



Foliar Heavy Metal Concentrations of 19 Tree Species Grown on a Phytocapped Landfill Site

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KV and NA have designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors have read and approved the final manuscript.

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ABSTRACT

An alternative landfill capping technique 'Phytocapping' (establishing plants on the waste directly, or on a layer of soil placed over the waste) was trailed at Rockhampton, Australia, as it is eco-friendly, less expensive and socially acceptable. In this capping trees are used as 'Bio-pumps and Screen' and soil cover as a 'Storage'. They together minimise water percolation into buried waste leading to reduced leachate production. Twenty one tree species were grown on two soil depths and monitored for their growth and their ability to restrict water infiltration through the buried waste. A very common question raised by most scientist and engineers is the heavy metal uptake by the tree species and its impact on flora and fauna. Hence to determine the heavy metal concentration in trees species and its cycle within the phytocapping system, foliar and foliar litter heavy metal concentrations were measured in all the tree species grown on the phytocapped landfill site. Results from this analysis suggest that heavy metal composition of the leaves show no real elevated concentrations except in *Glochidion lobocarpum* which showed high levels of cobalt and *Acacia harpophylla* and *Hibiscus tiliaceus* which showed higher levels of arsenic cadmium respectively.

Keywords: Phytocapping; landfill; heavy metals; litter fall; foliage litter.

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1. INTRODUCTION

Plants grown in landfills are affected by surface environmental conditions as well as the nutrient supply from the buried waste [1]. Waste in a typical Municipal Solid Waste (MSW) constitutes more than 50% organics [2] which are the major sources of nutrients for plants established on landfills. Other than organic waste, landfills also contain heavy metals such as arsenic, boron, cadmium, chromium, cobalt, copper, iron, manganese, mercury, lead, nickel and zinc [3,4]. Consequently, trees grown on these landfills will be exposed to the above chemicals [5,6] and may be released into the environment through the food chain [7-10].

In general, heavy metal uptake by plants is influenced by bio-availability of heavy metals [11], organic matter content of the soil and soil temperature [12]. Trees take up heavy metals and store them in the leaves and branches [13-15] to protect themselves from insects and fungi [16]. Heavy metals that are taken up by trees are eventually distributed to the environment via litter fall [12,17,18].

However heavy metal availability may vary from one landfill to another and also within landfills [19]. Heavy metal concentrations of the plants grown on phytocaps were assessed with the view to confirming if the established plants were healthy, and also to test if the same plants accumulate unusual levels of heavy metals that could adversely impact on the environment.

Foliar chemical analysis is a good method to assess plant nutritional stress [20,21] and heavy metal concentration [9]; both of which are indicators of processes occurring at the ecosystem level [22]. Plants require heavy metals such as zinc, copper, manganese and iron in trace amounts to grow [23]. However, excessive uptake by plants may cause serious health problems to plants and micro and macro fauna [6]. Most landfill soils contain elevated levels of heavy metals [3], which may be released into the environment via trees [24]. Leaves are a good indicator of heavy metal concentrations in the root-zone and soil [25] and hence the foliage of species grown in the phytocapping system was assessed for their heavy metal concentrations.

Several researchers have shown great concern about the flow of heavy metals into the environment through litter fall and/or the food

chain. There have been concerns about lead concentrations in landfill soil because lead is toxic even at low concentration [26]. Scrap tyres and mechanical parts of vehicles found in many MSWs are a good source of zinc, cadmium, nickel and chromium [3]. Adefemi and Awokumi (2009) also reported the presence of arsenic, chromium and copper associated with waste from sludge incineration and fly ash. Heavy metals released into the environment have an adverse impact on macro-fauna such as caterpillars, earthworms, beetles, birds [7,8,10] and plants as they affect photosynthesis [11] which subsequently affect growth rate of plants [27]. This effect will vary between species [28] as photosynthesis reduction is dependent on canopy class, stand management, canopy dimensions, infections and seasonality [14]. However, studies in the past have reported low toxicity symptoms by trees [29] suggesting their use of enhanced tolerance mechanisms by evolving ecotypes that help gain more tolerance to heavy metals in order to survive under harsh conditions [30]. The aim of this study was to assess the health of plants grown in a phytocapping system by examining heavy metal uptake and their release into the ecosystem via litter fall.

2. MATERIALS AND METHODS

2.1 Site Establishment

An experimental site of 5000 m² area at the Lakes Creek Road Landfill, Rockhampton, Australia was selected for this study. The experimental site was established in October 2003. The site had two soil depths treatments (Thick soil cover, 1400 mm and Thin soil cover, 700 mm; Fig. 1). These treatments were replicated twice (total 4 plots). In the Thin soil cover, only 300 mm of sandy loam soil and 100 mm of green waste mulch was placed over the pre-existing 400 mm uncompacted clay soil (total soil cover of 700 mm). In the Thick soil cover, four layers of soil were placed over the pre-existing 400 mm clay soil. This consisted of 200 mm of sandy loam, 300 mm of Yaamba clay and 300 mm of Andersite clay, 200 mm of sandy loam soil and 100 mm of green waste mulch (soil cover of 1400 mm). Both Thick and Thin soil cover treatments were mulched with a layer of shredded green waste (100 mm). Eighteen seedlings of 21 species were planted at 2 m x 1m spacing (Fig. 2) in each plot (1 plot x 4). Two tree species out of the 21 grown did not survive.



