Photosynthetic Pigments and Peroxidase Activity as Indicators of Heavy Metal Stress in the Grey Mangrove, *Avicennia marina* (Forsk.) Vierh.

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Mangroves have been observed to possess a tolerance to high levels of heavy metals, yet accumulated metals may induce subcellular biochemical changes, which can impact on processes at the organismal level. Six month-old seedlings of the grey mangrove, *Avicennia marina* (Forsk.) Vierh, were exposed to a range of Cu (0–800 µg/g), Pb (0–800 µg/g) and Zn (0–1000 µg/g) concentrations in sediments under laboratory conditions, to determine leaf tissue metal accumulation patterns, effects on photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids), and the activity of the antioxidant enzyme peroxidase. Limited Cu uptake to leaves was observed at low sediment Cu levels, with saturation and visible toxicity to Cu at sediment levels greater than 400 µg/g. Leaf Pb concentrations remained low over a range of Pb sediment concentrations, up to 400 µg/g Pb, above which it appeared that unrestricted transport of Pb occurred, although no visible signs of Pb toxicity were observed. Zn was accumulated linearly with sediment zinc concentration, and visible toxicity occurring at the highest concentration, 1000 µg/g Zn. Significant increases in peroxidase activity and decreases in photopigments were found with Cu and Zn at concentrations lower than those inducing visible toxicity. Significant increases in peroxidase activity only, were found when plants were exposed to Pb. Positive linear relationships between peroxidase activity and leaf tissue metal concentrations were found for all metals. Significant linear decreases in photosynthetic pigments with increasing leaf tissue metal concentrations were observed with Cu and Zn only. Photosynthetic pigments and peroxidase activity may be applicable as sensitive biological indicators of Cu and Zn stress, and peroxidase activity for Pb stress in *A. marina*. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: bioindicator; carotenoids; chlorophyll; heavy metals; mangrove; peroxidase.

Introduction

Heavy metals are common pollutants in urban aquatic ecosystems, and in contrast to most pollutants, are not biodegradable and are thus persistent in the environment. Many metals, a number of which are non-essential to plant and animal metabolism, are often toxic in low concentrations (Baker, 1981). Metal inputs arise from industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps and mining operations. The metals of greatest concern are copper (Cu), lead (Pb), and zinc (Zn), both because of the potential threat they pose to aquatic organisms and to human consumption (Luoma, 1990; MacFarlane, 2000).

Mangrove systems have the capacity to act as a sink or buffer and remove or immobilize heavy metals before they reach nearby aquatic ecosystems. Because mangrove sediments have a large proportion of fine particles, high organic content and low pH, they effectively trap heavy metals, often by immobilising them in the anaerobic sediments either by adsorption on ion exchange sites on the surface of sediment particles, incorporation into lattice structures of the clay particles, or precipitation as insoluble sulfides (Harbison, 1986).

Mangroves, however, appear to possess a great tolerance to relatively high levels of heavy metal pollution. A number of researchers have found high concentrations of accumulated metals in the tissues of numerous mangrove species in the field including *Kandelia candel*.
(L.) Druce, Rhizophora spp and Avicennia spp (Peters et al., 1997). For many mangrove species, essential metals (Cu and Zn) show some limited translocation to leaf tissue, while nonessential metals (Pb) mainly accumulate at the root level (Lacerda, 1998). This is thought to be the result of metals in sediment being in forms with low bio-availability, in conjunction with biological mechanisms including metal exclusion and sequestering processes in root tissue (Chiu and Chou, 1991). Although considered tolerant, uptake of metals in excess to nutritional requirements by mangroves may initiate a variety of subcellular responses, that is, metabolic reactions, which can cause damage at the cellular level, or possibly lead to wider phytotoxic responses (Vangronsveld and Clijsters, 1994).

