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An evaluation of the bioavailability and bioaccumulation of selected metals occurring in a wetland area on the volcanic island of Guam,

Bob Wilson<sup>1,\*</sup>, Brian Pyatt<sup>1</sup>, Gary Denton<sup>2</sup>

Western Pacific Ocean

School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, UK. E-mail: bob.wilson@ntu.ac.uk
 Water and Environmental Research Institute of the Western Pacific, University of Guam, UOG Station, Mangilao, Guam 62923, USA

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#### Abstract

This initial research examined the presence, distribution and bioavailability of Cu, Cr, Ni, Mn and Fe in a wetland area of southern Guam. The research sites are within an area covered with saporite, a soil type derived from volcanic deposits on the island. Leaf tissue of *Pandanus tectorius* was extracted and analysed to determine the bioaccumulation of the target metals. Metal accumulation at sites considered aerobic and anaerobic was investigated together with an attempt to correlate actual accumulation of the target metals in the plant tissue with a recognised bioavailability indicator, in this case, three step sequential extraction scheme. Manganese was found to be accumulated in relatively high concentrations and to a lesser extent Cu was also accumulated. Chromium, Ni and Fe however exhibited very low accumulation factors. Accumulation of Mn in particular was significantly affected by aerobic conditions whereas the converse effect was experienced by Cu. Significant correlation between various steps of a Sequential Extraction Scheme and actual accumulation was not achieved although the degree of aerobic conditions at each site and soil pH did affect concentrations of metals extracted by differing steps of SES. Results obtained suggest that further research in the area should be undertaken using different plant species and tissues.

**Key words**: Guam; bioaccumulation; sequential extraction; bioavailability; accumulation factor; heavy metals **DOI**: 10.1016/S1001-0742(08)62453-5

# Introduction

Guam is the southern most island of the Mariana chain in the Western Pacific Ocean and is a United States Territory. Volcanic rock forms the foundation of the island and is exposed over about 35% of the islands surface predominantly in southern Guam (Gingerich, 2003) where the Taelayag River basin is situated and this region constituted the research area (Fig. 1). The underlying rocks in the study area originate from the Eocene and Oligocene periods (Reagan and Meijer, 1984) and the permeability of the formations are considered low (Ward *et al.*, 1965). The watersheds of southern Guam are scattered with perennial and seasonal wetlands. Their location, configuration and long term evolution result from several hundred years of diverse land use superimposed on bedrock geology (Seigrist *et al.*, 1997).

In 1997, Seigrist *et al.*, researched the aqueous chemistry of the area and noted the predominance of *Pandanus tectorius*, a monocot tree, in the vicinity together with an analysis of the soil in various sample collection sites. Relatively high concentrations of Mn (> 1000 mg/kg), Ni (> 300 mg/kg) and Cr (> 350 mg/kg) were also reported

\* Corresponding author. E-mail: bob.wilson@ntu.ac.uk

in the soil at some of these sites. Additionally, their report contained indications that sites varied from aerobic to anaerobic conditions and with subsequent differing degrees of lability of metal cations in varying soil samples.

Research into the bioavailability of heavy metals and subsequent accumulation has been carried out in varying climatic conditions (Jung *et al.*, 2002; Wilson *et al.*, 2005a; Wilson and Pyatt, 2007a, 2007b, 2007c). Wilson and Pyatt in 2006 also investigated bioaccumulation of tungsten and associated metals at an abandoned tungsten mine and demonstrated recognised monitoring procedures produced varying indications of bioavailability of tungsten in particular which appeared to be related to pH values of extractants used. They also hypothesised that soil pH and redox potential would therefore affect the degree of bioaccumulation, by plants, of metals from soil in which plants were growing.

Work investigating the effects of soil pH on bioavailability and actual bioaccumulation by plants is ongoing, however it was decided to investigate three sites in the southern Guam wetlands to determine whether aerobic, anaerobic wetland and non-wetland conditions affect bioaccumulation of Cu, Cr, Ni, Mn and Fe by leaf tissue of *P. tectorius*. The wetland soil is composed of highly



Fig. 1 Location of Guam research area and position of sampling sites.