Fig. 1. Thick and Thin soil covers



Fig. 2. Tree species planted at 2 m x 1 m spacing

Detailed foliar chemical analysis was undertaken to determine nutrient composition of 19 species grown on Thick and Thin phytocapping systems. Foliar analysis was conducted twice during this study; once in 2005 and then in 2006. In the first instance, the youngest fully expanded leaves were analysed for nutrients and heavy metals. Then, in the second instance mature, young and the youngest fully expanded leaves were analysed for nutrients and heavy metals. Results from the heavy metal analysis were compared with the heavy metal concentrations of soils/plants [10,30,31] (Table 1).

2.2 Youngest Fully Expanded Leaf (2005)

The youngest fully expanded leaves were collected from 9 plants per species per plot in the trial. Fifty to sixty such leaves were collected randomly from the 2 year-old trees and placed in labelled plastic bags which were placed in on ice in an insulated storage container. To ensure removal of dust from the leaves, the samples were washed subsequently in a series of four buckets of distilled water. Once washed, the samples were blot dried and then oven dried at

70°C for up to 96 hours until they attained a constant dry weight. Once completely dried, the leaf samples were ground to <600 µm using the Mikro-Feinmuhle-Culatti (MFC) grinder. The finely ground samples were then placed in polycarbonate tubes, labelled and sent for chemical analysis. The foliage nutrient concentrations of these samples were compared with the standard nutrient concentrations reported by [31,36,37,38] with the view to detecting whether the observed concentrations were low, adequate or excessive for plant growth.

2.3 Mature, Young and Youngest Fully Expanded Leaves (2006)

A mixture of mature, young and the youngest fully expanded leaves were sampled from 9 plants per species per plot. In addition, 50 to 60 leaves were randomly collected from the top, bottom and middle layers of the canopy of the 3 year-old trees. A similar procedure was followed as described in section above.

2.4 Leaf Litter

A 50 cm x 50 cm quadrat was used for leaf litter sample collection. Senescing leaves that were about to fall from the plants were also collected during this process. Leaves were collected in the 2 & 3 year-old plantation. The quadrat was thrown randomly between stands of 9 plants in thick and thin phytocaps and in both replications and leaf litter samples were collected within those randomly selected quadrats. Undecomposed leaf litter was collected from three quadrats per species in each replication. The leaf litter was washed free of dust as per live leaves, dried, ground and sent for chemical analysis.

2.5 Statistical Analysis

Mineral composition data was statistically tested for outliers, normality and homogeneity of error variances before being subjected to analysis of

variance (ANOVA) using Genstat ver. 13 [39,40]. The effects of soil thickness, species and the interactions between soil thickness and species were tested. The effects of time were also tested for the leaf parameters that were measured repeatedly. Least significance differences (l.s.d) are presented where the treatment, capping, species, time or their interactions were significant ($P < 0.05$). Standard errors are provided where there were insufficient data available for ANOVA or when the F test was found not significant ($P < 0.05$).

3. RESULTS AND DISCUSSION

Foliar and leaf litter compositions were used to determine variability in the performance of each species over two soil thicknesses and over time. Results from ANOVA are presented in Table 2.

Table 1. Baseline heavy metal concentrations in soils and plants

Elements	Plant/soil	mg kg ⁻¹	Reference
As	Soil	7.2	[30]
Pb	Soil	19	[30]
Ni	Soil	19	[30]
Cr	Plant	18	[32]
Co	Plant	2.75	[31]
Cd	Soil/Plant	0.35–0.40	[10, 30]
Se	Soil	1	[33]
Mo	Plant	1	[34]
Hg	Plant	0.16	[35]

Table 2. ANOVA for leaf and litter nutrient and heavy metal compositions (2005 & 2006)

Parameter	ANOVA	d.f.	Significance (P)
Foliar (heavy metals)			
	Cap	1	<0.001
	Species	18	<0.001
	Year	1	0.43
	Cap. Species	18	<0.001
	Cap. Year	1	0.54
	Species. Year	18	1
	Cap. Species. Year	18	0.999
Litter * (heavy metals)			
	Cap	1	0.38
	Species	12	<0.001
	Year	1	1
	Cap. Species	12	0.777
	Cap. Year	1	0.21
	Species. Year	12	1
	Cap. Species. Year	12	0.136

*Nutrient (N, P, K, S, Na, Ca, Mg, Cu, Zn, Mn, Fe, B) and heavy metal (Cr, Co, Ni, As, Se, Mo, Cd, Hg, Pb) analysis was conducted in species that had significant quantity of litter in all plots/replications

3.1 Foliar and Leaf Litter Heavy Metal Composition

3.1.1 Foliar composition of heavy metals in 2 and 3 year-old trees

Overall, the 2 and 3 year-old stands showed no elevated concentrations of heavy metals (Table 3) except in *G. lobocarpum*, which showed high levels of cobalt. In this study, species differed significantly ($P < 0.001$) (Table 2) in heavy metal concentrations. This may be attributed to the ability of different tree species to translocate heavy metals from root to shoot. Zinc, cadmium and nickel are translocated to the leaves, while chromium, lead and copper are usually retained in the roots [41].

