RESPONSES OF *Avicennia marina* (FORSSK.) VIERH. TO CONTAMINATION BY SELECTED HEAVY METALS

By

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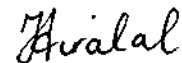
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PREFACE

The experimental work described in this dissertation was carried out at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville, from February 2004 to September 2008, under the supervision of Professor Gonasageran Naidoo and Dr. Yougasphree Naidoo.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Hiralal

Tisha Hiralal

DEDICATION

In dedication to my mother

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LIST OF ABBREVIATIONS

AAS	atomic absorption spectrophotometry
AB	abaxial
AD	adaxial
c	cuticle
CO_2	carbon dioxide
Cu^{2+}	copper ions
EC	epidermal cells
EDX	energy dispersive X-ray microanalysis
ESEM	environmental scanning electron microscopy
ETR	electron transport rate through PSII
Fv/Fm	photochemical efficiency of PSII
gb	globose
Hg^{2+}	mercury ions
Pb^{2+}	lead ions
PC	prismatic crystal
ppm	part per million
PSII	photosysytem II
SEM	scanning electron microscopy
SG	salt gland
SS	salt secretion/salt deposits
s	stomata
t	trichome
μgml^{-1}	microgram per millilitre
Zn^{2+}	zinc ions

ABSTRACT

Heavy metal contamination of mangroves is of critical concern due to its accumulative and adverse effects in aquatic ecosystems. This study was undertaken to investigate the effects of mercury (Hg^{2+}), lead (Pb^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) on plant responses, specifically growth and productivity, in *Avicennia marina* (Forssk.) Vierh. *A. marina* plants were grown for twelve months in pots contaminated with Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} at concentrations of 0, 40, 80, 120 and 160 ppm (1 ppm = 1 μgml^{-1}). Accumulation and distribution of the heavy metals in shoot and root tissues were determined using atomic absorption spectroscopy (Perkin-Elmer Model 303) while secretion of the heavy metals from leaves was studied using scanning electron microscopy and energy dispersive X-ray microanalysis. I hypothesized that heavy metals have deleterious effects on plant growth and that they are absorbed by roots and secreted from salt glands present on the leaves.

SEM X-ray microanalyses confirmed secretion of Cu^{2+} and Zn^{2+} ions as well as salt (NaCl) from glandular structures on both the adaxial and abaxial surfaces of leaves; however Hg^{2+} and Pb^{2+} were not detected in the secretion. Ion concentrations were significantly higher in plant roots than in shoots, particularly at 160 μgml^{-1} for all heavy metals. In addition, toxic levels of Hg^{2+} and Pb^{2+} were detected in the shoot tissue; however, Cu^{2+} and Zn^{2+} were within the normal ion concentration in the shoots. Plant height, number of leaves, biomass accumulation and chlorophyll content were significantly lower at 160 μgml^{-1} than the control values for all heavy metals. Carbon dioxide exchange, transpiration and leaf conductance generally decreased with increasing metal concentration. CO_2 exchange at a concentration of 160 μgml^{-1} was significantly lower than the control for all metals. CO_2 exchange at 160 μgml^{-1} for Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} were 49.6 %, 55 %, 47.6 % and 63.6 % respectively lower than the control values. Photosystem II (PS II) quantum yield, photochemical efficiency of PSII (Fv/Fm) and electron transport rate (ETR) through PS II generally decreased with increasing concentration for all heavy metals.

This study has shown that *A. marina* experiences dose-dependent stress responses to Cu^{2+}, Zn^{2+}, Hg^{2+} and Pb^{2+} in shoot and root tissue at a concentration of 160 $\mu g m l^{-1}$, evidenced by decreases in growth and photosynthetic performance. The results also indicate that Cu^{2+}, Zn^{2+}, Hg^{2+} and Pb^{2+} are taken up by roots and transported to shoots. In addition, only Cu^{2+} and Zn^{2+} are secreted via the glands while Hg^{2+} and Pb^{2+} accumulate within the shoots.

CHAPTER 1

INTRODUCTION

1.1 POLLUTION IN MANGROVES

Mangrove swamps are highly productive components of marine environments and are prominent features of the coastal zone (Potts, 1984). Mangroves are intertidal wetlands, common in tropical and subtropical coastal environments, especially in bays and estuaries (Tam and Wong, 2000). These forests support genetically diverse communities of terrestrial and aquatic organisms of direct and indirect socio-economic value. As the primary producer, mangroves supply food for marine animals; in addition mangroves provide habitats for birds, insects, fishes, algae and bacteria (Teas, 1979). However mangrove forests, located near or along estuaries, are often polluted by river-borne and marine-derived particles and pollutants.

Heavy metal contamination is of great concern, owing to the toxicity of metals and their accumulation in aquatic environments. Heavy metals such as zinc, copper, lead and mercury at concentrations above metabolic requirements may induce adverse effects on growth and productivity. Heavy metals are not biodegradable in comparison to most pollutants (Mallick and Mohn, 2003). They undergo a global ecological cycle wherein natural waters are the main pathways.

Mangroves experience both natural and human-induced disturbances. Natural disturbances may be caused by wave or current action, or by biological agents such as predatory activity, burrowing or excavating, accumulation of drift plants and animal debris which cause patchiness (Su et al., 1994; Wang et al., 2001). Human-induced disturbances include dredging, pollution, channeling of water, introduction of exotic plants and construction of structures. The developing industry of mining, smelting and metal treatment has resulted in the serious problem of heavy metal pollution (Wu et al., 1989; Liao, 1993; Guo, 1994; Su et al., 1994; Wang et al., 2001). These activities by human beings cause a high anthropogenic emission of heavy metals which then enter into the biosphere. Waste emissions, waste water and waste solid are the origin of heavy metal pollution to water, soil and plants (Cheng, 2003). These pollutants can have profound effects on the mangrove ecosystem.

According to Mallick and Mohn (2003), heavy metals are one of the most common non-biodegradable pollutants. They have further reported that these heavy metals interfere with the metabolism of photosynthetic organisms at different stages of growth at elevated concentrations in many parts of the world. Heavy metals are defined as metals with a density greater than 5.0 g cm^{-3} and are referred to as any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations (Seaward and Richardson, 1990). Cheng (2003) states that heavy metal pollution not only affects the production and quality of crops, but also influences the atmosphere and water bodies, and threatens the health and life of animals and human beings by way of the food chain. Consequently this type of pollution has a detrimental effect, and is long-term and non-reversible (Zhang, 1999; Tam and Wong, 2000).

Plants have a natural ability to extract elements from the soil and to distribute them between the roots and shoots depending on the biological processes in which the element is involved (Ximénez-Embún et al., 2002). In addition to the uptake of nutrients, toxic compounds such as heavy metals can also be taken up by the plants. These pollutants are a serious problem in estuarine and coastal environments due to their persistence, toxicity and bioaccumulation properties (Tam and Wong, 2000). Mangrove sediments are reduced and act either as sources or sinks of heavy metals such as Zn^{2+}, Pb^{2+}, and Cd^{2+} (Banus et al., 1975; Harbinson, 1986; Silva et al., 1990; De Lacerda et al., 1993b; Tam and Wong, 1993; 1995; 2000; McFarlane and Burchett, 1999). The salt marsh and mangrove sediments are anaerobic and rich in reduced sulphides and organic matter content (Naidoo and Chirkoot, 2004; Naidoo, 2006). Thus this favours the retention of the water-borne heavy metals.

Knowledge of the levels of such contaminants in mangrove sediments and plants is therefore important for understanding the degree of heavy metal pollution in aquatic systems, providing information on the anthropogenic sources of metal pollution, the transport, fate and bioavailability of the metals concerned (Japenga et al., 1990).

1.2 DESCRIPTION OF *AVICENNIA MARINA*

In Southern Africa, mangrove swamps occur only within the various estuaries along the east coast of KwaZulu-Natal, Transkei and Eastern Cape (Berjak et al., 1977; Ward and Steinke, 1982). *Avicennia marina*, a facultative halophyte is the most dominant mangrove species in

Southern Africa (Waisel *et al.*, 1986; Hutchings and Saenger, 1987). According to Palmer and Pitman (1973), Linnaeus named this genus after an oriental physician who lived over 1000 years ago – from 980 to 1036 – and whose name was Avicennia.

Avicennia marina has been described as an evergreen shrub or small bushy tree that grows to heights of 1-10 m high and is highly salt tolerant ((Little, 1983; Steinke, 1995; Naidoo and Chirkoot, 2004; Naidoo, 2006). It is highly adapted to the mangrove habitat because of its extensive, but shallow root system. The root system has no central tap root; instead numerous horizontal cable roots radiate out from the base of the trunk at a depth of 200-500 mm below the soil surface and serve to anchor the tree firmly in the loose substrate (Berjak *et al.*, 1977).

A characteristic feature of the tree is the presence of a large number of upright pneumatophores ('pencil-roots'), 10-15 cm high and 6 mm in diameter by which the subterranean part of the tree is able to obtain oxygen (Odum *et al.*, 1982; Little, 1983; Steinke, 1995). Pneumatophores (Fig. 1e) are soft and corky with small openings on their surface, called lenticels, which facilitate gaseous exchange of oxygen and carbon dioxide at low tide. In addition, pneumatophores produce tufts of fine rootlets, below ground level, which are particularly concerned with nutrient absorption (Berjak *et al.*, 1977). Masses of small air roots are often observed on the trunk; however there are no prop or stilt roots present. The bark is whitish to greyish or yellow-green in colour, smooth, often powdery with raised dots and scales. The inner bark is greenish and is lenticellate in younger parts (Little, 1983; Steinke, 1995).

Avicennia marina leaves occur in opposite pairs, are ovate, lanceolate to elliptical, acute at the apex and rounded or tapering towards the base (Little, 1983; Steinke, 1995). Leaves are numerous, particularly towards the very ends of the branches and they have a thick, tough leathery texture. The leaves are 3.5-12 cm in length and 1.5-5 cm wide with entire margins (Fig. 1d). Leaves are shiny olive green and glabrous on the upper surface while matt, and silver green with dense fine hairs or trichomes underneath (Berjak *et al.*, 1977; Little, 1983; Steinke, 1995; Naidoo and Naidoo, 2005). Specialized secretory glands are present on the leaf surface, particularly on the smooth adaxial surface from which an exudation of liquid is secreted from the leaves (Naidoo and Chirkoot, 2004; Naidoo and Naidoo, 2005)). The glandular exudate is very salty. The petioles are 5-10 mm long and glabrous (Little, 1983; Steinke, 1995).

Flowers are in bloom from September to February. Each inflorescence is a densely-clustered head or cyme comprising several individual flowers. Inflorescences are neither large nor colourful; however they produce an abundance of fragrant nectar (Berjak *et al.*, 1977). Flowers are sessile, few to many, 4 mm long and 5 mm across. The calyx is five-lobed, green and hairy. The corolla is tubular and white turning pale-yellow or orange with four nearly equal, short lobes. Corolla lobes are ovate-acute; there are 4 stamens; the ovary is superior and villous; the style is short and there are 2 stigmas (Little, 1983). Pollination is by insects, commonly bees (Berjak *et al.*, 1977).

The fruits (Fig. 1d) are most abundant in February and March. These pale green capsules or propagules (Fig. 1a) contain a single seed (Fig. 1b-c) and are borne in clusters. They are 12-15 mm long, ovoid in shape, apiculate, firm and velvety to the touch, compressed and viviparous (Little, 1983; Steinke, 1995).
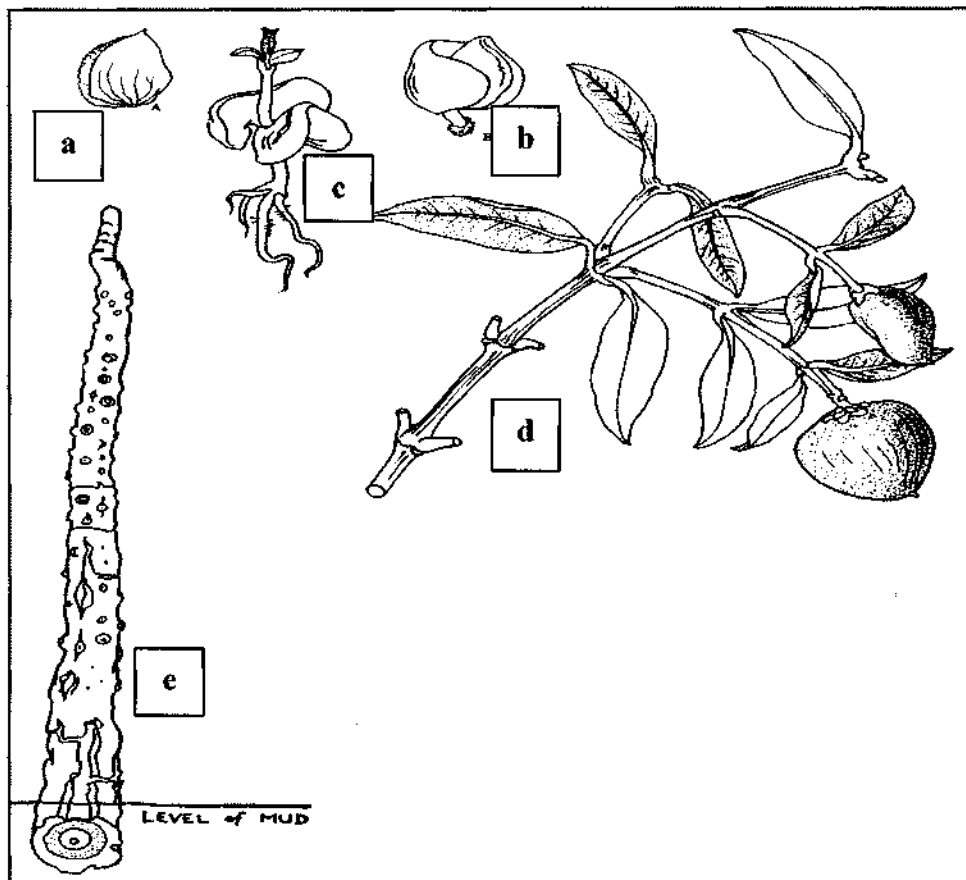
Fig. 1. *Avicennia marina*. a. The cotyledons appear thin-skinned. b. The cotyledons are folded with the radicle appearing. c. The young plant. d. Leaves and fruits.

e. A pneumatophore (Palmer and Pitman, 1973).

Avicennia marina is characterized by various adaptations for coping with hypersaline environments. These adaptations may affect uptake, distribution, loading and secretion of heavy metals such as zinc, mercury, lead, cadmium and copper within the plant. *A. marina* possesses numerous secretory glands on both the adaxial and abaxial leaf surfaces, which actively excrete salts rich in sodium chloride and potassium in order to maintain favourable osmotic gradients in a saline environment (Thompson, 1975; Drennan and Pammenter, 1982). According to Ernst (1972), other halophytes excrete heavy metals in conjunction with other solutes in similar leaf glandular tissues. In the present study, the responses of the mangrove, *Avicennia marina*, to contamination by selected heavy metals i.e. Cu^{2+}, Zn^{2+}, Pb^{2+}, and Hg^{2+} were investigated.

1.3 HYPOTHESES

We tested the following hypotheses:

1. Heavy metals are either chelated in the soil or roots or taken up by the plant.
2. Plant growth is adversely affected by heavy metals uptake.
3. Heavy metals transported into leaves are secreted *via* salt glands.

1.4 SPECIFIC OBJECTIVES OF THE STUDY

The major objectives of this study were to determine the following:
1. The effect of Cu^{2+}, Zn^{2+}, Hg^{2+} and Pb^{2+} on growth of *A. marina*.
2. Whether the heavy metals are absorbed and translocated within the plant.
3. If *A. marina* is adversely affected by heavy metals within the shoot tissues.
4. The distribution of metals in plant organs.
5. Whether metals are secreted *via* the glands.

1.5 THE POSSIBLE OUTCOMES OR EXPECTATIONS OF THIS STUDY

This study will provide information on the effects of heavy metals on the ecophysiology, ecotoxicology and leaf surface structure. The study will also indicate if the species can serve as an indicator for heavy metal tolerance. Data obtained from this study will contribute to a better understanding of the effects of Cu^{2+}, Zn^{2+}, Hg^{2+} and Pb^{2+} on plant responses and thus provide information for the conservation and environmental management of this pioneer species.

CHAPTER 2

LITERATURE REVIEW

"One perceives a forest of jagged, gnarled trees protruding from the surface of the sea, roots anchored in deep, black, foul-smelling mud, verdant crowns arching toward a blazing sun. Here is where land and sea intertwine, where the line dividing ocean and continent blurs ..." (Rutzler and Feller, 1996).

In this description of one of the world's most elaborate and intricate ecosystems, the fascinating mangrove plants thrive.

Meandering along the seaward fringe and other saltwater sources, a highly exclusive breed of plant has adapted and colonized the shorelines; they are known as the halophytes. The mangrove is seen as the most famous of the halophytes (Duke, 1992; Rutzler and Feller, 1996).

What is a mangrove?
A mangrove may be defined as a woody plant or a community of tropical trees, shrubs or palms which live amid the sea and the land in areas which are inundated by tides. A mangrove community is composed of plant species uniquely adapted for living in saline and flood conditions (Duke, 1992).

2.1. DISTRIBUTION, ZONATION AND HABITAT OF MANGROVES

Unlike most marginal ecosystems, mangroves are highly productive and dynamic. Healthy mangrove ecosystems are able to efficiently immobilize heavy metals (Vannucci, 2000). Mangrove stands, even if only isolated groups of just a few mangrove trees are immediately identifiable as a mangrove, whether they occupy many hectares of wetlands or just a tiny coastal tidally-inundated pool of brackish water. Due to their

ability to tolerate extreme environmental conditions, the species of mangrove are able to invade areas by competitive exclusion of other species of plants and animals that are unable to tolerate such harsh conditions.

Scientists have proposed that mangroves may have originated in the Indo-Malayan region (Quarto, 2000). The global distribution of mangroves (Fig. 2a) is divided into two hemispheres, namely, the Atlantic East Pacific, comprising of approximately 12 species, and the Indo West Pacific which contains approximately 58 species (Dawes, 1981; Duke, 1992; Quarto, 2000). Globally mangrove ecosystems are comprised of approximately 65 to 70 recognized mangrove species (Dawes, 1981; Duke, 1992). Species composition is also very different between the two hemispheres with only the mangrove fern common to both hemispheres (Duke, 1992). There are 12 genera of mangrove trees of common occurrence worldwide, and these belong to 8 different plant families (Berjak, 1977; http://www.enhg.org/b/b32/32_02.htm). Mangrove trees have unique seeds and dispersal mechanisms which have helped them to colonize a great deal of the tropical and sub-tropical intertidal shorelines, from latitudes 32° N to 38° S (Banks, 2003). Worldwide, mangroves are distributed along sheltered coastlines and cover an approximate area of 240 000 km^2 (Lugo *et al.*, 1990). Mangroves occur along the east coast of Africa, throughout south and south-east Asia, and into Australia. Asia is the region with the richest mangrove species diversity, with 44 reported species. (http://forest.and.nic.in/frst-mangroves1.htm).
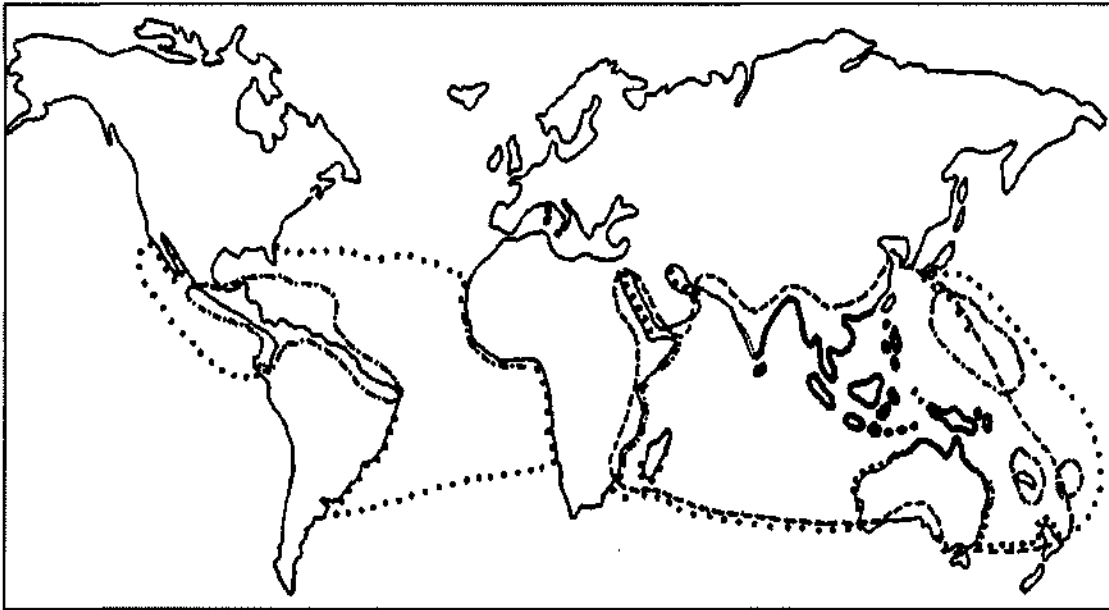
Fig. 2a. General world distribution of mangroves (Chapman, 1975).

In South Africa, there are 5 mangrove tree genera from 3 distinctive families (Fig. 2b). These are represented by *Avicennia* from the family Avicenniaceae, *Lumnitzera* from the Combretaceae family and the Rhizophoraceae family contains the genera *Bruguiera, Rhizophora* and *Ceriops*. *Avicennia* and *Bruguiera* are the most abundant mangroves within the mangrove belt region, followed by *Rhizophora* while *Lumnitzera* and *Ceriops* are generally found in highly localized areas within the region (Berjak *et al.*, 1977; Ward and Steinke, 1982).

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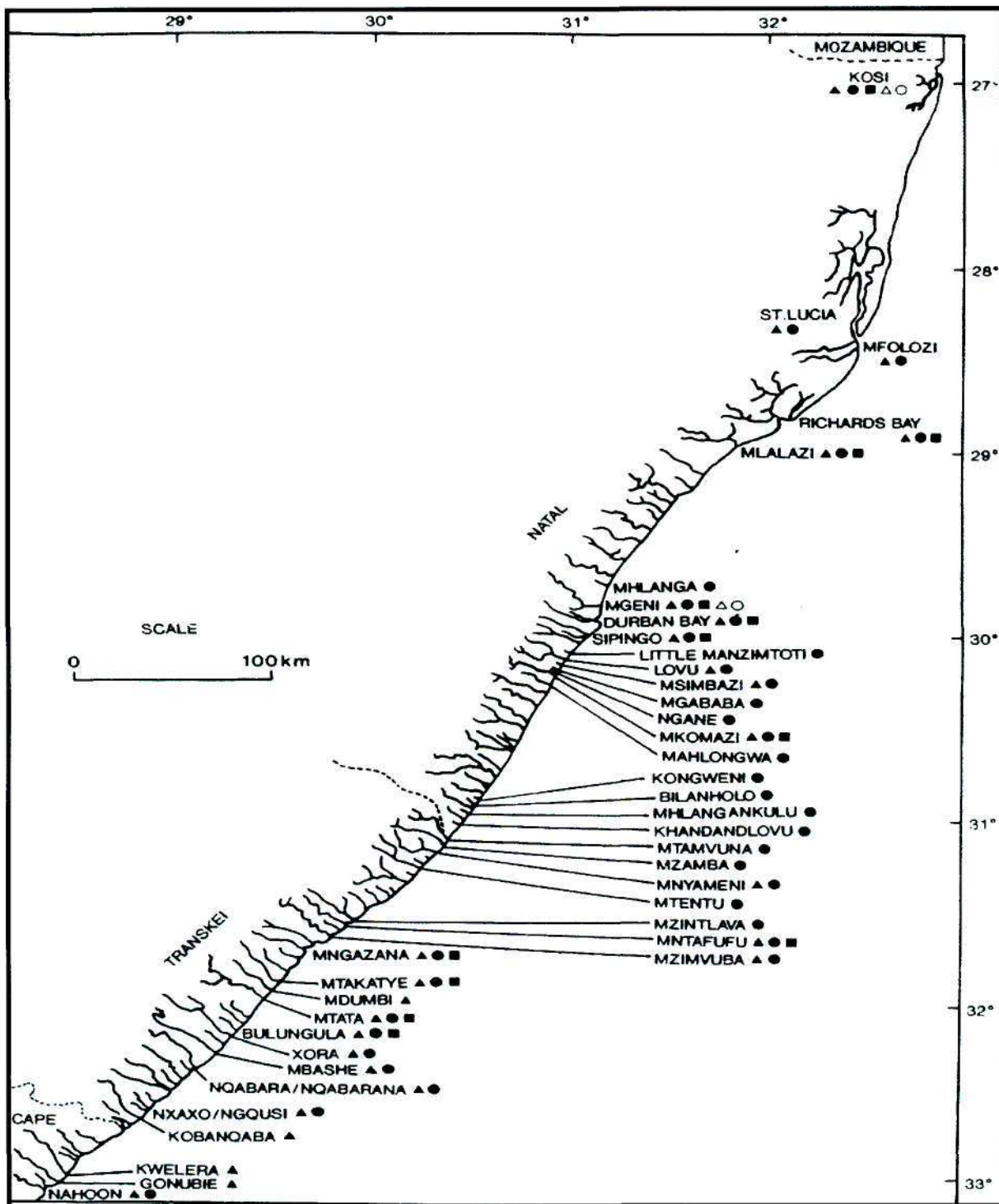
Fig. 2b. Distribution of mangroves in southern Africa (after Ward and Steinke, 1982);

▲ – *A. marina*; ● – *B. gymnorrhiza*; ■ – *R. mucronata*; △ - *C. tagal*;

○- *L. racemosa*
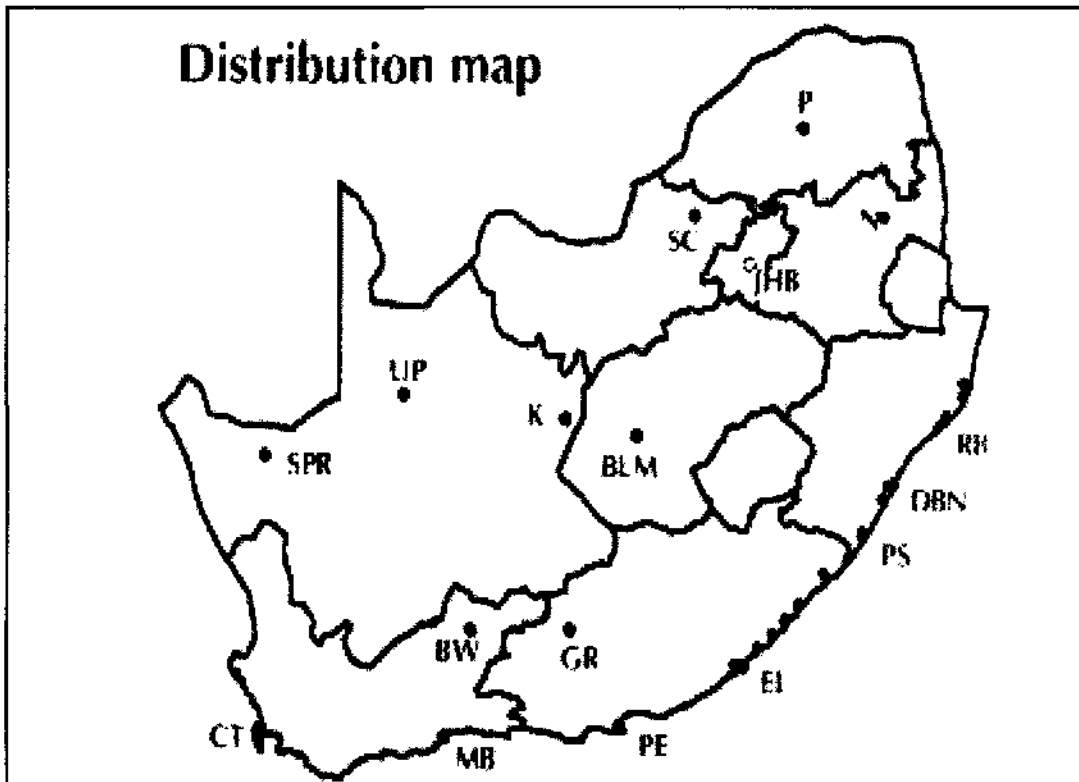
Fig. 2c. Distribution map of *A. marina* along the coastal regions of southern Africa (Palmer and Pitman, 1973).