 Table 1
 Samples collected from three sites in southern Guam with altitude

Sample ref.	Туре	Location	Altitude (m)
0508GU5	Pandanus leaf	Site 1	39
0508GU7	Soil (pH 6.94)	Site 1	
0508GU9	Pandanus leaf	Site 2	39
0508GU11	Soil (pH 7.05)	Site 2	
0508GU12	Pandanus leaf	Site 3	92
0508GU14	Soil (pH 5.2)	Site 3	

weathered saprolites and has a thickness of between 92 and 210 cm above weathered volcanic bedrock. It was subjected to a Sequential Extraction Scheme (SES) to investigate the mobility characteristics of the above metals in relation to levels found in *P. tectorius* leaves. The information obtained would determine whether more extensive research should be undertaken in the area.

# 1 Materials and methods

### 1.1 Sample collection and preparation

Samples of soil and *P. tectorius* leaves were collected from the wetland area at two sites. Site 1 was on the East bank of a stream considered to be anaerobic (Siegrist *et al.*, 1997) and from the West bank of a stream considered by the authors to be aerobic in nature. The sites were approximately 10 m apart and approximately 20 m upstream from the confluence of the two streams (Fig. 1). As shown in Fig. 1, a further set of samples was collected at site 3, an upland area approximately 400 m SE of sites 1 and 2, above the wetland area and of similar geochemical characteristics. Altitudes of the collection sites and sample types collected are shown in Table 1.

Leaves were collected from the same aspect of trees

of approximately the same diameter and height. Approximately 100 g of leaf samples were collected from each site and placed in clean polythene bags. All relevant soil samples were collected within 20 cm of the base of the *P. tectorius* trees using a stainless steel trowel. The initial 5 mm of soil in an approximately 100 cm<sup>2</sup> area was scraped off and discarded. Approximately 200 g of soil were collected to a maximum depth of 50 mm at each site and placed in a clean polythene bag. All samples were triple bagged to prevent transit damage and extraneous contamination.

Leaf samples were washed thoroughly with deionised distilled water and air dried for 24 h at 25°C in a positive pressure drying cabinet. The leaves were then further dried for 24 h at 70°C (Radojefic and Bashkin, 1999). Approximately 10 g of the dried foliage were ground for 10 min in a Pulverisette 2 (Fritsch, Germany), fitted with agate grinding equipment and final down force of 15 daN, before transfer to HDPE storage bottles. The soil samples were air dried for 7 d in a drying cabinet at 25°C and placed in an oven for 6 h at 40°C (Radojefic and Bashkin, 1999). After cooling the samples were passed through a 2-mm sieve and ground for 10 min in Fritsch Pulverisette 2 fitted with agate grinding equipment and at a down force of 10 daN, before transfer to clean HDPE storage bottles.

### 1.2 Soil pH determination

The 5.0 g dried soil was placed in a sample tube and 20 mL of demineralised distilled water added. The tube was shaken vigorously for 5 min and allowed to stand for 1 h before centrifuging at 4000 r/min for 20 min. The supernatant liquid was tested using a calibrated pH 210 Microprocessor pH meter (Hanna, UK), fitted with a HI 1230B probe.

### 1.3 Pseudototal metal extraction

Prepared soil  $(1.0 \pm 0.05)$  g  $((0.5 \pm 0.025)$  g vegetation) was weighed into a 50-mL conical flask, and then 10 mL of aqua regia were added and the flask simmered for 90 min on a hotplate in a fume cupboard. The flask was removed, cooled before a further 4 mL of aqua regia were added. After simmering for a further 30 min the flask was cooled and the contents filtered through a Whatman 541 filter paper into a clean 50 mL graduated flask. The 50 mL conical flask, filter paper and funnel were washed through thoroughly and the final volume made up with 0.5 mol/L HNO<sub>3</sub>. The extract was transferred to a HDPE bottle and stored in a refrigerator at 5°C until required for analysis (Wilson *et al.*, 2005b).