At the sampled growth stages (2 and 3 year-old), most species did not accumulate excessive amounts of heavy metals (Fig. 3), most likely due to very shallow penetration into the soil (approx. 600 mm) and the restricted location of metals into the roots and low uptake into foliage, which is a very common resistance trait of trees [42]. Overall, levels of mercury, cadmium, chromium, lead, and selenium were well within the threshold limits (Figs 3). However, the 3 year-old *E. grandis* showed slightly higher concentrations of mercury in the thin phytocap (Fig. 4) and *G.*

lobocarpum accumulated very high levels of cobalt in both Thick and Thin phytocaps (Fig. 4). The reason for high accumulation of cobalt by *G. lobocarpum* is unknown and requires further investigation on this species. Deeper root penetration and the possible access to heavy metals may vary from landfill to landfill and within landfills in space and time [19]. But, *G. lobocarpum* showed elevated concentrations of cobalt in both Thick and Thin phytocap, which may be associated with its genetic ability to hyperaccumulate cobalt. Numerous researchers have reported that the species that possess the ability to develop tolerance to heavy metals will take up heavy metals (hyperaccumulators; [43]. However, even at elevated levels of heavy metals in the soil, trees evolve a few metal-tolerant ecotypes [30] which restrict the uptake of heavy metals. The lack of toxicity symptoms in trees also indicates their tolerance to withstand higher heavy metal concentrations than for agricultural crops [29]. Several studies in the past have reported good growth rates of trees despite their root penetration into the spoil, waste and mine tailings [27]. In this study, however, the 3 year-old *H. tiliaceus* showed slightly higher levels of mercury (517mg/kg) in the Thin phytocap but the levels are not likely to affect the plant (Fig. 4). Mercury is readily available to plants [44] as it has a great affinity to organic matter [45].

Table 3. Lowest, highest and mean heavy metal concentrations (mg/kg) in 2 year and 3 year-old trees

		As	Cd	Co	Cr	Hg	Mo	Ni	Pb	Se
Leaves (2005)	Lowest	86.1	24.5	74.2	417.1	50.6	43.6	628.8	681.3	63.5
	Highest	1383.9	130.5	10208	1521.0	298.5	978.1	14202	5257.8	248.2
	Mean	380.0	11.4	755.0	770.4	127.3	253.5	3690.8	2250.4	123.7
Leaves (2006)	Lowest	101.1	10.5	86.2	415.1	51.6	47.6	625.8	684.3	65.5
	Highest	1398.9	134.5	10220	1519.0	299.5	982.1	14199	5260.8	250.2
	Mean	395.0	13.8	767.0	768.7	128.3	257.5	3687.8	2253.4	122.0
Leaf Litter (2005)	Lowest	220.5	24.5	166.7	681.6	65.9	140.3	963.5	1475.0	66.7
	Highest	3101.5	136.1	9609	1800.8	175.3	1067.8	6811.8	6238.5	166.0
	Mean	654.4	8.5	978.9	956.1	105.1	321.2	2967.9	2590.8	109.6
Leaf Litter (2006)	Lowest	211.5	5.2	129.1	744.8	68.3	142.8	868.3	1765.9	84.4
	Highest	4425.3	149.3	10824	1829.9	185.6	1276.5	5866.5	5726.5	179.4
	Mean	703.8	24.7	1005.4	1041.8	115.3	398.0	2355.8	2894.5	118.3

3.1.2 Effect of maturity on heavy metal composition

Seasonal variations in the foliar heavy metal concentrations in trees have been confirmed by various studies in the past, but results from this study revealed no significant (Table 2) changes in the foliar heavy metal concentrations over one year (at ages 2 and 3 years, respectively) (Table 3). It is too early to make any discrete statements on the observations made as the trees established in this system are in their initial growth phase and have shallow roots. However, based on previous reports and findings, roots of trees grown on landfills and landfill covers do not tend to develop deep roots due to high internal soil temperatures and landfill gases. However, trends in heavy metal uptake will vary as the trees mature and develop deep roots. Riddell-Black (1993) reported consistent increases in foliar heavy metal concentrations shortly before senescence in willow grown on a metal-contaminated substrate.

There was no significant increase (Table 2) in heavy metal concentrations over time as the roots were well within the soil profile and most roots did not penetrate the waste by year 3. However, this may not be the case as the trees mature. The roots of the trees may penetrate deep into the soil over time and they may access the waste below taking up heavy metals and releasing them into the environment. It is possible that the soil and trees in the landfill site may constitute a threat to the environment. However, these risks may not be as serious as the threats of trees grown on metal contaminated sites [46], mine sites [23,47], ultramafic mineral sites [48], agricultural sites [49], industrial sites [50], coastal areas and waterways [51] and in soils that contain naturally elevated levels of metals [51].

3.1.3 Leaf litter heavy metal concentration

Leaf litter of 3 year-old trees showed no elevated (Fig. 5) concentrations of heavy metals. Species varied significantly ($P < 0.001$) in their leaf heavy metal concentrations (Table 2). Overall, heavy metal concentration in leaf litter was higher than