Mangroves colonize sheltered coastal areas (Fig. 2c) such as deltas, estuaries, lagoons and islands. Mangrove development and zonation are influenced by many factors, including a number of edaphic, hydrographic, chemical, geological, meteorological, biological, and stochastic components (Tomlinson, 1986). These mangrove forests exist in relatively distinct zones, each dominated by different mangrove species. There are four common mangrove ecotypes, i.e. fringe, riverine, basin and scrub forests (Lugo and Snedaker, 1974; Twilley, 1998). These tropical trees or shrubs grow between near mean sea level and the high spring tide mark in accretive shores.

Mangroves are comprised of trees or shrubs and are commonly found growing in shallow and muddy salt water or brackish waters along quiet shorelines exposed during low tides. Some mangroves prefer a daily tidal flow while others find their optimum

conditions in shallow areas subject to infrequent high tides (http://forest.and.nic.in/frst-mangroves1.htm). This process governs their distribution. Mangroves do not grow on sandy beaches or rocky shores rather they favour a muddy substratum of varying depths and consistency for their growth. Mangroves are scarcely found near the open sea or the mouth of an estuary but prefer to shelter in places like creeks and estuaries. These ecosystems are highly fragile and subject to persistent stresses such as high salinity, anaerobiosis and wave action (http://forest.and.nic.in/frst-mangroves1.htm).

The dominant mangrove species, *A. marina*, is commonly known as the grey/white mangrove. This resilient mangrove species has a double zonation pattern, occurring as tall trees on the coastal fringe of the sea and as dwarf shrubs on the terrestrial edge depending on the salinity and drainage of the site. As the pioneer of the mangrove community, *A. marina* is generally the first species to putting up its distinctive 'pencil' roots or pneumatophores on newly-emerged mud banks (Berjak *et al.*, 1977).

Rhizophora species or red mangrove is usually located behind the *Avicennia* zone (Berjak *et al.*, 1977). Red mangrove trees line the banks of water courses where their long prop/stilt roots provide the trees with ventilation and a firm support against wind and wave action (Berjak *et al.*, 1977).

The next zone is inundated only by periodic spring tides during new and full moons and is therefore more saline. The more specialized yellow mangrove, *Ceriops* species, colonizes this drier, firmer sandy soil. Salt marshes or saline herblands with succulent plants also thrive here. The grey mangrove may appear again while the wet, fine, less saline mud in the swamp interior is covered with a thick forest of black mangrove trees i.e. *Bruguiera* species (Berjak *et al.*, 1977).

2.1.2. FACTORS INFLUENCING MANGROVE DISTRIBUTION

True mangroves have a multitude of adaptations, some of which include specialized dispersal mechanisms, respiratory roots and aerating devices, mechanical fixation in

loose soil and specialized mechanisms for coping with excess salt concentrations, thus enabling them to flourish in this ecotone between land and ocean (Odum *et al.*, 1982).

Four factors that appear to be the principal determinants for mangrove distribution are salinity, tidal fluctuation, climate and substratum (Odum *et al.*, 1982).

A) SALINITY

One main feature that distinguishes mangroves from others is its ability to survive in saline habitats. Salt is considered to be a toxic compound to most plants. However mangroves are often categorized by their salt tolerance mechanisms. Halophytes absorb large quantities of electrolytes, particularly in their leaves and the most important ions for osmotic adjustment in halophytes are Na^+, K^+ and Cl^- (Neales and Sharkey, 1981). Eudicotyledonous halophytes mainly accumulate Na^+ and Cl^- to lower water potentials and become succulents (Flowers and Yeo, 1986). Success of halophytic species in saline environments is determined by their ability to lower tissue water potentials sufficiently to maintain water uptake and growth (Waisel, 1972; Hsiao, 1973).

Mangroves are facultative halophytes, i.e. salt water is not a physiological requirement *per se* for plant growth and reproduction (Bowman, 1917; Egler, 1948). Therefore, mangroves have the following three basic strategies for coping with salt.

The first mechanism of defense is to completely prevent salt from entering the system. In salt-excluding mangrove species, the mangrove root system is adapted to filtering out about 90 % of seawater salt, while the tree is anchored in saline soil (Quarto, 2000). *Rhizophora mangle* L., for example, is a salt-excluding species with a root system which separates freshwater from seawater by means of a non-metabolic ultra-filtration system (Scholander, 1968). Other 'salt-excluders' are *Ceriops, Bruguiera* and *Osbornia* species (Berjak *et al.*, 1977; Scholander, 1968).

The second mechanism is the occurrence of glandular cells, *viz.* salt glands on the surface of the leaves, which is the most active salt-secreting system known for the excretion of excess salt (Hogarth, 1999). *Avicennia marina*, the species under investigation, is an excellent example of a salt-secreting species whereby salt glands on both the adaxial and abaxial leaf surfaces are used to excrete excess salt (Scholander *et al.*, 1962). Examples of other 'salt-secretors' include *Acanthus* and *Sonneratia* species (Scholander *et al.*, 1962). Some succulent mangrove plants, however, increase their water capacity in their vacuoles to reduce salt toxicity (Scholander *et al.*, 1962).

A third method involves the accumulation of salt into the bark or older leaves and upon senescence; they drop off, removing the salt with it (Tomlinson, 1986). In addition to these mechanisms, the leaves have other morphological features such as an extremely thick cuticle or dense hairs or stomata which are often sunken below the leaf surface in order to reduce transpiration, and thus water loss (Tomlinson, 1986). Some mangroves may use only one method for coping with salt; however, most mangroves use two or more (Scholander *et al.*, 1962; Scholander, 1968; Hogarth, 1999).

Although mangroves are faced with coping with excess salt, salinity is important in reducing competition from other vascular plant species that are physiologically intolerant of these saline environments (Kuenzler, 1974). The amount of evolutionary energy spent on these adaptations is small in comparison to the energy saved in competition. Mangrove species, namely *A. marina*, *B. gymnorrhiza* (L.) Lam. and *R. mangle* (L.) are the few tree species that have adapted to these extreme conditions and have low energy expenditures on living space and minimal offspring mortality rates from competition (Vannucci, 2000).

B) TIDAL FLUCTUATION

In the habitats where mangroves are found, mainly in the low-lying intertidal zones of the tropics and subtropics, the sea periodically inundates the community with relatively large variations in seawater levels (Naidoo, 2006). However, at low tide, particularly

during periods of high rainfall, mangroves may be exposed to floods of fresh water (http://www.enhg.org/b/b32/32_02.htm). *A. marina* thrives along the shoreline which is regularly inundated twice daily by tides (Naidoo, 2006).

Tidal fluctuations greatly influence the amount of seawater and nutrients that enter an estuary thus facilitating the establishment of mangrove stands. The highest concentration of mangrove species is generally found at the mouth of tidal creeks and rivers where salt and fresh water mix. Floodwaters deposit material to build up the banks and *R. stylosa* is frequently found here. Additionally, tidal ebb aids in the removal of organic carbon, reduced sulphur compounds and detritus and also allows for the dispersal of propagules (Odum *et al.*, 1982).

During high tides some of the smaller mangroves are completely immersed, some have only the lower foliage covered while the fully-grown trees have their canopies exposed above water level. In addition to altering the salinity levels, tidal fluctuations in water can alter temperatures (Odum *et al.*, 1982).

C) CLIMATE

Geographically, temperature limits the distribution of various mangrove species (Odum *et al.*, 1982). The richest mangrove communities are found in tropical and sub-tropical areas where the water temperature is greater than 24 °C. The annual rainfall exceeds 1250 mm and mountain ranges close to the coast are greater than 700 m high. http://www.epa.qld.gov.au/nature_conservation/habitats/mangroves_and_wetlands/mang roves/.

Along the North Atlantic coast of America and Africa where the sea surface temperature is approximately 27 °C, mangroves like *Avicennia* species in the western hemisphere are less tolerant of cold than the species in the eastern hemisphere. Mangroves can endure air temperatures much below 27 °C, but have low tolerance for frost and low temperature (5 °C), which adversely affect their growth and productivity (Tomlinson, 1986).

Higher latitudinal occurrence of mangroves into southern Africa is mainly due to the warm Agulhas Current that flows close in-shore southwards down the Mozambique Channel to about 33° S where it dissipates eastwards into the Indian Ocean (Chapman, 1975). According to Berjak *et al.* (1977), water temperature rather than ambient temperature is the major environmental factor that controls distribution.

D) SUBSTRATUM

Mangroves do not grow on sandy beaches or rocky shores. These plants prefer a substrate of fine-grained mud composed of heavy silt and clay with large amounts of organic material as well as a substrate of varying depth and consistency for their growth (Lugo and Snedaker, 1974; Kuenzler, 1974; Odum *et al.*, 1982). The depth of the mud and silt is governed by the maturity of the stands. The substrate is shallow during the early formative years but increases gradually as it becomes older until it is quite deep. As the stands grow, their outer limits expand as they reclaim more land from the sea. The dense mangrove mud is generally waterlogged, unstable and mostly oxygen deficient http://www.epa.qld.gov.au/nature_conservation/habitats/mangroves_and_wetlands/mang roves/.

The mud of the substrate emits a strong sulphur-like odour (Lugo and Snedaker, 1974; Kuenzler, 1974; Odum *et al.*, 1982). This is hydrogen sulphide that is produced below the mud surface by bacteria living there without light or oxygen. Plants that are unable to adapt and develop a mechanism in which to cope with these saline and anaerobic conditions will not survive. However, mangroves, along with other plants of the swamps, have developed systems to withstand such conditions. Mangroves have a highly adapted root system consisting of above-ground cable, prop, stilt or pencil-like roots (Odum *et al.*, 1982). These radiating aerial roots are covered with special cells called lenticels which, at low tide, allow oxygen to diffuse into the plant and down to the roots through aerenchyma tissue. However, at high tide, the lenticels are hydrophobic and exclude water from entering the aerenchyma tissue (Scholander, 1968). In addition, an extensive root system is necessary to support the trees in an unstable, semi-fluid soil.

2.1.3. REPRODUCTION IN MANGROVES

Mangrove flowers are generally pollinated by animals such as bats, insects, birds, etc. (Bawa and Hadley, 1990). Some mangroves produce lone seeds, whilst the other mangroves produce propagules, i.e. a long pencil-like tissue that encases the seeds. Mangrove forests are the only true viviparous plants, where the seed remains attached to the parent plant and germinates into a protruding embryo (propagule) before falling from the tree (Rey, 1999). This adaptation enables them to immediately begin germinating when they come into contact with the soil (Dawes, 1981). Some of the seedlings may become trapped in the network of pneumatophores beneath the parent tree while others are swept away by tides to near or distant new sites where, if conditions are favourable, a new community is established. Thus these propagules have an essential feature, i.e. the ability to float and they may remain viable for up to a year (Robertson, 1992). According to Saenger et al. (1983), at the age of ten, the pioneer mangrove can produce a whole mangrove community, given the proper conditions.

2.1.4. USES AND BENEFITS OF MANGROVES

Inhabitants of the forest interior and coastal waters may depend on the mangrove environment for their entire lives. Others may utilize mangroves only during specific life stages (Yañez-Arancibia et al., 1988). Mangroves offer shelter and are suitable nursery grounds for a diverse community of fish and shrimp. Almost 75 % of commercially caught fish and prawns spend some time in the mangrove ecosystem. They either seek shelter, food, or mating grounds (Robertson, 1992). The barracuda is the typical example.

Mangrove trees and shrubs serve as erosion blockers owing to their extensive roots, which shield the coast by absorbing the energy of storm driven waves and wind and siltation (Hogarth, 1999). Siltation is known to cause algal blooms, which in turn result in the death of coral reefs, sea grass and kelp beds (Hogarth, 1999; Quarto, 2000). These aerial roots also provide a substrate for colonization by algae; woodborers; and fouling

organisms such as barnacles, oysters, mollusks, anemones and sponges. Nutrient assimilation facilitates the colonization by epiphytic algae on which plankton feed on, and in turn, larger organisms feed off the plankton (Quarto, 2000).

Other invertebrates such as arthropods, crustaceans and molluscs play a significant role in mangrove ecosystems. Crabs and snails help break down leaf litter through grazing, however, some species of crabs are referred to as propagule predators and can therefore influence mangrove forest structure (Smith, 1987). The dense variety of smaller organisms thus serve as a food source for larger organisms, such as, sharks, crustaceans, barracudas, jellyfish etc. (Hogarth, 1999).

The terrestrial ecosystem is of great importance as it provides an abode for numerous insect species. Insects play a vital role as mangrove pollinators, herbivores, predators and are also a food source for other animals (Hogarth, 1999). Many bird species use the mangroves for refuge, nesting and feeding purposes. Amphibians and reptiles such as frogs, snakes, lizards and crocodiles may be found in mangrove ecosystems (Ewel *et al.*, 1998).

A variety of medicines are derived from mangroves. Ashes or bark infusions of certain species may be used in the treatment of skin disorders and sores including leprosy. Mangrove plants are traditionally used to treat headaches, rheumatism, snakebites, boils, ulcers, diarrhoea, haemorrhages and many more conditions http://www.epa.qld.gov.au/nature_conservation/habitats/mangroves_and_wetlands/mang roves/.

The cedar mangrove, cannonball mangrove and the grey mangrove are well known for their hardwood. They are used for boat building, cabinet timber and tools such as digging sticks, spears and boomerangs. The bark of *A. marina* trees produces a brown dye and the leaves are used as camel fodder around the Red Sea (Duke, 1983). Branches are cut and fed to cattle in India and Australia (Duke, 1983).

The fronds of the nypa palm are used for thatching and basket weaving. An assortment of barks are used for tanning; pneumatophores are also used as fishing floats while the wood from the yellow mangroves, *Ceriops* species, can burn even when wet. http://www.epa.qld.gov.au/nature_conservation/habitats/mngroves_and_wetlands/mangr oves/.

2. 1. 5. PHOTOINHIBITION

Mangrove species can tolerate a very wide range of salinities (Bowman, 1917; Egler, 1948; Quarto, 2000). This adaptation provides them with an advantage over other plant species. However mangrove species, like many other plant species, have one small-predisposed weakness, i.e. their susceptibility to photoinhibition (Ball and Anderson, 1986; Ball, 1996).

Photoinhibition is a light-dependent reduction in the photochemical efficiency of PSII when more light is absorbed than can be used in photosynthetic biochemistry causing the plant to cease carbon fixation (Osmond, 1994). When plants are not able to adapt to the prevailing light conditions, photoinhibition may result (Long *et al.*, 1994). Mangroves generally have low photosynthetic rates that become light-saturated at photon flux densities ranging from 30 to 50 % of incident sunlight, thus requiring dissipation of considerable excess excitation energy when exposed to direct sunlight (Ball, 1996).

Photoinhibition can result from direct photodamage to PSII and from photoprotection, wherein excess excitation energy is deflected away from PSII and dissipated harmlessly as heat (Osmond, 1994). Photoinhibition can be reversible by protecting the photosynthetic systems; however it can also be irreversible by reflecting damage that has already occurred in the photosynthetic apparatus. Naturally occurring pigments and enzymes in plants can prevent photoinhibition, while pre-adaptation to non-ideal conditions can improve tolerance to a certain stress factor (Alves *et al.*, 2002).

In nature, plants have diverse responses to light surplus occurring between damage and non-damage and respond to different time scales (Osmond, 1994). Photoinhibition can be

caused by ultraviolet light (UV), visible light (V) and by their interaction (Powles, 1984). Ball and Anderson (1986) found that PS II of *A. marina* and *Pisum sativum* was sensitive to NaCl in the chloroplasts; thereby suggesting that accumulation of ions of either salt-tolerant or salt-sensitive species would result in rapid damage of PS II, particularly in the light.

2. 1. 6. HEAVY METALS AND THEIR EFFECTS ON PLANTS

The degree of toxicity of heavy metals on plants is dependent on the nutritional status of the plant (Wallace, 1984). Heavy metal ions such as Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+} and Co^{2+} are essential micronutrients for plant metabolism but when present in excess amounts, together with non-essential metals such as Cd^{2+}, Hg^{2+} and Pb^{2+}, can become extremely toxic (Williams *et al.*, 2000). They may exhibit symptoms such as chlorosis and necrosis, stunting, leaf discolouration and inhibition of root growth (Williams *et al.*, 2000; Wang, 2004). The toxicity symptoms of excessive amounts of heavy metals in plants may be due to a range of interactions at the cellular or molecular level. According to Van Assche and Clijsters (1990), toxicity may result from the binding of metals to sulphydryl groups in proteins, which could lead to an inhibition of enzyme activity or protein structure, or from the displacement of an essential element resulting in deficiency effects (William *et al.*, 2000). In addition, an overload of heavy metal may disrupt cell transport processes and stimulate the production of free radicals and reactive oxygen species, leading to oxidative damage (Dietz *et al.*, 1999; William *et al.*, 2000; Schützendübel and Polle, 2002).

The critical values of heavy metals are defined as the lowest tissue concentration at which a metal has toxic effects (Kabata-Pendias and Pendias, 1984; Bahlsberg-Pahlsson, 1989; Clemens *et al.*, 2002). Plant cells subjected to heavy metals rapidly synthesize phytochelatins, i.e. metal-binding polypeptides whose function is to sequester and detoxify excess metal ions. Failure to synthesize these peptides results in growth inhibition or apoptosis (Kahle, 1993). A wide array of metals, namely, Cd^{2+}, Pb^{2+}, Zn^{2+}, Sb^{3+}, Ag^{3+}, Ni^{2+}, Hg^{2+}, AsO_4, Cu^{2+}, Sn^{4+}, SeO_3, Au^{3+}, Bi^{3+} and Te^{4+} is known to induce phytochelatin

production, however, binding of metals to phytochelatins has only been demonstrated for Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+} (Grill *et al.*, 1987; Grill, 1989).

Heavy metals are non-biodegradable and are thus pollutants in the environment. Mangrove systems have the capacity to act as a sink or buffer and remove or immobilize heavy metals before they reach nearby aquatic ecosystems. Mangrove sediments have a large proportion of fine particles, high organic content and low pH which make them effective in trapping heavy metals and immobilizing them in the anaerobic sediments (MacFarlane and Burchett, 2001). Mangroves however, are tolerant of relatively high levels of heavy metal pollution (Peters *et al.*, 1997) but extreme heavy metal contamination may induce metabolic reactions which can result in damage at the cellular level or lead to greater phytotoxic responses (Vangronsveld and Clijsters, 1994). The sediment metal levels that induce toxicity in *A. marina* and that cause biological effects in estuarine sediments are as follows: Cu^{2+} = 65, Pb^{2+} = 50, Zn^{2+} = 200 µg/g (Long *et al.*, 1995; Anzecc and Armcanz, 1999). Metal levels for toxic effects on terrestrial plants for the following metals are: Cu^{2+}= 60-125, Pb^{2+}= 100-400, Zn^{2+}= 70-400 µg/g (Kabata-Pendias and Pendias, 1984). The current sediment guidelines are therefore adequate for the protection of the species on individual exposure to Cu^{2+}, Pb^{2+} and Zn^{2+}. The guidelines suggest that *A. marina* has a greater tolerance to heavy metals compared to many other terrestrial plants (MacFarlane and Burchett, 2002).

The distribution of heavy metals within plant tissues is dependent on the ability of the metals to be transported. Metal ions are mobilized by secretion of chelators and by acidification of the rhizosphere (Clemens *et al.*, 2002). Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall. Transport systems and intracellular high-affinity binding sites then mediate and drive uptake across the plasma membrane. The rates of accumulation are necessarily governed by physiological requirements rather than toxicity. Trace elements such as Cu^{2+} and Zn^{2+} pose a specific dilemma to organisms. Their ions are essential for a vast number of metabolic processes yet are potentially dangerous (Clemens *et al.*, 2002). For many mangrove species, essential metals such as Cu^{2+} and Zn^{2+} show some limited

translocation to leaf tissues, while non-essential metals (Pb^{2+} and Hg^{2+}) mainly accumulate at the root level (De Lacerda *et al.*, 1995). MacFarlane and Burchett (2002) confirmed that *A. marina* showed the accumulation pattern of an indicator species, with tissue concentrations reflecting environmental concentrations and thus may be employed as a possible biological indicator of environmental heavy metal concentrations (Baker, 1981). Wittig (1993) reported however, that field data is inconsistent and may be influenced by a number of environmental factors such as temperature, sediment physiochemical properties, air pollutants and water shortage.

A) COPPER

Copper, unlike other heavy metals, such as Cd^{2+}, Pb^{2+} and Hg^{2+}, is not readily bioaccumulated and therefore its toxicity to man and other mammals is relatively low Kabata-Pendias and Pendias, 1984; Bahlsberg-Pahlsson, 1989; Fernandes and Henriques, 1991). On the contrary, plants in general are very sensitive to Cu^{2+} toxicity. Plants exhibit metabolic disturbances and growth inhibition at Cu^{2+} concentrations only slightly higher than the normal levels in the tissues (Fernandes and Henriques, 1991).

Copper (II) is the most frequently used toxic heavy metal for industrial purposes (Ma *et al.*, 2003; Andrade *et al.*, 2004; Perales-Vela *et al.*, 2007). Naturally occurring and man-made sources are responsible for its presence in aquatic systems. A range of sources of Cu^{2+}, including industrial and domestic wastes, agricultural practices, copper mine drainage, copper-based pesticides, and antifouling paints have contributed to a progressive increase in Cu^{2+} concentrations in aquatic environments (Ma *et al.*, 2003; Andrade *et al.*, 2004; Perales-Vela *et al.*, 2007). Worldwide estimates of total anthropogenic discharge of copper to surface waters range from 35×10^3 to 90×10^3 metric tons per year (Nriagu and Pacyna, 1988). Large algal blooms in lakes and water reservoirs are most often controlled by the application of copper sulphate, however these copper treatments can result in potentially high levels of Cu^{2+} in the surface waters, accumulation of copper in the sediment as well as water quality problems (Haughey *et al.*, 2000).

Copper is an essential trace element for normal plant metabolism. Copper participates in a number of electron transport reactions in both photosynthesis and respiration, while a wide range of enzymes either contain or are activated by Zn^{2+} and Mn^{2+} (Marchner, 1995). Copper is required in cell wall lignification, enzyme systems related to photosystem II electron transport, carbohydrate metabolism and protein synthesis (Verkleij and Schat, 1990; Williams *et al.*, 2000; MacFarlane and Burchett, 2002). However, at elevated concentrations, these metals can become dangerously toxic, as do the non-essential metals, causing symptoms such as chlorosis and necrosis, leaf discolouration, stunting and inhibition of root growth (Van Assche and Clijsters, 1990; Marchner, 1995). Furthermore, Cu^{2+} is important to seed production, disease resistance and the water relations in the plant (Bussler, 1981).

The normal range of Cu^{2+} content in plant tissues is reported to be 8-13 μgml^{-1} by Howeler (1983), 3-10 μgml^{-1} by Clarkson and Hanson (1980), and 5-20 μgml^{-1} by Stevenson (1986). Nriagu (1979) suggests 12 μgml^{-1} as a global value for Cu^{2+} concentrations in plant biomass, but refers to a lower value (3.5 μgml^{-1}) for marine plants. The critical leaf tissue concentrations of Cu^{2+} whereby most species are adversely affected ranges between 15 and 25 μgml^{-1} dry weight, but corresponding concentrations of Cu^{2+} in culture solutions have been shown to vary considerably (Beckett and Davis, 1977; Davis and Beckett, 1978; MacNicol and Beckett, 1985). Some species may respond to below 10 μgml^{-1} of leaf tissue, whereas other species such as *Picea sitchensis* is comparatively insensitive, enduring 88 μg Cu ml^{-1} dry weight of the needles (Burton *et al.*, 1983). Gupta (1979) and Stevenson (1986) indicated that inhibitory effects arise at Cu^{2+} concentrations in tissues higher than 20 μgml^{-1}, while Folsom *et al.* (1981) reported that the toxicity threshold in sedges is 575 μgml^{-1}.

The degree of toxicity of heavy metals on plants is dependent on the nutritional status of the plant. Phosphorus deficiency in the plant may increase the toxicity of Cu^{2+} (Wallace, 1984). In the study by Heale and Ormrod (1982), four woody plant species were grown in culture solutions supplied with two concentrations of Cu^{2+} (4000 and 20 000 μgml^{-1}).

Visible symptoms were similar to those mentioned in the first paragraph of the previous page. The Cu^{2+} concentration in the needles was 16 μgml^{-1} dry weight and in the roots more than 3000 μgml^{-1} Cu^{2+} dry weight. The dry weight of the other species studied was also markedly affected; however, the absorption of Cu^{2+} differed significantly among the species. Correspondingly, Burton *et al.* (1986) also found that similar concentrations of Cu^{2+} affected seedlings of sitka-spruce (*Picea sitchensis*). Shoot and root growth was significantly decreased by 5000 μgml^{-1} Cu^{2+} in the growth medium.

Copper has both a positive and negative effect on algal growth. It plays an important role in biological reactions as an enzymatic cofactor and electron carrier in the photosynthetic and respiratory processes although, when in excess, it becomes highly toxic (Andrade *et al.*, 2004; Dewez *et al.*, 2005). Investigations on algae showed the effect of sub-lethal concentrations of Cu^{2+} on the growth and metabolism of *Scenedesmus incrassatulus* (Chlorophyceae). Perales-Vela *et al.* (2007) have shown that growth, photosynthetic pigments and metabolism had different sensitivities to Cu^{2+} concentrations. Growth and photosynthetic activities decreased with increasing Cu^{2+} concentration. Nalewajko and Olaveson (1995) found different sensitivities between growth and photosynthesis in *Scenedesmus acutus*, that growth was the most sensitive process to Cu^{2+}, followed by photosynthesis and respiration. Studies conducted by Brown and Newman (2003) on *Gracilariopsis longissima* (Rhodophyceae) indicated that exposure to increasingly higher Cu^{2+} treatments, significantly reduced growth; and photosynthetic oxygen evolution and the maximum quantum yield of PSII was reduced. Cid *et al.* (1995) concluded that under Cu^{2+} stress, there is a higher consumption of ATP required to circumvent the toxic effects of Cu^{2+}, maintaining cellular integrity and activating metabolic processes related to cell growth.