## 1.4 Sequential extraction scheme

The SES used during this research was an optimised BCR three step procedure (Pueyo *et al.*, 2001) and used by Prez and Valiente (2005) to demonstrate the potential for this procedure when monitoring an abandoned mine site.

The metal concentrations determined in each step of the BCR SES are classified as: (1) acid soluble; (2) reducible; (3) oxidizeable. Any remaining metals after SES were termed residual and were unlikely to be available for bioaccumulation for a considerable time (Sutherland and Tack, 2003). The soil  $(1.0 \pm 0.05)$  g was weighed into a 50-mL centrifuge tube and subjected to the following SES.

Step 1: the tube was rotated on a SB3 rotator (Stuart Ltd., UK) at 25 r/min and at  $(22 \pm 2)^{\circ}$ C for 16 h with 20 mL of 0.11 mol/L acetic acid. The sample was centrifuged for 15 min at 4000 r/min. And the supernatant decanted and saved in a HDPE bottle for analysis. Twenty milliliters of water were added to tube and shaken for 20 min before being again centrifuged at 4000 r/min for 15 min and the supernatant was discarded.

Step 2: the tube was shaken for 16 h with 20 mL of 0.5 mol/L hydroxyl ammonium chloride containing 25 mL of 2 mol/L HNO<sub>3</sub> per liter. Analyte reclamation and washing procedures were carried out as in Step 1.

Step 3: 10 mL of 30% hydrogen peroxide was added to the residue in each tube, in small aliquots to avoid possible violent reaction. The tube was loosely covered and left at room temperature for 1 h, shaking occasionally. The digestion was continued by placing tubes in a water bath at  $(85 \pm 2)^{\circ}$ C, with occasional manual shaking for the first 30 min. The volume of the liquid was reduced to less than 0.5 mL over a period of approximately 6 h. Twenty millilitres of 1.0 mol/L ammonium acetate adjusted to pH 2.0 was added to each tube, and shaken for 16 h as in the other stages. Analyte was reclaimed as in other steps.

#### 1.5 Analysis

In all cases, six replicates of each soil and vegetation sample were extracted and analysed using a Optima 2100 ICP-OES (Perkin Elmer, USA) fitted with an AS-90 Plus autosampler. The instrument was calibrated using mixed Cu, Cr, Ni, Mn and Fe solution of 0.5 mg/L and 5.0 mg/L prepared from 1000 mg/L certified standards, diluted with processed extractant which was also used as a blank during calibration. Standard operating parameters of the instrument used are shown in Table 2.

## 2 Results and discussion

### 2.1 Metal concentrations

Table 3 indicates the metal concentrations determined in leaf and soil samples collected from all sites. High concentrations of Fe were to be expected however Mn concentrations are also relatively high in soil and leaf tissue. In contrast the Cr levels noted in soil were not reflected in *P. tectorius* leaves.

In order to put these values in perspective the authors use a value which they have termed accumulation factor (Wilson and Pyatt, 2006, 2007b) and is expressed as a percentage of the metals contained in the soil in which the plant examined was growing.

The highest accumulation factor at site 1, is achieved by Cu, however, at sites 2 and 3 accumulation factors are highest for Mn. Iron exhibits very low values at all sites.

 
 Table 2
 Standard wavelength and survey range for analysing target metals on Perkin Elmer 2100 ICP-OES

Element	$\lambda(nm)$	Survey low (nm)	Survey up (nm)	Peak algorithmm	Points per peak
Cu	327.393	327.244	327.542	Area	7
Cr	267.716	267.599	267.833	Area	7
Ni	231.604	231.501	231.707	Area	7
Mn	257.610	257.725	257.495	Area	7
Fe	238.204	238.096	238.612	Area	7

#### 2.2 Sequential extraction

Table 4 illustrates the metal concentrations extracted at each stage of SES when applied to the three soil samples collected on this occasion together with pH values. Percentage of total Cu extracted is the greatest during step 2 and is of the same order of magnitude for all sites. Copper extracted during step 1 from soil at site 3 however is significantly higher than at other sites. Percentage of Mn extracted from soil at sites 2 and 3 are much higher than that at site 1 particularly during step 2 extraction. Total iron extraction during all stages of SES is low when compared to Ni and Mn, and the extracted concentration is highest at site1, the anaerobic site.

