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FICUS STRANGLERS AND *MELASTOMA MALABATHRICUM*: POTENTIAL TROPICAL WOODY PLANTS FOR PHYTOREMEDIATION OF METALS IN WETLANDS

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ABSTRACT. – Hydroponic experiments were performed on rooted cuttings of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* over five weeks in different combinations of Hoagland's solutions and singly-added metal concentrations of cadmium, lead, copper, and zinc to characterize metal accumulation and translocation to aboveground biomass. From total dry mass, ratio of shoot to root dry masses, and metal concentrations in plants, *Ficus microcarpa* was shown to have generally low metal content and the highest growth sensitivity to nutrient levels, and thus considered to be the least suitable species for use in oligotrophic waters. *Ficus benjamina* and *Melastoma malabathricum* are good candidates for phytoextraction of lead, and *Ficus virens* and *Melastoma malabathricum* are good candidates for phytoextraction of zinc. For phytoextraction of cadmium, *Ficus microcarpa* had the least favourable efficiency, while the other three species should be investigated further. No significant specific difference was detected for copper, although all species accumulated favourably high levels compared to that reported in other woody, non-hyperaccumulator species. All the species accumulated much lower levels of lead than earlier reports of a non-hyperaccumulator with potential for phytoextraction. *Ficus benjamina*, *Ficus virens*, and *Melastoma malabathricum* should be further investigated for their potential use in tropical phytoremediation wetlands for cadmium, copper, and zinc.

KEY WORDS. – Heavy metals, hydroponics, *Ficus* strangler, *Melastoma malabathricum*, phytoextraction, tropical.

INTRODUCTION

Kivaisi (2001) has defined wetlands as areas bordering land and water, which can be classified according to salinity and the vegetation cover (swamp: woody shrubs and trees; marsh: herbaceous macrophytes; and bog: mosses). Hammer & Bastian (1989) have reviewed the functions of natural wetlands to include ecological functions, such as supporting a diversity of wild life by acting as stopping points for migratory birds and spawning grounds for fish and shellfish, having hydrological functions such as the attenuation of erosion, and as discharge areas for groundwater. Furthermore, these wetlands support biodiversity that may provide a further source of human enjoyment (Sather, 1989). These services, which are hard to value economically, are what constructed wetlands seek to replicate.

However, most current work on constructed wetlands is focused on constructing herbaceous marshes because of their faster growth. They can be established and brought up to full operational performance in the shortest timeframe compared to the other alternatives (Hammer & Bastian, 1989). Water hyacinth is one example of a herbaceous plant successfully applied for nitrogen (N) removal, and to a lesser extent, phosphorous (P) removal, in tropical and subtropical regions. In addition, it has the potential for biogas production (Kivaisi, 2001).

Dependence on herbaceous species for phytoremediation, however, has certain disadvantages. Introduction of these fast-growing species outside of their native ranges may generate problems stemming from their invasiveness, thus requiring costly management measures such as physical removal (Pimentel et al., 2004). Furthermore, as woody wetlands have more complex structures, they may support more complex communities as compared to herbaceous marshes, which may be good for the conservation of biodiversity. Lastly, woody plants have the benefit of forming more persistent biomass that can accumulate over longer intervals between harvests. This is advantageous compared to the herbaceous biomass that can decompose or be consumed by herbivores quickly, especially in a warm and equable tropical climate that does not enforce dormancy on either process.

Glaringly, even though about half of the world's wetlands occur in the tropics (Neue et al., 1997), the adoption of the constructed wetland technology is slow (Kivaisi, 2001), and usually takes the form of commercial spin-offs of technologies from developed, donor countries (Denny, 1997). To put this into perspective, tropical zone terrestrial ecosystems make up the world's largest terrestrial ecosystems, with a third of the world's soils, and supporting three-quarters of the human population (Saxena & Misra, 2010). Therefore, the danger stemming from the implementation of unsuitable or unoptimised technologies, or non-action because of the lack of information, may be greater in the tropics than anywhere else.

Though willows (*Salix* species) have been used for phytoremediation of wetlands in temperate countries, being a largely temperate genus, its usefulness is probably limited in the tropical context. While there has been a search for tropical hyperaccumulators (Reeves, 2003), a good portion of the candidates may have the same shortcomings as their temperate counterparts, such as being slow-growing, having narrow cultural requirements, and accumulating small and often herbaceous biomasses. Thus, there is an urgency to discover tropical woody species among the more diverse non-hyperaccumulators, which may fulfill the equivalent functions as *Salix*.

Among the potential species, we would like to propose the use of figs (*Ficus* species). With over 600 species worldwide (Berg & Corner, 2005), which compares well against the 450 species of *Salix* (Argus, 1997), we hope to find, among them, phytoremediation equivalents to *Salix* species. We have reasons to be hopeful as the genus has varied life forms coming from a range of successional stages, and can be found in varied habitats (Berg & Corner, 2005).

Last but not least, there have been concerns over invasions by introduced plant species in wetlands endangering native biodiversity and threatening natural ecosystems, often incurring tangible economic costs (Pimentel et al., 2000). Zedler & Kercher (2004) have attributed the greater vulnerability of wetlands to invasion by exotic species partly to their being landscape sinks. Whatever the cause, we should strive to minimize the introduction of exotic species whenever native species with equivalent functions exist for use in the creation of artificial wetlands. With a specious genus such as *Ficus*, we should be able to source for native species with phytoremediation potential for this application, and thus minimize any risk of unintended introductions.

Presently, the research is focused on *Ficus* stranglers as they have the following advantages:

1. Ease of mass propagation by stem cuttings
2. Wide tolerance for salinity and waterlogging in some species
3. Good coppicing ability that allows repeated harvesting, if required. The removal of biomass would be less frequent than for herbaceous plants in similar applications.
4. Formation of a dense canopy cover that, once established, could potentially suppress weedier species that may predominate in arrested succession, especially in eutrophic tropical wetlands (Kent et al., 2000).
5. Profusion of adventitious roots that allows plants to penetrate extensively into the substrate and increase the efficiency of phytoremediation processes
6. The potential for plants to be grown on dry land with roots trailing into adjacent wetlands as a novel mode of phytoremediation

Following this strategy, we hope to be able to find tropical non-hyperaccumulator species with the potential for use in phytoremediation of wetlands. We anticipate that more woody species from this genus as well as from other tropical genera could be found with desirable characteristics for such applications should they be needed in the tropics.

MATERIAL AND METHODS

Species used in the study. – For the present study we have shortlisted the following fig species: *Ficus benjamina* L., *Ficus microcarpa* L.f., and *Ficus virens* Aiton. *Ficus microcarpa* is known to grow in the landward side of mangrove forest and freshwater swamps (Berg & Corner, 2005), thus it is expected to be the most useful for wetlands, being tolerant of waterlogging and a wide range of salinity (see Tan et al. (2010) for a broader discussion of the species, including its value as an ornamental plant and its potential phytoremediation ability). *Ficus virens* has also been reported to occur from coastal to inland forests (Berg & Corner, 2005), but its tolerance for waterlogging is presently unknown. *Ficus benjamina* is a weedy, commonly planted species usually found in urban tropical environments, growing on concrete structures or wayside trees (pers. obs.), thus it may have a narrower ecological tolerance compared with the other two species. For comparison, we have included a common, native, non-*Ficus* shrub, *Melastoma malabathricum* L., as it is readily propagated from cuttings, and is known to tolerate a range of well-drained to waterlogged soils (W. F. Ang, pers. comm.). The species is of interest as it is a known aluminum accumulator (Watanabe et al., 1998). Thus, it will be interesting to ascertain if it could also tolerate and accumulate other metals.