Copper reduces growth and inhibits respiratory and photosynthetic activities with the latter appearing more susceptible, resulting in a decrease in the activity of photosystem I more than photosystem II and a decrease in electron transport rates ((Bahlsberg-Pahlsson, 1989; Fernades and Henriques, 1991; Nalewajko and Olaveson, 1995; Mallick and Mohn, 2003). The key enzymes in photosynthesis, phosphoenolpyruvate

carboxylase (PEPC) and ribulose-1,5-bisphosphate carboxylase (RuBPC) are inhibited by Cu^{2+} (Stiborova *et al.*, 1986a, b). Chlorophyll content is also decreased in the leaf cells (Bahlsberg-Pahlsson, 1989). Symptoms of Cu^{2+} toxicity and yield reductions in plants are thought to be a consequence of tissue damage, peroxidation of chloroplast membrane lipids, disruptions to carbohydrate metabolism and protein synthesis, alteration of membrane permeability and inhibition of photosynthetic electron transport (Woolhouse, 1983).

Copper aids in cell function, structural stability of chromosomes and energy transfer. However, when in excess, Cu^{2+} affects viscosity of the plasma membrane resulting in cell abnormalities and functional disorders e.g. root tip mitosis (Singh and Sharma, 1980; Fiskesjö, 1988). In non-tolerant clones of *Agrostis capillaries*, both root growth and cell elongation were seriously retarded when treated with 64 μgml^{-1} Cu^{2+} in the growth medium. Similar results have been obtained with onion (*Allium cepa*) roots. Copper increases the permeability of the cell membranes, leading to leakage of nutrients such as K^{+}, sugars and amino acids (Wainwright and Woohouse, 1977; Nag *et al.*, 1980; Fiskesjö, 1988).

B) ZINC

Zinc is considered to be the least toxic of the heavy metals (Bahlsberg-Pahlsson, 1989). Like Cu^{2+}, Zn^{2+} is an essential micronutrient required for normal plant growth and development in higher plants and plays a role in several metabolic processes (Hall, 2002). Zinc ions are key structural and catalytic components in DNA binding proteins and hydrolytic enzymes, respectively and are important in the biosynthesis of plant growth hormones (Collins, 1981; Balsberg-Pahlsson, 1989; Ernst *et al.*, 1992; Clemens *et al.*, 2002). Growth symptoms of Zn^{2+} toxicity in plants are similar to those of Zn^{2+} deficiency. Large concentrations of Zn^{2+} affect both root and shoot growth with the latter becoming stunted and chlorotic (Bahlsberg-Pahlsson, 1989). Moreover, the epidermis of roots and the epidermal cells may become lignified (Päivöke, 1983). In addition, Zn^{2+} and Cu^{2+} uptake and allocation to plant organs such as photosynthetic leaf tissue are high

whereas Pb^{2+}, Cd^{2+} and Hg^{2+} are non essential to plant growth and are of very low solubility (Balsberg-Pahlsson, 1989; McFarlane and Burchett, 2000).

Critical Zn^{2+} concentrations varied from 60 to 900 μgml^{-1} dry weight in tops and leaves of different species, however, most species, including trees are sensitive, in the range 200 to 300 μgml^{-1} (Balsberg-Pahlsson, 1989). Accordingly, 226 μgml^{-1} dry weight was found to be critical in the needles of sitka-spruce (*Picea sitchensis*), whereas 250 μgml^{-1} was required for minimum toxicity in *Quercus rubra* (Jordan, 1975; Burton et al., 1983). These levels have been found in leaves of naturally growing trees in unpolluted areas (Balsberg-Pahlsson, 1989). Kabata-Pendias and Pendias (1984) reported that Zn^{2+} toxicity at higher concentrations may result in changes in membrane permeability, decreased leaf chlorophyll content disruption of enzyme systems and respiration. It is also reported that it decreases in leaf chlorophyll content and damage to the photosynthetic apparatus leading to reductions in yield and lethality or cessation of emergence (Van Assche et al., 1979; Porter and Sheridan, 1981). Other well documented effects of Zn^{2+} toxicity are increased permeability of root membranes, which will cause leakage of nutrients from the roots (Kabata-Pendias and Pendias, 1984; Balsberg-Pahlsson, 1989). Thus, 650 μg Zn ml^{-1} was reported to enhance leakage of K^{+} from maize roots (Collins, 1981).

Stiborova et al. (1986b) found that phosphoenolpyruvate carboxylase (PEPC), a key enzyme of photosynthesis in C_4-plants, was more sensitive to Zn^{2+} than the corresponding enzyme, ribulose-1,5-bisphosphate carboxylase (RuBPC) in C_3-plants. In addition, Van Assche and Clijsters (1986) observed that lethal concentrations of Zn^{2+} negatively affect photosynthetic electron transport and photophosphorylation in bean (*Phaseolus vulgaris*) seedlings. Root growth and elongation are affected at relatively low levels (1000 μgml^{-1}) in the growth medium (Balsberg-Pahlsson, 1989). It is well known from Zn^{2+} treatment studies that aberrations and abnormalities increase during root tip mitosis in *Allium cepa* (Nag et al., 1980; Singh and Sharma, 1980).

Some metals also play a role in plant metabolism, and can be considered nutrients. This is the case for Zn^{2+}, Mn^{2+} and Ni^{2+}, which are involved in major functions (Welch, 1995). Zinc is involved in membrane integrity, enzyme activation and gene expression (Page et al., 2006). In contrast, some heavy metals such as Cd^{2+} are not required by the plant, but are also absorbed (Römer et al., 2000, 2002; Ximénez-Embún et al., 2002; Zornoza et al., 2002). Both nutrients and pollutants, can accumulate in excess in the plant to levels undesirably high for human or animal nutrition, and may even become toxic to plants at a certain concentration (William et al., 2000). Thus, uptake of heavy metals by the roots, their transport in the different parts of the root system, their release to the shoot and further redistribution within the shoot are important processes for (i) redistribution of heavy metals in the plant/soil system; (ii) supply of shoot parts with nutrients; and (iii) quality of harvested plant parts (Page et al., 2006).

The transport and mobility of heavy metals have been studied in plants including bean and wheat. For example, Zn^{2+} can be transported rapidly in the phloem (Herren and Feller, 1994, 1996; Pearson et al., 1995; Haslett et al., 2001; Erenoglu et al., 2002; Page and Feller, 2005). Cadmium, which is chemically very similar to Zn^{2+} is transported in plants by similar pathways (Chesworth, 1991; Grant et al., 1998; Ernst and Nelissen, 2000). However, Cd^{2+} has a mobility different from Zn^{2+} in wheat (Page and Feller, 2005; Riesen and Feller, 2005).

Page et al. (2006) investigated the translocation of three essential heavy metals, namely ^{54}Mn, ^{63}Ni and ^{65}Zn; a non essential heavy metal and ^{109}Cd and ^{57}Co in Lupinus albus (Sauvé et al., 2000; Lugon-Moulin et al., 2004). Findings suggest that ^{54}Mn, ^{63}Ni, ^{57}Co, ^{65}Zn and ^{109}Cd behaved differently with regard to root-to-shoot transfer and redistribution within the shoot and root system. ^{65}Zn and ^{63}Ni were probably transported via the xylem to transpiring leaves and then redistributed to newly formed leaves. Furthermore, ^{65}Zn and ^{63}Ni were transported via the phloem from older leaves to younger expanding leaves (Page et al., 2006). It can be assumed that since ^{65}Zn and ^{63}Ni are micronutrients, they would move from roots to shoots in the xylem to reach the leaves (Welch, 1995). The rapid re-translocation of ^{65}Zn and ^{63}Ni from old to young

leaves via the phloem contributes to the supply of these micronutrients to rapidly growing plant parts. Cadmium, a non essential and pollutant heavy metal, found in different concentrations in soils (Sauvé et al., 2000; Lugon-Moulin et al., 2004). It showed a specific behaviour and remained in the root system (Römer et al., 2000, 2002; Ximénez-Embún et al., 2002; Zornoza et al., 2002). Since Cd^{2+} is recognized as a toxic compound at the root level, and is not required in the shoot, L. albus sequestered it in the root to avoid damage to the shoot. Cadmium is known to have several lethal effects on plants (Sanità di Toppi and Gabbrielli, 1999).

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 sp. and Avicennia sp. (Silva et al., 1990; Peters et al., 1997). For many mangrove species, essential metals (Cu^{2+} and Zn^{2+}) show some limited translocation to leaf tissues, while non-essential metals (Pb^{2+} and Hg^{2+}) mainly accumulate at the root level (De Lacerda et al., 1995). This is also evident in some marsh plants.

C) LEAD

Lead is considered to be a non essential metal to plants therefore the phytotoxicity of Pb^{2+} to plants is relatively low, due to very limited availability and uptake from the soil and soil solutions (Bahlsberg-Pahlsson, 1989). However, plant roots are usually able to take up and accumulate large quantities of Pb^{2+} in the soil and culture solutions but translocation to aerial shoots is generally limited, due to binding at the root surfaces and cell walls (Bahlsberg-Pahlsson, 1989). With respect to associated anions in the rooting medium, Pb^{2+} uptake was found to be higher if applied as a nitrate rather than as a carbonate or sulphate (Zimdahl, 1976). The effect of Pb^{2+} varies inversely with the phosphate concentration of the nutrient solution and with the phosphate status of the plant. Other factors of great importance are plant age and time of treatment (Koeppe, 1981).

Lead is considered to be one of the most hazardous heavy metal pollutants and is derived from a variety of sources such as burning of coal, mining and smelting of lead ores, effluents from storage battery industries, metal plating and finishing operations, fertilizers, pesticides, automobile exhausts and additives in pigments and gasoline (Pichtel *et al.*, 2000; Verma and Dubey, 2003; Kadukova and Kalogerakis, 2007). Symptoms of Pb^{2+} toxicity include smaller leaves and stunted growth. Leaves may become chlorotic and reddish with necrosis and the roots turn black (Balsberg-Pahlsson, 1989). The contribution of Pb^{2+} from direct aerial pollution of leaf surfaces compared to that taken up by the roots is usually greater. Most foliar applied Pb^{2+} has proved to be effectively immobilized at the leaf surface (Zimdahl and Arvik, 1973). The limit for estuarine/coastal aquatic life for lead is 5.80 μgml^{-1} dry weight (USEPA, 1995).

Lead is well known to affect various physiological and biochemical processes. Exposed plants show decreased photosynthetic and transpiration rates with increasing supply of the metal (Bazzaz *et al.*, 1974). Chlorophyll biosynthesis is also inhibited by Pb^{2+} leading to lowered chlorophyll content (Hampp and Lendzian, 1974). Since roots are effective barriers against further transport of Pb^{2+} to the shoots, very high levels of Pb^{2+} are needed to affect photosynthesis in intact plants. Other physiological processes such as water and nutrient uptake are sensitive to heavy metals. Thus fairly elevated levels of Pb^{2+} are needed to affect these processes (Oberländer and Roth, 1978; Päivöke, 1983).

The activity of several enzymes is influenced when Pb^{2+} reacts with other important functional groups, some of which are fundamental to photosynthesis and nitrogen metabolism (Bahlsberg-Pahlsson, 1989). Hydrolytic enzymes and peroxidases become altered resulting in enhanced senescence in plants treated with Pb^{2+} (Bahlsberg-Pahlsson, 1989). Lee *et al.* (1976) observed increased soluble protein and free amino acid contents in Pb^{2+} treatments, resulting in an increased degradation of proteins. Due to the limited transport of Pb^{2+} from roots to shoots, the biochemical, like the physiological responses, is often more pronounced in the roots. With increasing concentrations of Pb^{2+} and time of exposure, cell division becomes inhibited and subcellular organelles, particularly the mitochondria, are known to be Pb^{2+} sensitive (Sekerka and Bobak, 1974).

González and Ramírez (1995) conducted an investigation to determine the distribution of Ni^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} on core sediment samples and leaves of the red mangrove (*Rhizophora mangle*) from Levisa Bay, Cuba. This area was affected by nickel mining and metallurgical activities. Results indicated that sediments were heavily polluted with Ni^{2+}, Fe^{2+}, Co^{2+} and Mn^{2+}. Their concentrations decreased with increasing distance from the discharge sources. Results also revealed that *R. mangle* is considered to be a useful bioindicator of heavy metal pollution in the ecosystem.

D) MERCURY

The distribution of metals within plant tissues is dependent on the ability of the metals to be transported (Bahlsberg-Pahlsson, 1989). Some metals such as Hg^{2+}, Cr^{2+} and Pb^{2+} have low translocation rates and are therefore much more concentrated in roots than in stems or leaves as they are not known to have any function in plants (Alberts *et al.*, 1990; Baker and Walker, 1990; De Lacerda *et al.*, 1995; De Lacerda, 1998). Mercury is fixed in the sediment. Many studies have shown that plant roots accumulate Hg^{2+} when exposed to Hg-contaminated soils (Lenka *et al.*, 1992; Coquery and Welbourne, 1994; Ribeyre and Boudou, 1994; Bersenyi *et al.*, 1999; Kalac and Svoboda, 2000; Wang, 2004). In aquatic plants, Hg^{2+} is bioaccumulated and is usually stored in roots and stems and only small fractions are found in the leaves (Ribeyre and Boudou, 1994; Lenka *et al.*, 1992; Kalac and Svoboda, 2000; Wang, 2004). It is severely toxic to aquatic organisms. The limit for estuarine/coastal aquatic life for Hg^{2+} is 0.025 μgml^{-1} dry weight (USEPA, 1995).

All physiological and biochemical processes in plants may be negatively affected by Hg^{2+} when plant are exposed to Hg^{2+}-contaminated soil, water or air (Patra and Sharma, 2000; Wang 2004). Because almost all proteins contain sulphydryl groups, Hg^{2+} can disturb almost any function in which proteins are involved in plants (Clarkson, 1972). Mercury compounds can also bind to RNA, several synthetic polyribosomes, and DNA (Katz and Santilli, 1962; Kawade, 1963; Cavallini *et al.*, 1999). Mercury is also known

to affect photosynthesis, mineral nutrient uptake, and transpiration (Barber *et al.*, 1973; Godbold and Hütterman, 1988; Godbold, 1991, 1994; Patra and Sharma, 2000; Wang, 2004). Plants can generally sequester toxic ions in complexes at the cytoplasm and then transport them to vacuoles (Rajesh *et al.*, 1996; Zenk, 1996) to defend against phytotoxicity (Wang, 2004).

According to Weis *et al.* (2002), *Spartina alterniflora* leaves contained 2-3 times more Hg^{2+}, Pb^{2+} and Cr^{2+} than *Phragmites australis* leaves. It was concluded that since *S. alterniflora* leaves possess salt glands, twice the amount of metals are excreted into estuaries compared to *P. australis* and that Hg^{2+} excretion correlates with Na^+ release.

The response of spruce seedlings, *Picea abies*, to Hg^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} was examined in short-term experiments in solution culture (pH 4.5) (Godbold *et al.*, 1987). Results revealed that root elongation was greatly inhibited by 6 and 12 μgml^{-1} Zn (as $ZnSO_4$) after only 24 hours of treatment, while Pb^{2+} ($PbCl_2$) inhibited root elongation at solution concentrations of 0.1-0.4 μgml^{-1} Pb^{2+} over a 7-day treatment. Mercury, applied as $HgCl_2$ was found to be considerably more toxic than Zn^{2+}, Cd^{2+} or Pb^{2+}. Root elongation was severely depressed by 0.02 and 0.1 μgml^{-1} Hg^{2+} after 24 hours. Root elongation ceased completely within 24 hours at 1 and 3 μgml^{-1} Hg^{2+}, and shrinkage was observed 1-5 mm behind the root tip. The degree of toxicity to spruce seedlings was $Hg^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+}$, with Hg^{2+} being over 100 times more lethal than Zn^{2+} (Godbold *et al.*, 1987).

2. 1. 7. EFFECTS OF HEAVY METALS ON WETLAND PLANTS

A number of laboratory based studies have looked at growth responses and the accumulation of Cu^{2+}, Pb^{2+} and Zn^{2+} at sub-lethal metal levels in mangrove species. Most mangrove species show a high tolerance to heavy metal exposure. McFarlane and Burchett (2002) conducted studies on *A. marina* seedlings under laboratory conditions to determine the effects of Cu^{2+}, Pb^{2+}, Zn^{2+} and a combination of Zn^{2+} and Pb^{2+} together on germination, growth and accumulation for a period of six months. Growth responses

showed significant reductions in seedling height, leaf number and area with significant increases in Cu^{2+} concentrations to tissues at 100 μg/g sediment Cu^{2+}. At 400 μg/g, a stasis in Cu^{2+} accumulation to tissues was observed. In addition total biomass decreased and root growth was inhibited. Emergence was retarded and inhibited with increasing Cu^{2+} concentrations and 100 % mortality was recorded at the 800 μg/g Cu^{2+} treatments. Similar phytotoxic responses to Cu^{2+} were reported for numerous plant species (Woolhouse and Walker, 1981). Less conspicuous negative effect on growth was observed for Pb^{2+} treatments with no delays in emergence and mortality of seedlings being reported due to the limited accumulation and transport of Pb^{2+} in the plants. Root growth inhibition was only recorded at the highest treatment of 800 μg/g Pb^{2+}. It was also observed that growth inhibition occurred with excess Zn^{2+} accumulation, and at 1000 μg/g Zn^{2+} treatment total mortality occurred. Significant decreases in seedling height, leaf number, area and biomass and root growth inhibition were found at concentrations of 500 μg/g sediment (MacFarlane and Burchett, 2002).

Chiu, et al. (1995) observed growth reductions in young Kandelia candel (L.) Druce seedlings repeatedly exposed to 0-400 μg/g of Zn^{2+} and Cu^{2+} under laboratory conditions for 12 weeks. Results showed significant root inhibition and leaf growth (dry mass) reductions at 400 μg/g treatments for both Cu^{2+} and Zn^{2+}. Zinc concentrations in the soil were linearly related to Zn^{2+} concentrations in the roots and leaves while Cu^{2+} levels were linearly related to concentrations of Cu^{2+} in the roots only. Leaf levels of Cu^{2+} remained constant, suggesting restricted translocation. Root metal concentrations were higher than surrounding sediment concentrations, while metal concentrations in the leaves were much lower. Additionally, a rise in salinity levels resulted in the reduction of toxicity and accumulation for both metals.

Further studies were conducted by Thomas and Eong (1984) on Rhizophora mucronata Lam. and Avicennia alba Bl. seedlings treated with 50-250 mg/ml Pb^{2+} and 10-500 mg/ml Zn^{2+} in sediment, twice during a 10 week exposure period. Less conspicuous adverse effects were observed on the growth of seedlings. Both species showed higher Zn^{2+} accumulation in the roots. Zinc leaf tissue levels of up to 45 μg/g for R. mucronata

and 260 µg/g for *A. alba* were recorded in soil treated with 500 µg/ml Zn^{2+}. Lead was found to accumulate in root tissue, with negligible translocation to shoots. Accumulation of Zn^{2+} and Pb^{2+} was significantly higher in *A. alba* than *R. mucronata*. Similarly, Walsh *et al.* (1979) exposed young seedlings of *Rhizophora mangle* Lam. to sediments treated with a range of 0-250 µg/g Pb^{2+} twice over a 3-week period. At these concentrations, there was no effect of Pb^{2+} on the total weight and size of hypocotyls, stems, roots or leaves. Seedlings treated with 250 µg/g Pb^{2+}, exhibited accumulation of Pb^{2+} in roots but no significant translocation to shoots.

Important insight has been obtained from the study by MacFarlane and Burchett (2000) on the distribution and excretion of Cu^{2+}, Pb^{2+} and Zn^{2+} in the root and leaf tissue of *A. marina*. Mature leaves contained significantly higher amounts of Zn^{2+} and Cu^{2+} than control plants after one month, suggesting excretion of both metals from the glandular trichomes. In addition, salt crystals exuded from the glands on the adaxial surface of mature leaves were composed of alkaline metals: Zn^{2+} in Zn^{2+}-treated plants and Cu^{2+} in Cu^{2+}-treated plants. Leaf tissue in seedlings dosed with 4 g/l Zn^{2+} showed a decreasing gradient of the metal from xylem tissue, through photosynthetic mesophyll, to hypodermal tissue, with a subsequent increase in concentration in the glandular tissue. A similar gradient was observed for Cu^{2+} treated seedlings.

Tamarix smyrnensis plants were exposed to Pb^{2+} in soil with and without the addition of salt for a period of 10 weeks (Kadukova and Kalogerakis, 2007). A statistical difference was observed among the groups treated with lead and different salt concentrations. Reduced plant biomass was found in the Pb^{2+} treatments without salt. Chaoui *et al.* (1997) have suggested that toxic metals are generally responsible for the reduction of biomass. A significant shoot Grade of Growth Inhibition (GGI) was observed in plants treated with lead without salt addition. Plant growth was inhibited in this group by 50 %. Apart from chlorophyll content, all other parameters i.e. shoot length and plant biomass, used to determine the physiological status of the plants, were the lowest in the group treated only with lead without salt addition. This suggests that the combination of Pb^{2+} and salt has a less damaging effect on plants than only lead in soil (Kadukova and

Kalogerakis, 2007). The roots were the main accumulation site of Pb^{2+} in *Tamarix smyrnensis*. The large differences between root and leaf Pb^{2+} concentrations indicate an important restriction of the internal transport of metal from the roots toward stems and leaves (Kadukova and Kalogerakis, 2007).

There are well documented studies on the inhibition of photosynthesis by heavy metals in higher plants, especially in those which utilize a C_3 photosynthetic pathway, including *A. marina* (Ball, 1985; Clijsters and Van Assche, 1985; Basak *et al.*, 1996; Prasad and Strzalka, 1999; MacFarlane and Burchett, 2001). Exposure to heavy metals such as Cu^{2+} (Van Assche and Clijsters, 1990), Pb^{2+} (Wozny and Krzeslowska, 1993; Kastori *et al.*, 1998), and Zn^{2+} (Krupa *et al.*, 1996), caused a marked reduction in the levels of photosynthetic pigments, including chlorophyll *a* and *b* and accessory pigments such as carotenoids (MacFarlane and Burchett, 2001). *Kandelia candel* (L.) Druce exhibited reductions in chlorophyll *a* and *b* content when exposed to wastewater containing a mixture of heavy metals (Chen *et al.*, 1995). Changes in photosynthetic pigments in response to metal stress may be used to indicate damage to the photosynthetic apparatus capacity. Other consequences of changes in photosynthetic pigments may be reduced carbon assimilation, growth, survival and reproduction (Vangronsveld and Clijsters, 1994; MacFarlane and Burchett, 2001).

In other laboratory investigations, 6 month-old *A. marina* seedlings were exposed to a range of Cu^{2+}, Pb^{2+} and Zn^{2+} concentrations, to determine leaf tissue metal accumulation patterns, effects on photosynthetic pigments and peroxidase activity (MacFarlane and Burchett, 2001). Peroxidase induction is a common response of higher plants to various environmental stressors including heavy metals (Dietz *et al.*, 1999). Findings suggest that significant increases in peroxidase activity and decreases in photosynthetic pigments were found with Cu^{2+} and Zn^{2+} at concentrations of 400 μgml^{-1} and 1000 μgml^{-1} respectively. Significant increases in only peroxidase activity were found in plants exposed to Pb^{2+}. Positive linear relationships between peroxidase activity and leaf tissue metal concentrations were found for Cu^{2+}, Pb^{2+} and Zn^{2+}. Significant linear decreases in photosynthetic pigments with increasing leaf tissue metal concentrations were observed

with Cu^{2+} and Zn^{2+} treatments only (Chen *et al.*, 1995MacFarlane and Burchett, 2001). Thus, these biochemical parameters may be used as indicators of metal exposure and may serve as early-warning signals of plant stress prior to visible damage (MacFarlane, 2002).

Results obtained in further studies on *A. marina* by MacFarlane (2002) demonstrated that peroxidase activity may be a suitable biomarker for Zn^{2+} or total metal accumulation in leaf tissue, and that the chlorophyll *a/b* ratio is a suitable biomarker of Zn accumulation. It was found that peroxidase activity best reflected the total phytotoxic effect from the combined metal stress of Cu^{2+}, Pb^{2+} and Zn^{2+} in the leaves.

Aust *et al.* (1985) showed that metals such as Zn^{2+}, Pb^{2+} and Cu^{2+} are efficient generators of active oxygen species and thus are important factors in heavy metal toxicity, resulting in the generation of oxidative stress. Plants have a series of detoxifying antioxidants involving both non-enzymatic and enzymatic mechanisms to avoid accumulation of these toxins. A close correlation was seen in peroxidase activity with changes in physiological processes such as respiration and photosynthesis, with associated growth and fitness consequences. As a consequence, an increase in the activity of peroxidase on exposure to heavy metals in the cell may play a major role in facilitating cellular defense mechanisms against metal toxicity (Van Assche and Clijsters, 1990; MacFarlane and Burchett, 2001). Therefore peroxidase activity has the potential to serve as a sensitive indicator of compromised metabolic activity (Verkleij and Schat, 1990).

Plant species and populations differ widely in their ability to accumulate heavy metals. Metals absorbed by plant roots together with the uptake of mineral nutrients may be incorporated into the above-ground tissues. Distribution of metals within the plant depends on the ability of these metals to be transported. Toxic substances (Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+}) accumulated in plant tissues could also impair normal physiological functioning of the plants (Pryzwra and Stepniewski, 1999).

MacFarlane and Burchett (2002), in further investigations on $A.$ $marina$ seedlings, found that Cu^{2+} and Zn^{2+} showed the greatest uptake, compartmentation, greater toxic effects on growth responses and total inhibition of emergence at higher concentrations. In contrast Pb^{2+} exhibited limited uptake, minimal mobility and little toxic effects on growth responses. Zinc and Pb^{2+} were accumulated in roots in proportion to sediment concentrations, while Cu^{2+} was regulated. Translocation of Cu^{2+}, Pb^{2+} and Zn^{2+} from the root to the leaves was restricted. The physiological requirement for Cu^{2+} resulted in some accumulation at lower sediment concentrations with an exclusion or saturation mechanism occurring at higher concentrations (Baker, 1981). Consequently, Zn^{2+} translocation to leaves showed a dose-response relationship with both root and sediment Zn^{2+} levels. Zinc uptake and mobility to the tissues was high thus indicating the essential role that this micronutrient plays in plant function (Baker and Walker, 1990). Lead was excluded from the leaves at concentrations of up to 400 µg/g, above which some transport of Pb^{2+} was observed (MacFarlane and Burchett, 2002).

Other published laboratory-based studies have reported on the effects of a range of heavy metal concentrations to mangrove seedlings in synthetic wastewaters and sewage. The mangroves, $Kandelia$ $candel$ and $Aegiceras$ $corniculatum$ (L.) Blanco, showed tolerance to low metal levels in wastewater. Seedlings showed higher Cu^{2+}, Pb^{2+} and Zn^{2+} accumulation in the roots with limited translocation of Cu^{2+} and Zn^{2+} only to the leaves (Chen et $al.$, 1995; Chiu et $al.$, 1995; Wong et $al.$, 1997; Shenyu and Chen, 1998). Several mangrove species studied under laboratory conditions exhibited accumulation at the root level, with limited translocation of Cu^{2+} and Zn^{2+} and exclusion of Pb^{2+} to aerial parts of the plant thus showing tolerance to elevated levels of soil metals (Yim and Tam, 1999; Takemura et $al.$, 2000; MacFarlane and Burchett, 2002; Ye et $al.$, 2003; Zhang et $al.$, 2007).