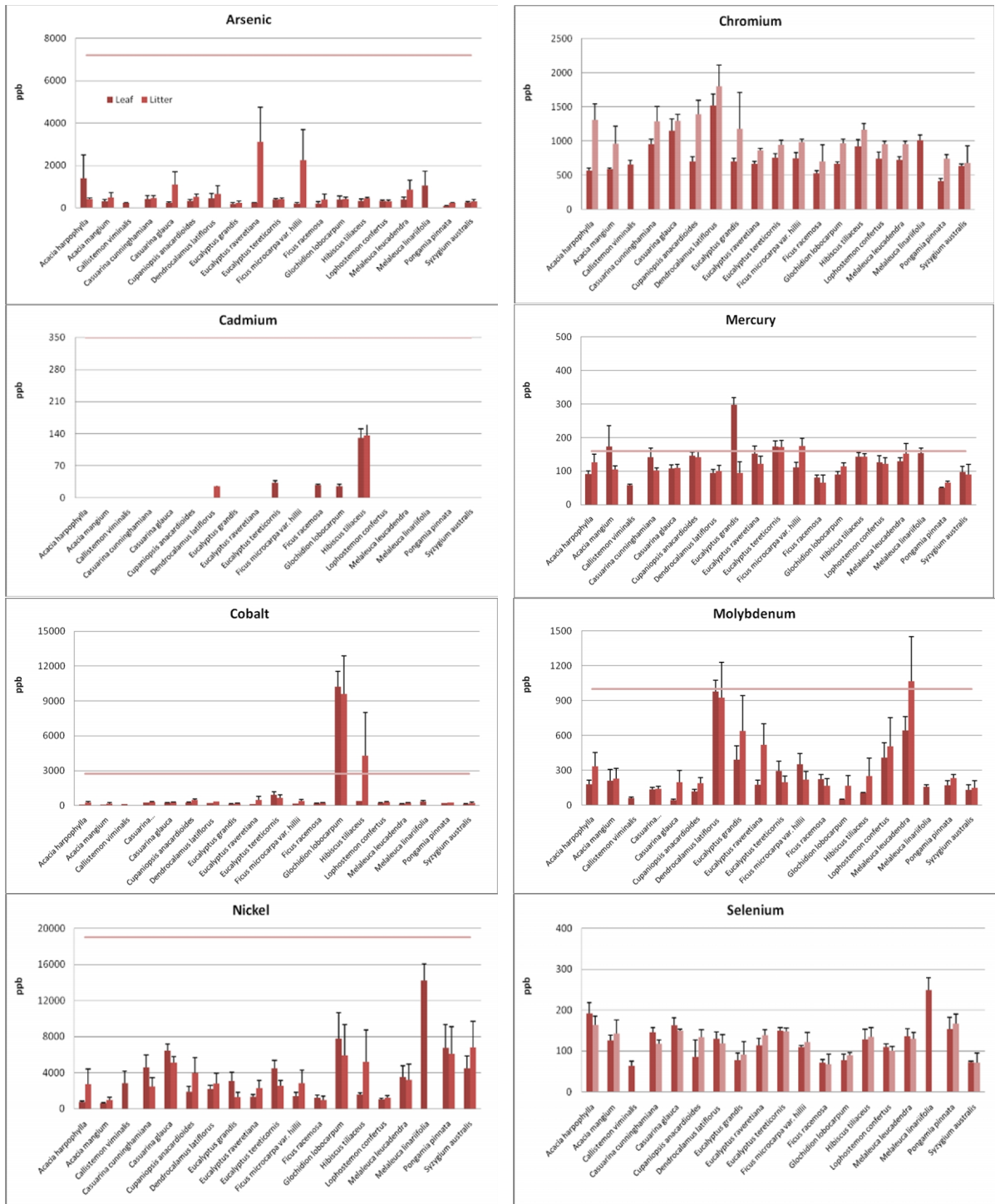
that found in live tissues of leaves (Table 3). *Eucalyptus tereticornis* had high concentrations of arsenic compared to other species (Fig. 5), but levels were well below the threshold limit (2700 ppb). Similarly, leaf litter cadmium composition of *H. tiliaceus* and *L. confertus* were higher (Fig. 5) than those in other species, but was well within the acceptable limit. *Acacia harpophylla* and *H. tiliaceus* showed higher levels of arsenic and cadmium (Fig. 5), respectively, than other species. Overall, the leaf litter from the majority of the plants did not accumulate heavy metals in excessive quantity and the current concentrations are not expected to have an adverse impact on soil, flora and fauna in the phytocapping system. However, cobalt accumulation of *G. lobocarpum* is of some concern as the high levels were also found in the leaf litter (Figs 5 and 6). Overall, levels of heavy metals being recycled into the system via leaf litter fall are well within the limits the limits reported to affect the environment.

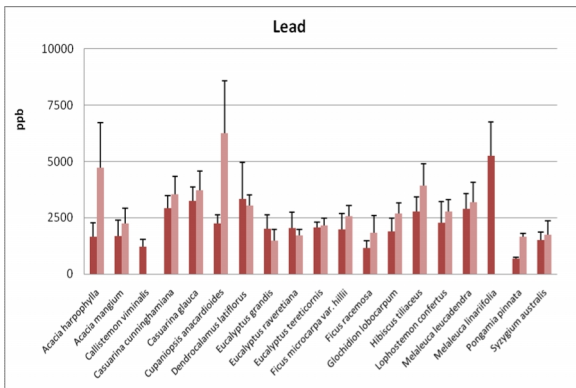
3.1.4 Effect of soil depth on heavy metal composition

Heavy metal concentrations varied significantly ($P < 0.01$) between Thick and Thin phytocaps. Trees grown in the Thin soil cover contained slightly elevated levels of heavy metals compared to those grown in the Thick soil cover (Figs 3 and 4) and this may be associated with closer proximity of their roots to the buried waste. At this stage, the trees have developed shallow roots to avoid high soil temperature and anaerobic conditions and also due to irrigation supply to support their growth in the initial stages. Hence the availability of water in the upper layers of the soil may not have encouraged the roots to penetrate into the buried waste.