Hydroponics experiment. – Stem cuttings of the four species were made and rooted in aerated water before being individually grown in 500 ml of solutions in polyethylene containers containing various additions of a single metal (Cu, Zn, Cd, or Pb at 0, 0.1 and 1 mg l^{-1}) in various Hoagland's solution concentrations (Hoagland's nutrient solutions at 1×, 0.1×, and 0.01× concentration, CK backgrounds for short) adjusted to pH 6.5. For each *Ficus* species, effort was made to collect all materials from a single individual to rule out genotypic variability. However, the same could not be done for *Melastoma malabathricum*, as a single shrub was too small to yield the number of cuttings required. The cuttings were made from about 12-cm lengths of young stems, yet to be fully covered by periderm, with at least three nodes. The duration of rooting varied between species, taking four weeks for *Ficus benjamina*, *Ficus microcarpa*, and *Melastoma malabathricum*, and six weeks for *Ficus virens*.

The composition of 1× Hoagland's solution consists of the following: 6 mM KNO_3, 4 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2 mM $MgSO_4 \cdot 7H_2O$, 1 mM $NH_4H_2PO_4$, 5.00 mg l^{-1} Fe in the form of $FeNa(EDTA)_2$, 2.86 mg l^{-1} H_3BO_3, 0.22 mg l^{-1} $ZnSO_4 \cdot 7H_2O$, 0.029 mg l^{-1} $NaMoO_4 \cdot 2H_2O$, 1.81 mg l^{-1} $MnCl_2 \cdot 4H_2O$, and 0.02 mg l^{-1} $CuSO_4 \cdot 5H_2O$. The metals tested, Cu, Zn, Cd, and Pb, were added in the form of $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $3CdSO_4 \cdot 8H_2O$, and $Pb(NO_3)_2$. Three replicate plants were used in each treatment. The solutions were aerated, topped up to volume with water, and changed once a week to ensure stability in their concentrations and pH. The plants were grown for a period of five weeks before being harvested, and thereafter separated into roots, leaves, and stems, rinsed with deionized water, and dried to constant weight in an oven at 60 °C. The parts were then weighed, before being crushed and digested, with about 0.2 g of dried plant material in 15 ml of concentrated nitric acid at 95 °C using SC154 Hotblock (Environmental Express, U.S.A.). The concentrations of the metals were determined after digestion using Varian SpectrAA 55B (Varian Analytical Instruments, U.S.A.) with an air-acetylene flame.

Total dry mass and ratio of shoot to root dry masses were used to detect any significant effect of the treatments on the growth within a species. Shoot (leaves plus stem), root, and overall concentrations of the metals, translocation factor from root to shoot, and shoot accumulation factor for metals were also compared between the species. Translocation factor of a metal was calculated as the percentage of metal concentration in the shoot over that in the root, while accumulation factor was calculated as the metal concentration in the plant (mg kg^{-1}) over that in the solution (mg l^{-1}).

Statistical analyses. – Analysis of variance (ANOVA) tests were done to detect effects of different concentrations of Hoagland's solution and metal levels on the variables mentioned above. Where differences were detected, Fisher's least significant difference (LSD) test was used at a 5% level of significance to show how the values differ. Different letters were used on the graphs to indicate where the values were significantly different from one another. Lowercase letters on top of the bars indicated statistical differences within the same Hoagland's background, while uppercase letters above the charts indicated statistical differences among the different Hoagland's treatments. All statistical analyses were performed using CoStats and the graphs plotted using CoPlot (CoHort Software, U.S.A.).

RESULTS

Effects of treatments on dry mass and ratio of shoot to root dry masses. – From the dry mass accumulation, *Ficus microcarpa* showed the greatest sensitivity to the treatments for Cd and Pb. ANOVA indicated that for this species, dry mass accumulation was significantly positively related to Hoagland's solution concentration for Zn ($0.05 \geq p > 0.01$), Cd ($0.01 \geq p > 0.001$), and Pb ($0.01 \geq p > 0.001$) (Fig. 1). ANOVA also showed that *Ficus virens* was significantly affected by Cu (undetermined direction, $0.05 \geq p > 0.01$) and Zn concentrations (positively, $0.05 \geq p > 0.01$), while in all other cases, metal concentrations had non-significant effects. Furthermore, *Ficus benjamina* was the least affected by Hoagland's solution concentration, with no significant effect for all metal treatments except for Pb ($0.01 \geq p > 0.001$), with higher Hoagland's solution concentrations promoting biomass accumulation. Lastly, *Melastoma malabathricum* was sensitive to the concentration of Hoagland's solution when treated with Pb ($0.01 \geq p > 0.001$) and Cu ($0.05 \geq p > 0.01$), with total dry mass positively related to the concentration.

For the ratio of shoot to root dry masses, *Ficus microcarpa* again showed the most instances of significant increase with an increase in Hoagland's solution concentration for Cd ($0.05 \geq p > 0.01$), Cu ($p < 0.001$), and Zn ($0.05 \geq p > 0.01$), with the exception of Pb, for which 0.1× Hoagland's solution concentration had the highest ratio of shoot to root dry masses ($0.05 \geq p > 0.01$) (Fig. 2). Interestingly, for *Ficus virens* and *Melastoma malabathricum*, only the interaction between Hoagland's solution concentration and Cd levels had detectably significant effect on the ratio ($0.05 \geq p > 0.01$). Lastly, *Ficus benjamina* was the only species in which the ratio of shoot to root dry masses was not shown by ANOVA to be significantly affected by the concentration of Hoagland's solution or the metals. Thus, in general, both dry mass accumulation and the ratio of shoot to root dry masses indicate that *Ficus microcarpa* is the most sensitive species to the treatments, and *Ficus benjamina*, the least.

Effects of treatments on shoot, root and overall metal concentrations. – Overall, root and shoot metal concentrations were shown by ANOVA to be significantly dependent on both the concentrations of Hoagland's solution and the metals, generally increasing with increasing metal concentration in solution, and decreasing with increasing Hoagland's solution concentration, unless otherwise stated. An exception was noted for *Ficus benjamina* treated with Pb, for which overall total concentration of the metal in the plant was only dependent on its concentration in solution ($0.05 \geq p > 0.01$) (Fig. 3). Exceptions were also observed for root metal concentrations of Pb for *Ficus benjamina*, for which neither factor had a significant effect; and that of Cd for *Ficus microcarpa* ($0.05 \geq p > 0.01$), Pb for *Ficus virens*, ($p < 0.001$), and Cd for *Melastoma malabathricum* ($0.01 \geq p > 0.001$), which only rose with metal concentration; as well as that of Zn for *Melastoma malabathricum*, which peaked at 0.1×Hoagland's ($0.05 \geq p > 0.01$), and rose with metal concentration in solution ($p < 0.001$) (Fig. 4). For shoot concentration, *Ficus benjamina* showed non-significant

