue	Nickel	Copper	Cobalt	Chromium
Annonaceae	*Haplosticanthus lanceolatus*	Leaves	141	3	bdl	bdl
		Stems	80	10	bdl	bdl
Combretaceae	*Terminalia pellucida*	Leaves	161	8	3	8
		Stems	143	5	2	4
Pinaceae	*Pinusmerkusii*	Leaves	118	5	2	10
		Stems	47	5	1	4
Rubiaceae	*Mussaenda chlorantha*	Leaves	177	5	2	bdl
		Stems	62	17	1	bdl
Sapotaceae	*Diploknema ramiflora*	Leaves	139	4	1	2
		Stems	166	14	1	2

Table 4. Nickel hemiaccumulator species from Acoje, Zambales (200 – 999 µg/g Ni in dry matter). Tissue levels of copper, cobalt and chromium are also included.Bdl = below detection limit.

Family	**Species**	**Tissue**	**Nickel**	**Copper**	**Cobalt**	**Chromium**
Phyllanthaceae	*Breyniaracemosa*	Leaves	839	7	19	19
		Stems	140	4	4	`4
Rubiaceae	*Antirhea*sp.	Leaves	487	5	2	4
		Stems	185	7	1	1
Ochnaceae	*Brackenridgea fascicularis*	Leaves	434	3	2	4
		Stems	87	3	1	2
Anacardaceae	*Swintoniaacuta*	Leaves	403	3	2	<1
		Stems	75	5	6	<1
Rubiaceae	*Timonius arboreus*	Leaves	365	7	bdl	bdl
		Stems	104	5	2	bdl
Rubiaceae	*Psychotria ilocana*	Leaves	334	5	2	bdl
Dilleniaceae	*Dillenia luzonensis*	Leaves	327	7	2	1
		Stems	212	5	1	bdl
Fabaceae	*Dalbergia sp.*	Leaves	318	25	3	10
		Stems	98	23	3	bdl
Combretaceae	*Maranthes corymbosa*	Leaves	315	2	2	<1
		Stems	111	3	6	<1
Ebenaceae	*Diospy rosferrea*	Leaves	284	2	bdl	1
		Stems	312	6	bdl	bdl
Goodeniaceae	*Scaevola micrantha*	Leaves	99	6	13	-
		Stems	88	5	24	-
Lauraceae	*Litsea sp.*	Leaves	245	16	2	6
		Stems	63	3	1	4
Verbenaceae	*Vitex* sp.	Leaves	226	7	2	2
		Stems	58	6	1	<1
Myrtaceae	*Syzygium pallidum*	Leaves	208	4	bdl	7
		Stems	79	3	bdl	bdl

Acknowledgements

Financial support for this research was provided by a grant from the Philippines Department of Science and Technology(DOST) - Philippine Council for Industry and Energy Research and Development(PCIERD) for Dr MO Quimado and Prof ES Fernando and a travel grant from DOST Balikscientist program(BSP) for Dr AI Doronila. Sincere thanks to Ms Alicia Follosco, Senior researcher, GIS expert, Cordillera Studies Centre, University of the Philippines-Baguio College for producing the map of the study site.

References

Anon. 2008. A preliminary feasibility study of the Acoje Laterite project, Executive summary.Rusina Mining Co/Pl 90p.

Baker, A.J.M., Proctor, J., van Balgooy, M.M.J. and Reeves, R.D. 1992. Hyperaccumulation of nickel by the ultramafic flora of Palawan, Republic of the Philippines. In: The Vegetation of Ultramafic (Serpentine) Soils. Eds. J.Proctor, A.J.M Baker and R.D Reeves.pp. 291–304. Intercept Ltd., Andover, U.K.

Baker, A.M. and Brooks, R.R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. *Biorecovery* 1: 81-126.

Baker, A.J.M., McGrath, S.P., Reeves, R.D. and Smith, J.A.C. 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In N. Terry,G. Bañuelos, eds, Phytoremediation of Contaminated Soil and Water. CRC Press LLC, Boca Raton, Florida, pp 85-108.

Boyd, R.S. 2007. The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant and Soil* 293: 153-176.

Boyd, R.S. 2004. Ecology of metal hyperaccumulation. *New Phytologist* 162: 563-567.

Brooks, R.R. 1987. Serpentine and its vegetation: A multidisciplinary approach. Dioscorides Press, Portland, Oregon.

Brooks, R.R. 1998. Plants that Hyperaccumulate Heavy Metals. CAB International, Oxon & New York.

Brooks, R.R., Morrison, R.S., Reeves, R.D., Dudley, T.R. and Akmans, Y. 1979. Hyperaccumulation of nickel by *Alyssum* Linnaeus (Cruciferae). Proceedings of the Royal Society of London B 203: 387-403.