The accumulation factors determined for *P. tectorius* leaf tissue (Table 5) highlight a distinct difference for Cu and Mn. Copper is accumulated to a greater concentration at wetland sites 1 (anaerobic) and 2 (aerobic) site than upland site 3 (aerobic).

The accumulation factor for Cu at site 3 is also the highest recorded and this may be due to the lower pH (5.2) of the soil at this site since Wilson and Pyatt (2007c) recorded this phenomenon at various abandoned mining sites. Conversely Mn accumulation factor in *P. tectorius* from site 2 was twice that recorded at site 1. Gal *et al.* (2007) proposed that low pH produced comparatively aerobic conditions and high pH produced comparatively anaerobic conditions and the data recorded here appears to confirm their hypothesis. Iron accumulation factors are low at all sites but this is a reflection of the very high concentrations of Fe in soil from each site.

Total percentage of Mn extracted by all stages of SES (Table 4) is much higher (82%–91%) at the aerobic sites when compared with the anaerobic site (21.5%). The highest percentage per step of SES of Mn from soil at aerobic sites was extracted during step 2 whereas step 3 extracted the highest percentage at the anaerobic site. This agrees with the proposition made by Sutherland and Tack (2003) that step 2 is the reducible element and step three is the oxidizeable element of the procedure. Small percentages of Fe were extracted at each site which reflect the accumulation factors in Table 4. However, a relatively high percentage of Ni (18%-28%) was extracted from soil at all sites but this was not reflected by the accumulation factors achieved in P. tectorius leaf tissue. Only small percentages of Cr were extracted by all three stages of SES relative to Cu and Mn although step 3 extracted a significantly higher percentage than steps 1 and 2. This is also reflected in the accumulation factors for the same leaf tissue.

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Table 3	Pseudo metal	concentration i	n soils and	d P tectorius	leaves collected	at three sites	in South	Guam
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Sample number	Туре	Cu mean (mg/kg)	Cu RSD (%)	Cr mean (mg/kg)	Cr RSD (%)	Ni mean (mg/kg)	Ni RSD (%)	Mn mean (mg/kg)	Mn RSD (%)	Fe mean (mg/kg)	Fe RSE (%)
0508GU5	P. tectorius leaf	8.47	3.1	1.39	2.7	1.45	6.5	1309	5.7	120	3.3
0508GU7	Soil	90.1	1.84	209	1.5	123	1.7	14667	1.2	22800	0.5
0508GU9	P. tectorius leaf	6.80	4.8	1.46	11.2	1.12	7.5	712	2.5	98.8	1.5
0508GU11	Soil	143	1.8	369	2.4	259	3.8	3697	3.5	99000	0.9
0508GU12	P. tectorius leaf	10.2	1.4	1.19	1.1	2.96	1.3	473	2.9	59.3	5.7
0508GU14	Soil	147	2.5	280	2.4	124	5.6	3037	1.6	99900	1.8

Table 4 Metal concentrations extracted Guam wetland samples at various stages of SES together with relative RSD and pH values