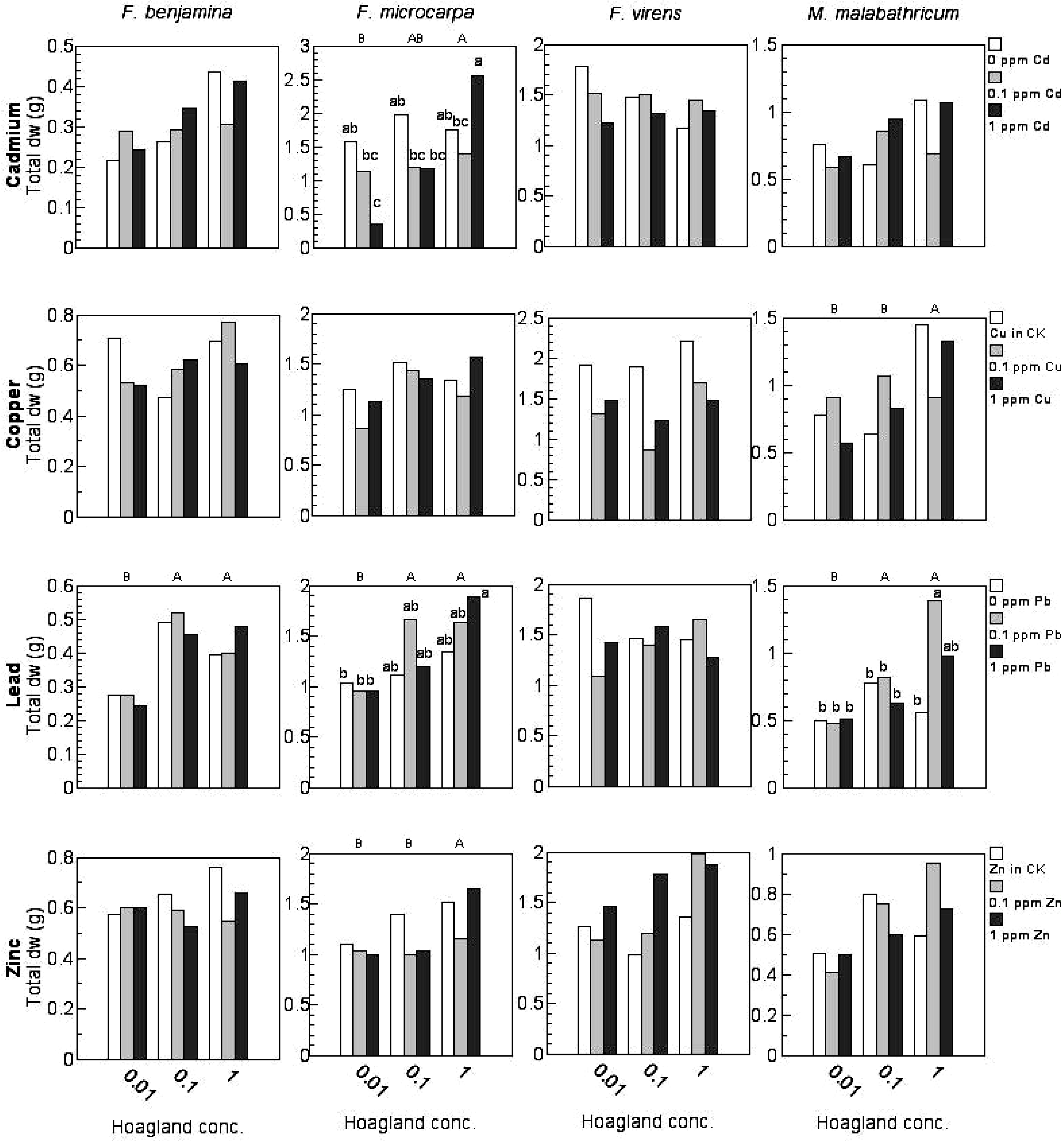

Fig. 1. Total dry mass of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution.

dependence on both the factors for Pb, while shoot concentrations of Pb ($0.01 \geq p > 0.001$), and Cu ($0.05 \geq p > 0.01$) for *Ficus virens* were only significantly positively dependent on the metal concentration in solution (Fig. 5). ANOVA showed significant specific differences in overall metal concentrations of Cd ($p < 0.001$), Pb ($0.01 \geq p > 0.001$), and Zn ($p < 0.001$), as well as root and shoot concentrations of Zn (both $p < 0.001$) (Table 1 and Fig. 3). *Ficus microcarpa* was the lowest accumulator of Cd on the basis of overall dry weight (1.79–419 mg kg^{-1}); *Ficus benjamina* and *Melastoma malabathricum* were jointly the highest accumulators for Pb (8.68–2100 mg kg^{-1} and 37.5–2390 mg kg^{-1} respectively); and *Melastoma malabathricum* was the highest for Zn (31.6–1380 mg kg^{-1}). *Melastoma malabathricum* also had the highest shoot and root concentrations for Zn (29.6–722 mg kg^{-1} and 41.2–5760 mg kg^{-1} respectively). Cu was the only metal for which no significant specific difference was found.

As we were interested in how the metals are accumulated in the aboveground biomass, the shoot accumulation factors were compared between treatments for each species, and found to be significantly increasing with decreasing Hoagland's solution and metal concentrations, unless otherwise stated. For the Cd treatment, the shoot accumulation

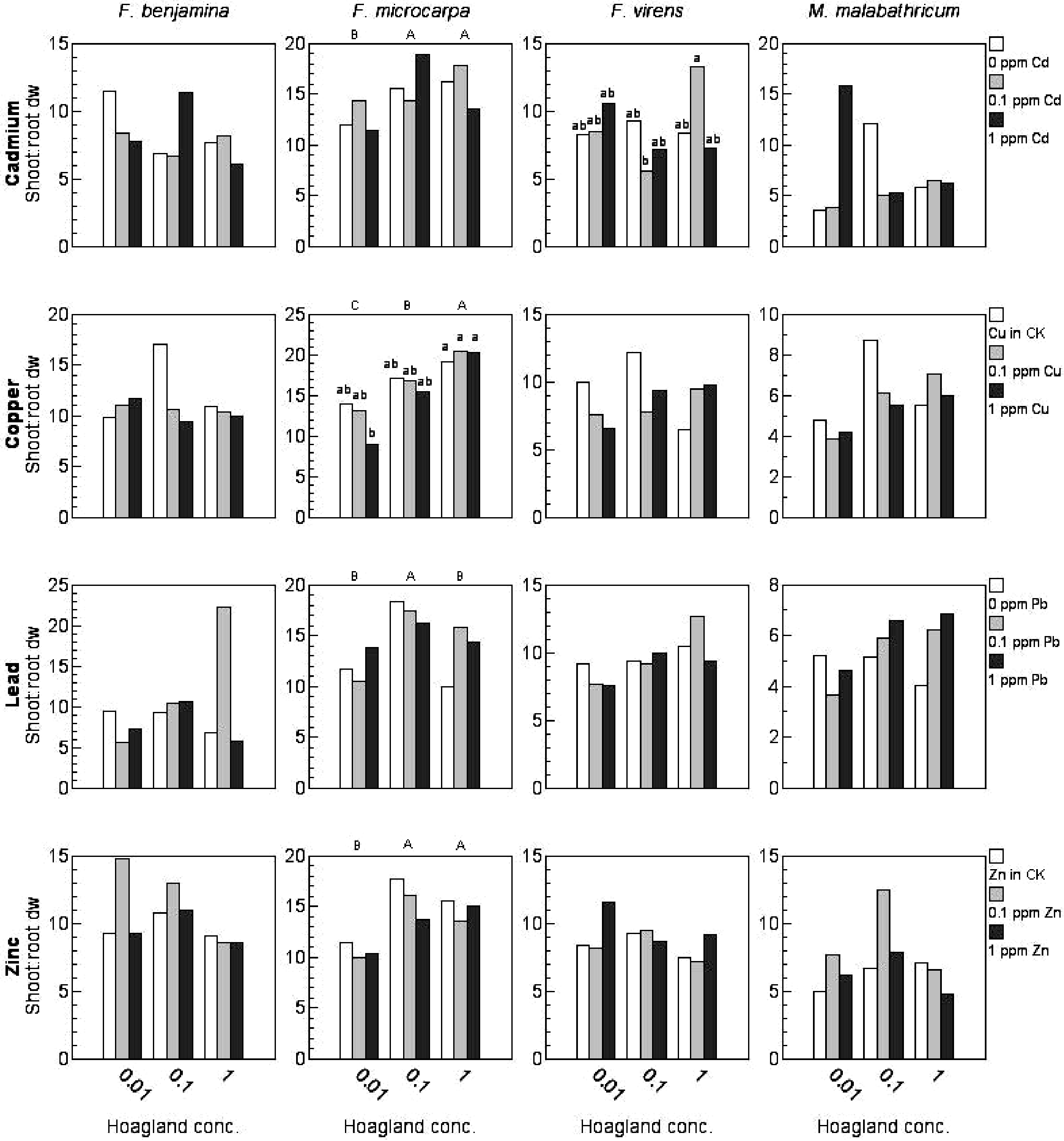

Fig. 2. Ratios of shoot to root dry masses of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution.

factor was dependent on both concentrations of metal ($p<0.001$) and Hoagland's solution ($p<0.001$) only for *Ficus benjamina,* while other species were only dependent on Hoagland's solution concentration ($p<0.001$) (Fig. 7).