The formation of free radical species (all forms of active oxygen), which may be initiated directly or indirectly by metals, can cause severe damage to different cell components, particularly biological membranes (Van Assche and Clijsters, 1990; Dietz et al., 1999). Metals such as Cu, Pb and Zn are efficient generators of active oxygen species and thus an important factor in these metals’ toxicities is the generation of oxidative stress (Aust et al., 1985). To avoid the accumulation of these toxic intermediates (hydrogen peroxide, superoxide radical and hydroxyl radical), plant tissues have a series of de-toxifying antioxidants (reductants and radical scavengers including glutathione, ascorbic acid, tocopherols, hydroquinones and flavonoids) involving both non-enzymatic and enzymatic mechanisms. Peroxidases catalyze quenching reactions of dehydrogenation with the transfer of hydrogen from an antioxidant donor to hydrogen peroxide (Vangronsveld and Clijsters, 1994). In consequence, the increase in peroxidase activity on exposure to heavy metals in the cell may play a key role in the cellular defence mechanism against metal toxicity (Van Assche and Clijsters, 1990).

Peroxidase activity levels have been found to show a significant positive correlation in tissues of a number of plant species with the levels of Cu (Mocquot et al., 1996; Mazhoudi et al., 1997), Pb (Lee et al., 1976; Wozny and Krzeslowska, 1993) and Zn (Van Asshe et al., 1988; Wecks and Clijsters, 1997). The increased production of peroxidase is considered to be due to the phytotoxic metal fraction, or free metal not bound to cell walls or accumulated in vacuoles. The free metal fraction may also produce secondary effects such as growth inhibition (Van Assche and Clijsters, 1987). Peroxidases have various physiological roles in plant cells and participate in many reactions including lignification, cross linking of cell wall polysaccharides, oxidation of indole-3-acetic acid, regulation of cell elongation and phenol oxidation, all linked to growth reductions (Mocquot et al., 1996). Peroxidase activity shows a close correlation with changes in physiological processes such as, respiration, photosynthesis, CO₂-fixation, transpiration and gas exchange with obvious growth and fitness consequences, and therefore has the potential to serve as a sensitive indicator of compromised metabolic activity (Verkleij and Schat, 1990).

Inhibition of photosynthesis by heavy metals in higher plants is well documented (Clijsters and Van Assche, 1985; Prasad and Strzalka, 1999), especially in those which utilize a C₃ photosynthetic chemistry, including A. marina (Ball, 1985; Basak et al., 1996). Reduction in the levels of photosynthetic pigments, including chlorophylls a and b and accessory pigments such as carotenoids, on exposure to heavy metals have been observed in many species for Cu (Van Assche and Clijsters, 1990), Pb (Wozny and Krzeslowska, 1993; Kastori et al., 1998), and Zn (Krupa et al., 1996). In terms of mangrove species responses, K. candel has been found to exhibit reductions in chlorophylls a and b when exposed to wastewater containing a mixture of heavy metals (Chen et al., 1995).

Mechanisms directly concerned with the reduction of photosynthetic pigments include inhibition of enzymes involved in chlorophyll biosynthesis including δ-aminolevulinic acid (ALA)-dehydratase (EC 4.2.1.24) (Van Assche and Clijsters, 1990) and protochlorophyllide reductase (De Filippis and Pallaghy, 1994). Substitution of the central magnesium ion by heavy metals can also be an important damage mechanism, where substitution prevents photosynthetic light harvesting and results in chlorophyll decay and a breakdown in photosynthetic activity (Prasad and Strzalka, 1999). The inhibition of both photosystem II and photosystem I generating lipid peroxidation from active oxygen radicals, may also result in the destruction of photosynthetic pigments and thylakoid membrane structure (Droppa and Horvath, 1990). Monitoring changes in photosynthetic pigments in response to metal stress not only indicates damage to the photosynthetic apparatus and accompanying changes in photosynthetic capacity, but also has consequences for reduced carbon assimilation, growth, survival and reproduction (Vangronsveld and Clijsters, 1994).

Thus, both peroxidases and photosynthetic pigments may have the potential to be employed as sensitive indicators of metal stress (Dietz et al., 1999), and may predict subsequent events at the organism level (Van Assche et al., 1988). Very little work is published on the effects of heavy metals on angiosperms in estuarine environments, which are unique and very different in terms of their adaptations to an often highly saline environment (Peters et al., 1997). This manuscript is the first to address metal accumulation in Avicennia spp. under laboratory conditions, and the first to examine subcellular effects of metals on the photosynthetic pigments and peroxidase content in mangroves, which have been considered to be relatively tolerant to metals.