3.4 Overall Trend

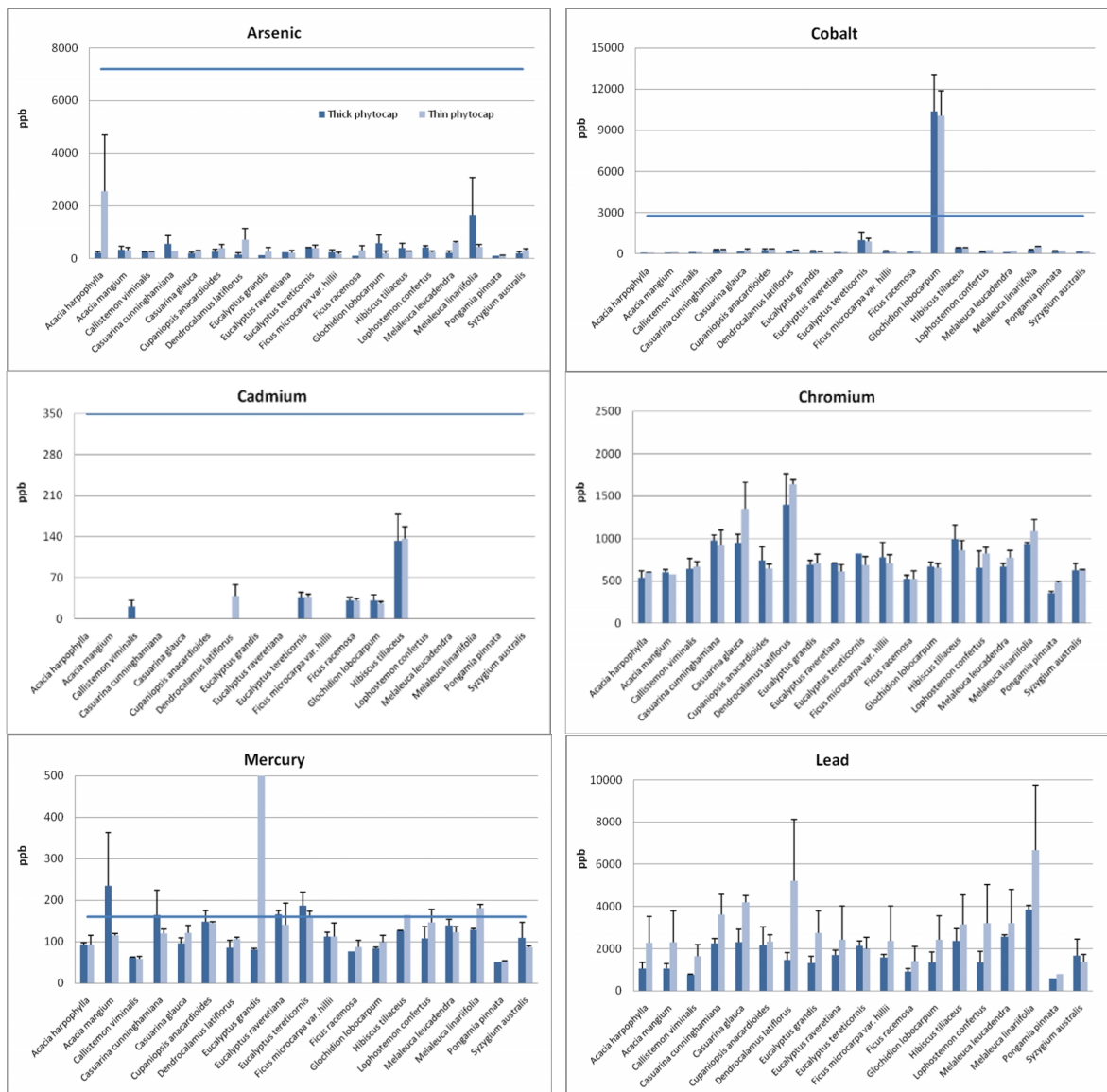
An overall trend in heavy metal concentrations in foliage and leaf litter of 2 and 3 year-old trees established in the phytocapping system is summarised in Table 4.





Note: Threshold concentration for Cr, Pb & Se is 18000, 19000 & 1000 ppb respectively

Fig. 3. Foliar and leaf litter heavy metal concentrations in 2 year-old species averaged over two phytocapping systems
 Bars represent standard errors. The horizontal line shows the optimum levels recommended for heavy metals in plants/soil (Table 1)