Exposure of $Azolla$ sp., the aquatic fern, to heavy metals resulted in reduced growth rate and water content. High levels of Cr^{2+}, Cu^{2+} and Ni^{2+} were found in the root tissue of $Azolla$ species with relatively small amounts translocated to the shoots. Conclusively, this plant reflects low mobility of these ions (Sela et $al.$, 1989). The discovery that the water hyacinth ($Eichhornia$) can accumulate up to 500 ppm (parts per million) of Cd^{2+},

Pb^{2+} or Hg^{2+} has led to the suggestion that aquatic plants may prove very effective in the removal of such heavy metals from the environment (Chigbo et al., 1982).

Thus plants have adapted a range of potential mechanisms for metal ion homeostasis at the cellular level that might be involved in detoxification of polluted waters (Clemens, 2001). Several plant species such as *Armeria maritime, Tamarix aphylla, Rhizophora stylosa, Lygeum spartum, Hordeum distichum, Carex rostrata, Paspalum distichum, Elodea canadensis* and *Potamogeton crispus* have developed tolerant races that can survive and thrive on such metalliferous soils (Zheng and Chen, 1997; Hall, 2002; Stoltz and Greger, 2002; Shu et al., 2002; Samecka-Cymerman and Kempers, 2007; Conesa et al., 2007; Dos Santos Utmazian and Wenzel, 2007; Battaglia et al., 2007).

On the basis of the information which is gathered from this literature review, the author was able to select the appropriate methods which were relevant to test the chosen hypotheses in the introduction. She was also able to pursue the specific objectives of the study. Thus the following methodologies were used:

(i)   Experimental Design: controlled study

(ii)   Heavy metal exposure

(iii)   Plant height,, number of leaves and biomass accumulation

(iv)   Ion analyses

(v)   Gas exchange

(vi)   Chlorophyll fluorescence

(vii)   Chlorophyll content

(viii) Scanning electron microscopy (SEM)

(ix)   Energy Dispersive X-Ray microanalysis (EDX)

(x)   Environmental scanning electron microscopy (ESEM)

CHAPTER 3

MATERIAL AND METHODS

3.1. EXPERIMENTAL DESIGN: CONTROLLED STUDY

Approximately 120 propagules of *Avicennia marina* were collected from the Beachwood Mangrove Nature Reserve, Isipingo Estuary and Durban Bay Heritage Site and cultivated individually in 15 cm X 15 cm (diameter x depth) plastic pots without drainage holes. Each pot contained 2000 g of soil composed of a 1:1:1 mixture of river sand, potting soil and compost, oven dried at 70 °C for several days and initially watered with 1000 ml of deionised water in order to acclimatize the plants. In addition, plants were fertilized once with 100 ml half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950). Thereafter, seedlings were watered twice a day with 100-150 ml of deionised water for approximately twelve months and 20 % seawater was added every 2-3 weeks, in order to mimic typical estuarine sediment. Pots were covered with black plastic bags to prevent penetration of light and algal growth. All plants were grown under normal light in an air-conditioned glasshouse at 24 °C.

3.2. HEAVY METAL EXPOSURE

Seedlings were allowed to acclimatize in the glasshouse for approximately one month. Thereafter, uniform plants were exposed to four heavy metal treatments, namely, $HgSO_4$, $PbSO_4$, $CuSO_4$ and $ZnSO_4$ respectively, each with 6 replicate pots per treatment. One hundred and twenty plants were watered with 200 ml of metal solution containing $HgSO_4$, $PbSO_4$, $CuSO_4$ and $ZnSO_4$ respectively. *A. marina* seedling were grown for approximately eleven months in pots contaminated with Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} at concentrations of 0, 40, 80, 120 and 160 μgml^{-1} (1 μgml^{-1} = 1 ppm). The six replicate pots were located randomly for each treatment and concentration.

From this cohort, five replicate plants at concentrations of 0, 40, 80, 120 and 160 μgml^{-1} for each treatment (a total of 100 plants) were selected for physiological investigations only. The sixth replicate pot in each treatment (a total of 20 seedlings) was used for scanning electron microscopy investigations and EDX- microanalyses.

3.3. PLANT HEIGHT, NUMBER OF LEAVES AND BIOMASS ACCUMULATION

At the end of the experiment (after 12 months), the height and the number of leaves of Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} treated seedlings and the control were measured and recorded to monitor plant growth. Thereafter seedlings were harvested and the roots were washed with deionized water to remove soil particles. Excess moisture was blotted with paper towels. The roots and shoots were separated and fresh weight recorded. Roots and shoots from all five replicates in each treatment, at each concentration, were then dried in an oven at 72 °C for approximately 5 days. Thereafter, the total, root and shoot dry weights were determined.

3.4. ION ANALYSES

Dried shoot and root samples were milled through a 1-mm screen and stored in plastic vials. Soil samples from control treatments were also weighed to 1 g and stored in plastic vials. The dried and powdered plant samples and soil samples were weighed to approximately 0.5 g in 50 ml conical flasks. All samples were pre-digested with 10 ml of concentrated nitric acid which was added to the samples and allowed to stand for 24 h. Subsequently, all samples were heated for 1 – 1.5 hours at 200 °C, except mercury, where a temperature <120 °C was used. During the digestion and cooling processes, the volume of nitric acid was reduced. The samples were still too acidic for analysis and therefore, diluted with sterile deionised water. These were then transferred to glass tubes, washed in concentrated nitric acid and analyzed. Concentrations of Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} in the extracts were determined by atomic absorption spectrophotometry (Perkin-Elmer Model 303, USA).

Similarly, three batches of leaves and root samples collected from approximately five *A. marina* trees at the Durban Bay Heritage Site were analyzed by atomic absorption to

conduct a comparative investigation of levels of heavy metals in natural populations of mangroves.

3.5. GAS EXCHANGE

Gas exchange readings were taken for the Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} treatments at concentrations of 0, 40, 80, 120 and 160 μgml^{-1} prior to harvesting. A portable, infrared gas analyzer (LICOR-6400 Licor, Lincoln, Nebraska, USA) was used to record the measurements. Measurements were carried out at saturating natural light of

>1000 $\mu molm^{-2}s^{-1}$ at a temperature of 30 °C. Five replicate pots were selected for each treatment, i.e. Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+}. Two fully expanded leaves were randomly selected from each replicate pot per treatment for measurements. Thus, a total of ten measurements were recorded for Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} respectively for each concentration. The leaf was clamped into the leaf chamber which was then tilted to maximum PAR (photosynthetically active radiation) incident on the enclosed leaf. An electronic flow meter measured the flow rate (499.5 ml per hr) of air going through the chamber. Net photosynthesis and transpiration were computed by measuring the air-flow rate, the incoming and chamber CO_2 and H_2O concentrations and leaf area.

3.6. CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence measurements were made on the same leaves used for gas exchange using a portable, pulse amplitude modulated fluorometer (PAM 2100, Walz, Effeltrich, Germany). In this technique a pulse of very weak modulated measuring beam of red light and a saturating pulse of white light is sent through a fibreoptics to the leaf.

Photosystem II (PSII) quantum yield (yield), electron transport rate (ETR) through PSII, and photochemical efficiency of PSII (Fv/Fm) were measured after 30 minutes dark adaptation with dark leaf clips. Before a measurement was taken, the shutter was opened and Fv/Fm measured by sending a pulse of modulated beam through the fibreoptics.

3.7. CHLOROPHYLL CONTENT

Chlorophyll content measurements were made on the same leaves used for gas exchange. Chlorophyll content was determined with a hand-held chlorophyll absorbance meter (CCM-200) (Opti Sciences, Tyngsboro, MA, USA). Five replicate pots were selected for each treatment, i.e. Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+}. Thus, five measurements were recorded for Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} respectively for each concentration.

3.8. STATISTICAL ANALYSES

All data were subjected to one-way analysis of variance (ANOVA) and means separated using Tukey-Kramer Multiple Comparisons test ($P<0.05$) using GraphPad Instat Version 3.00 (Mustek) and GraphPad Prism Version 2.00. All data were analyzed and plotted to determine differences in plant height, biomass accumulation, gas exchange, chlorophyll fluorescence and ion absorption amongst the treatments at different concentrations.

3.9. SCANNING ELECTRON MICROSCOPY (SEM)

Fully expanded leaves were harvested from Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} treatments at increasing concentrations of 0, 40, 80, 120, 160 μgml^{-1} after 11 months of exposure to heavy metals and from control seedlings to investigate leaf surface morphology. The leaf blade was cut into three regions, namely, the apex; middle and basal regions. The material was rapidly quenched in liquid nitrogen and freeze-dried in an Edwards Modulyo freeze-drier at -60 °C at a vacuum of 10^{-2} Torr for 5 days. Samples were secured onto brass stubs with carbon conductive tape and sputter coated with gold in a Polaron SC500 for 4 minutes. Both adaxial and abaxial leaf surfaces were viewed with a Jeol 6100 SEM and a Philips SEM 500 operated at 12 kV and a working distance of 15 mm.

3.10. ENERGY DISPERSIVE X-RAY MICROANALYSIS (EDX)

Leaves from the highest concentration of 160 µgml^{-1} for all metals and control plants were selected for analysis. The surface of leaf samples, namely the apex, mid-region and basal regions, were analyzed for elemental composition by energy dispersive X-ray microanalysis (EDX) using the Jeol SEM 6100 interfaced with the Noran system six microanalysis system. During analyses, the accelerating voltage was maintained at 15 kV, data collection time was 60 s and the working distance 15 mm. Area analyses were performed at a magnification of 500× with an area diameter of 50 µm. The Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} were further localized by X-ray mapping.

3.11. ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY (ESEM)

Fresh leaf samples from the highest concentration of 160 µgml^{-1} for Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} treatments and control plants were harvested for viewing with the Philips XL30 Environmental Scanning Electron Microscope (ESEM) under low vacuum mode to validate the appearance of samples prepared using the freeze-drying and conventional SEM. During analyses, the accelerating voltage was maintained at 15 kV and the working distance at 10 mm. Spot analyses were performed at a magnification of approximately 250X with an area diameter of 200 µm.

CHAPTER 4

RESULTS

4.1. PHYSIOLOGY

4.1.1 PLANT GROWTH

a) TOTAL DRY BIOMASS ACCUMULATION

Highly significant decreases in total dry biomass accumulation were observed for Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} treatments with increasing metal concentrations. Seedlings exposed to 80, 120 and 160 μgml^{-1} of Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} had significantly lower dry biomass than the control values. At 160 μgml^{-1} of Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+}, total dry biomass was significantly lower than the 0 and 40 μgml^{-1} treatments (Figs. 3A-D). Percentage decreases in dry biomass accumulation in the 160 μgml^{-1} Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} treatments were 42.5 %, 36.7 %, 41.5 % and 40 % respectively compared to the control values (Figs. 3A-D).
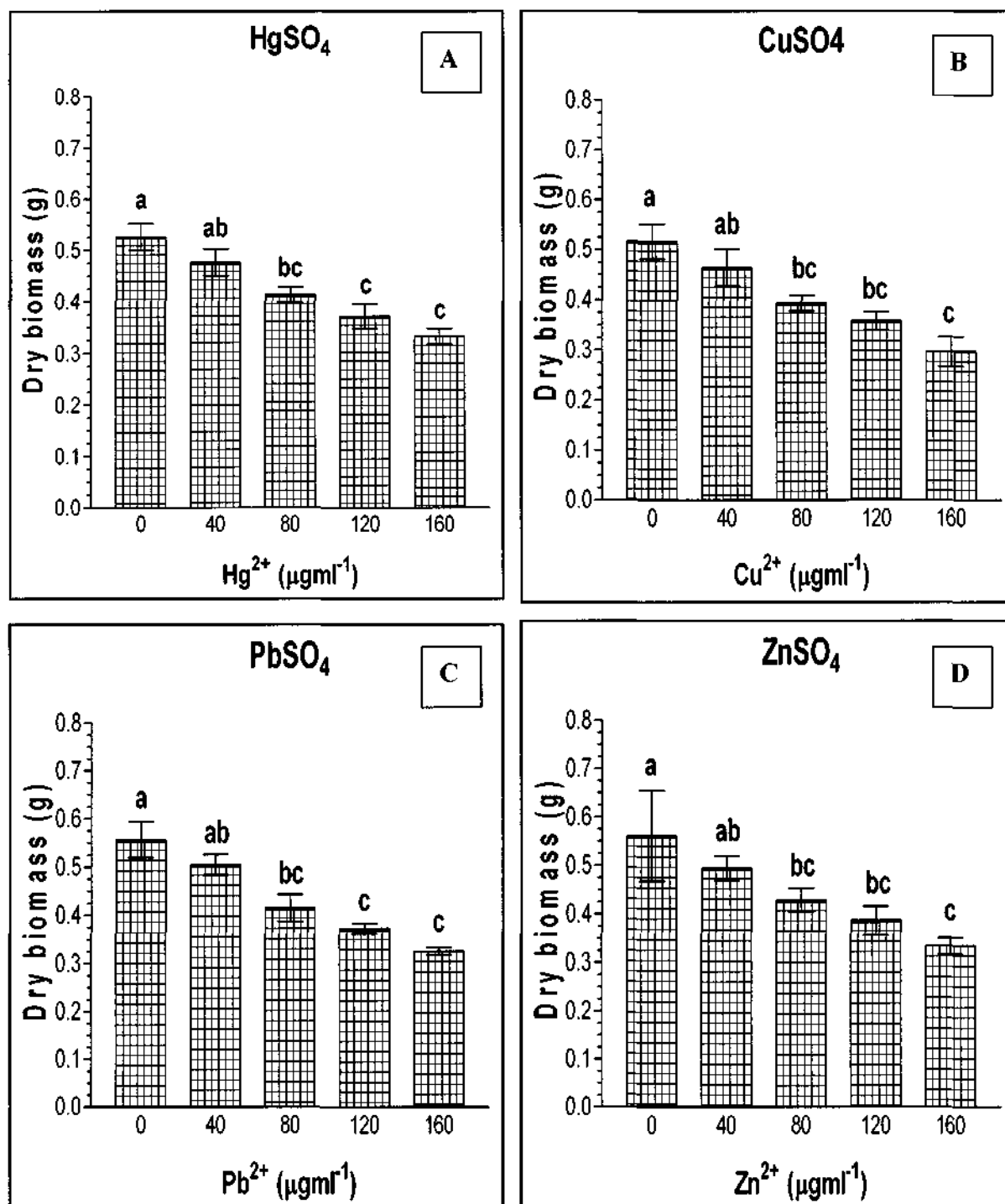
Figs. 3A-D. Total dry biomass accumulation of *A. marina* at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

c) DRY SHOOT BIOMASS ACCUMULATION

In the Hg^{2+} treatment, dry shoot biomass at 80, 120 and 160 μgml^{-1} Hg^{2+} were significantly lower than the control (Fig. 4A). Dry shoot biomass decreased with increasing concentrations of metal in the Cu^{2+} treatment. Dry shoot biomass in the 160 μgml^{-1} Cu^{2+} treatment was significantly lower than the control and 40 μgml^{-1} treatments (Fig. 4B). In plants contaminated with Pb^{2+} and Zn^{2+} a significant decrease in dry shoot biomass was observed at dosages of 120 and 160 μgml^{-1} compared to the control and 40 μgml^{-1} treatments (Figs. 4C-D). Percentage decreases in dry shoot biomass in the 160 μgml^{-1} Hg^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} treatments were 35.7 %, 47 %, 39.8 % and 35.6 % respectively compared to the control values (Figs. 4A-D).
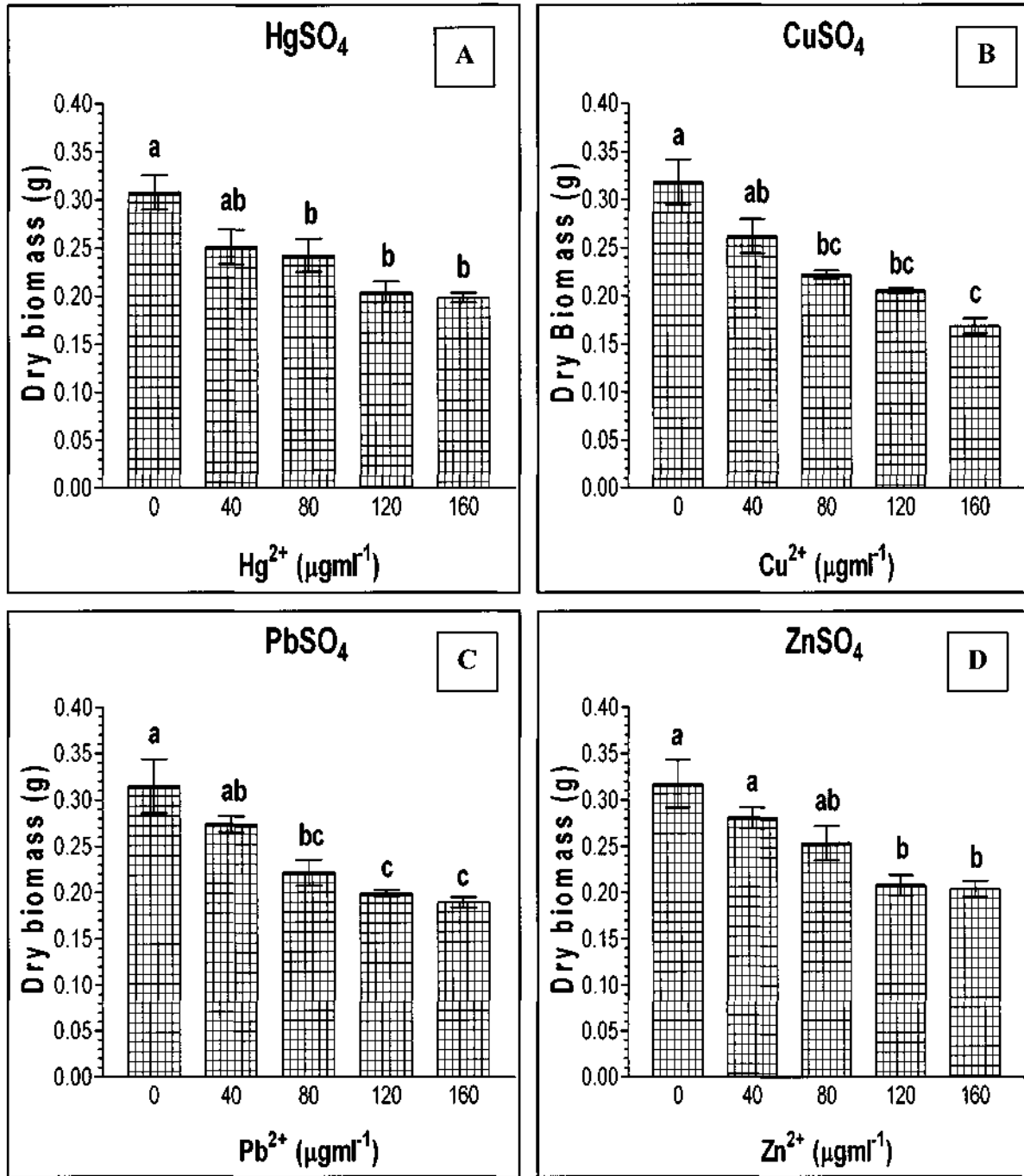
Figs. 4A-D. Dry shoot biomass accumulation of *A. marina* at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

b) DRY ROOT BIOMASS ACCUMULATION

The dry biomass of roots for all metals followed trends similar to those of total dry biomass, decreasing with increasing metal concentrations. Dry biomass in the 120 and 160 μgml^{-1} Hg^{2+} treatments were significantly lower than the control and 40 μgml^{-1} treatments (Fig. 5A). Dry root biomass in the 160 μgml^{-1} Cu^{2+} treatment (Fig 5B) was significantly lower than the control. Dry root biomass of Pb^{2+} in the 160 μgml^{-1} treatment was significantly lower than those at 0, 40 and 80 μgml^{-1} Pb^{2+} (Fig. 5C). At 160 μgml^{-1} of Zn^{2+}, dry root biomass was significantly lower than those at 0 and 40 μgml^{-1} treatments (Fig. 5D). Percentage decreases in dry root biomass in the 160 μgml^{-1} Hg^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} treatments were 38.3 %, 44.4 %, 42.1 % and 48.1 % respectively compared to the control values (Figs. 5A-D).

Figs. 5A-D. Dry root biomass accumulation of *A. marina* at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.
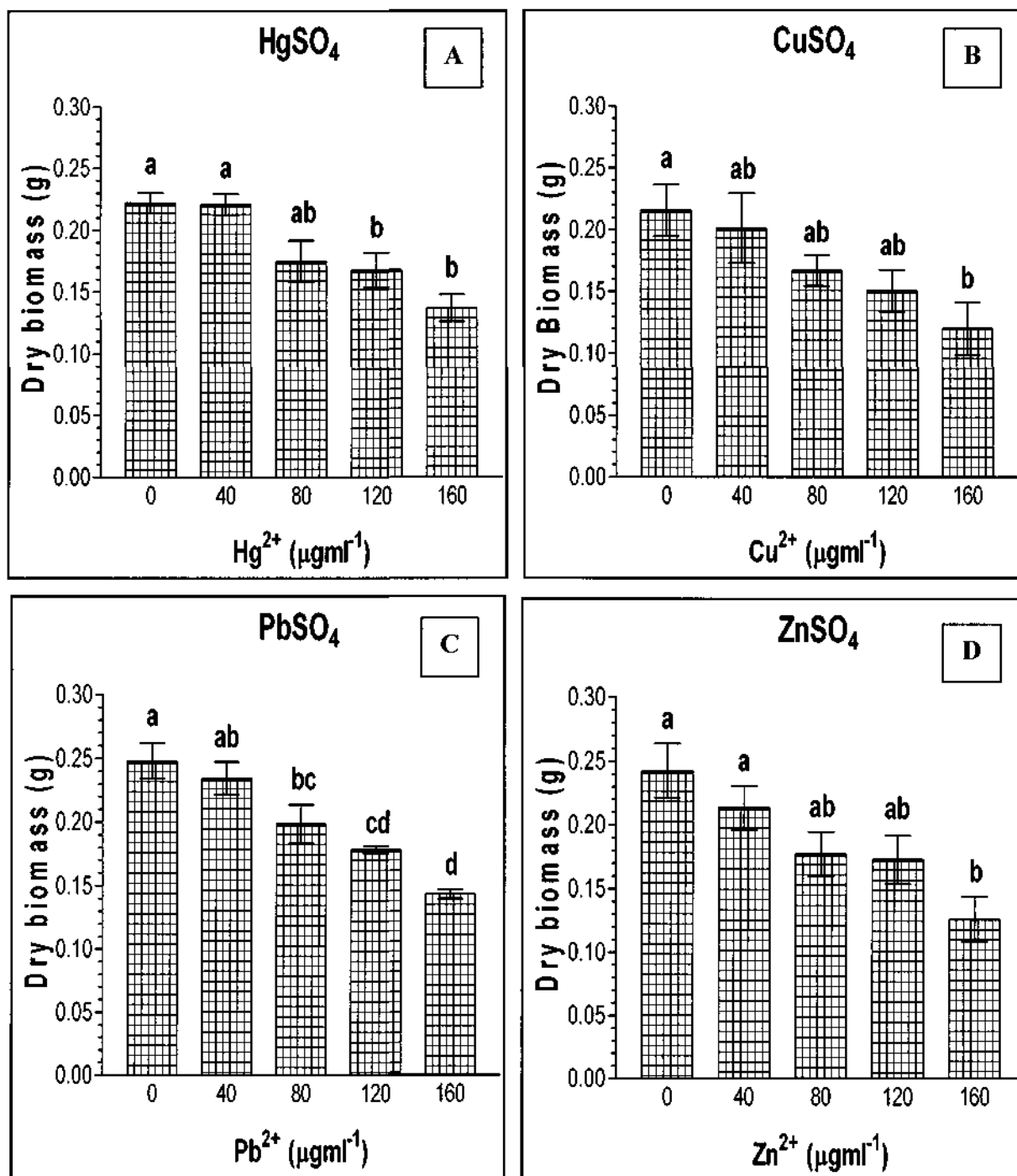
d) PLANT HEIGHT

In the Pb^{2+} treatment, plant height at 120 and 160 μgml^{-1} Pb^{2+} was significantly lower than the control (Fig. 6A). In the Hg^{2+} treatment, plant height decreased with increasing concentrations of metal. Plant height at 160 μgml^{-1} Hg^{2+} was significantly lower than the control and 40 μgml^{-1} treatments (Fig. 6B).

In plants contaminated with Zn^{2+} and Cu^{2+}, a significant decrease in plant height was observed at a dosage of 160 μgml^{-1} compared to the control (Figs. 6C, D). No significant differences in height were observed in plants exposed to 40, 80 and 120 μgml^{-1} for Zn^{2+} and Cu^{2+} (Figs. 6C, D). Percentage decreases in plant height in the 160 μgml^{-1} Pb^{2+}, Hg^{2+}, Zn^{2+} and Cu^{2+} treatments were 36.6 %, 60 %, 36.7 % and 46 % respectively compared to the control values (Figs. 6A-D).

Fig. 6A-D.   Plant height of *A. marina* seedlings at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

e) NUMBER OF LEAVES

With increasing metal concentration, the number of leaves in the Pb^{2+}, Cu^{2+}, Hg^{2+} and Zn^{2+} treatments were significantly reduced.

Seedlings exposed to 80, 120 and 160 μgml^{-1} of Pb^{2+} had a significantly lower number of leaves than the control (Fig. 7A). In the Cu^{2+} treatments, the number of leaves at 120 and 160 μgml^{-1} Cu^{2+}, was significantly lower than the 0 and 40 μgml^{-1} treatments (Fig. 7B). At 80, 120 and 160 μgml^{-1} Hg^{2+}, the number of leaves was significantly lower compared to the control treatment (Fig. 7C). In addition, Zn^{2+} treatments had a significantly lower number of leaves for the 120 and 160 μgml^{-1} Zn^{2+} treatments than the control (Fig. 7D). Percentage decreases in the number of leaves in the 160 μgml^{-1} Pb^{2+}, Cu^{2+}, Hg^{2+} and Zn^{2+} treatments were 46.3 %, 45.4 %, 50 % and 45.2 % respectively compared to the control values (Figs. 7A-D).

Figs. 7A-D. Number of leaves of *A. marina* at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

f) CHLOROPHYLL CONTENT IN LEAVES

Generally, for all heavy metals, chlorophyll content in the leaves decreased with increasing metal concentrations from 0 to 160 μgml^{-1}.

Similar trends were observed for Cu^{2+}, Pb^{2+} and Hg^{2+} treatments with increasing metal concentrations. Chlorophyll content in the leaves of Cu^{2+}, Pb^{2+} and Hg^{2+} treatments were significantly lower than the controls (Figs. 8A, C-D). In the Cu^{2+} treatment, chlorophyll content at 80, 120 and 160 μgml^{-1} Cu^{2+} was significantly lower than the control treatment (Fig. 8B). Percentage decreases in chlorophyll content in the 160 μgml^{-1} Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} treatments were 55.6 %, 57.9 %, 49.1 % and 49.7 % respectively compared to the control values (Figs. 8A-D).