We hypothesize that A. marina seedlings will accumulate Cu, Pb and Zn in leaf tissue on exposure to sediment borne metals. In addition, accumulated metals will produce decreases in photosynthetic pigments and increases in peroxidase content.
Thus the aims of the current study have been:
1. to determine if Cu, Pb, and Zn are accumulated in leaf tissue on exposure to sediment borne metals,
2. to investigate the effects of Cu, Pb and Zn exposure on leaf chlorophyll \( a \), chlorophyll \( b \), total chlorophylls and the \( a/b \) ratio, carotenoids, and peroxidase activities, and
3. to quantify the relationships between metal exposure and effects on photosynthetic pigments and peroxidase activity under laboratory conditions.

Materials and Methods

Field collection and germination
Mature *A. marina* propagules were collected from Powells Creek, Homebush Bay, Sydney, Australia, adjacent to the Olympics 2000 site, in December 1996. Only complete, undamaged propagules with intact and no emergent hypocotyl or radicle were selected for planting. Propagules were sorted into 17 weight classes of 0.5 g increments, which ranged from 3 to 11.5 g fresh weight. The propagules selected were from the most abundant weight class, 10–10.4 g fresh weight. Propagules were grown for 6 months under glasshouse conditions in a commercially prepared soil (50% silty clay loam, 20% washed river sand, 30% organic peat moss) in 200 l seawater, with the fertilizer Aquasol (0.8% w/v), in order to mimic an estuarine sediment. The seedlings were planted in separate 140 × 140 mm plastic pots immersed within 2 l plastic containers (as holding trays) to minimize drainage and simulate anoxic, waterlogged conditions. Seedlings were watered manually. Levels of water in the holding trays were maintained at 300 ml and re-percolation was carried out once a week, where the contents of the holding tray were poured through the soil medium and the percolate collected in the same holding tray, creating a closed system to maintain salinity (and metal levels) in the sediment.

Heavy metal exposure
Ninety-six individual six-month old seedlings were chosen for experimentation. All seedlings were similar in apparent health, height (318 ± 29 mm), and leaf number (8 ± 0.86) (mean ± SE). Seedlings were randomly allocated to each treatment (\( n = 6 \)). To each individual pot in each treatment, an appropriate solution of the metal salt was added to arrive at 6 replicates across concentration ranges, respectively: 0, 50, 100, 200, 400 and 800 \( \mu \)g Cu (as CuCl\(_2\) · 2H\(_2\)O) per gram of dry sediment; 0, 100, 200, 400 and 800 \( \mu \)g Pb (as Pb(II)Cl\(_2\)) per gram of dry sediment; and 0, 125, 250, 500 and 1000 \( \mu \)g Zn (as ZnCl\(_2\)) per gram of dry sediment. Metal concentrations were chosen because these levels are indicative of heavy metal contaminated sediments in the field (Luoma, 1990). Metals were added as the chloride salt to minimize adverse effects of anion toxicity, mangroves being adapted to high levels of Cl in estuarine sediments (Burchett *et al.*, 1984). As sediments were treated with 20% seawater, the increase in chloride ion concentration from chloride salt addition was minimal. Dosed replicates were block randomized and maintained in the glasshouse for a period of 8 weeks prior to harvest.

Heavy metal content in leaf tissue and sediment
After harvest, 500 mg samples (dry weight) of washed, oven dried and homogenized second leaf pairs, and sediment, were weighed into acid washed (10% HNO\(_3\)) beakers (100 ml) and digested for heavy metal analysis with a hot mixture of concentrated acids, after the method of Krishnamurti et al. (1976). This procedure involved the addition of up to 10 ml of concentrated HNO\(_3\), with refluxing, on a sand bath for two hours at 100°C. The digests were allowed to cool prior to the addition of up to 6 ml of hydrogen peroxide to oxidize any remaining organic matter. Once efervescence had subsided, the beakers were returned to the sand bath for another hour. Digests were then filtered through a Whatman 41 filter paper into 50 ml volumetric flasks and made to volume with MilliQ grade water.

Heavy metal analyses were carried out on the resulting digestions using atomic absorption spectroscopy (air/acetylene, Varian AA-1275, Australia). Standards used were matrix matched and international plant/sediment standards (NBS standard reference materials; 1646/estuarine sediment; 1572/citrus leaves) were used to check percentage recovery of metals (Sediment Cu = 94%, Pb = 82%, Zn = 84%; Leaf tissue Cu = 93%, Pb = 97%, Zn = 94%).