Sample	Metal data	SES step 1 (mg/kg)	Total (%)	SES step 2 (mg/kg)	Total (%)	SES step 3 (mg/kg)	Total (%)	Total extract (mg/kg)	Total (%)	Total (mg/kg)
0508GU7	Cu mean	0.89	1.0	9.90	11.0	6.89	7.6	17.68	19.6	90.2
рН 6.94	Cu RSD (%)	4.7		2.0		4.3				
(Site 1)	Cr mean	0.28	0.1	4.40	2.1	6.34	3.0	11.02	5.3	209
	Cr RSD (%)	2.1		2.3		1.8				
	Ni mean	2.07	1.7	13.5	10.9	7.39	6.0	22.96	18.5	124
	Ni RSD (%)	1.0		1.0		1.8				
	Mn mean	461	3.1	1556	10.6	1140	7.8	3157	21.5	14667
	Mn RSD (%)	1.04		4.9		3.2				
	Fe mean	8.81	0.003	15424	6.7	7018	3.1	22451	9.8	228488
	Fe RSD (%)	5.1		2.0		3.3				
0508GU11	Cu mean	0.87	0.6	21.4	15.0	6.47	4.5	28.74	20.1	143
pH 6.84	Cu RSD (%)	2.3		2.8		1.1				
(Site2)	Cr mean	0.18	0.05	3.07	0.8	17.0	4.6	20.25	5.5	369
	Cr RSD (%)	2.6		2.8		2.9				
	Ni mean	2.19	0.8	29.9	11.5	22.3	8.6	54.39	21.0	259
	Ni RSD (%)	2.0		4.1		4.5				
	Mn mean	79.6	2.2	2563	69.3	402	10.8	3045	82.4	3697
	Mn RSD (%)	2.0		4.1		2.8				
	Fe mean	1.94	0.002	3044	3.1	592	0.6	3638	3.7	98896
	Fe RSD (%)	6.1		3.0		1.9				
0508GU14	Cu mean	7.07	4.8	26.0	17.7	12.0	8.2	45.07	30.6	147
pH 5.2	Cu RSD (%)	1.4		2.7		3.0				
(Site 3)	Cr mean	0.21	0.07	1.74	0.62	14.2	5.1	5.8		280
	Cr RSD (%)	3.6		1.4		0.9				
	Ni mean	1.90	1.5	15.1	12.1	18.5	14.9	35.5	28.6	124
	Ni RSD (%)	0.5		1.3		4.1				
	Mn mean	184	6.0	2257	74.3	331	10.9	2772	91.2	3038
	Mn RSD (%)	1.1		0.9		1.6				
	Fe mean	1.13	0.001	1316	1.5	273	0.32	1590	1.9	85283
	Fe RSD (%)	4.7		0.8		4.7				

 Table 5
 Accumulation factors (AF) (%) for P. tectorius leaf samples collected at Southern Guam wetland sites

Sample	Location	Туре	Cu (AF) (%)	Cr (AF) (%)	Ni (AF) (%)	Mn (AF) (%)	Fe (AF) (%)
0508GU5	Site 1	P. ectorius leaf	9.4	0.66	1.1	8.9	0.05
0508GU9	Site 2	P. tectorius leaf	4.8	0.40	0.43	19.3	0.1
0508GU12	Site 3	P. tectorius leaf	6.9	0.42	2.4	15.6	0.06

The only significant correlation determined by various steps of SES is exhibited by step 1 for Ni, all other data do not significantly correlate (Table 6). Of course only three samples were compared on this occasion but it would appear that SES values obtained do not reflect the actual accumulation taking place.

# **3** Conclusions

*P. tectorius* leaves accumulated significant concentrations of Mn and to a lesser extent Cu and Fe from soil in a wetland area of Guam. Soil conditions in terms of redox potential appear to affect the bioavailability of some metals, in particular Mn, although Cr was not accumu**Table 6** Regression values  $(R^2)$  (p = 0.05) obtained when comparingmetal concentration data sets for steps of SES soil extraction and metalconcentration in leaf tissue of *P. tectorius* grown in Guam wetland soils(n = 3)

Parameter	Cu	Cr	Ni	Mn	Fe
Step 1 vs. leaf	0.761	0.007	0.9362	0.7279	0.6814
Steps $(1 + 2)$ vs. leaf	0.2436	0.4819	0.3323	0.679	0.7883
Steps $(1 + 2 + 3)$ vs. leaf	0.3609	0.035	0.0791	0.7883	0.6749

lated readily by this plant. Various steps of a three step SES procedure indicated that soil condition appears to affect total metal extracted and the concentration extracted during each step. The results obtained here indicate that further research should be carried out to investigate whether different tissues of *P. tectorius* accumulate different concentrations of the metals occurring in the soil, that is, will partitioning occur and if different soil conditions affect this situation. More extensive sampling of soils and differing vegetation would also determine if correlation exists between differing bioavailability indicators.