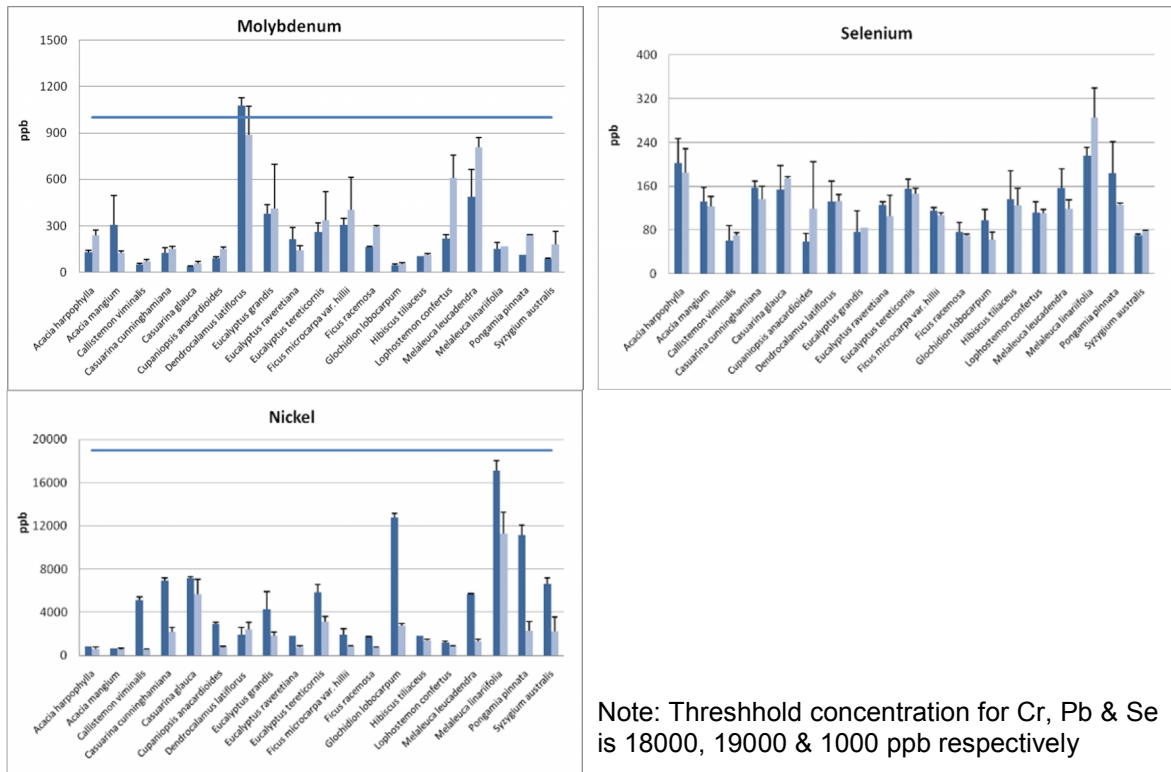
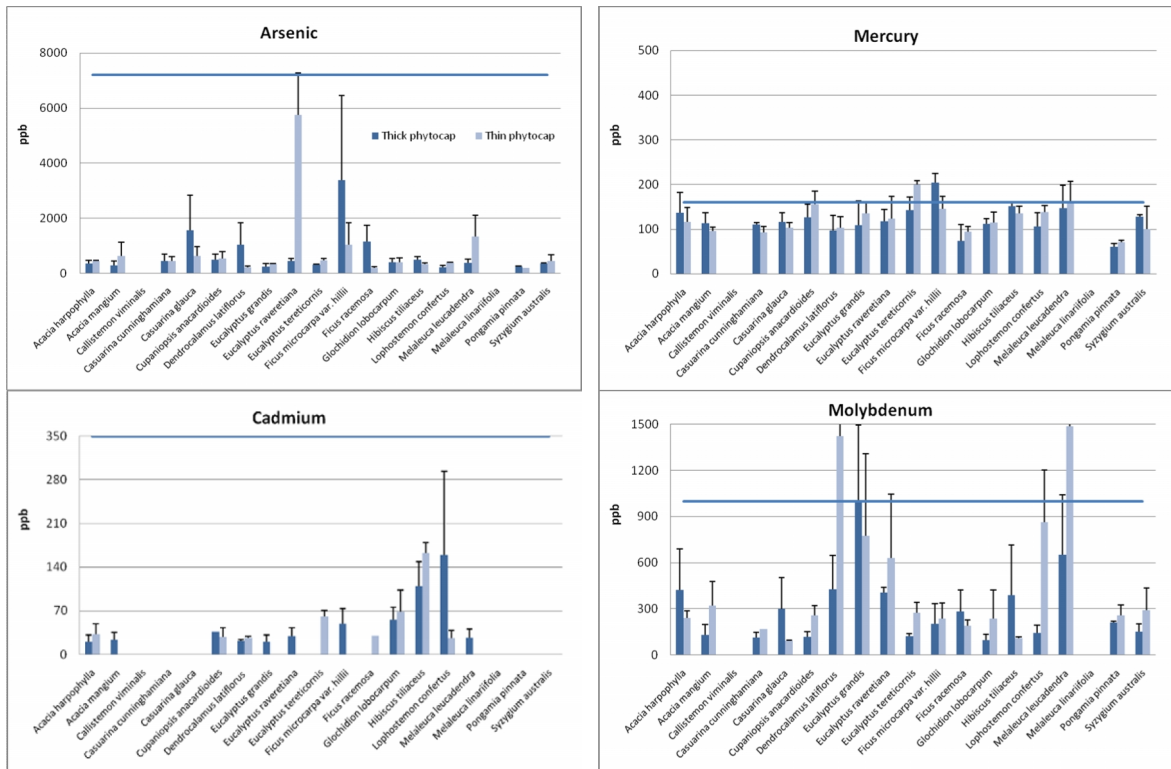


Fig. 4. Foliar heavy metal concentrations in the 3 year-old species averaged over the Thick and Thin phytocapping systems
 Bars represent standard errors. The horizontal line shows the optimum levels recommended for heavy metals in plants/soil (Table 1)