For the Pb treatment, only for *Ficus benjamina* did shoot accumulation factor not show any significant dependence on either factor. The shoot accumulation factor for *Ficus microcarpa* ($0.05\geq p>0.01$), and *Melastoma malabathricum* ($0.05\geq p>0.01$) were dependent only on Hoagland's solution concentration. For *Ficus virens*, it was dependent only on metal level ($0.01\geq p>0.001$). For Cu and Zn treatments, the dependence of shoot accumulation factor on both factors was almost uniformly highly significant for all species ($p<0.001$), except for *Ficus virens* for Cu, for which none of the factors was significant.

Effects of treatments on translocation factor and shoot accumulation factor. – The translocation factor was found to generally increase with increasing Hoagland's solution concentration and decrease with increasing metal concentration of the hydroponic solution, and the effect was significant unless otherwise stated. Exceptions included all species for Cd; *Ficus virens* for Pb, for which both factors were non-significant; and *Ficus benjamina* for Zn, which was

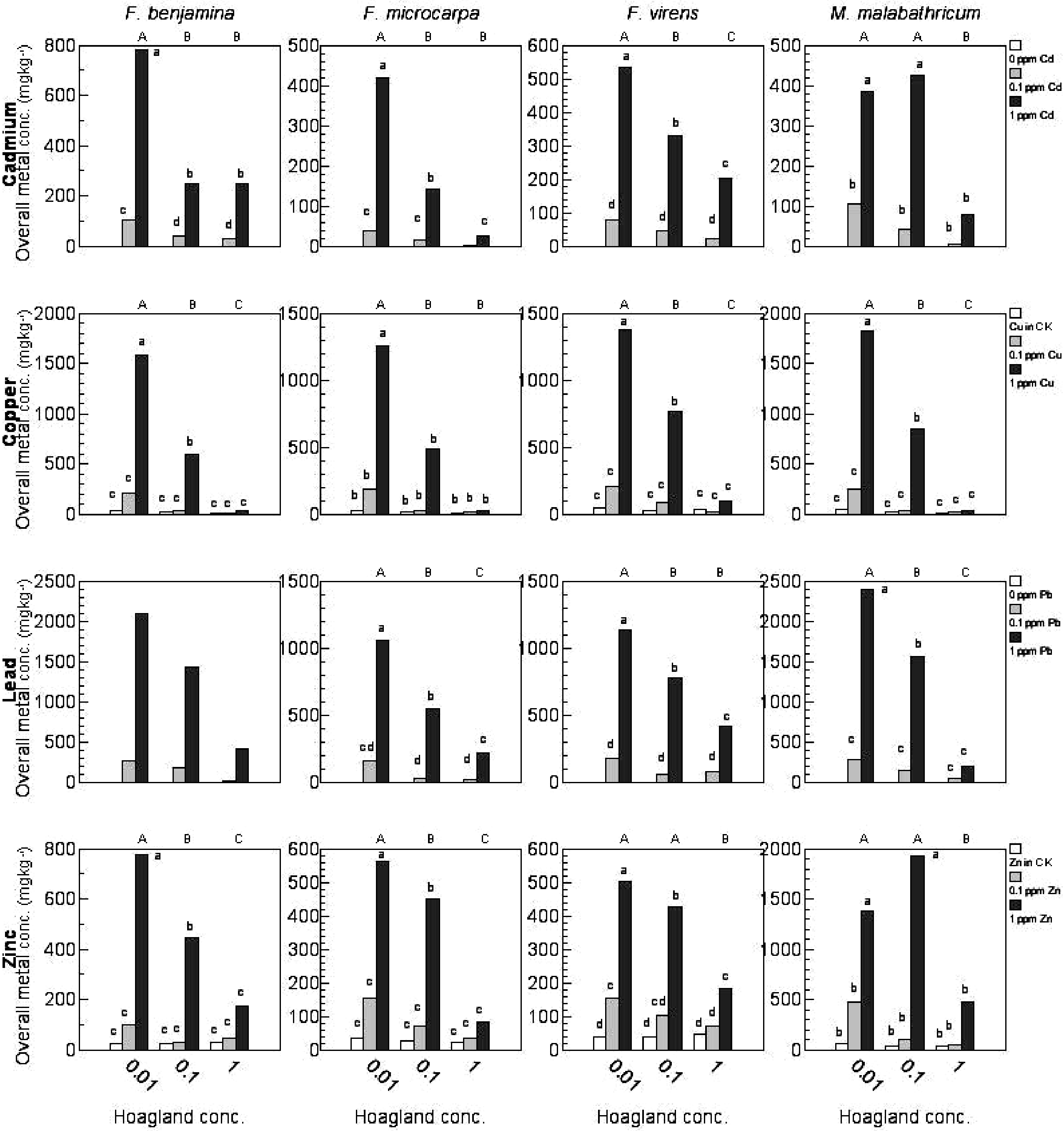

Fig. 3. Total metal concentrations of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens* and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution.

dependent on both Hoagland's solution concentration ($0.05 \geq p > 0.01$) and metal levels ($p < 0.001$), but peaking at 0.1× Hoagland's solution concentration (Fig. 6). Other instances of translocation factors showing only dependence on metal level but not Hoagland's solution concentration, included *Ficus microcarpa* for Pb ($p < 0.001$) and Zn ($0.01 \geq p > 0.001$), *Ficus virens* for Cu ($0.01 \geq p > 0.001$) and Zn ($p < 0.001$), and *Melastoma malabathricum* for Pb ($0.01 \geq p > 0.001$) and Zn (undetermined direction, $0.05 \geq p > 0.01$). The translocation of the micronutrients Cu and Zn were also much higher than that for the non-nutrients Cd and Pb.