Photosynthetic pigments
Upon harvest, further samples of second leaf pairs from each seedling (\( n = 6 \) per treatment) were taken for determination of chlorophyll \( a \), chlorophyll \( b \), the derived parameters (total chlorophyll and chlorophyll \( a/b \) ratio), and carotenoids by the method of Inskeeper and Bloom (1985), using extraction in \( N,N \)-dimethylformamide (DMF) and spectrophotometric estimation. Leaf tissue samples of 50 mg (fresh weight), were finely sliced with stainless steel scissors, to increase the surface area of tissue exposed to the extractant, and placed in a 15 ml amber glass screw cap bottle containing 10 ml DMF. The bottles were placed in a darkened container in the refrigerator for 7 days prior to spectrophotometric determination in glass cuvettes (1 cm path length) on a UV/VIS spectrophotometer (LKB Ultraspec II UV/VIS, model 4050, England). Spectral resolution 1.00 nm). Wavelengths chosen for analysis were 647 and 664 nm for the chlorophylls, and 480 nm for the carotenoids. Pigment contents were calculated in mg/g dry weight by applying the absorption coefficient equations described by Wellburn (1984).

Peroxidase activity
The peroxidase activity (donor H\(_2\)O\(_2\) — oxidoreductase, EC 1.11.1.7) measured in the current experiment includes a group of non-specific enzymes from different
sources, which is generally referred to as non-specific or guaiacol peroxidase. The peroxidase (POD) activity was determined following the methodology of Putter (1974) (guaiacol assay), with a number of modifications. Preliminary experiments were performed to determine the optimum conditions of incubation for A. marina leaf tissue, in order to obtain a proportional increase in the rate of guaiacol oxidation with increased peroxidase content of the sample. The peroxidase extractions were carried out in the presence of polyvinylpyrrolidone in order to neutralize the effect of phenol in leaf tissue. The final protocol developed for the determination was as follows: 300 mg of frozen green leaf tissue (~80°C) from the second leaf pairs of each seedling (n = 6), was macerated in a tissue homogenizer (OMNI 5000, USA) for 30 min in 0.1 M Tris-HCl buffer, containing 4 ml of 8.75% polyvinylpyrrolidone (MW 40,000) (w/v), 2 ml of 0.1 M KCl, and 0.1 ml of 0.28% Triton X-100, in a total volume of 35.1 ml. The homogenate was centrifuged (Orbital 400, Clements, UK) for 30 min at 4000 rpm, and the supernatant was filtered through a 0.45 µm membrane filter. The processing was carried out at 0–4°C. Peroxidase activity was assayed spectrophotometrically (LKB Ultraspec II UV/VIS, model 4050, England) in an incubation mixture containing, 200 µl of 1% v/v guaiacol, 100 µl of 0.18% v/v hydrogen peroxide, 2 ml of standard K phosphate buffer, and 0.5 ml (±0.25 ml) of sample supernatant. The rate of increase in absorbance was measured at 470 nm at room temperature. The activity was calculated as the rate of increase of absorbance per minute, and expressed as units/g dry weight.

**Statistical analysis**

Leaf metal levels, photosynthetic pigment concentrations and peroxidase activity levels were compared separately by a one factor analysis of variance (ANOVA), a routine of SYSTAT (Wilkinson, 1989). When the ANOVA identified a significant difference for a main effect of the metal treatment (p < 0.05), a post hoc pairwise comparison of the sample means was performed with the Tukey’s honestly significant difference test (HSD) test. This test was used to identify which treatments were most similar. Relationships between metal concentrations in sediments, accumulated metal concentrations in leaf tissue, leaf pigments and peroxidase activity were determined using single regression analyses, including linear, sigmoidal and exponential models in SigmaPlot (1997).