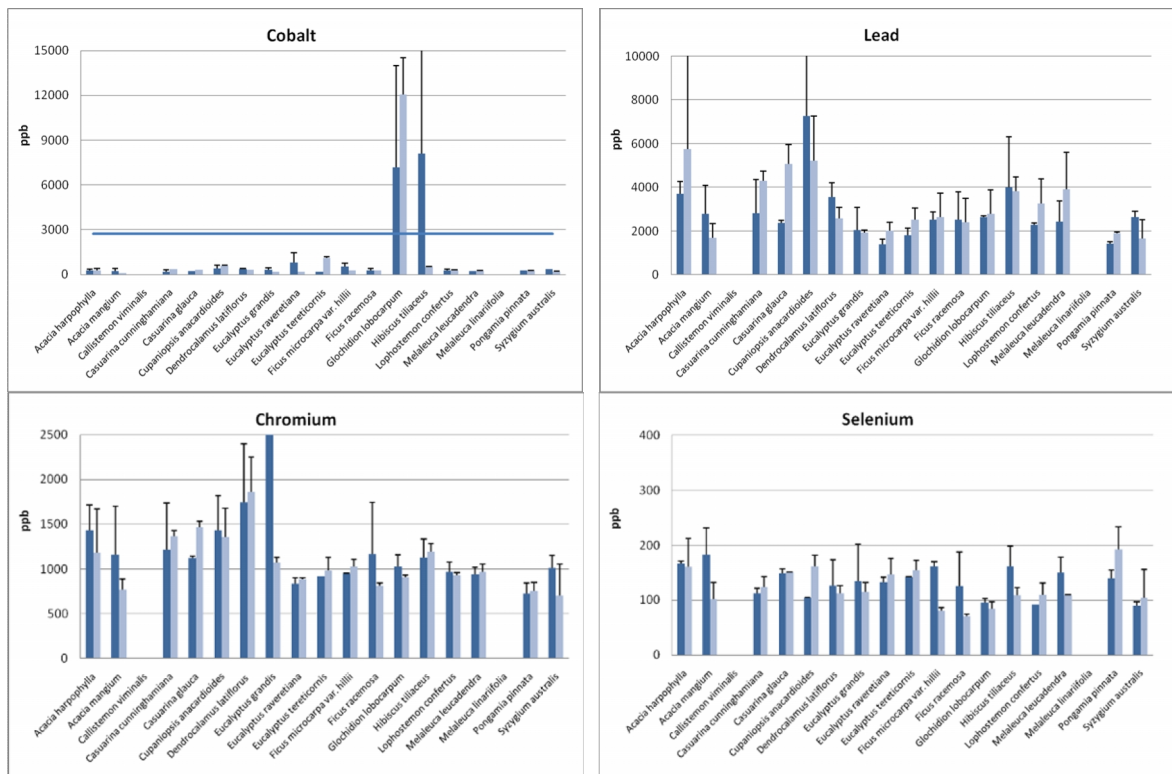
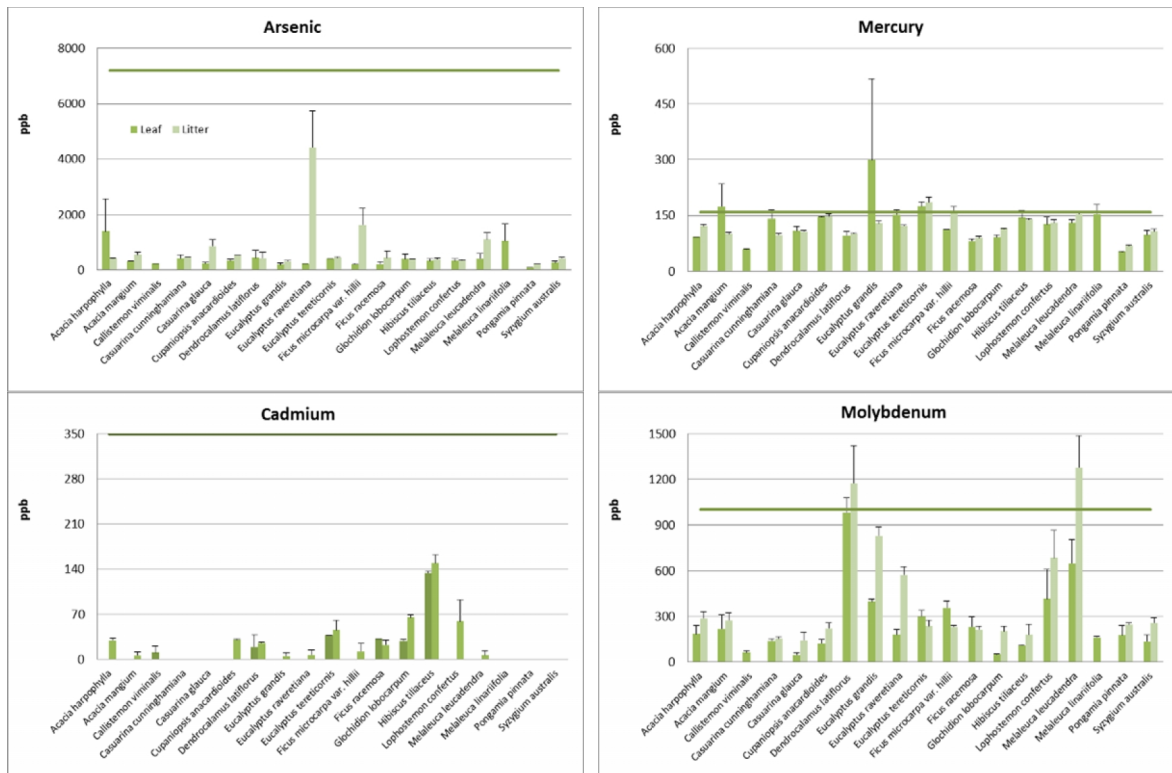


Fig. 5. Leaf litter heavy metal concentrations in 3 year-old species averaged over the Thick and Thin phytocapping systems
 Bars represent standard errors (n=4). The horizontal line shows the threshold levels recommended for heavy metals in plants/soil (Table 1)