Significant specific differences were shown for shoot accumulation factors for all metals except Cu, while *Melastoma malabathricum* was shown to have the highest translocation factor for Zn ($0.01 \geq p > 0.001$, 2.83–399%). No significant specific difference was found for translocation factors in the other metals. Comparison of shoot accumulation factors showed that the interaction between species and Hoagland's solution concentration to be significant for Cd ($0.01 \geq p > 0.001$), with *Ficus benjamina* having the highest value (9.29–308 l kg^{-1}), followed by a tie between *Ficus microcarpa* (2.68–281 l kg^{-1}) and *Melastoma malabathricum* (13.3–150 l kg^{-1}), and with *Ficus virens* having the lowest shoot accumulation factor (16.6–163 l kg^{-1}). Specific differences in shoot accumulation factors were also found

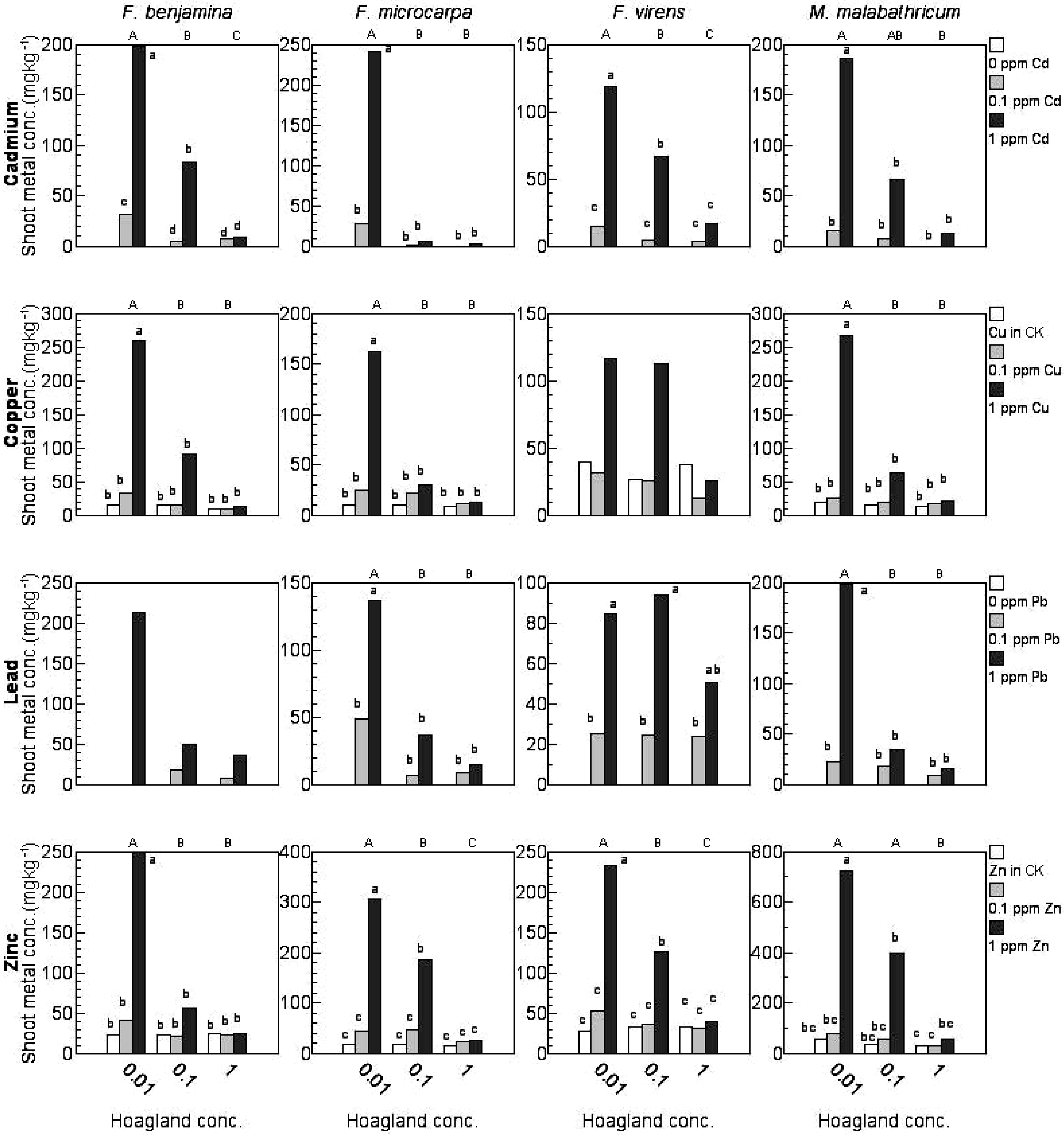

Fig. 4. Root metal concentrations of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution.

for Zn ($p<0.001$), with *Melastoma malabathricum* having the highest accumulation (53.7–111000 l kg^{-1}), followed by *Ficus virens* (37.1–56200 l kg^{-1}). No significant specific difference was found for Cu and Pb.

DISCUSSION

Both total dry mass and ratio of shoot to root dry masses showed *Ficus microcarpa* to be the species most dependent on nutrient level for the growth and maintenance of aboveground to belowground balance in development. In contrast, *Ficus benjamina* was shown to be the least sensitive under the same criteria. Therefore, *Ficus microcarpa* may be the least suitable for use in the phytoremediation of oligotrophic water bodies, and its high nutrient requirement is of no surprise given the high nutrient availability expected of the back mangrove and estuarine natural habitats of this species. However, its responsiveness to nutrient level suggests a greater potential of this species for use in tropical constructed wetlands with higher productivity. It may grow faster in response to a nutrient increase, thus allowing it to keep up with, and not be overgrown by, weedier species, the predominance of which in tropical wetlands may result in arrested succession (Kent et al., 2000). Furthermore, a canopy-forming species such as *Ficus microcarpa* may be beneficial in

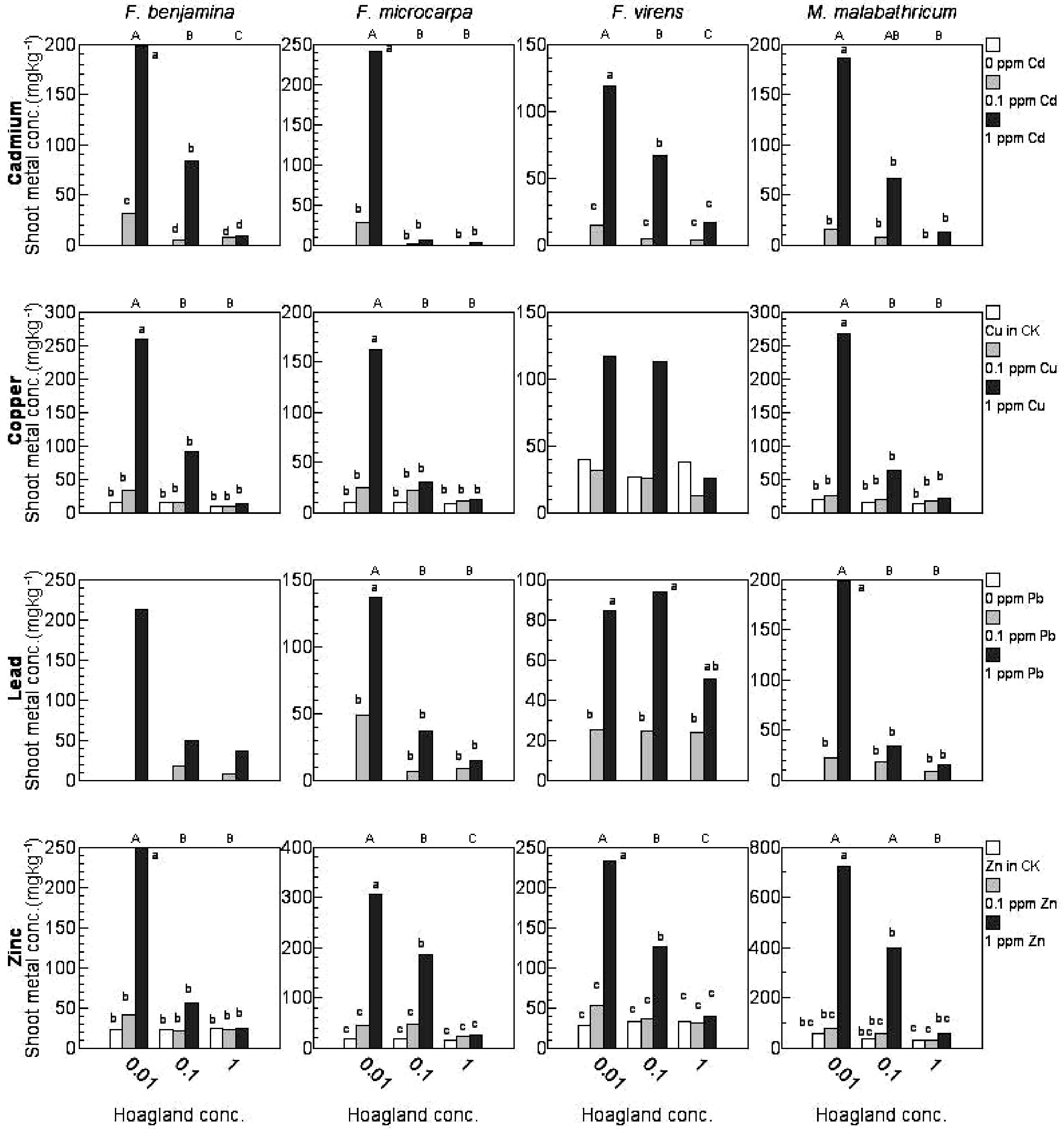

Fig. 5. Shoot metal concentrations of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution.