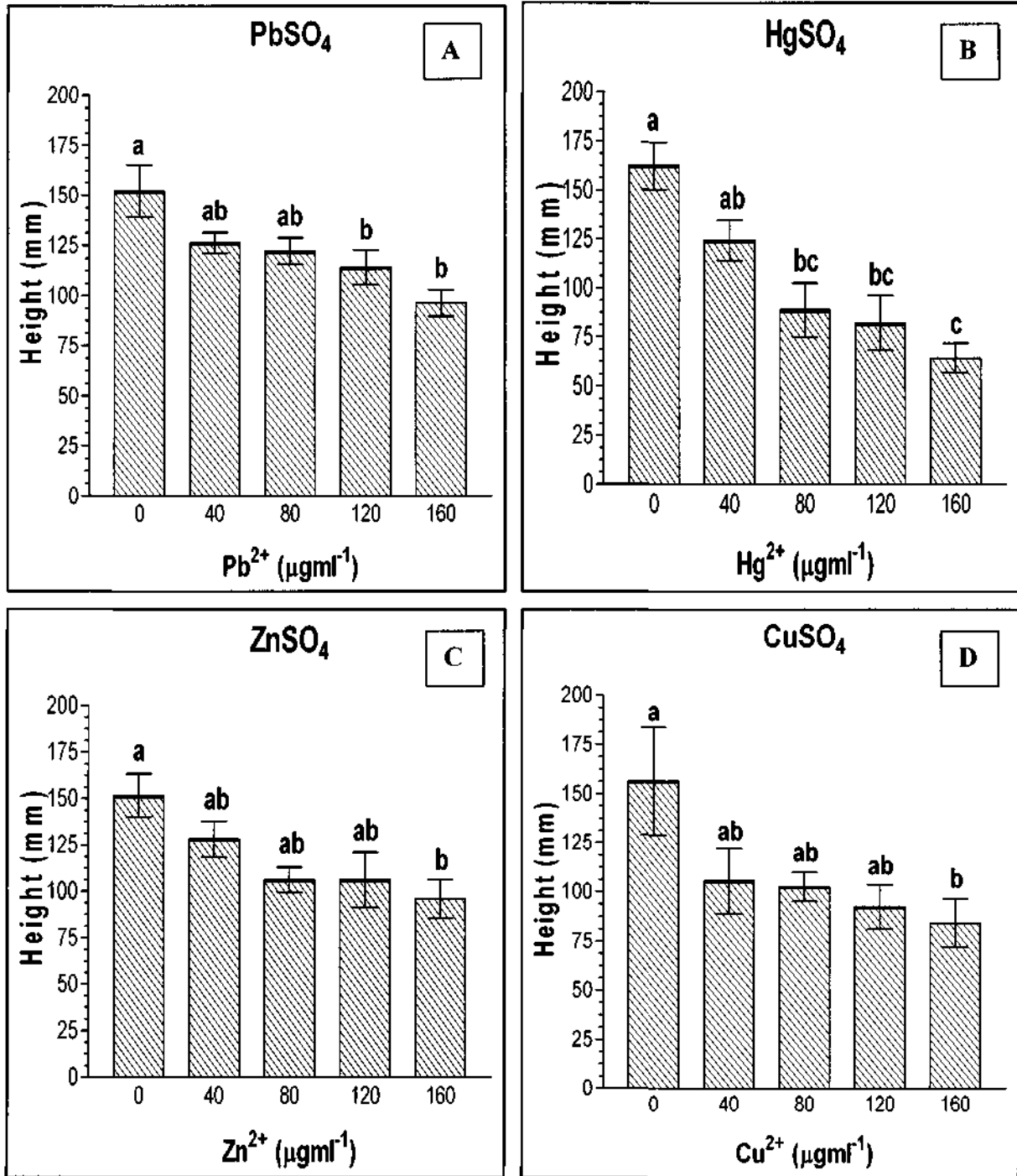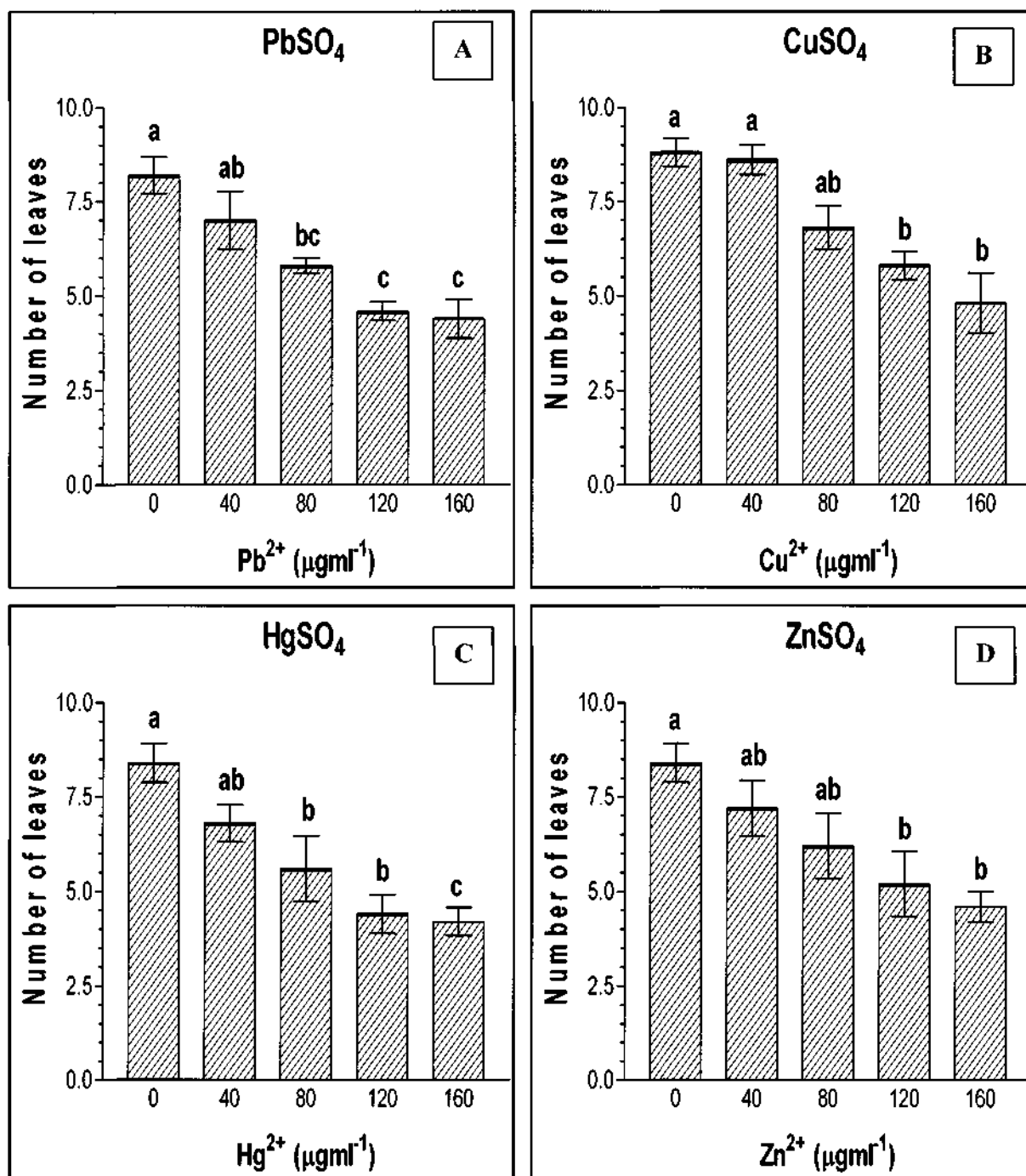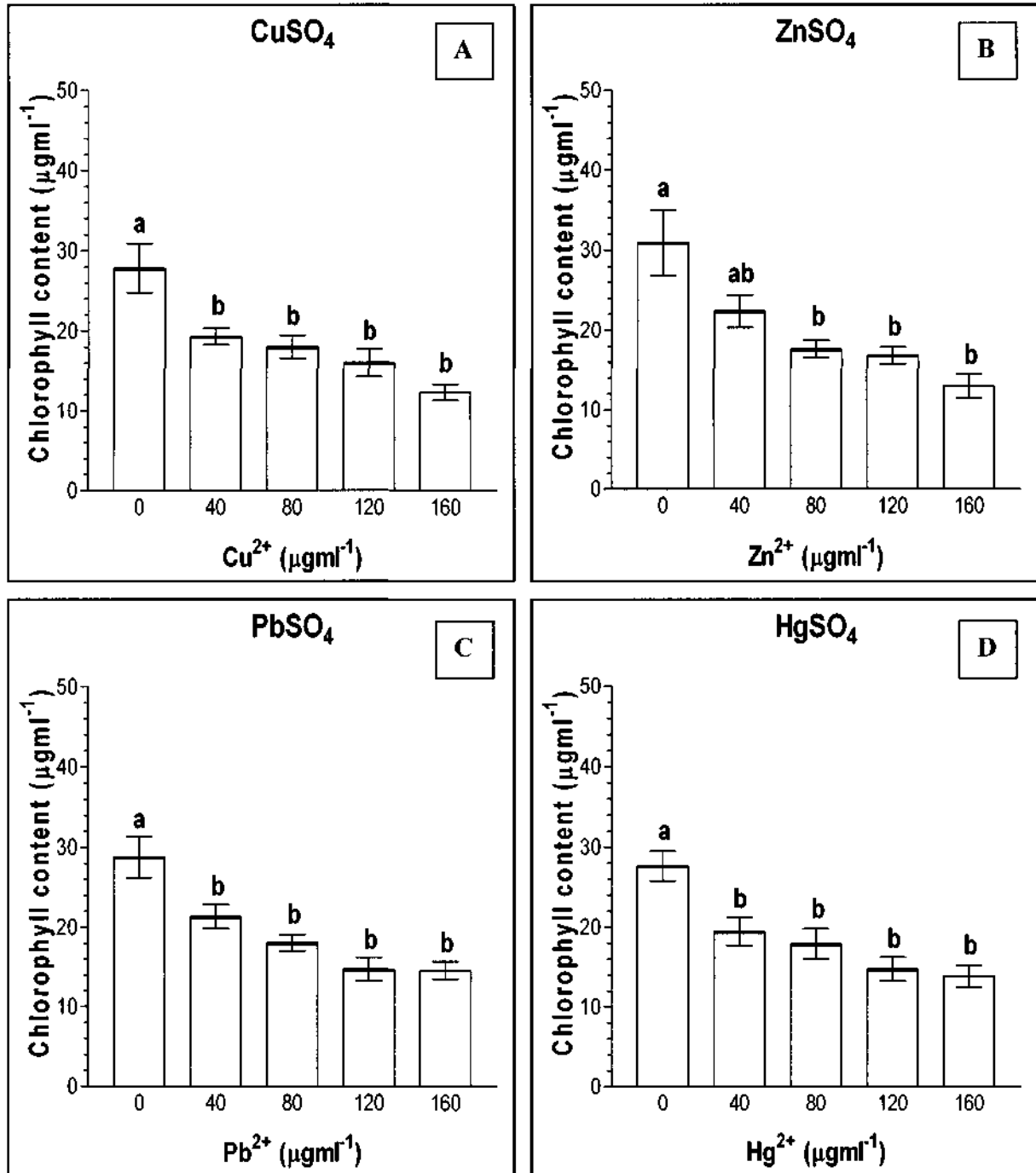**Figs. 8A-D.** Chlorophyll content of leaves of *A. marina* at increasing metal concentrations. Means ± S.E. are given (n = 5). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

g) ION ANALYSIS

- GLASSHOUSE ANALYSIS

Copper concentrations were higher in roots than in shoots (Figs. 9, 10). The concentration of Cu^{2+} in roots and shoots increased with increasing metal concentrations. The concentration of Cu^{2+} in the roots at 160 μgml^{-1} was significantly higher than those at 0, 40 and 80 μgml^{-1} (Fig. 9). In the 160 μgml^{-1} Cu^{2+} treatment concentrations in roots and shoots were higher by 73 % and 56.6 %, respectively, compared to the control values.

Similarly, Zn^{2+} concentrations were higher in the roots than in shoots and increased with increasing metal concentrations (Figs. 11, 12). Root concentrations of Zn^{2+} were significantly higher at 160 μgml^{-1} compared to 0, 40 and 80 $\mu g\ ml^{-1}$ (Fig. 11). At 160 μgml^{-1} Zn^{2+}, metal concentrations in roots and shoots were higher by 69 % and 49.8 %, respectively, compared to the controls.

Mercury was not detected in root and shoot tissues of control plants (Figs. 13, 14). However, Hg^{2+} accumulation increased with increasing metal concentration being considerably higher in roots than shoots. In roots and shoots the concentration of Hg^{2+} at 160 μgml^{-1} was significantly higher than the others (Figs. 13, 14). Consequently, Hg^{2+} accumulation, at 160 μgml^{-1} Hg^{2+}, was above threshold levels in the shoot and root tissues. The limit/threshold level for Hg^{2+} uptake in aquatic plants is 0.025 μgml^{-1} dry weight respectively (USEPA, 1995).

Lead accumulation increased significantly with increasing metal concentrations in roots and shoots (Figs 15, 16). Concentration of Pb^{2+} at 160 μgml^{-1} was significantly higher than those at 0, 40 and 80 μgml^{-1} in roots and shoots (Figs. 15, 16). Similar to Hg^{2+}, Pb^{2+} accumulation, at a concentration 160 μgml^{-1}, was also above threshold levels in the shoots and roots. The limit/threshold level for Pb^{2+} accumulation in aquatic plants is 5.80 μgml^{-1} dry weight respectively (USEPA, 1995).

Soil samples from control pots (Fig. 17) were analyzed for the presence of Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+}. Zinc and Cu^{2+} (essential micronutrients) were present at acceptable levels in the soil, whereas Pb^{2+} and Hg^{2+} (non-essential elements) were not detected.
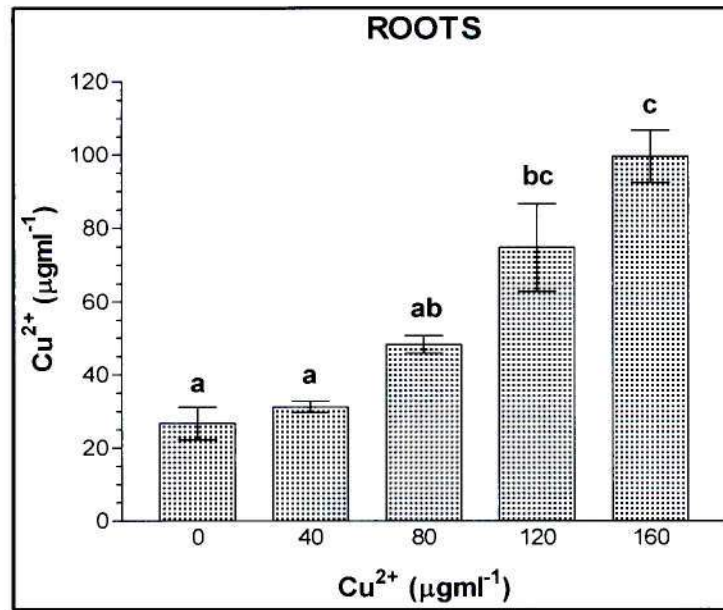
Fig. 9.   Concentration of Cu^{2+} in roots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Cu^{2+}.   Values are means ± S.E. (n = 3).   Bars with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.
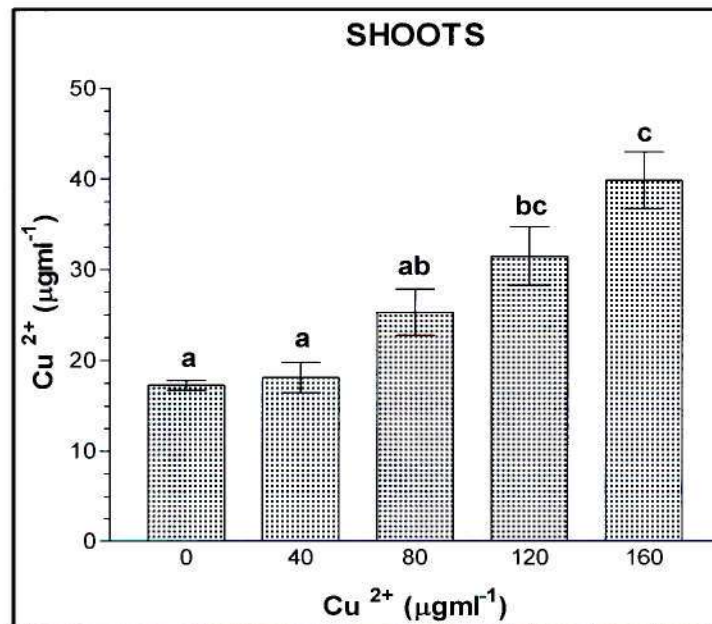
Fig. 10.   Concentration of Cu^{2+} in shoots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Cu^{2+}.   Values are means ± S.E. (n = 3).   Bars with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.
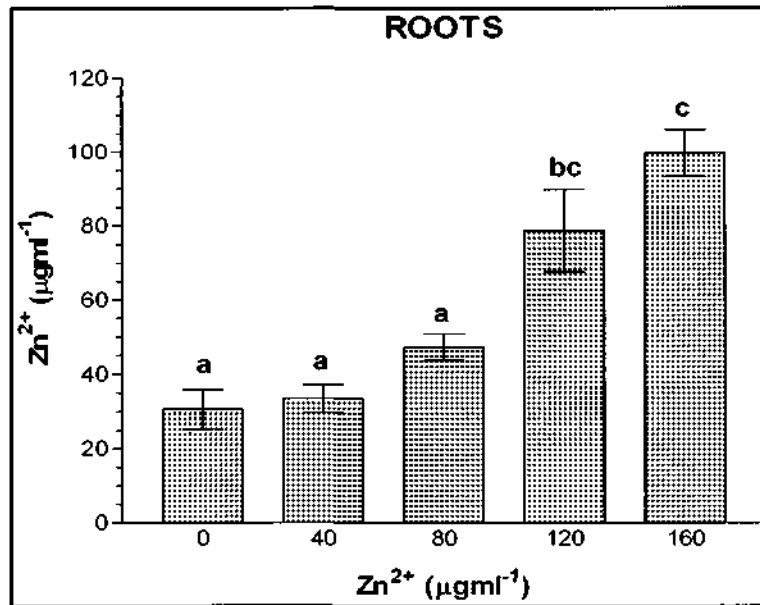
Fig. 11. Concentration of Zn^{2+} in roots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Zn^{2+}. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.
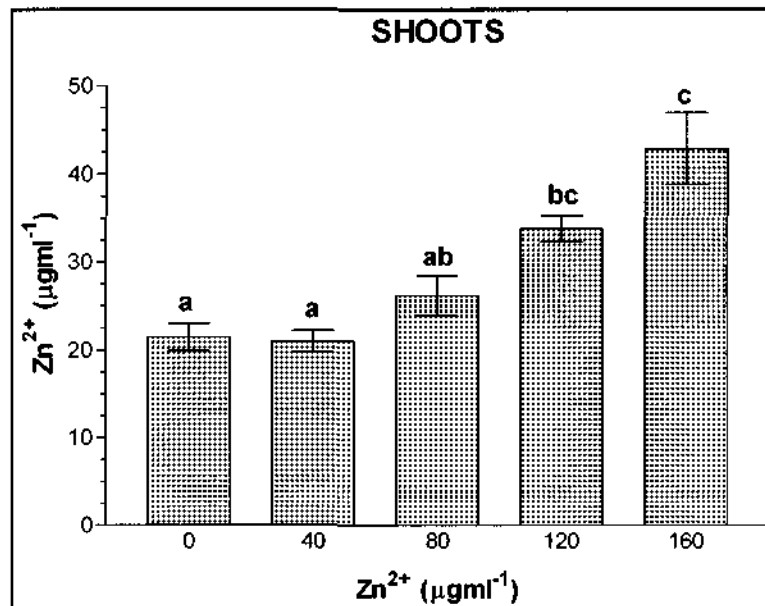
Fig. 12. Concentration of Zn^{2+} in shoots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Zn^{2+} Values are means ± S.E. (n = 3). Bars with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

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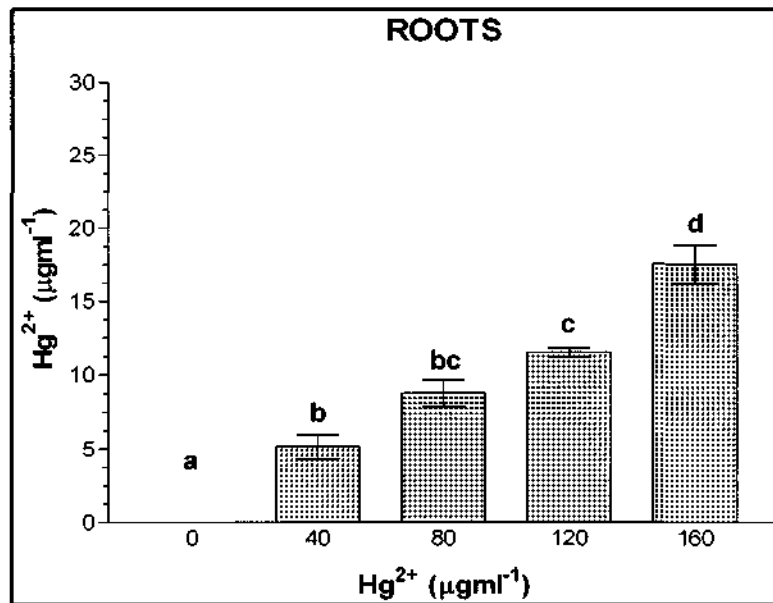
Fig. 13. Concentration of Hg^{2+} in roots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Hg^{2+}. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.
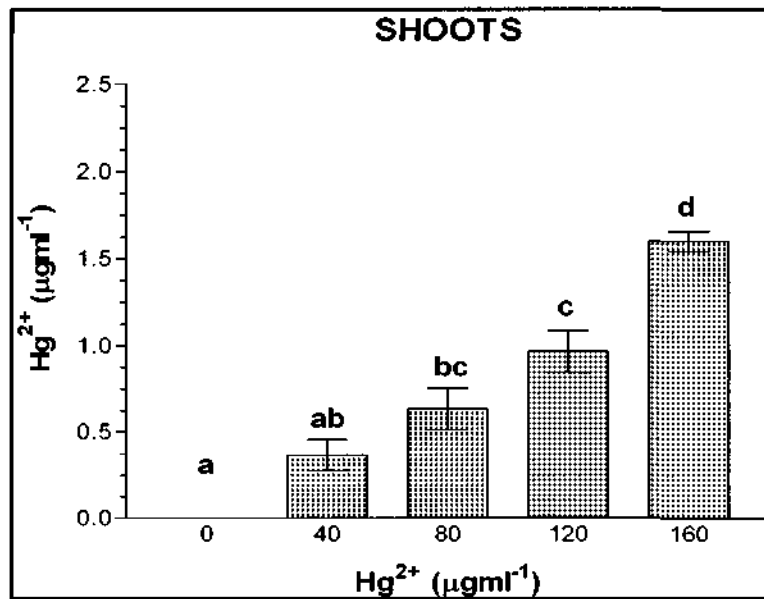
Fig. 14. Concentration of Hg^{2+} in shoots of *A. marina* seedlings treated with 0, 40, 80, 120 and 160 μgml^{-1} Hg^{2+}. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.
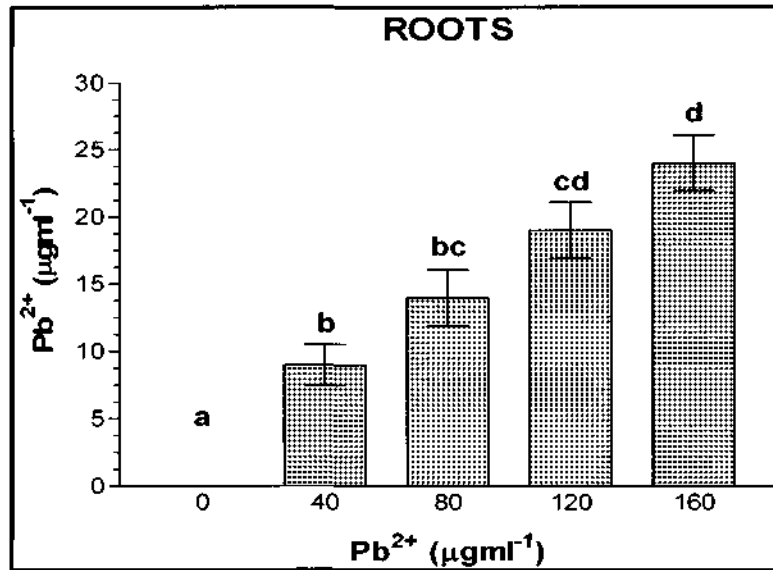
Fig. 15. Concentration of Pb^{2+} in roots of *A. marina* seedlings treated with 0, 40, 80 120 and 160 µgml⁻¹ Pb^{2+}. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.
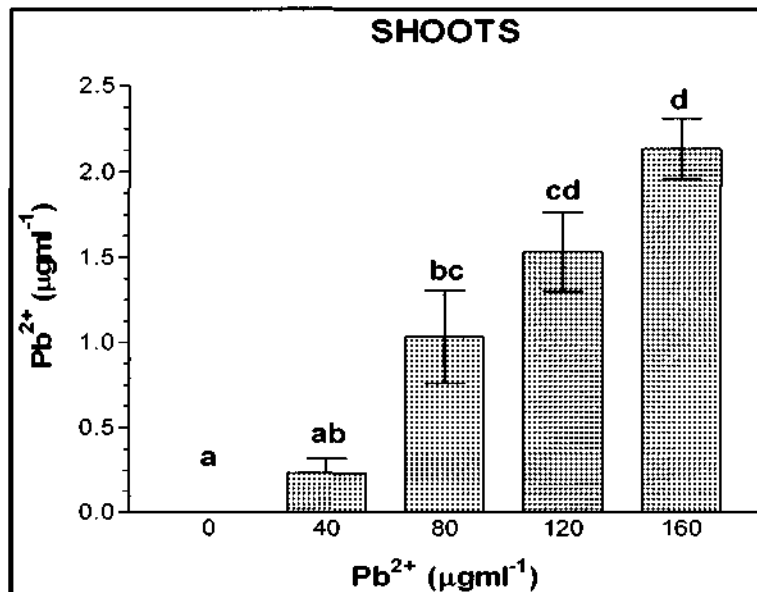
Fig. 16. Concentration of Pb^{2+} in shoots of *A. marina* seedlings treated with 0, 40, 80 120 and 160 µgml⁻¹ Pb^{2+}. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.
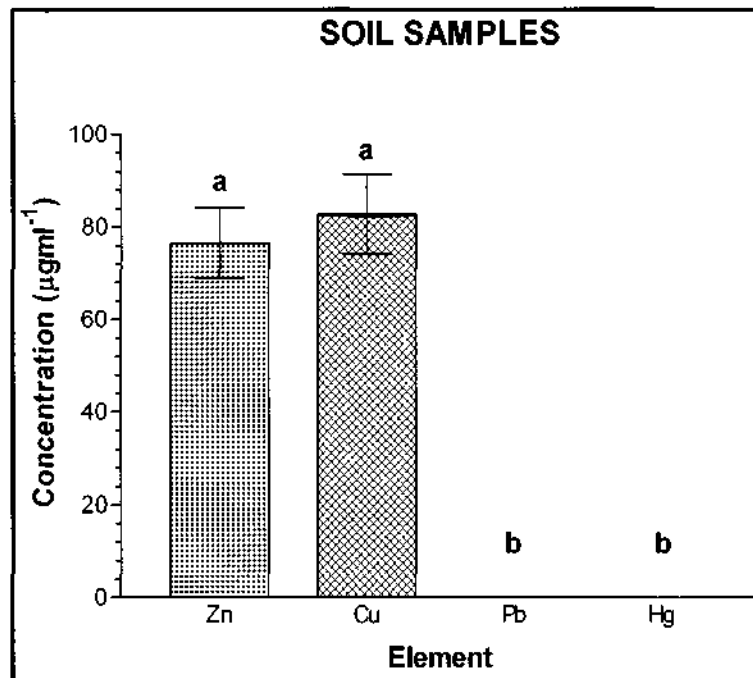
Fig. 17. Concentration of Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+} in soil samples of the control treatments. Values are means ± S.E. (n =43). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

- FIELD ANALYSIS

Root samples, collected from mature *A. marina* trees at the Durban Bay Heritage Site, revealed significantly high levels of Zn^{2+}, Cu^{2+} and Pb^{2+} (Fig. 18) compared to the controlled study. Leaf concentrations of heavy metals were considerably lower than those in roots (Fig. 19). In the leaves, concentrations of Zn^{2+} and Cu^{2+} were significantly higher than those in the controlled study.