### References

- Gal J, Hursthouse A, Cuthbert S, 2007. Bioavailability of arsenic and antimony in soils from an abandoned mining area, Glendinning (SW Scotland). *Journal of Environmental Science and Health, Part A*, 42: 1263–1274.
- Gingerich S B, 2003. Hydrologic resources of Guam. Honolulu: US Geological Survey, Report 03–4126.
- Jung M C, Thornton I, Chon H T, 2002. Arsenic, Sb and Bi contamination of soils, plants, waters and sediments in the vicinity of the DalsungCu-W mine in Korea. *Science of the Total Environment*, 295: 81–89.
- Pérez G, Valiente M, 2005. Determination of pollution trends in an abandoned mining site by application of a multivariate statistical analysis to heavy metals fractionation using SM&T-SES. *Journal of Environmental Monitoring*, 7: 29– 36.
- Pueyo M, Rauret G, Luck D, Yli-Halla M, Mutau H, Quevauviller P H et al., 2001. Certification of the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn in a freshwater sediment following a collaboratively tested and optimised three step sequential extraction procedure. *Journal of Environmental Monitoring*, 3(2): 243–250.
- Radojefic M, Bashkin V N, 1999. Practical Environmental Analysis. Cambridge: The Royal Society of Chemistry. 293.
- Reagan M K, Meijer A, 1984, Geology and geochemistry of early arc-volcanic rocks from Guam. Boulder: Geological Society of America. Bulletin v.95. 701–713.
- Seigrist Jr H G, Denton G R W, Wood H R, Conception L, Lewis R R, 1997. Aqueous chemistry of a perennial wetland in Southern Guam. Guam, Water and Energy Research Institute of the Western Pacific. Technical Report 79.
- Sutherland R A, Tack F M G, 2003. Fractionation of Cu, Pb and Zn in certified reference soils SRM 2710 and SRM 2711 using an optimized BCR sequential extraction procedure. *Advanced Environmental Research*, 8: 37–50.
- Ward P E, Hoffard S H, Davis D A, 1965. Hydrology of Guam. Guam. US Geological Survey Professional Paper 403-H.
- Wilson B, Lang B, Pyatt F B, 2005a. The dispersion of heavy metals in the vicinity of Britannia Mine, British Columbia, Canada. *Ecotoxicology and Environmental Safety*, 60: 269– 276.
- Wilson B, Braithwaite A, Pyatt F B, 2005b. An evaluation of procedures for the digestion of soils and vegetation from areas with metalliferous pollution. *Toxicology and Environmental Chemistry*, 87: 335–344.
- Wilson B, Pyatt F B, 2006. Bio-availability of tungsten in the vicinity of an abandoned mine in the English Lake District and some potential health implications. *Science of the Total Environment*, 370: 401–408.
- Wilson B, Pyatt F B, 2007a. Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, UK. *Ecotoxicology and Environmental Safety*, 66: 224–231.

Wilson B, Pyatt F B, 2007b. Heavy metal bioaccumulation by the

important food plant, *Olea europaea* L., in an ancient metalliferous polluted area of Cyprus. *Bulletin of Environmental Contamination and Toxicology*, 78: 390–394.

Wilson B, Pyatt F B, 2007c. Bioaccumulation and partitioning of heavy metals in vegetation growing in the vicinity of abandoned copper and tungsten mines. In: Progress in Environmental Science and Technology (Wang Y, Li S, Huang P, Yang Y, An Y, Sun X, eds.). Beijing: Science Press Inc. 1, 151–159.