suppressing the weeds by shading them out, thus allowing a wetland to develop more fully towards a woody successional stage.

From the specific differences in shoot, root, and overall concentrations, and translocation and shoot accumulation factors of the metals, it can be tentatively concluded that *Ficus benjamina* and *Melastoma malabathricum*, in having higher overall concentrations of Pb, are better candidates for the phytoextraction of the metal. While *Ficus virens*, based on shoot accumulation factor, and *Melastoma malabathricum*, based on all the above variables, would be better candidates for the phytoextraction of Zn. As *Melastoma malabathricum* is a known hyperaccumulator of aluminum (Watanabe et al., 1998), it will also be interesting to find out if the metals share similar mechanisms of transport and accumulation implicated in aluminum accumulation (Watanabe & Osaki, 2002). Interestingly, no species performed better than others in Cu accumulation. On the basis of overall metal concentrations, *Ficus microcarpa* appears to be the worst at accumulating Cd. Taken together with the results showing *Ficus microcarpa* to have the lowest overall metal concentration for Cd and Zn (tying with *Ficus benjamina*), and not doing significantly better at accumulating Cu, it seems to be the least useful species for phytoextraction.

Table 1. Range of overall, root and shoot metal concentrations, translocation and shoot accumulation factors of metals in the species studied.

Species		***Ficus benjamina***		***Ficus microcarpa***		***Ficus virens***		***Melastoma malabathricum***	
Overall concentration (mg kg^{-1})		Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Metals	Cd	780	29.7	419	1.79	533	21.1	426	6.27
	Cu	1580	9.65	1260	9.51	1370	17.3	1820	12.8
	Pb	2100	8.68	1050	20.5	1140	53.4	2390	37.5
	Zn	775	25.8	561	20.8	503	40.0	1380	31.6
Root concentration (mg kg^{-1})		Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Metals	Cd	5270	215	2400	32.4	4730	242	3690	35.9
	Cu	15900	16.5	10900	18.4	9600	25.8	7870	16.4
	Pb	12000	35.1	13400	202	9050	312	12600	206
	Zn	5810	59.4	3090	95.8	3610	107	5760	41.2
Shoot concentration (mg kg^{-1})		Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Metals	Cd	199	4.75	241	nd	118	3.75	187	nd
	Cu	259	9.04	163	9.02	117	12.6	266	12.2
	Pb	213	7.50	137	6.66	93.6	23.7	199	8.15
	Zn	249	20.6	304	15.2	233	28.1	722	29.6
Translocation factor (%)		Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Metals	Cd	4.49	0.547	252	0.0915	3.05	1.15	10.0	2.95
	Cu	57.0	1.56	49.2	1.09	150	1.61	77.3	1.36
	Pb	21.4	0.446	3.50	0.450	20.8	0.983	4.36	0.287
	Zn	40.5	1.34	16.9	4.04	31.2	2.93	399	2.83
Shoot accumulation factor		Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
Metals	Cd	308	9.29	281	2.68	163	16.6	150	13.3
	Cu	279000	13.1	210000	12.9	769000	25.5	367000	21.2
	Pb	213	35.8	490	14.8	247	50.3	225	14.8
	Zn	43700	23.6	32500	25.5	56200	37.1	111000	53.7

Lastly, the micronutrients, Cu and Zn, have higher translocation factors than Pb or Cd for the species studied (Fig. 6). This could mean that phytoextraction might be more feasible for Cu and Zn, while rhizofiltration or phytostabilisation are better options for Cd and Pb. This remains to be ascertained by soil-based studies and field trials.

It is clear that the species investigated in this study are non-hyperaccumulators by the criteria of Reeves (2003). Much of the work on the phytoremediation potential of woody non-hyperaccumulators was carried out on temperate species. Experimental details such as the nutrient composition and concentration, concentrations of metals, whether metals were added singly or in a mixture, the duration of experiment, type of hydroponic set up and the cultural conditions often differ among the studies. Therefore, comparisons made should be mindful of the differences in the experimental details. For a quick assessment of whether the tropical species studied here are comparable to these potentially useful woody species, comparisons of root and shoot metal concentrations were made. Table 2 summarises some of the values reported for the woody species gathered from literature, and includes some relevant information on the experimental conditions. It should be mentioned that this list is not meant to be a comprehensive review.

For Zn, the shoot concentrations of *Ficus microcarpa*, and *Melastoma malabathricum* are at the higher range of the values reported for shoot concentrations of *Salix viminalis* (Landberg & Greger, 2002), and branch concentrations of *Kandelia candel* (Chiu et al., 1995). Therefore these two species seem suited for the phytoextraction of Zn. The root concentrations of the metal were higher in all the species studied compared to the two reference species. But we are wary of making a direct comparison, because no chemical was used to desorb the metal from the roots in the present study, as was also the case with the work done on *Kandelia candel*. This is justified on the ground that in rhizofiltration or phytostabilization applications, the adsorbed fraction would still contribute toward phytoremediation, and therefore should not be discounted. It should also be noted that *Kandelia candel* is a mangrove plant, and therefore grows better in high salinity nutrient solutions that would ameliorate metal toxicity and decrease metal uptake (Chiu et al., 1995). However, the same saline condition cannot be uniformly used in this present study as none of the species are mangrove species, except for *Ficus microcarpa*.

For Cu, all four species studied exceeded the two reference species stated above in the shoot and root concentrations of the metal. It should be noted that the *Salix viminalis* study used a much lower Cu concentration than this study. But