Mercury accumulation in the root tissues of field plants was significantly higher (Fig. 18) than those in the controlled study. The field root samples contained considerably higher amounts of Hg^{2+} compared to the leaves in the field study. In addition, significantly high levels of Pb^{2+} were also found in the leaves. However, 0.9 μgml^{-1} and 0.36 μgml^{-1} of Hg^{2+} were detected in the root (Fig. 18) and leaf tissues (Fig. 19) respectively. Consequently, Hg^{2+} and Pb^{2+} accumulation was above threshold levels in the leaf and root tissues i.e. 0.025 μgml^{-1} (USEPA, 1995).
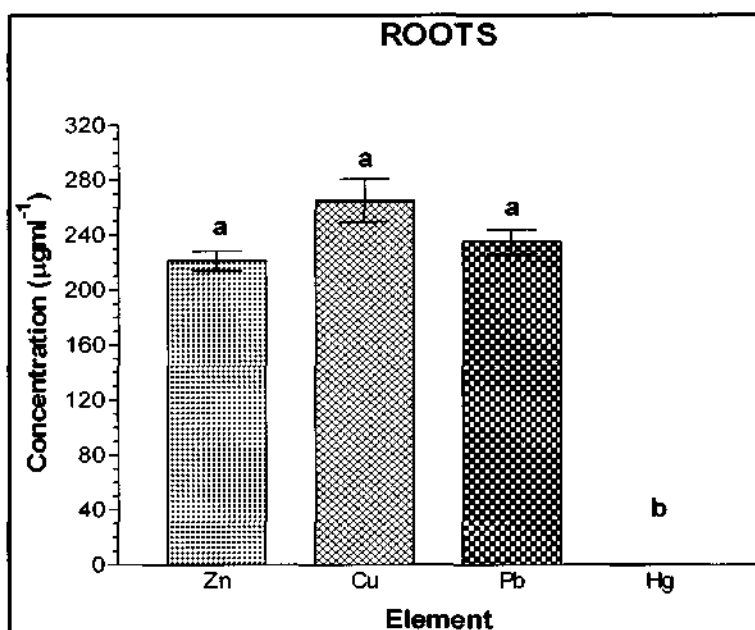
Fig. 18. Concentration of Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+} in roots of mature *A. marina* trees at the Durban Bay Heritage Site. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test
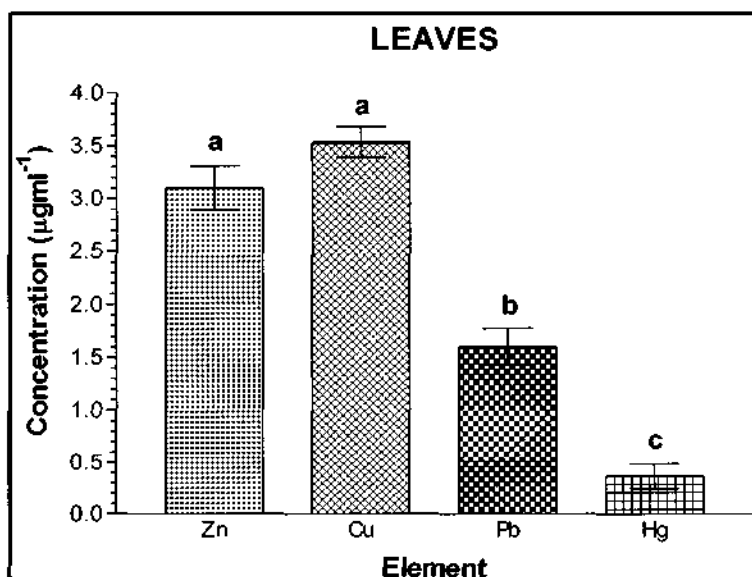

Fig. 19. Concentration of Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+} in mature leaves of *A. marina* trees at the Durban Bay Heritage Site. Values are means ± S.E. (n = 3). Bars with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

4.1.2 GAS EXCHANGE

a) CO_2 EXCHANGE

CO_2 exchange generally decreased as heavy metal concentration increased for all metals (Figs. 20A-D). For Pb^{2+} treatments (Fig. 20A), CO_2 exchange at 160 μgml^{-1} was significantly lower than the other concentrations. For Hg^{2+} (Fig. 20B) and Cu^{2+} (Fig. 20C) treatments, CO_2 exchange at concentrations of 120 and 160 μgml^{-1} were significantly lower than the control. For Zn^{2+} (Fig. 20D), CO_2 exchange at 160 μgml^{-1} was significantly lower than those at 0, 40 μgml^{-1}. Carbon dioxide exchange at 160 μgml^{-1} for Pb^{2+}, Hg^{2+}, Cu^{2+} and Zn^{2+} were 55 %, 49.6 %, 47.6 % and 63.6 % respectively lower than the control values (Figs. 20A-D).
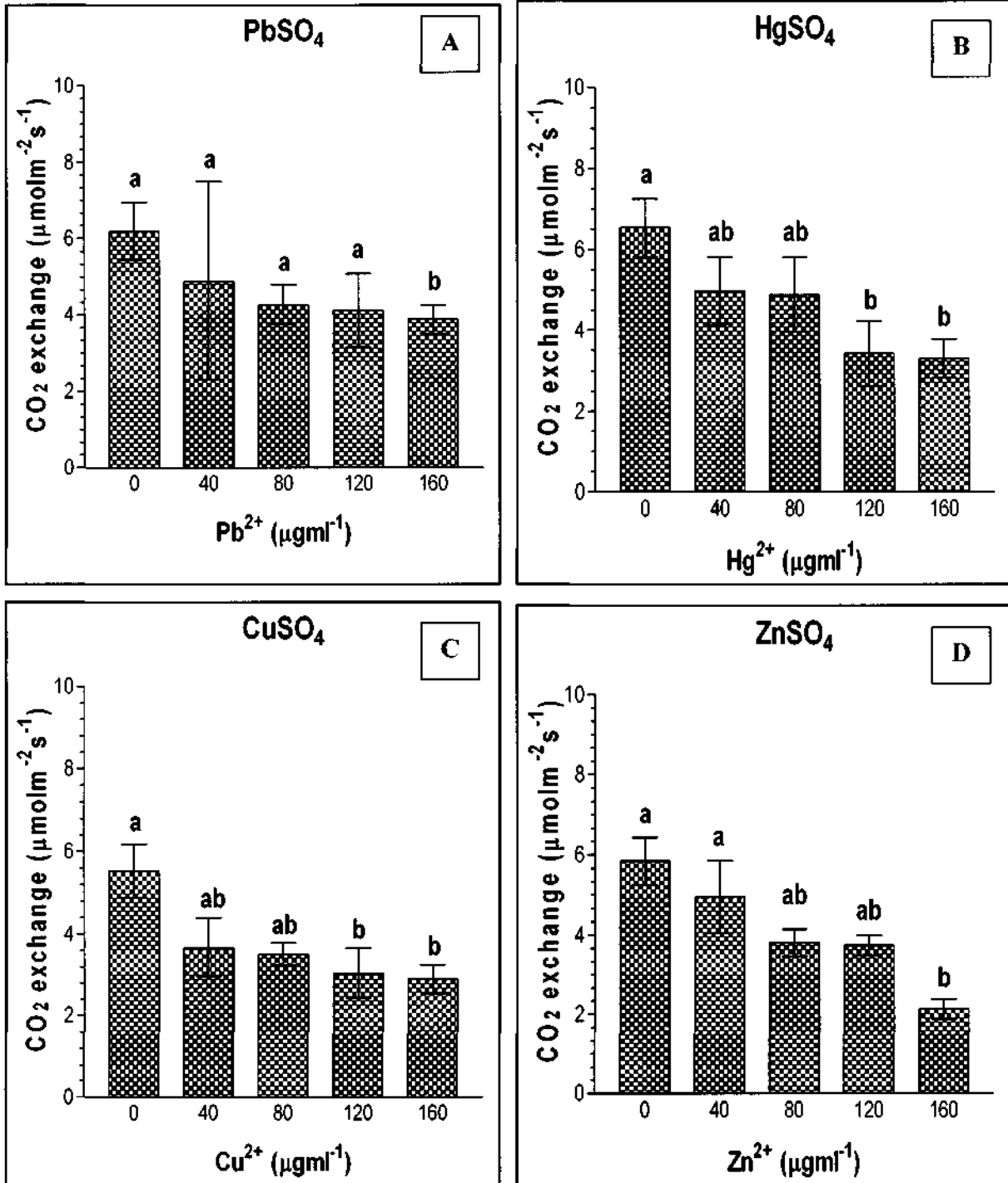
Figs. 20A-D. CO_2 exchange rates of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 10). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

b) TRANSPIRATION

Transpiration rates followed trends similar to those for CO_2 exchange decreasing with increases in heavy metal concentration for all metals (Figs. 21A-D). In Hg^{2+} treatments (Fig. 21A), transpiration rates at the various concentrations were significantly different from each other. Transpiration rates in Pb^{2+} treated plants (Fig 21B) at 120 and 160 μgml^{-1} were significantly lower than those at 0 and 40 μgml^{-1}. In Cu^{2+} treatments (Fig. 21C), transpiration rates at 160 μgml^{-1} was significantly lower than the other concentrations while at 120 μgml^{-1} it was significantly lower than those of 0, 40 and 160 μgml^{-1}. The rate of transpiration for Zn^{2+} treatments (Fig. 21D) at 160 μgml^{-1} was significantly lower than those at 0, 40 and 80 μgml^{-1}. Consequently, transpiration rates at 160 μgml^{-1} for Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} were 95 %, 29.8 %, 74.7 % and 64 % respectively lower than the control values (Figs. 21A-D).
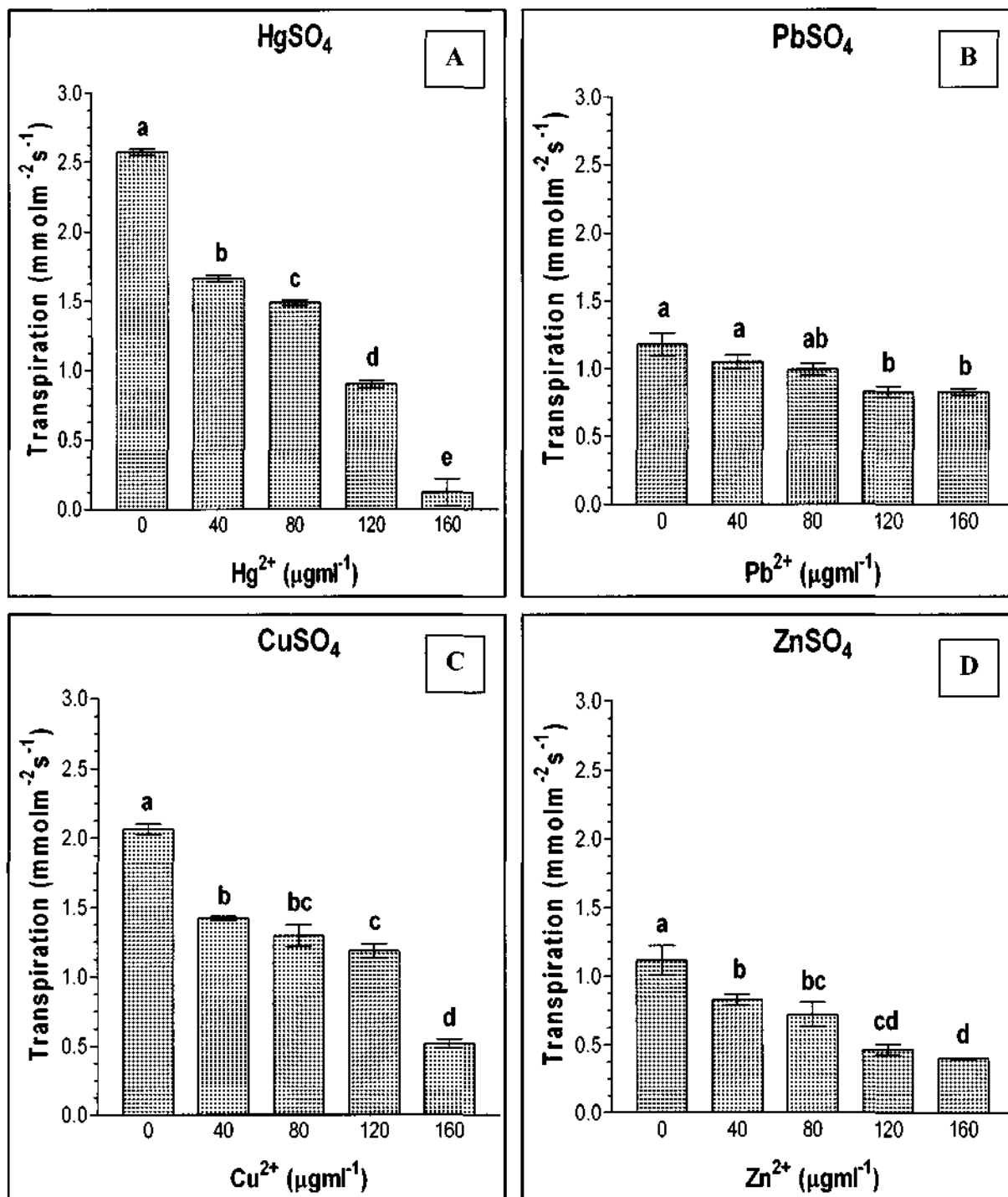
Figs. 21A-D. Transpiration rates of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 10). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

c) LEAF CONDUCTANCE

Similar to CO_2 exchange and transpiration, leaf conductance decreased with an increase in metal concentration for all metals (Figs. 22A-D). Leaf conductance in Pb^{2+} treatments (Fig. 22A) at 120 and 160 μgml^{-1} was significantly lower than the others. No differences in leaf conductance were detected between 0 and 80 μgml^{-1} Pb^{2+}. Mercury (Fig. 22B) and Cu^{2+} (Fig. 22C) treatments showed significantly lower leaf conductance at 160 μgml^{-1} than at the other concentrations. Leaf conductance at 160 μgml^{-1} Zn^{2+} (Fig. 22D) was significantly lower than those at 0, 40 and 80 μgml^{-1}. Percentage decreases in leaf conductance at 160 μgml^{-1} for Pb^{2+}, Hg^{2+}, Cu^{2+} and Zn^{2+} were 45 %, 68 %, 76.5 % and 69.9 % respectively compared to the control values (Figs. 22A-D).
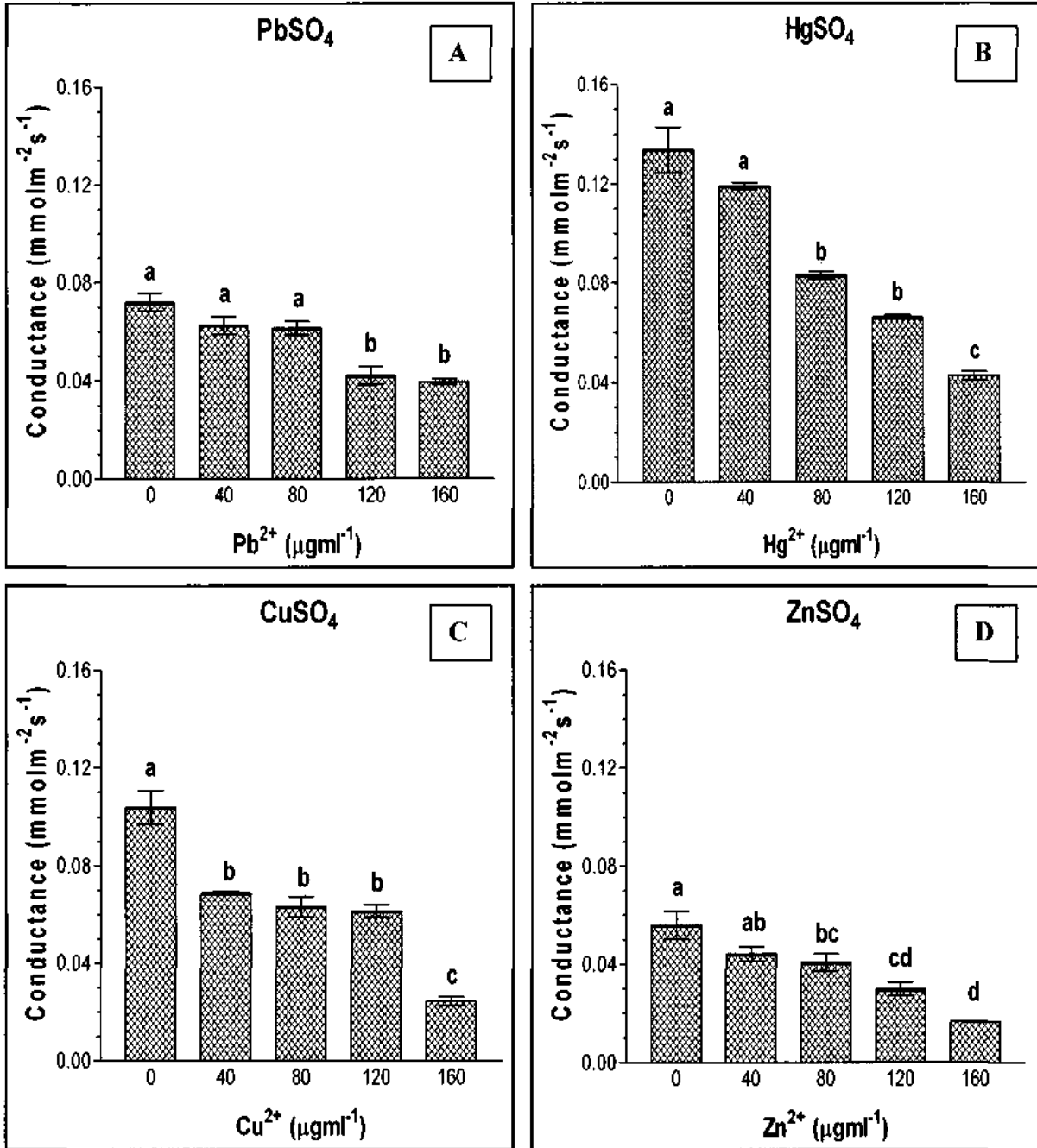
Figs. 22A-D. Leaf conductance of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 10). Means with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

4.1.3 CHLOROPHYLL FLUORESCENCE

a) YIELD

Photosystem II quantum yield decreased with increasing metal concentrations for all treatments (Figs. 23A-D). For Zn^{2+} treatments (Figs 23A), the highest yield was recorded for the control. Yield for all Zn^{2+} treatments were lower than the control with no differences amongst the treatments. For Pb^{2+}, Cu^{2+} and Hg^{2+} (Figs. 23B-D) the yield at 160 μgml^{-1} was significantly lower than the other concentrations. PSII quantum yield at a concentration of 160 μgml^{-1} for Zn^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+} treatments were 53 %, 50 %, 48.6 % and 54.6 % respectively, lower than the control values (Figs. 23A-D).

Figs. 23A-D. Quantum yield of PS II of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 15). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

b) ELETRON TRANSPORT RATE (ETR)

Trends for ETR were similar to those for quantum yield. All Zn^{2+} concentrations had significantly lower ETR values than the control with no differences amongst the treatments (Figs 24A-D). For Pb^{2+} and Cu^{2+} (Figs. 24B-C) ETR at 160 μgml^{-1} was significantly lower than the other concentrations. Seedlings treated with 120 and 160 μgml^{-1} Hg^{2+} (Fig. 24D) showed significantly lower ETR values than the control. ETR at 160 μgml^{-1} for Zn^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+} was 49 %, 37 %, 42 % and 27.6 % respectively, lower than the control values (Figs. 24A-D).
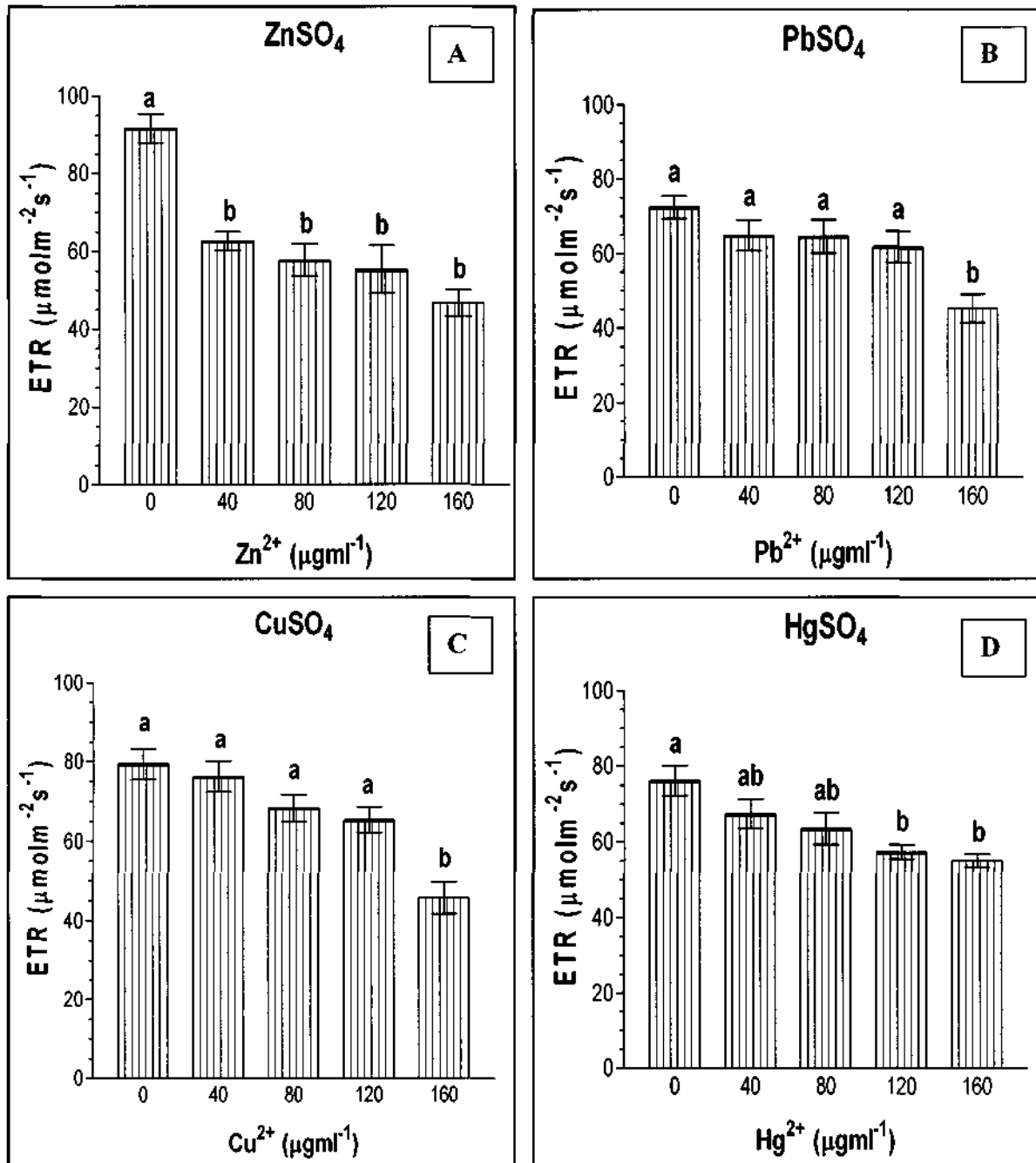
Figs. 24A-D. Electron transport rates through PS II (ETR) of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 15). Means with different letters are significantly different at $P<0.05$, using Tukey-Kramer Multiple Comparisons Test.

c) PHOTOCHEMICAL EFFICIENCY OF PS II (Fv/Fm)

All metals had similar effects on Fv/Fm (Figs. 25A-D). Increases in concentration of metals from 0 to 120 μgml^{-1} had no effect on Fv/Fm. At 160 μgml^{-1}, however, all metals significantly reduced Fv/Fm. Percentage decreases in Fv/Fm at 160 μgml^{-1} for Zn^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+} were 11 %, 14 %, 13 % and 16 % respectively, compared to the control values (Figs. 25A-D).