**Results**

**Copper**

It was found that symptoms of phytotoxicity became apparent in treatments of 400 µg/g Cu and above, with visible signs of desiccation, leaf epinasty and chlorosis. Seedlings in these treatments were harvested one week from initial application of Cu, to enable biochemical analyses. Leaf tissues were found to accumulate between 20% and 50% of the Cu concentrations in sediments, with seedlings exposed to 100 µg/g Cu and above accumulating significantly more Cu than the control leaves (Table 1). The accumulation of Cu increased with sediment Cu to the 200 µg/g treatment, after which further increases in sediment Cu resulted in only small increases in...

### TABLE 1

Photosynthetic pigments, peroxidase activity and accumulated metals in leaf tissue of A. marina after an 8 week exposure period to Cu, Pb and Zn.\(^a\)

<table>
<thead>
<tr>
<th>Sediment metal concentrations</th>
<th>Peroxidase activity (units/g)</th>
<th>Chlorophyll a (mg/g)</th>
<th>Chlorophyll b (mg/g)</th>
<th>Total chlorophyll (mg/g)</th>
<th>Chlorophyll a/b ratio (mg/g)</th>
<th>Carotenoids (mg/g)</th>
<th>Leaf metals (µg/g)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/g Cu control</td>
<td>1148 ± 67(^a)</td>
<td>2.04 ± 0.29(^b)</td>
<td>1.19 ± 0.17(^b)</td>
<td>3.22 ± 0.47(^b)</td>
<td>1.71 ± 0.03</td>
<td>0.52 ± 0.05(^b)</td>
<td>19.2 ± 3.9(^b)</td>
</tr>
<tr>
<td>50 µg/g Cu</td>
<td>1038 ± 31(^b)</td>
<td>1.96 ± 1.15(^b)</td>
<td>1.14 ± 0.08(^b)</td>
<td>3.11 ± 0.22(^b)</td>
<td>1.73 ± 0.10</td>
<td>0.60 ± 0.02(^b)</td>
<td>24.2 ± 5.5(^b)</td>
</tr>
<tr>
<td>100 µg/g Cu</td>
<td>1207 ± 27(^a)</td>
<td>1.72 ± 0.13(^b)</td>
<td>1.06 ± 0.08(^b)</td>
<td>2.78 ± 0.20(^b)</td>
<td>1.63 ± 0.03</td>
<td>0.61 ± 0.04(^b)</td>
<td>39.5 ± 5.7(^b)</td>
</tr>
<tr>
<td>200 µg/g Cu</td>
<td>1341 ± 49(^c)</td>
<td>1.27 ± 0.10(^b)</td>
<td>0.67 ± 0.06(^b)</td>
<td>1.94 ± 0.17(^b)</td>
<td>1.91 ± 0.05</td>
<td>0.51 ± 0.03(^b)</td>
<td>46.5 ± 9.9(^b)</td>
</tr>
<tr>
<td>400 µg/g Cu</td>
<td>1458 ± 63(^b)</td>
<td>1.13 ± 0.06(^b)</td>
<td>0.65 ± 0.08(^b)</td>
<td>1.88 ± 0.12(^b)</td>
<td>2.08 ± 0.39</td>
<td>0.52 ± 0.03(^b)</td>
<td>82.9 ± 5.6(^b)</td>
</tr>
<tr>
<td>800 µg/g Cu</td>
<td>1744 ± 89</td>
<td>0.93 ± 0.10(^b)</td>
<td>0.61 ± 0.06(^b)</td>
<td>1.54 ± 0.16(^b)</td>
<td>1.53 ± 0.07</td>
<td>0.35 ± 0.07(^b)</td>
<td>67.0 ± 2.6(^b)</td>
</tr>
</tbody>
</table>

ANOVA F

| 22.4** | 10.7** | 10.2** | 11.2** | 2.16 | 5.93** | 15.7** |

ANOVA F

| 28.8** | 1.54 | 0.51 | 0.52 | 2.52 | 1.66 | 20.2** |

ANOVA F

| 48.4** | 32.4** | 29.8** | 31.4** | 5.58** | 37.3** | 44.1** |

\(^a\) Mean values ± SE (n = 6). Results of a one way ANOVA. ** Significant difference at p < 0.01. * Significant difference at p < 0.05. Treatments identified as similar, according to Tukey’s HSD multiple comparison, are linked by identical letters.

\(^b\) Accumulated leaf metals correspond with sediment metal applied.
in accumulation of Cu in leaves, a sigmoidal regression best explained the accumulation relationship (Fig. 1(a)).