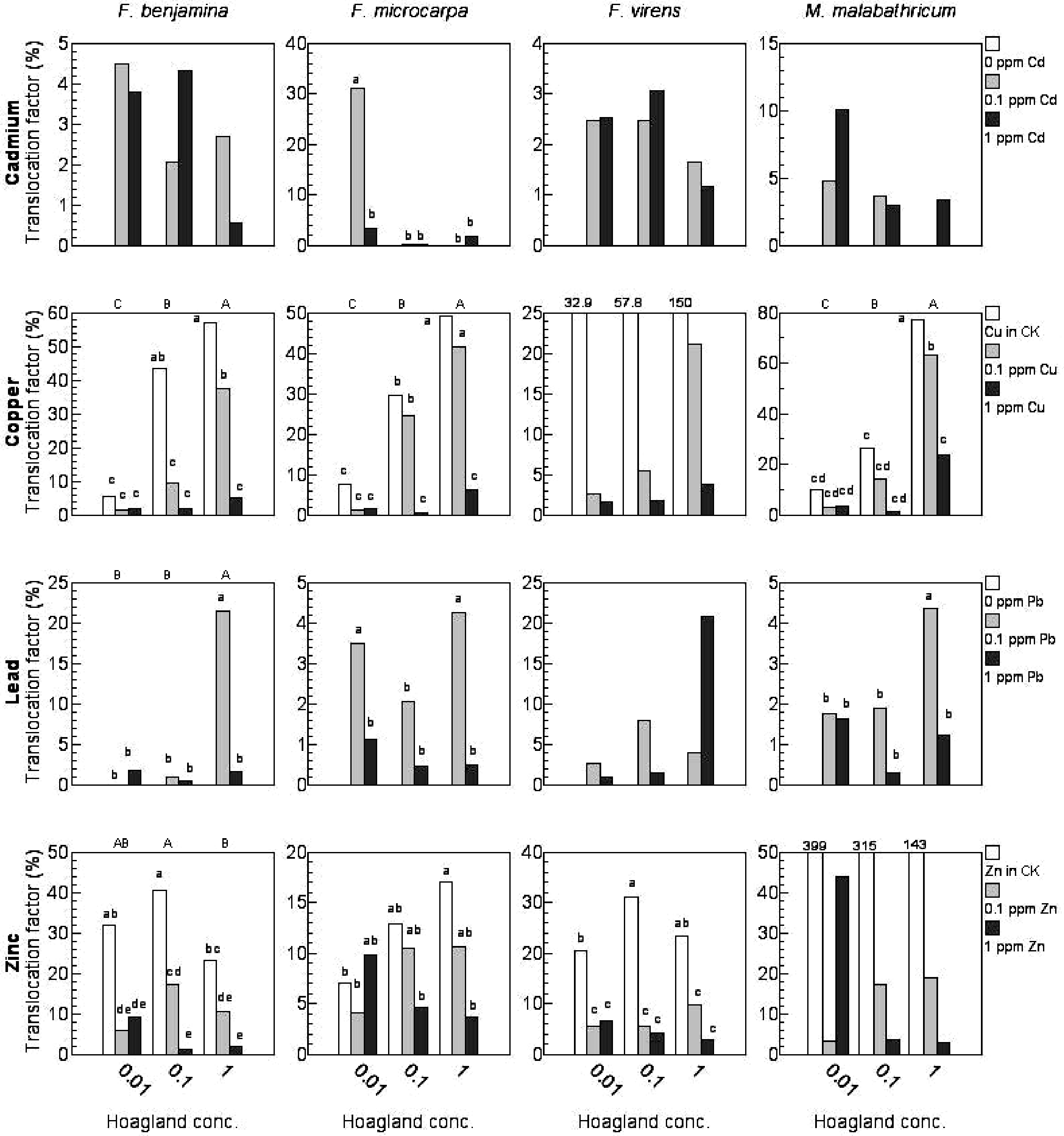

Fig. 6. Translocation factors of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution. Some charts have the extremely high bars truncated to allow lower values to be seen.

interestingly, even at the lowest Cu level provided by the Hoagland's solutions, both shoot and root concentrations still exceeded that in *Salix viminalis*. As for the *Kandelia candel*, the comparison should be made bearing in mind the high salinity of the nutrient solutions. Though all four species studied appeared to have similar Cu phytoextraction capabilities, the levels of Cu accumulation were above the range previously reported for woody plants.

For Cd, all the four species studied exceeded the values reported for the *Salix* species in Landberg & Greger (1996), but did not perform as well compared to what was recorded for *Populus* and *Salix* species by Zacchini et al. (2009). However, the Cd concentration used in the former work was almost 10 times lower than that used in the present study, while the latter work used a concentration more than five times higher. Therefore for this metal, it is difficult to conclude if the plant species accumulated amounts comparable to the reference plants, unless a comparison is drawn between the 0.1 mg l^{-1} Cd treatments used in the present work and comparable treatment concentrations used by Landberg & Greger (1996), in which case the species studied here still had shoot and root concentrations higher than the *Salix* species.

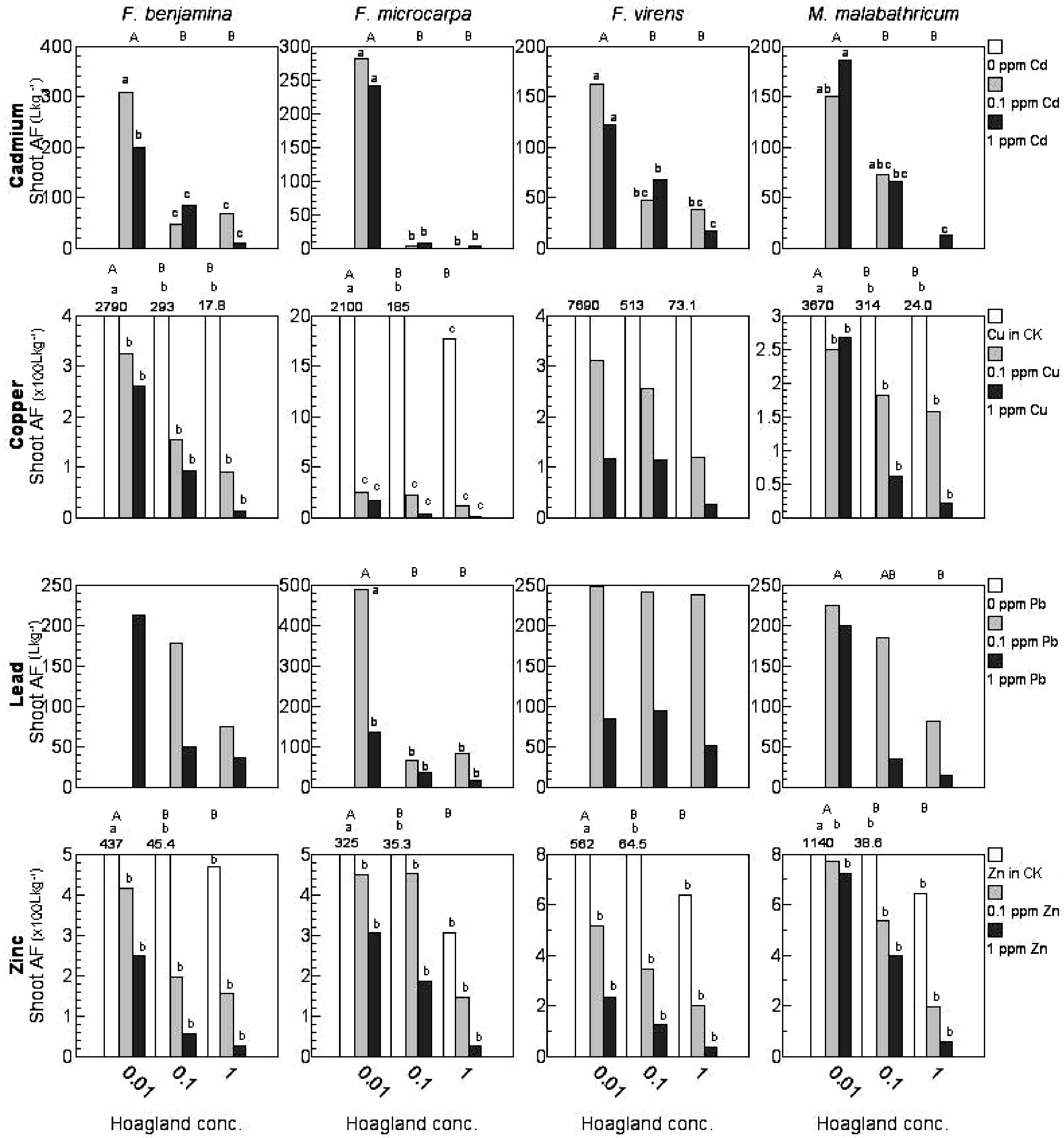

Fig. 7. Shoot accumulation factors of *Ficus benjamina*, *Ficus microcarpa*, *Ficus virens*, and *Melastoma malabathricum* under different concentrations of metal and Hoagland's solution. Some charts have the extremely high bars truncated to allow lower values to be seen.