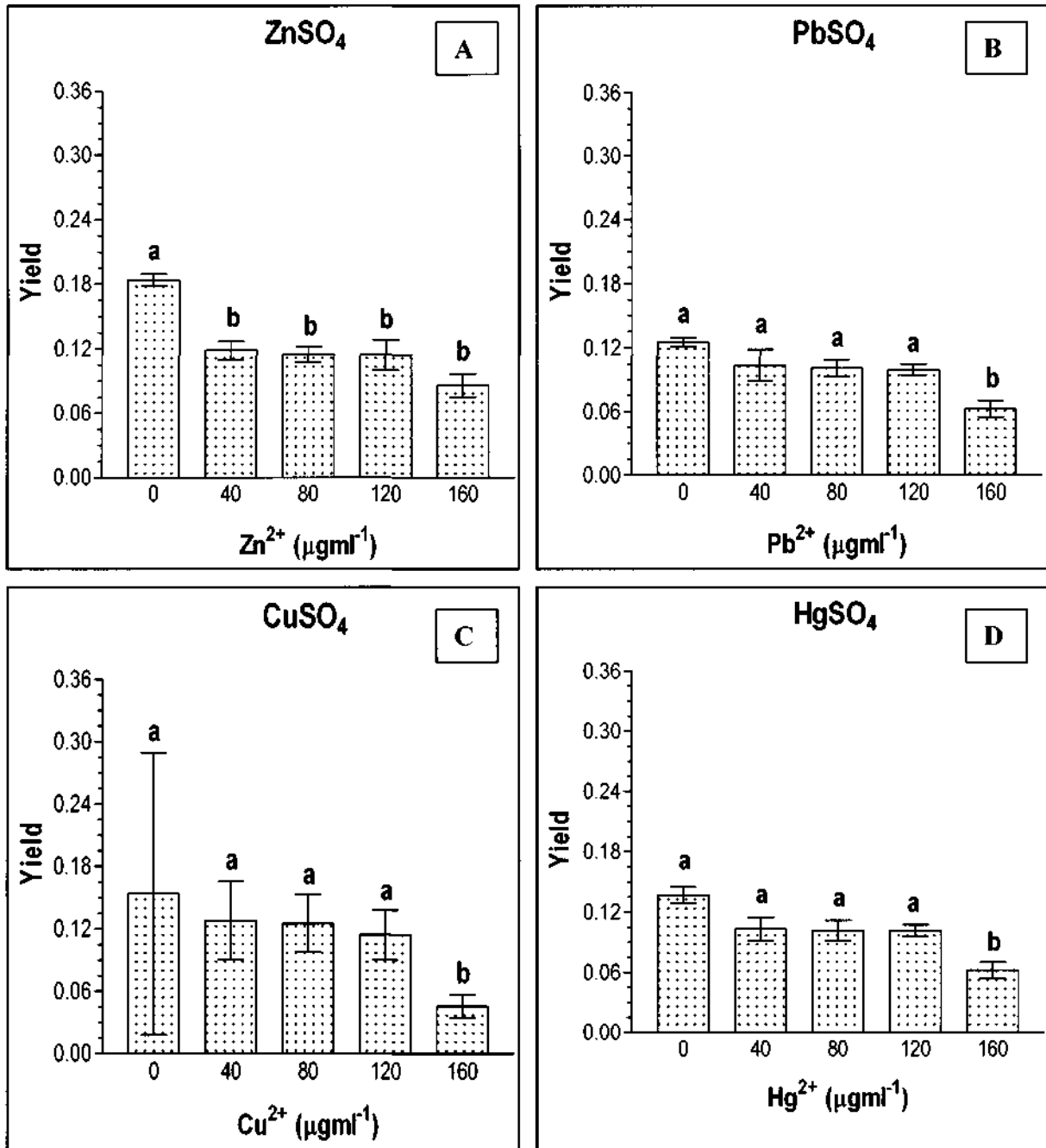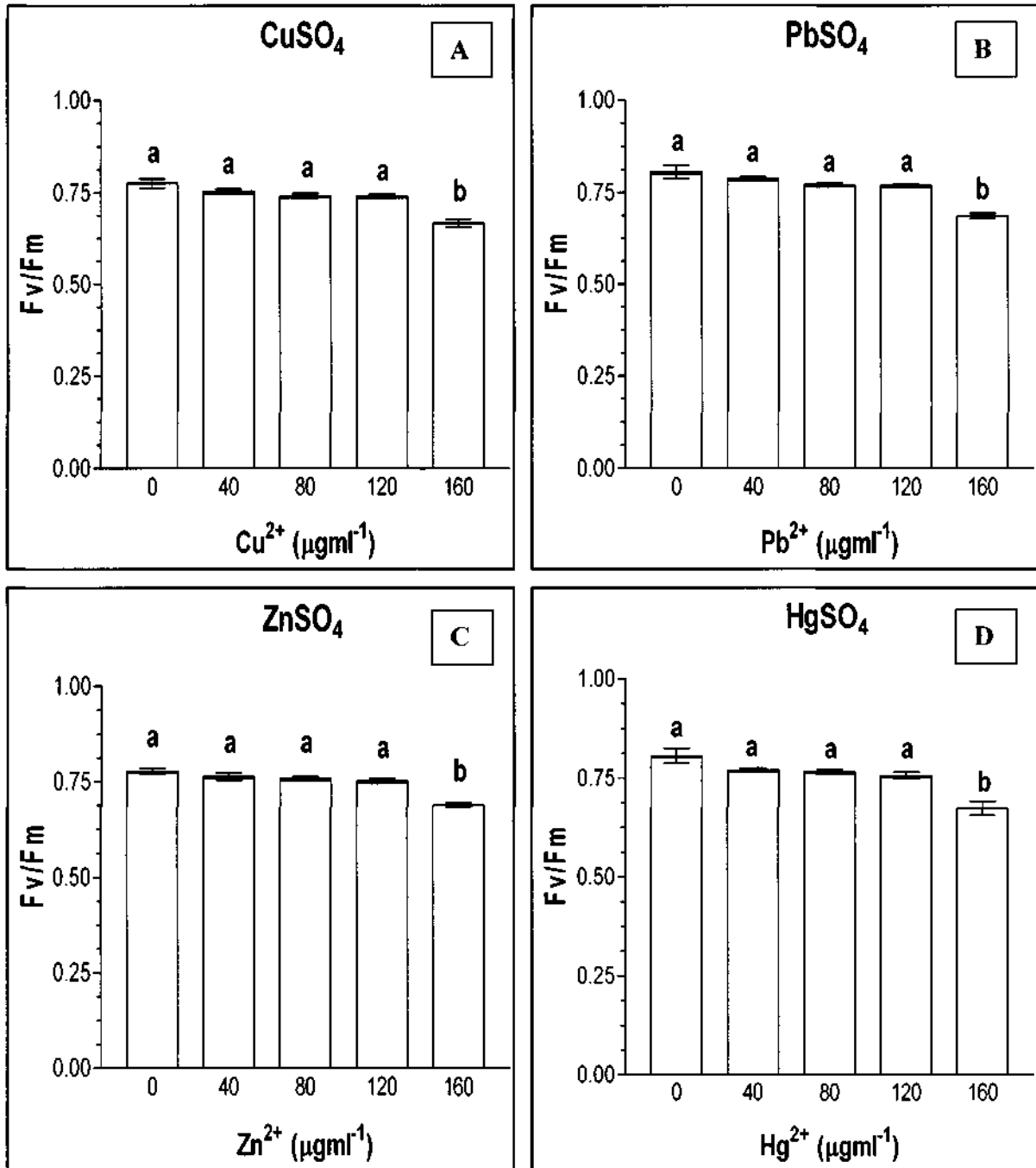Figs. 25A-D. Photochemical efficiency of light energy conversion (Fv/Fm) values of *A. marina* leaves at increasing metal concentrations. Means ± S.E. are given (n = 15). Means with different letters are significantly different at *P*<0.05, using Tukey-Kramer Multiple Comparisons Test.

4.2 MICROSCOPY

4.2.1 MORPHOLOGY

At the end of the experimental period, *A. marina* (Figs. 26a-b) in the control had ± 8 young leaves which were about 4 – 7 cm in length and 1.5 – 3 cm in width along the mid region of the leaf. Similarly, plants treated with heavy metals had an average of 4 – 6 leaves. In the control and heavy metal treatments, the upper surface of the leaves appeared shiny green and glabrous while the under surface was a lighter silver green with a dense coat of trichomes.

In control plants, the adaxial surface of the leaf was typically covered by a thick, smooth cutinized layer (Fig. 27) with conspicuous protruding salt glands occurring at regular intervals within shallow crypts and surrounded by regularly-shaped epidermal cells (Fig. 28). Salt glands generally appeared round; however degeneration of glands occurred with age and heavy deposits of secretions (Figs 29-30). In addition, SEM and ESEM observations indicated that stomata were absent from the upper leaf surface (Fig. 27). Salt crusts and crystalline deposits (Fig. 27) were commonly found on the cutinized surface of the glands and epidermal cells thus resulting in occlusion of the glands within their crypts (Figs. 29-30). The gland was often depressed (Figs. 29-30) when the cuticle ruptured under pressure, releasing the exudates. This is a possible mode for secretion in *A. marina* plants.

Observations from the ESEM revealed that leaves were hypostomatous and sheltered on the abaxial surface by a dense mat of overlapping, multicellular peltate trichomes (Fig.32). The stomatal apparatus is protected by two kidney-shaped guard cells, each supported by a swollen subsidiary cell (Fig. 31). Numerous bulb-like salt glands are localized on the abaxial surface (Figs. 33a-b). The salt glands are not sunken and often appear large with distinct quandrants, however they are obscured by the occurrence of the overlapping unseriate peltate hairs. (Figs.33a-b). Leaves of *A. marina* bear a dense indumentum of non glandular

hairs which support an abundance of heavy crystalline deposits on their cup-like structures (Figs. 34a-d, 35).

ESEM images were given in Figure 28, 32 and 36, while standard SEM applied for the other images. Secretions emanating from the glands on both surfaces were initially in the form of small droplets (Fig. 28) which subsequently formed large prismatic crystals (Figs. 34a-d; 35; 36a; 37a-c). The abaxial surface also displayed clusters of large, globose crystalline deposits evident on the peltate trichomes (Fig 34a) as well as a lattice appearance of salt crystals on the capitate hairs (Fig. 36b).

The overview of the leaf structure of *Avicennia marina* revealed that little or no external morphological changes occurred due to excessive exposure to essential micronutrients (Cu^{2+} and Zn^{2+}) and non-essential ions (Hg^{2+} and Pb^{2+}).

Figs. 26 a-b.   Experimental design of *A. marina* seedlings under glasshouse conditions taken at the termination of the experiment.
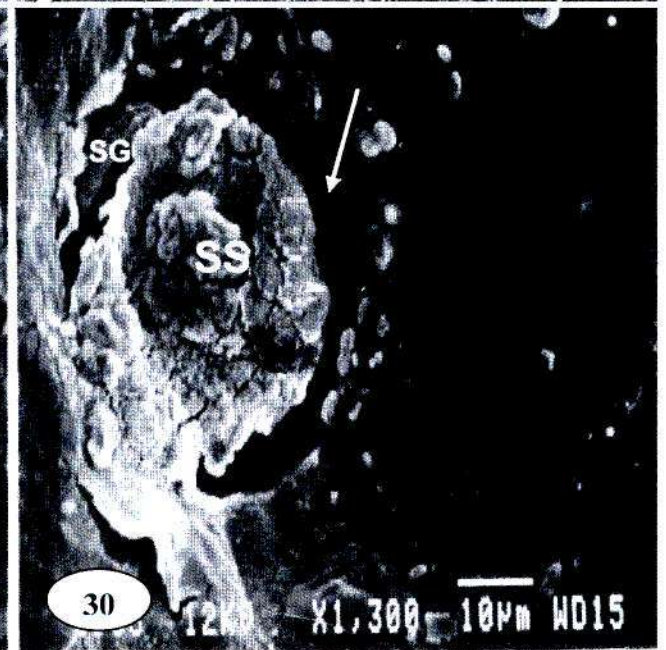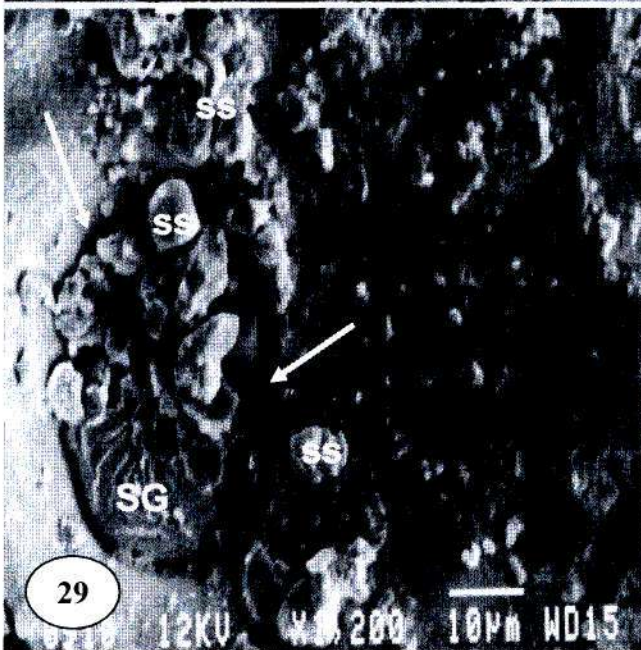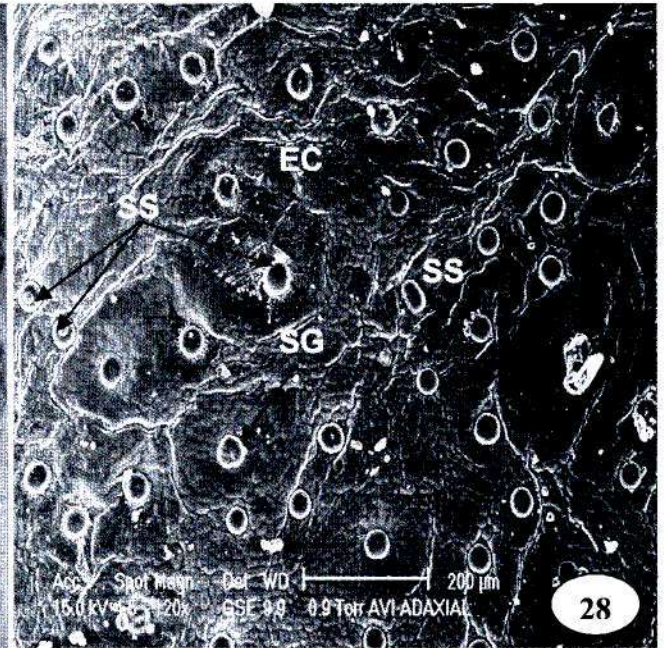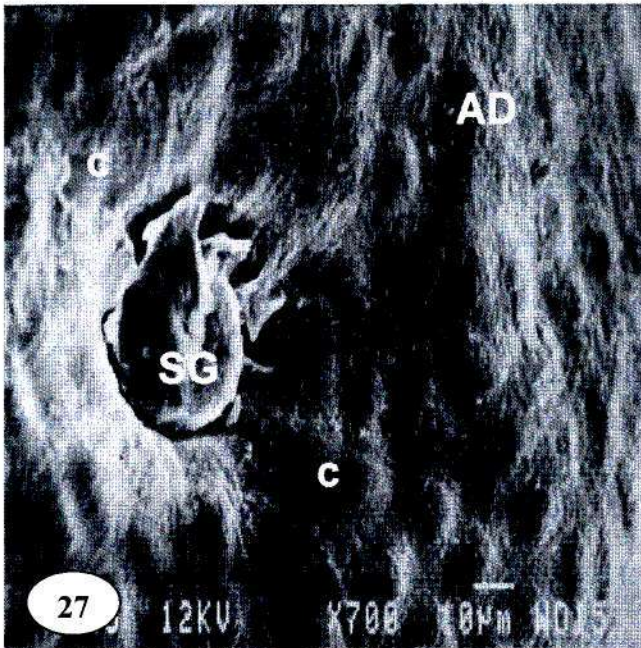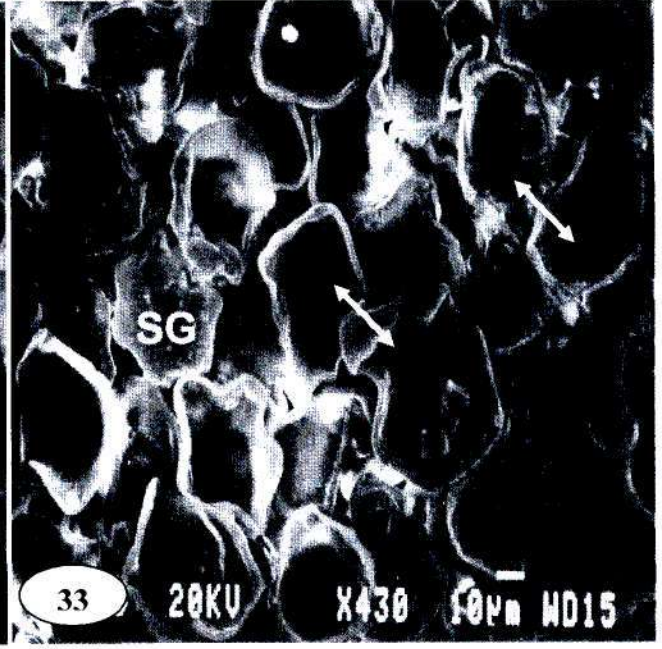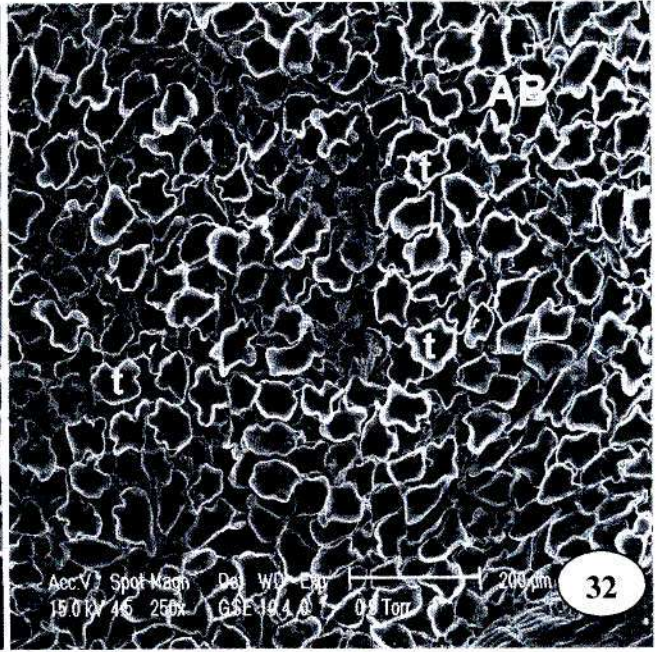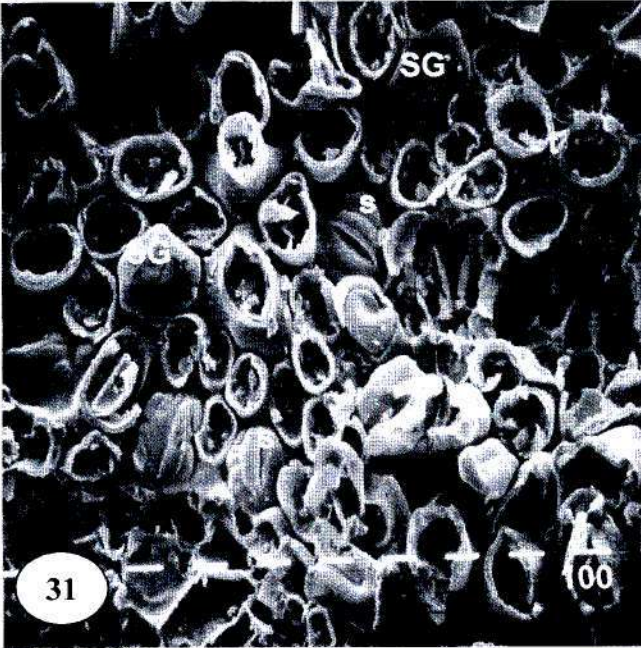
PLATE 3

Figure 34-35   SEM of secretions emanating from the glands on the abaxial leaf surface

of *A. marina* in control plants.

Figure 34 a-d   The abaxial surface displaying clusters of large, globose mass of

secretory deposits (gb) and prismatic crystals (PC) over peltate

trichomes (t) in control plants.

Figure 35   Distinct prismatic crystals (PC) lining umbrella-like trichomes (arrows).
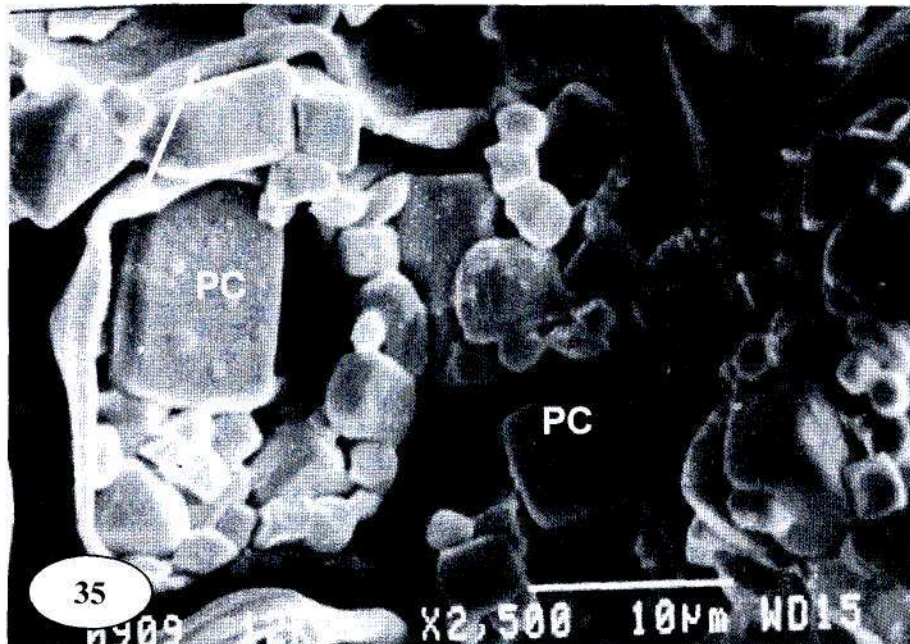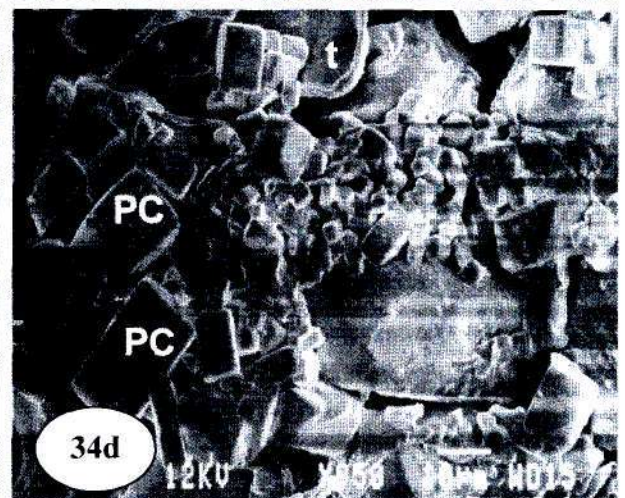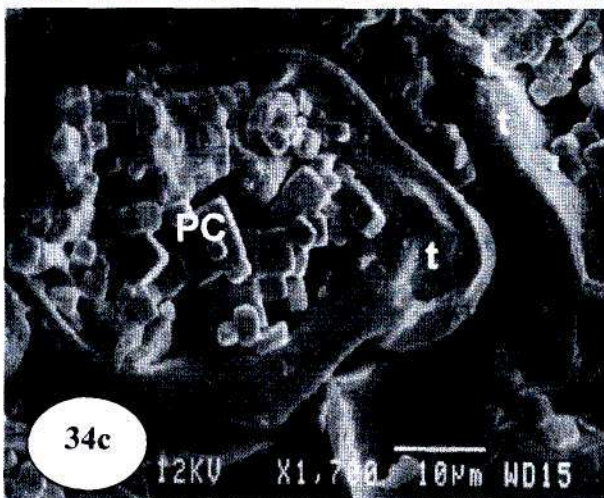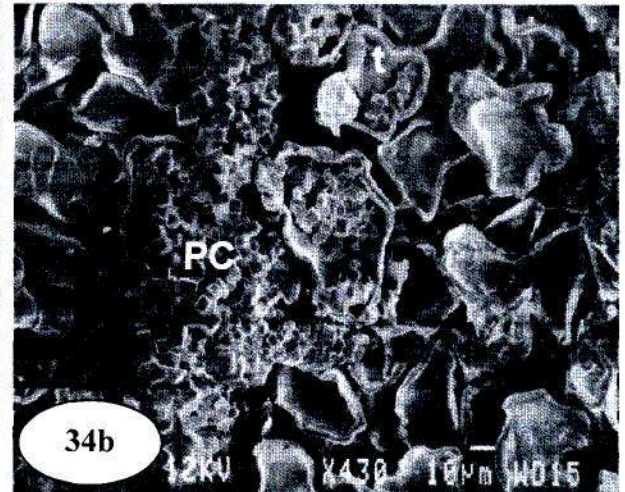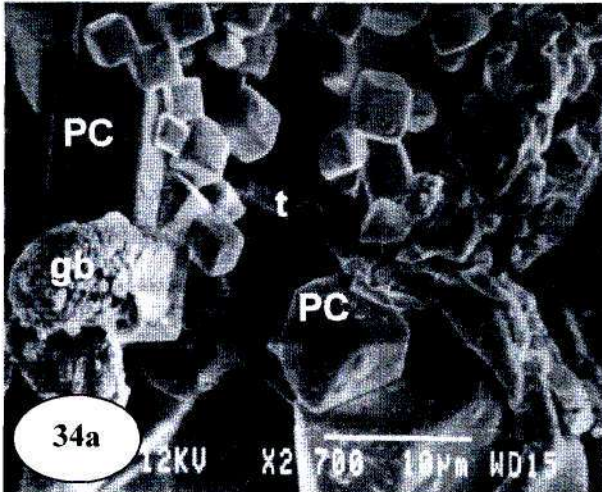
PLATE 4

Figure 36 a-b   ESEM of secretions emanating from the glands on the adaxial surface of

A. marina in control plants.

Figure 36a   Distinct prismatic crystals (PC) emanating from salt glands obscured by

a dense indumentum of trichomes (t) on the abaxial surface (AD).

Figure 36b   Non glandular trichome (t) displaying a salt lattice (arrows) within its

cup-shaped structure.

36a

36b
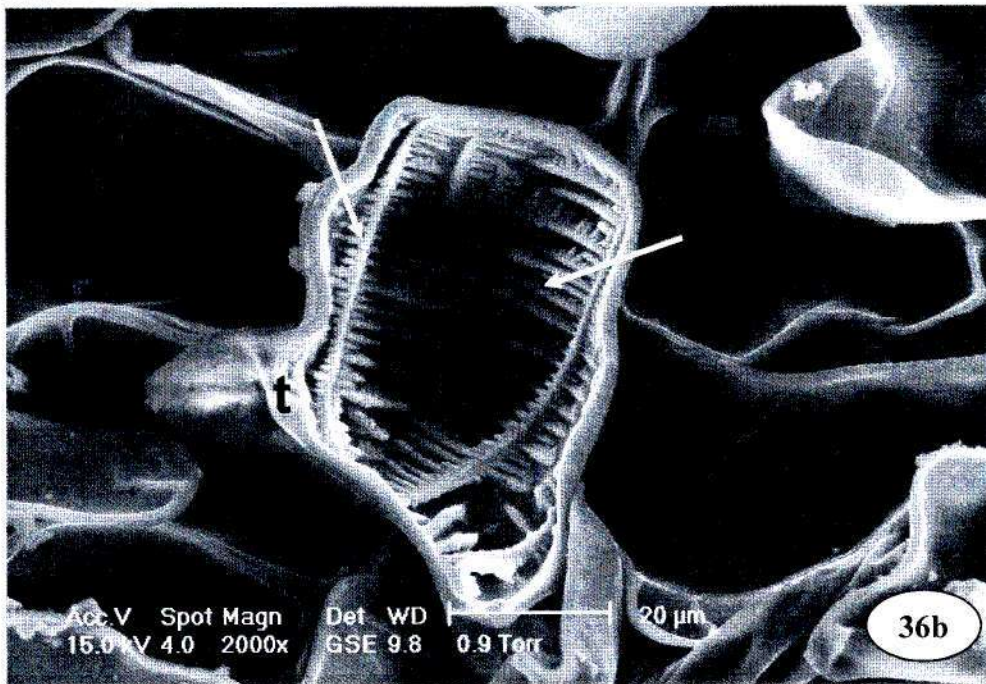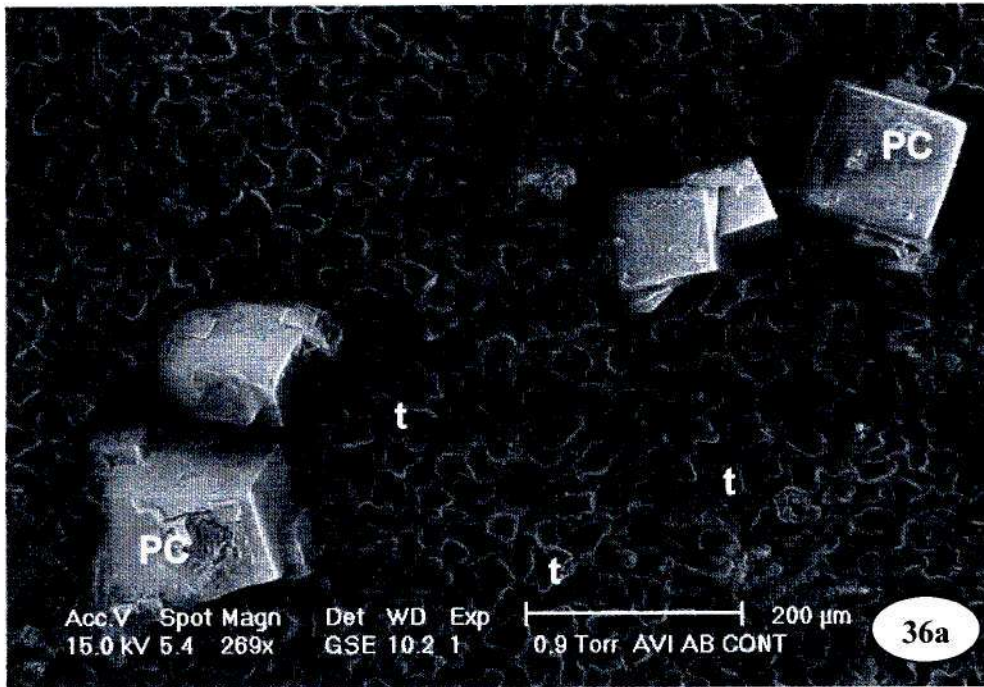
PLATE 5

Figure 37 a-c   SEM of secretions emanating from the glands on the adaxial leaf surface of $A.$ $marina$ in control and Pb^{2+} and Hg^{2+} treated plants.