Significant increases in peroxidase activity were induced on exposure to Cu, with seedlings exposed to 200 µg/g Cu and above producing significantly higher peroxidase levels than control plants, and the 800 µg/g Cu treatment producing significantly higher peroxidase levels than all other treatments (Table 1). As Cu concentrations in leaf tissue increased, peroxidase activity increased proportionally (Fig. 1(b)). Significant declines in the overall contents of pigments with increased Cu exposure were evident. Chlorophylls, carotenoids, and total chlorophylls experienced significant declines in Cu treatments of 200 µg/g and above. The chlorophyll a/b ratio remained constant over the Cu treatment range. Carotenoids were significantly reduced at the highest sediment Cu concentration (800 µg/g) (Table 1). Increasing Cu concentrations in leaf tissue produced weak proportional increases in all photosynthetic pigments (Fig. 1(g)).

**Lead**

Seedlings were found to be tolerant to Pb in the concentration range applied, with no visible signs of toxicity being observed across all treatments. Accumulation of Pb to leaf tissue was low, leaf tissue ranging between 1% and 2.5% the Pb content of the sediments. Seedlings exposed to 400 µg/g Pb and above were found to accumulate significantly more Pb in the leaves than control plants (Table 1). An exponential regression model was found to best explain the pattern of accumulation for Pb to leaf tissue from sediments (Fig. 1(c)).

Significant increases in peroxidase activity were produced by exposure to Pb. Treatments of 200 µg/g Pb and above showed significant increases in peroxidase activity compared with control seedlings (Table 1). Increases in leaf Pb were associated with proportional increases in peroxidase activity (Fig. 1(d)). However the accumulated Pb in leaf tissues showed no significant inhibitory effects on any of the photosynthetic pigments measured. Chlorophylls, carotenoids and associated pigment ratios remained constant across the Pb concentration range applied (Table 1).

**Zinc**

Seedlings were found to be tolerant to the addition of zinc in sediments, with no visible signs of toxicity being apparent for plants in concentrations up to 500 µg/g Zn in sediments. Acute toxicity was observed, however, in the 1000 µg/g treatment. Within one week of zinc addition, seedlings exposed to this treatment displaying severe stress symptoms, including desiccation, chlorosis, blackening of leaf tissue and premature leaf abscission. Plants in this treatment were harvested one week after initial metal exposure. Seedlings exposed to zinc were found to accumulate between 20% and 33% of the zinc contained in sediments. Seedlings exposed to 125 µg/g Zn and above were found to accumulate significantly more zinc in leaf tissues than the control treatment (Table 1). The accumulation of zinc in leaf tissue was found to be highly proportional to sediment Zn levels (Fig. 1(e)).

Significant increases in peroxidase activity in leaf tissue of seedlings were found with exposure to zinc. Seedlings exposed to 125 µg/g Zn and above produced significantly higher peroxidase levels than control plants, with plants in the 1000 µg/g zinc treatment showing the greatest enzyme response, i.e. producing significantly higher peroxidase activity levels than all other treatments. Increasing Zn concentrations in leaf tissue produced proportional increases in peroxidase activity (Fig. 1(f)). There was a significant decline in the overall pigment content with increased Zn exposure. Chlorophylls, carotenoids and total chlorophylls showed significant declines in Zn treatments of 500 µg/g and above. The chlorophyll a/b ratio significantly increased in Zn treatments of 500 µg/g and above, indicating a relatively greater depletion of chlorophyll b. Carotenoids were significantly reduced at the greatest Zn exposure concentration (1000 µg/g) only (Table 1). Increasing Zn concentrations in leaf tissue produced proportional decreases in all photosynthetic pigments (Fig. 1(h)).