Doronila, A.I., Unson, J.R.S., Penaranda, M.C.R., Gotera, K.M.C. and Claveria, R.J.R. 2010. The discovery of a nickel hyperaccumulator plant, *Breynia*sp. in the ultramafic terrains of Zambales, Philippines.Proceedings of the 1st

National Conference on Bioremediation. 26-27 Oct 2010. Traders Hotel, Pasay City, Philippines. p18.

Gambi, O.V., Pancaro, L. and Gabbrielli, R. 1979. Investigations on a nickel accumulating plant: *Alyssum bertolonii* Desv. II. phosphorous, potassium, iron and trace element content and distribution during growth. *Attidella Societa Toscana di Scienze Naturali, Serie B* 86: 317-329.

Homer, F.A., Reeves, R.D., Brooks, R.R. and Baker, A.J.M. 1991. Characterization of the nickel-rich extract from the nickel hyperaccumulator *Dichapetalum gelonioides*. *Phytochemistry* 30: 2141-2145.

Krämer, U., Cotter-Howells, J.D,, Charnock, J.M., Baker, A.J.M. and Smith, J.A.C. 1996. Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379: 635-638.

Krämer, U., Grime, G.W,, Smith. J.A.C., Hawes, C.R. and Baker, A.J.M. 1997. Micro-PIXE as a technique for studying nickel localization in leaves of the hyperaccumulator*Alyssum lesbiacum*. *Nuclear Instruments and Methods in Physics Research B-Beam Interactions with Materials and Atoms* 130: 346-350.

Krämer, U., Pickering, I.J., Prince, R.C., Raskin, I. and Salt, D.E. 2000. Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. *Plant Physiology* 122: 1343-1353

Küpper, H., Lombi, E., Zhao, F., Wieshammer, G. and McGrath, S.P. 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspigoe singense*. *Journal of Experimental Botany* 52: 2291-2300.

Lombi, E., Zhao, F.J., Dunham, S.J. and McGrath, S.P. 2001. Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. *Journal of Environmental Quality* 30: 1919-1926.

Macnair, M. 2003.The hyperaccumulation of metals by plants. *Advances in Botanical Research* 40: 63-105.

Macnair, M.R., Bert, V., Huitson, S.B., Saumitou-Laprade, P. and Petit, D. 1999. Zinc tolerance and hyperaccumulation are genetically independent characters. *Proceedings of the Royal Society of London* 266: 2175-2179.

Martens, S.N. and Boyd, R.S. 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia* 98: 379-384.

McGrath, S.P. 1998. Phytoextraction for soil remediation. In R. R. Brooks, ed, Plants That Hyperaccumulate Heavy Metals. CAB International, Oxon & New York, pp 261-287.

Minguzzi, C. and Vergnano, O. 1948. Il contenuto di nichelnelleceneri di *Allysum bertolonii* Desv.considerazionibotaniche e geochimiche. *Attidella Societa Toscana di Scienze Naturali, Serie A* 55: 1-28.

PAGASA. 2012. Philippine atmospheric, geophysical and astronomical services administration. http://www.pagasa.dost.gov.ph/index.php?option=com_content&view=article&id=157:philippine-climate&catid=93:climate-data-and-statistics&Itemid=127.

Pollard, A.J. 2000. Metal hyperaccumulation: a model system for coevolutionary studies. *New Phytologist* 146: 179-181.

Proctor, J. 2003. Vegetation and soil and plant chemistry on ultramafic rocks in the tropical Far East. *Perspectives in Plant Ecology, Evolution and Systemati*cs 6: 105-124.

Reeves, R.D., Baker, A.J.M, Borhidi, A. and Berazaín, R. (1996). Nickel-accumulating plants from the ancient serpentine soils of Cuba. *New Phytologist* 133(2): 217-224.

Reeves, R.D. and Baker, A.J.M. 2000. Metal-accumulating plants. In IRaskin, BD Ensley, eds, Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. John Wiley and Sons, New York, pp 193-221.

Reeves, R.D. 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil* 249: 57 – 65.

Robinson, B.H., Lombi, E., Zhao, F.J. and McGrath, S.P. 2003. Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheyacoddii*. *New Phytologist* 158: 279-285.

Whiting, S.N., Reeves, R.D., Richards, D., Johnson, M.S., Cooke, J.A., Malisse, F., Paton, A., Smith, J.A.C., Angle, J.S., Chaney, R.L., Ginocchio, R., Jaffrè, T., Johns, R., McIntyre, T., Purvis, O.W., Salt, D.E., Schat, H., Zhao, F.J. and Baker, A.J.M. 2004. Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology* 12: 106-116