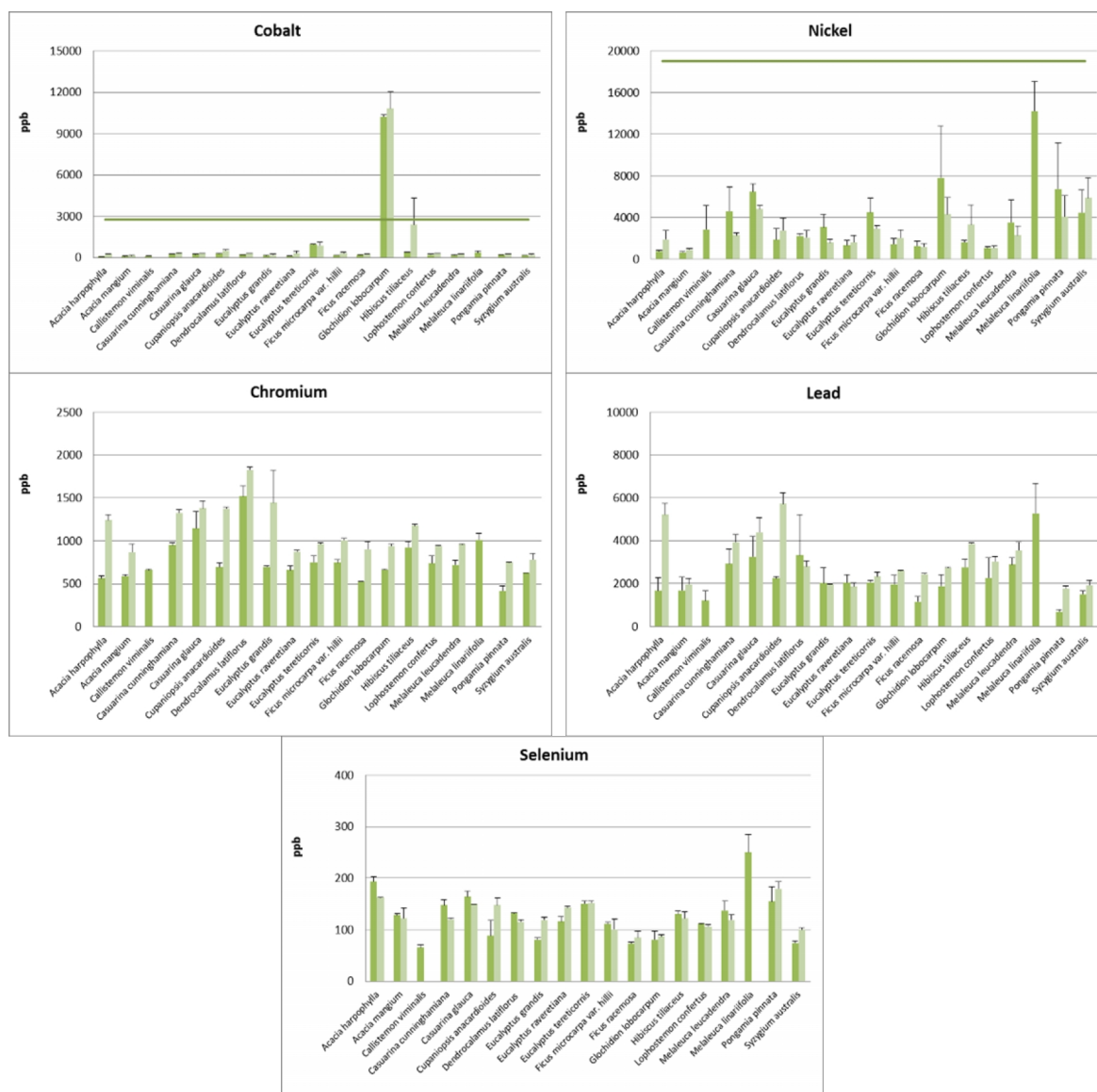


Fig. 6. Comparison between foliar and leaf litter heavy metal concentrations in 3 year-old species grown in the phytocapping systems
 Bars represent standard errors (n=2). The horizontal line shows the Threshold levels recommended for heavy metals in plants/soil (Table 1)

Table 4. Overall trends in foliar and leaf litter nutrient and heavy metal concentrations in the phytocapping system (at 2 and 3 years)

Element	Foliar (2005)			Foliar 2006			Leaf litter (2006)			Remark
	Normal	Low	High	Normal	Low	High	Normal	Low	High	
Mo	*			*			*			Slightly high in six species Very high in <i>G. lobocarpum</i>
Co	*			*			*			
As	*			*			*			
Cd	*			*			*			
Hg	*			*			*			
Ni	*			*			*			
Pb	*			*			*			
Cr	*			*			*			
Se	*			*			*			

5. CONCLUSIONS

At 3.5 years of age, the roots of the trees grown in the phytocapping system are shallow and are yet to penetrate the buried waste. However, trees may develop tolerance to heavy metals contained in the waste. With time, trees grown on the Thin soil cover are expected to accumulate larger quantities of heavy metals than those grown in Thick soil cover.

Leaf litter from the majority of the species accumulates low levels of heavy metals, and therefore is unlikely to affect the soil, flora or fauna in the phytocaps. It will be interesting to see if the heavy metal concentrations of the leaf litter will increase as the trees mature. Further tests on mature trees will establish the role of trees in mobilising heavy metals from the soil and releasing these metals into the environment. However at this stage the established trees do not pose any threat to the environment.

Cobalt accumulation by *G. lobocarpum* is of some concern and this needs to be investigated further, particularly for ecological implications, as the leaves of this species may be completely decimated by caterpillars (10) and predation of these caterpillars by birds may lead to adverse ecological consequences. For the time being, it is recommended that this species be not used in phytocaps.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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