For Pb, a comparison was made with the semi-woody shrub, *Ricinnus communis*, used in the work done by Romeiro et al. (2006), owing to the lack of studies using a woody plant model free from chelating agents. None of the species studied here accumulated even up to the lower range reported for *Ricinnus communis*. However, it should be noted that even the lowest concentration of metal used in the study was more than twenty times of that used in the present work, and that the nutrients were administered separately from the Pb solution, thus preventing any precipitation of the Pb^{2+} cations by the components of the nutrient solution used. Though for Pb the four species did not perform up to mark of the reference non-hyperaccumualtor species, given the confounding factors, this does not conclusively show that the plants species in the present study are unsuitable for Pb phytoremediation.

Some limitations of the present study to be addressed by future work. – It is a concern that the short duration of the study could not fully demonstrate the adverse effect of low nutrient levels and presence of heavy metals on plant growth. It was observed that cholorsis started to show in plants that were given the lowest nutrient level about halfway through the experiments, even though the effect did not generally translate into significantly lower dry mass

Table 2. Root and shoot concentrations of non-hyperaccumulator species studied in hydroponics reported in earlier studies. For comparison, 1 ppm concentration of the metals used in the present study corresponds with: 8.90 µM Cd, 15.7 µM Cu, 4.83 µM Pb, and 15.3 µM Zn.

Species	Metal solution concentration	Duration of expt.	Plant concentration	Desorption agent used	Reference
Kandelia candel	0.02–5 ppm Cu Supplemented with NaCl	12 weeks	Root: 19.28–248.81 ppm Branch: 16.01–38.18 ppm	No	Chiu et al. (1995)
Kandelia candel	0.08–50 ppm Zn Supplemented with NaCl	12 weeks	Root: 37.95–2430.11 ppm Branch: 35.80–377.05 ppm	No	Chiu et al. (1995)
Populus species	50 µM Cd	21 days	Root: 9962 ppm Shoot: 293 ppm	Yes	Zacchini et al. (2009)
Ricinus communis	100–400 µM Pb	28 days	Root: 7000–24000 ppm Shoot: 320–550 ppm	No	Romeiro et al. (2006)
Salix species	1 µM Cd	20 days	Root: < 300 ppm Shoot: < 7 ppm	No	Landberg & Greger (1996)
Salix species	50 µM Cd	21 days	Root: 4296 ppm Shoot: 651 ppm	Yes	Zacchini et al. (2009)
Salix viminalis	0.3 µM Cd, 0.1 µM Cu, 3 µM Zn (singly or mixture)	20 days	Root: < 25 ppm Cd, < 90 ppm Cu, < 1300 ppm Zn Shoot: < 3 ppm Cu, < 350 ppm Zn	Yes	Landberg & Greger (2002)

accumulation, except for the case of *Ficus microcarpa*. It is believed that the short duration of the study probably limited its ability to detect the effect on growth rates of the woody plant species, thus longer-term studies are needed.

Furthermore, the interactions between the different metals affecting uptake were also not investigated, though previous work on *Salix* and *Populus* by Dos Santos Utmazian et al. (2007), and *Salix viminalis* by Landberg & Greger (2002) showed metal uptake to be different when administered in a mixture compared to single administrations. This was not addressed in the present study, as it was meant more as a first screening for potential candidates for phytoremediation applications. Certainly, more work dealing with metal mixtures will be needed after a number of potentially suitable plants are found, to account for realistic situations where metals do co-occur in polluted waters or soils.

More importantly, Dos Santos Utmazian & Wenzel (2007), in comparing the results from soil-based trials with their hydroponic study (Dos Santos Utmazian et al., 2007) on *Populus* and *Salix,* have shown that hydroponic tests may underestimate the translocation of metals. Limitations of hydroponic experiments have also been discussed by Stoltz & Greger (2002), as well as Watson et al. (2003). However, Watson et al. (2003) have also shown that hydroponic screening results do correspond broadly to performance in the field. Furthermore, interactions with rhizosphere organisms were also not addressed in the present work, though these are important determinants of plant uptake of the metals, as Burd et al. (2000) and Khan et al. (2000) have shown for bacteria and mycorrhizae respectively. These factors overlooked in the present work can only be properly addressed using soil-based pot trials or field trials.

Lastly, only *Ficus microcarpa* and *Melastoma malabathricum* are known to be tolerant of waterlogging, a precondition for use in phytoremediation wetlands, while the tolerance of *Ficus virens* and *Ficus benjamina* remain untested. However, the real usefulness of these species can only be known with soil-based trials that try to replicate wetland-like conditions. Furthermore, though the current study is interested in finding species suitable for the phytoremediation of water, these plants, being non-obligate wetland species, may be shown in future trials to be suitable for remediating non-waterlogged soils as well.

CONCLUSIONS

As Pulford & Dickinson (2005) have highlighted, the genetic and phenotypic variability of Salicaceae hold a lot of potential for phytoremediation applications. Similar claims, awaiting confirmation, can be made here for the genus *Ficus* of the Moraceae, with more than 600 species worldwide (Berg & Corner, 2005). To date, we know of no other study that tries to ascertain if there are any tropical woody species that may be used in similar applications.

Furthermore, there may be other genera worth looking into, as our present study of *Melastoma malabathricum* has shown. As woody plants in the tropics include many more species than in the temperate regions, there may be many more potentially useful species to be discovered, and a quick screening method using hydroponics could be employed to aid this process. Such basic research will have to be done before a list of potential tropical plants can be drawn, compared and field tested. Furthermore, until the information for tropical plants becomes available, comparison with, and extrapolation from, temperate counterparts may not be reliable owing to differences in climate, growth conditions, edaphic factors, etc. Indeed, we have a very far way to go to make up for the lag in the state of our knowledge compared to that of temperate regions.

In conclusion, *Ficus benjamina*, *Ficus virens*, and *Melastoma malabathricum* could be useful for the phytoextraction of Cd, and all four species should be further investigated for Cu. *Ficus benjamina* and *Melastoma malabathricum* should be further investigated for the phytoextraction of Pb, while *Ficus virens* and *Melastoma malabathricum* should be investigated for Zn. Of all the metals investigated, only for Pb did the plant species studied here not compare well against reference species reported to have phytoextraction potential for these metals. However, even *Ficus microcarpa*, the worst performing species in most measures, should not be ruled out for its potential to be used to phytoremediate eutrophic waters until further study is conducted. Though none of the species are hyperaccumulators of the metals, their more vigorous production of biomass compared to slower-growing hyperaccumulators may ultimately remove more metals. This is the line of reasoning followed by Dickinson & Pulford (2005) in proposing the use of fast growing *Salix* clones for extracting Cd in a short-rotation coppice system.

With these results, we hope to show that tropical non-hyperaccumulator species with suitable qualities may be found for use in the phytoremediation of metals. We hope that this will be a motivation to search for potentially better candidates so that a list of species could be tested for their phytoremediation ability in more realistic and scaled-up trials to show the applicability of phytoremediation in artificial wetlands in the tropical context. Although the focus of this work has been on finding species for phytoremediation in wetlands, it should be noted that the candidates are not obligate wetland species, and may prove in further tests to be useful even for the phytoremediation of polluted terrestrial environments. However, like in the case of wetland phytoremediation, knowledge is lacking in the tropics and thus more work in these areas will be beneficial.

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