**Discussion**

*Avicennia marina* seedlings were found to be relatively tolerant to the three metals applied, although Cu and Zn showed a greater mobility to the leaf tissue and a greater phytotoxic effect, including visible signs of damage evident at higher concentrations. Conversely, Pb produced no visible signs of toxicity across the concentration range applied. The greater accumulations of Cu and Zn suggest that the plants actively take up these elements as they are required for metabolic (including photosynthetic) processes (Baker and Walker, 1990). When uptake exceeds metabolic requirements however, a toxic impact may be expected. The accumulated metal levels in leaf tissues were an order of magnitude lower than sediment metal concentrations, suggesting an exclusion mechanism to accumulation. *Avicennia marina* can actively sequester much of the accumulated metals in root tissue with limited translocation to leaf tissue (MacFarlane et al., 1999). Cu and Zn, although mobile, show restricted translocation to the shoot due to the endodermal casparian strip, while Pb is actively excluded at the root epidermis (MacFarlane and Burchett, in press). Accumulation patterns of metals in the leaf tissue of *A. marina* were different for each metal examined. Cu was accumulated in leaf tissue in a linear fashion at lower sediment concentrations, but at concentrations of 400 µg/g Cu and higher, no further increases in leaf Cu levels were evidenced (Fig. 1(a)). Thus for Cu it appears that the physiological requirements for Cu result in some accumulation at low sediment concentrations, but that an exclusion or saturation mechanism is operating at higher sediment concentrations, accounting for the
Significant relationships between sediment metal, accumulated metals and induced changes in peroxidase activity and photosynthetic pigments in *A. marina*. Regression models best describing the relationships are given with the adjusted determination coefficient ($r^2$) and significance level ($p$). The relationship between (a) sediment Cu and accumulated leaf Cu, $y = 76.3/(1 + \exp(-(x - 105.2)/74.2))$, $r^2 = 0.77$ $p < 0.01$; (b) accumulated leaf Cu and peroxidase activity, $y = 950 + 7.99x$, $r^2 = 0.61$ $p < 0.05$; (c) sediment Pb and accumulated leaf Pb, $y = \exp(0.0038x)$, $r^2 = 0.85$ $p < 0.01$; (d) accumulated leaf Pb and peroxidase activity, $y = 1372 + 35.8x$, $r^2 = 0.65$ $p < 0.01$; (e) Sediment Zn and accumulated leaf Zn, $y = 7.67 + 0.25x$, $r^2 = 0.86$ $p < 0.01$. (f) accumulated leaf Zn and peroxidase activity, $y = 1385 + 7.96x$, $r^2 = 0.92$ $p < 0.01$; (g) accumulated leaf Cu and chlorophyll $a$ ($\bullet$) $y = 1.90 - 0.008x$, $r^2 = 0.13$ $p < 0.05$, chlorophyll $b$ ($\bigcirc$) $y = 1.1 - 0.005x$, $r^2 = 0.14$ $p < 0.05$, total chlorophyll ($\triangledown$) $y = 3.02 - 0.013x$, $r^2 = 0.14$ $p < 0.05$; (h) accumulated leaf Zn and chlorophyll $a$ ($\bullet$) $y = 2.49 - 0.004x$, $r^2 = 0.40$ $p < 0.01$, chlorophyll $b$ ($\bigcirc$) $y = 0.93 - 0.004x$, $r^2 = 0.41$ $p < 0.01$, total chlorophyll ($\triangledown$) $y = 3.4 - 0.006x$, $r^2 = 0.38$ $p < 0.05$, chlorophyll $a/b$ ratio ($\bigtriangledown$) $y = 2.60 + 0.003x$, $r^2 = 0.52$ $p < 0.01$, total carotenoids ($\bigstar$) $y = 0.78 - 0.001x$, $r^2 = 0.43$ $p < 0.01$.

Accumulation pattern and toxicity evidenced (Baker, 1981). Zn was accumulated in a linear fashion across the sediment zinc concentration range applied (Fig. 1(e)). *A. marina* may be classified as an indicator species for Zn.
accumulation (Baker and Walker, 1990). Pb was excluded from leaf tissue at lower sediment concentrations, with some uptake occurring at sediment concentrations of 400 µg/g Pb and above (Fig. 1(c)). A marina showed the accumulation pattern of a typical excluder for Pb. Accumulated leaf Pb concentrations are maintained constant and low over a wide range of Pb sediment concentrations, up to a critical sediment value, above which the exclusion mechanism breaks down and apparently unrestricted transport of the element results (Baker, 1981). The low levels of accumulated Pb across the sediment concentration range also reflects the much lower solubility of Pb.

Strong linear relationships were observed between each of the accumulated metals and the peroxidase activity of the leaf tissue, with Zn showing the strongest dose–response relationship (Fig. 1(b), (d), and (f)). Furthermore, significant increases in peroxidase activity were observed before any visible signs of phytotoxicity were apparent. Increases in peroxidase activity are regarded as a reliable indicator of heavy metal impact (Dietz et al., 1999), the increases in peroxidase activity being a response to increases in oxidative reactions, corresponding to an increase in peroxides, disruption of the plasmalemma by lipid peroxidation and free radicals produced from exposure to the phytotoxic fraction of accumulated metals, in this case Cu, Pb or Zn (Van Asshe and Clijsters, 1990). Zinc shows the strongest relationship with peroxidase induction, possibly due to its essentiality to the plant and thus its relatively greater mobility in cells and tissues (Hagemeyer, 1999).

The levels of chlorophyll a and chlorophyll b, and to a lesser degree carotenoids, decreased in a dose-dependant fashion with increasing exposure levels of both Cu and Zn, Zn showing the strongest linear relationship with pigment decreases (Fig. 1(g)-(h)). Significant decreases in chlorophyll pigments were evident at concentrations of Cu and Zn lower than those which induced visible signs of toxicity. Decreases in pigments suggest that the chlorophyll synthetising system and chlorophyllase activity were affected at higher exposure concentrations (Van Asshe and Clijsters, 1990), while iron depletion, or substitution of mangenese with Cu and Zn may also be a contributing damage mechanism (Prasad and Strzalka, 1999). Changes in membrane permeability and chloroplast ultrastructure may also contribute to declines in pigment levels due to lipid peroxidation, which is supported by the increases in peroxidase activity and the depletion of other antioxidants such as the carotenoids (Dietz et al., 1999). Decreases of these pigments imply direct reductions in photosynthetic activity, and hence reduced carbon fixation and possible effects at the whole plant level (Baker and Walker, 1990). A significant increase was seen in the chlorophyll a/b ratio for Zn alone, suggesting that the chlorophyll b pool is more sensitive to Zn exposure. Greater decreases in chlorophyll b potentially compromise the energy trapping efficiency of photosystem II and reduce electron transport (Falkowski and Raven, 1997). Greater depletions of chlorophyll b have been observed upon metal exposure in other mangrove species (Chen et al., 1995). No significant inhibitory effects on any photosynthetic pigments were evidenced on exposure to Pb, which has often been found to be less damaging to the photosynthetic apparatus unless applied in very high concentrations (Ahmed and Tajmir-Riahi, 1993). Pb is not required for growth and development, and A. marina effectively excludes most Pb at the root epidermis (MacFarlane and Burchett, in press). Furthermore, accumulated Pb is possibly sequestered in vacuoles to minimize the toxic impact on photosynthetic pigments and to minimize membrane damage (Wozny and Krzeslowska, 1993).

The increases of peroxidase and decreases in photosynthetic pigments may be used to evaluate the potential phytotoxicity of metal-contaminated estuarine sediments to resident plant species such as A. marina. A variety of sediment physiochemical parameters including pH, salinity, and organic content can modify the availability of metals to plants (Harbison, 1986). Metals may be phytotoxic separately, or they may mutually interact in an antagonistic, synergistic or additive fashion (Hagemeyer, 1999). The physiological response of a plant in terms of peroxidase activity and photochemistry can reflect the total phytotoxic effect, integrating metal bioavailability and combined metal impact within the plant (Van Assche and Clijsters, 1990). In other higher plants, different isoenzymes of peroxidase can be distinguished upon exposure to different metals, which may also possibly be employed to identify specific metal impacts in mangroves (Van Assche and Clijsters, 1990; Vangronsveld and Clijsters, 1994). Thus peroxidase activity and photosynthetic pigment levels may be suitable biological indicators of individual metals or combined metal stress under field conditions. Changes in peroxidase and photpigments however, are not produced in response to heavy metals alone, but may be induced by a variety of environmental factors including temperature, water deficit, wounding, air pollutants and senescence, and thus care must be taken in the interpretation of results (Verkleij and Schat, 1990). We are currently conducting field studies to examine the relationships between peroxidase, photosynthetic pigments and accumulated metals in A. marina under natural environmental conditions.

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