Figure 37 a   Adaxial surface with secretory material, i.e. prismatic crystals (PC) found on and around the sunken salt gland of Pb^{2+} treated plants.

Figure 37 b   Large prismatic crystal (PC) secreted on depressed salt glands on the adaxial surface (AD) of control plants.

Figure 37 c   Cluster of prismatic crystals (PC) embedding and completely occluding the gland within the crypt on the adaxial surface (AD) of Hg^{2+} treated plants.
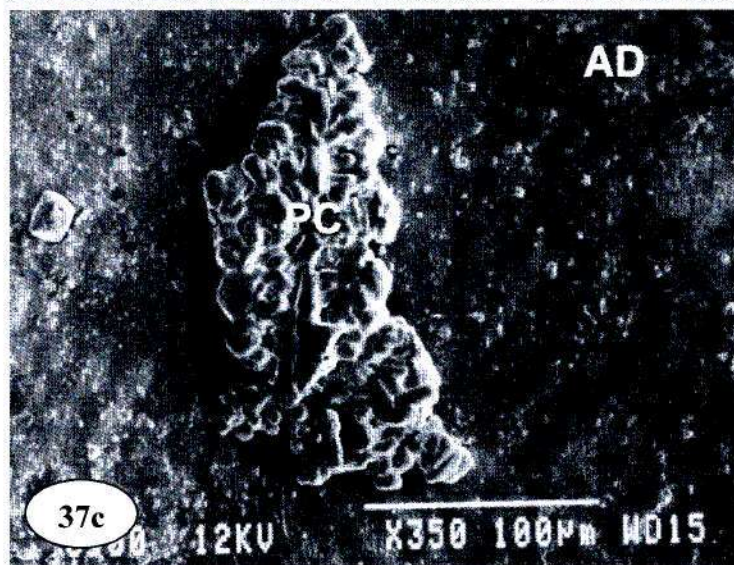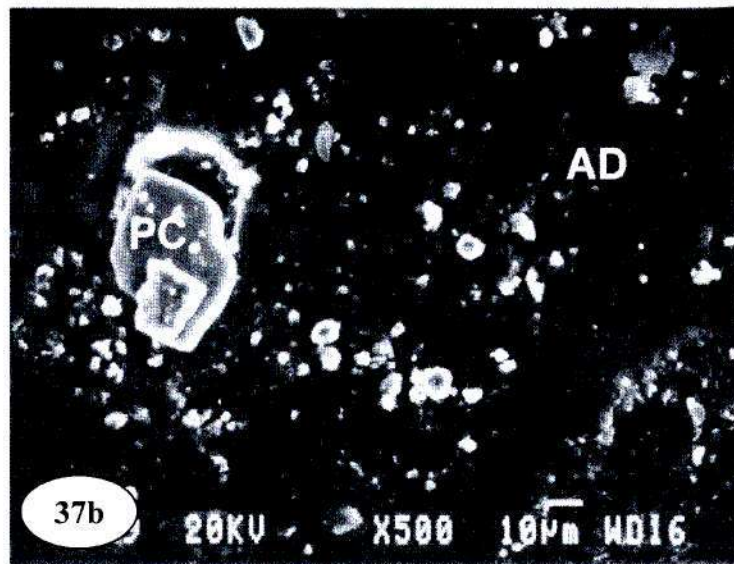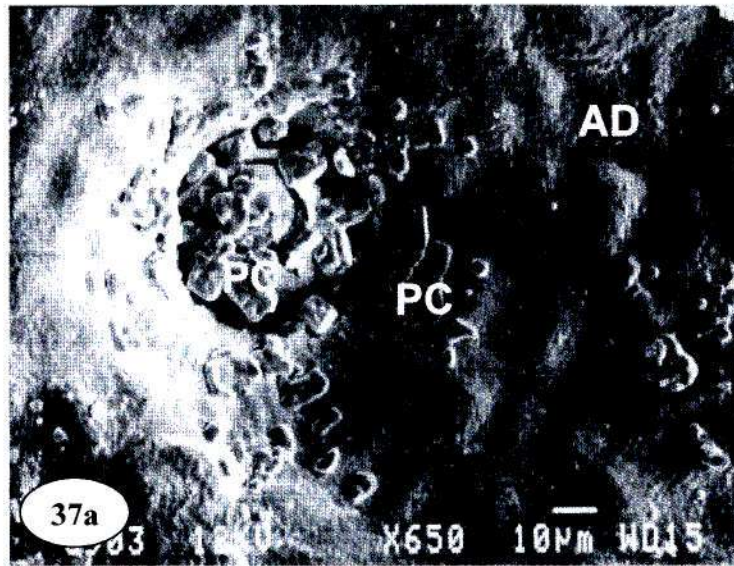
4.2.2 EDX MICROANALYSIS

Qualitative energy dispersive X-ray microanalyses were conducted on the apex, middle and basal regions of the leaf to detect for the presence of Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+}. The spectra data revealed that Zn^{2+} and Cu^{2+} were present in all three leaf regions on both leaf surfaces of treated plants (Figs. 38-39), however, Hg^{2+} and Pb^{2+} were absent from the leaf surface. Higher elemental weights of Zn^{2+} and Cu^{2+}, as seen in the tables of figures 38-39 (tables), were found on the middle and basal regions of leaves with lower quantities localized at the apex eg. Cu^{2+} apex = 0.06 wt %, Cu^{2+} mid. reg. = 0.1 wt % and Cu^{2+} base = 0.14 wt %. Zinc and Cu^{2+} were also found in trace amounts on the leaf surface of control plants (Figs. 40 a-b; elemental weights in tables) while Hg^{2+} and Pb^{2+} were absent.

In control plants, EDX microanalysis on the crystalline deposits, over the glands on the upper surface (Fig. 41a) and on the trichomes on the lower surface (Fig. 41b), revealed that the ions were predominantly Na^+ and Cl^+ (Fig. 41a, b). Other elements such as Zn^{2+} (adaxial = 0.06 wt %, abaxial = 0.04 wt %), Cu^{2+} (adaxial = 0.09 wt %, abaxial = 0.07 wt %), Mg^{2+} (adaxial = 0.88 wt %, abaxial = 0.4 wt %), Ca^{2+} (adaxial = 3.78 wt % , abaxial = 0.67 wt %), K^+ (adaxial = 0.93 wt %, abaxial = 1.07 wt %), Si^{4+} (adaxial = 2.49 wt %), and P^+ (adaxial = 0.65 wt %) were detected in the deposits, however, their concentrations were considerably lower than those of Na^+ (adaxial = 2.88 wt %, abaxial = 3.61 wt %) and Cl^+ (adaxial = 6.48 wt %, abaxial = 6.43 wt %). The predominant elements (Na^+ and Cl^+) were further localized by X-ray mapping in Cu^{2+}, Zn^{2+} and Hg^{2+} treatments (Figs. 42, 43, 44). In addition, Cu^{2+} (Fig. 42) and Zn^{2+} (Fig. 43) were localized by X-ray mapping. The red and yellow colours on the maps indicate the presence of NaCl (Figs. 42, 43) while the turquoise and green colours indicate the presence of Cu^{2+} and Zn^{2+} respectively. Figures 42 and 23 clearly indicate that Cu^{2+} and Zn^{2+} were present in the secretion from both adaxial and abaxial surfaces. In contrast, the map indicates that Hg^{2+} (purple colour-absent) (Fig. 44) and Pb^{2+} (cobalt blue colour-absent) were not detected on the leaf surface.
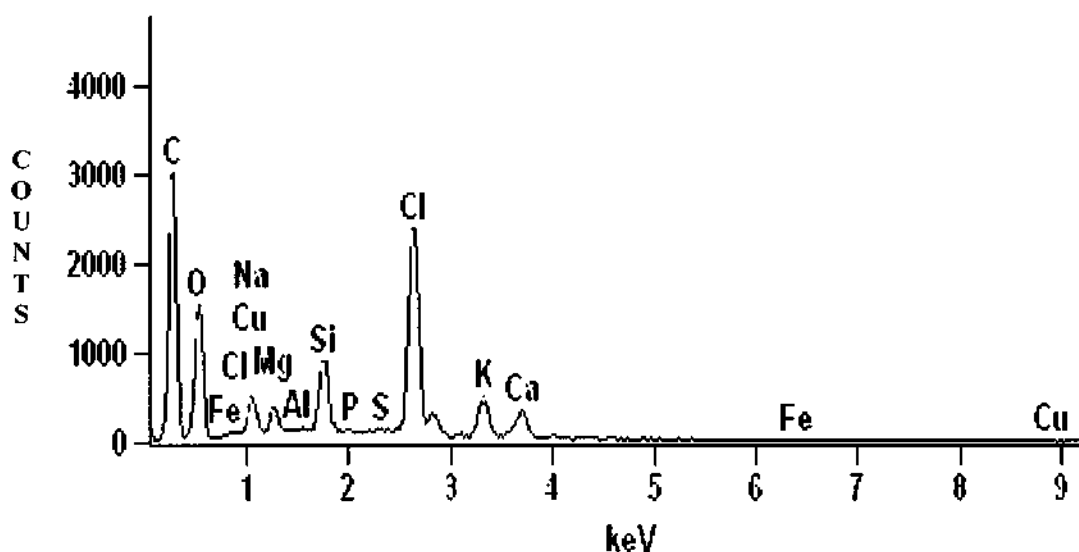
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.764	2.601	66.8	+/-0.52	75.12
O K	0.081	9.285	25.71	+/-0.38	21.7
Na K	0.01	3.367	1.13	+/-0.06	0.66
Mg K	0.005	2.306	0.4	+/-0.02	0.22
Si K	0.018	1.457	0.9	+/-0.02	0.43
S K	0.001	1.22	0.03	+/-0.01	0.01
Cl K	0.084	1.219	3.49	+/-0.04	1.33
K K	0.019	1.205	0.79	+/-0.03	0.27
Ca K	0.015	1.174	0.61	+/-0.03	0.21
Fe K	0.001	1.309	0.04	+/-0.02	0.01
Cu K	**0.001**	**1.389**	**0.06**	**+/-0.04**	**0.01**
Total			100		100

Fig. 38a. Representative EDX spectrum displaying relative elemental composition on the apex of the abaxial leaf surface of the 160 μgml^{-1} Cu^{2+} treatment. Spectrum indicates the presence of Cu^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.
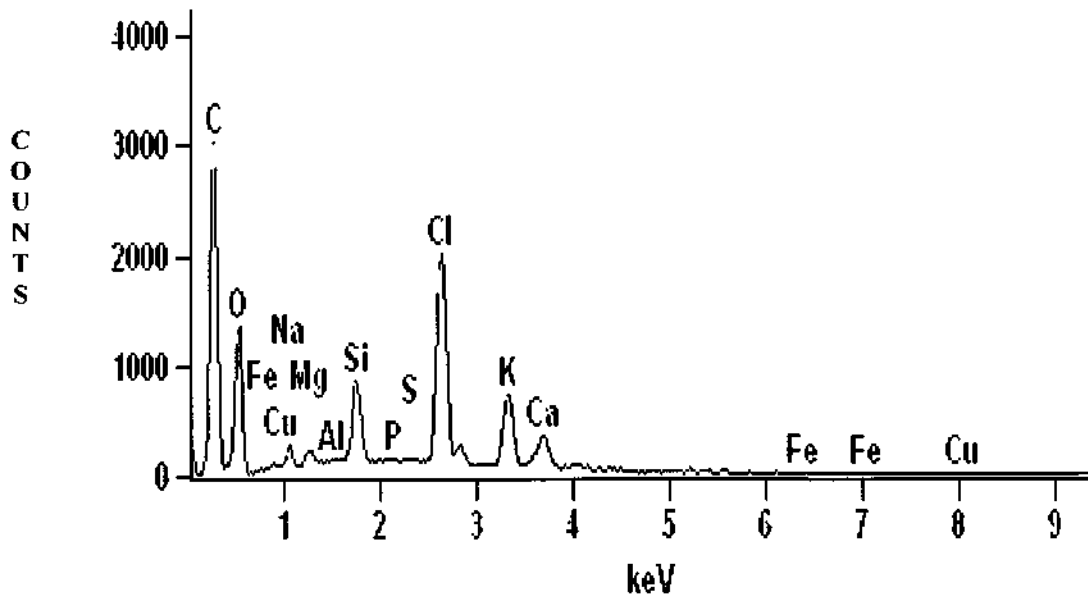
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.843	2.194	68.63	+/-0.54	75.91
O K	0.068	10.54	26.74	+/-0.39	22.2
Na K	0.003	4.537	0.51	+/-0.05	0.3
Mg K	0.002	2.931	0.23	+/-0.01	0.12
Si K	0.011	1.615	0.67	+/-0.01	0.31
S K	0.001	1.246	0.03	+/-0.01	0.01
Cl K	0.043	1.211	1.93	+/-0.02	0.72
K K	0.017	1.164	0.74	+/-0.02	0.25
Ca K	0.009	1.133	0.39	+/-0.02	0.13
Cu K	**0.002**	**1.322**	**0.1**	**+/-0.02**	**0.02**
Total			100		100

Fig. 38b. Representative EDX spectrum displaying relative elemental composition on the middle region of the abaxial leaf surface of the 160 μgml^{-1} Cu^{2+} treatment. Spectrum indicates the presence of Cu^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.
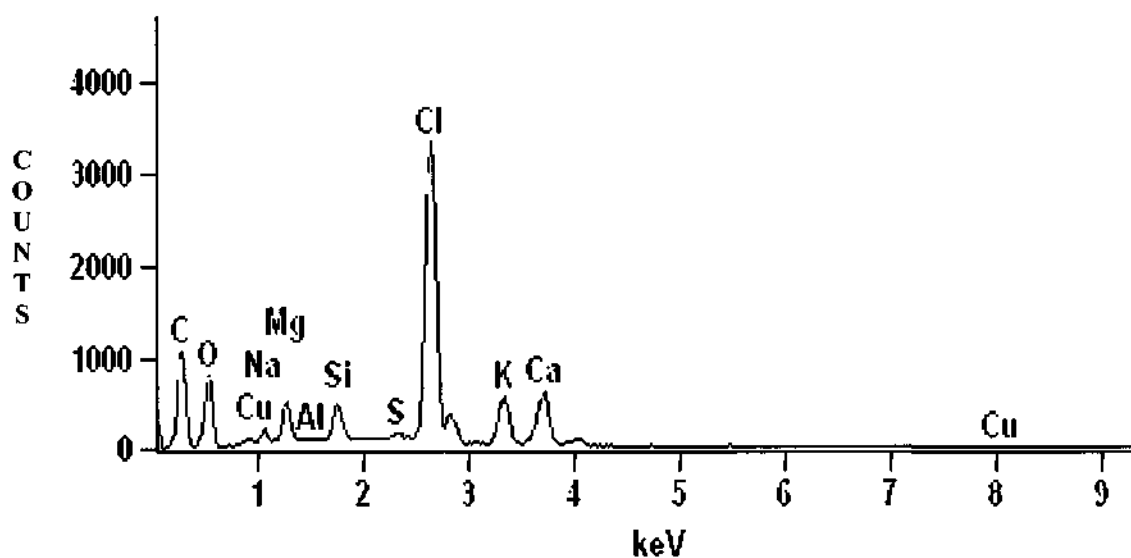
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.647	3.4	60.9	+/-0.84	70.86
O K	0.089	10.318	28.1	+/-0.54	24.55
Na K	0.004	4.618	0.54	+/-0.08	0.33
Mg K	0.012	2.961	1.05	+/-0.05	0.6
Al K	0	2.243	0.03	+/-0.01	0.02
Si K	0.013	1.661	0.68	+/-0.02	0.34
S K	0.003	1.255	0.1	+/-0.01	0.04
Cl K	0.162	1.219	6.01	+/-0.05	2.37
K K	0.029	1.226	1.07	+/-0.03	0.38
Ca K	0.038	1.181	1.38	+/-0.04	0.48
Cu K	**0.003**	**1.314**	**0.14**	**+/-0.03**	**0.03**
Total			100		100

Fig. 38c. Representative EDX spectrum displaying relative elemental composition on the base region of the abaxial leaf surface of the 160 µgml^{-1} Cu^{2+} treatment. Spectrum indicates the presence of Cu^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 µm and data collection time 60 s.
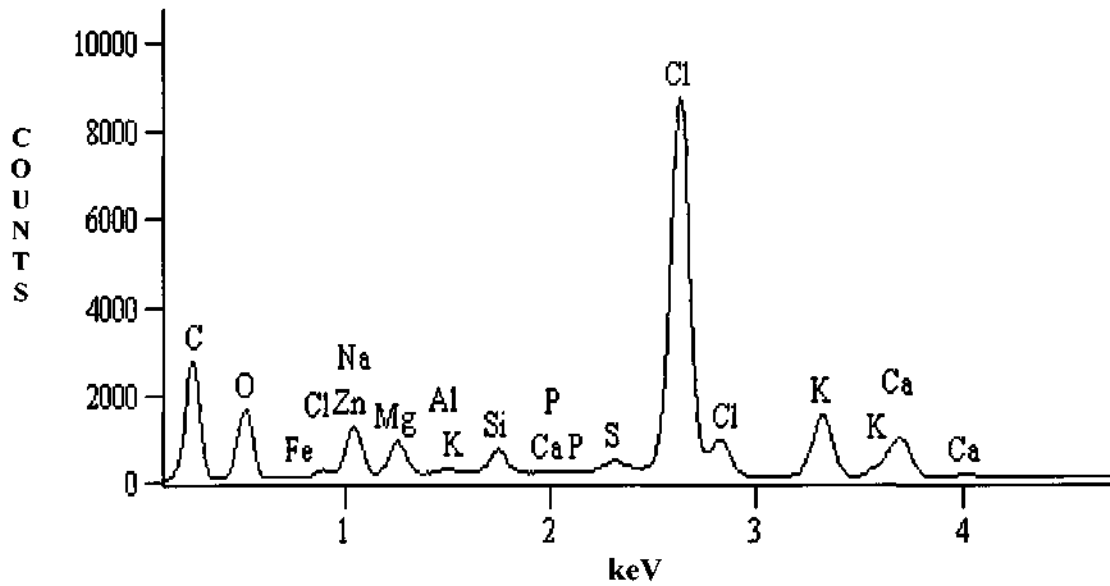
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.677	3.434	63.35	+/-0.51	73.24
O K	0.074	10.574	24.9	+/-0.33	21.61
Na K	0.017	4.421	2.33	+/-0.04	1.41
Mg K	0.006	3.01	0.62	+/-0.02	0.35
Si K	0.006	1.665	0.31	+/-0.01	0.15
S K	0.004	1.246	0.17	+/-0.01	0.07
Cl K	0.161	1.215	6.21	+/-0.03	2.43
K K	0.031	1.229	1.19	+/-0.02	0.42
Ca K	0.021	1.185	0.8	+/-0.02	0.28
Fe K	0.001	1.253	0.02	+/-0.01	0
Zn K	**0.002**	**1.32**	**0.06**	**+/-0.02**	**0.01**
Total			100		100

Fig. 39a. Representative EDX spectrum displaying relative elemental composition on the apex of the abaxial leaf surface of the 160 μgml^{-1} Zn^{2+} treatment. Spectrum indicates the presence of Zn^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.
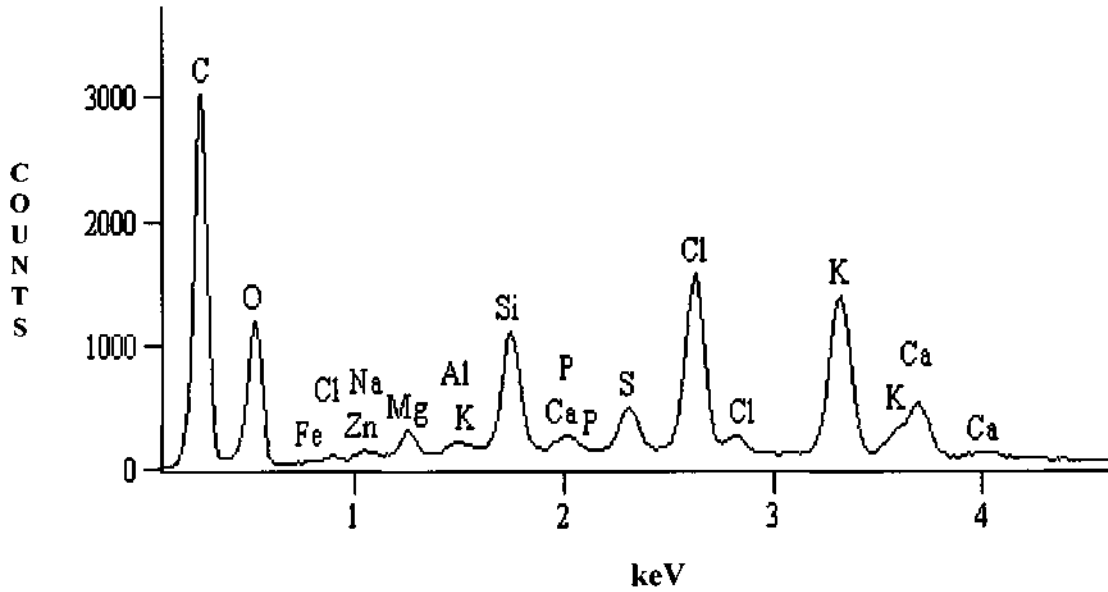
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.831	2.181	70.33	+/-0.39	77.85
O K	0.057	10.874	24.03	+/-0.39	19.97
Na K	0.001	4.294	0.11	+/-0.03	0.06
Mg K	0.002	2.749	0.24	+/-0.02	0.13
Al K	0.001	2.052	0.07	+/-0.01	0.03
Si K	0.014	1.559	0.87	+/-0.02	0.41
P K	0.002	1.413	0.12	+/-0.01	0.05
S K	0.008	1.234	0.39	+/-0.01	0.16
Cl K	0.032	1.218	1.51	+/-0.02	0.57
K K	0.035	1.164	1.56	+/-0.03	0.53
Ca K	0.012	1.146	0.52	+/-0.02	0.17
Fe K	0.001	1.257	0.03	+/-0.01	0.01
Zn K	**0.004**	**1.338**	**0.22**	**+/-0.03**	**0.05**
Total			100		100

Fig. 39b. Representative EDX spectrum displaying relative elemental composition on the middle region of the abaxial leaf surface of the 160 μgml^{-1} Zn^{2+} treatment. Spectrum indicates the presence of Zn^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.
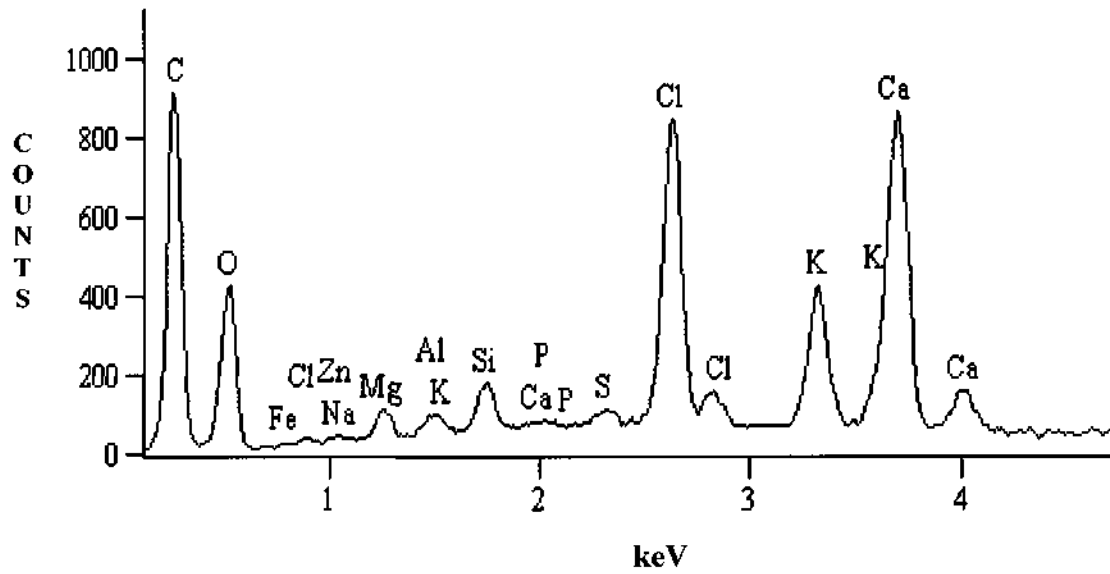
Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.766	2.31	66.52	+/-0.65	75.4
O K	0.062	10.921	25.41	+/-0.67	21.62
Na K	0.001	4.656	0.11	+/-0.04	0.07
Mg K	0.003	2.968	0.33	+/-0.02	0.19
Al K	0.002	2.187	0.17	+/-0.02	0.08
Si K	0.005	1.643	0.31	+/-0.03	0.15
P K	0.001	1.438	0.05	+/-0.01	0.02
S K	0.002	1.245	0.12	+/-0.02	0.05
Cl K	0.05	1.209	2.29	+/-0.05	0.88
K K	0.026	1.158	1.12	+/-0.05	0.39
Ca K	0.07	1.137	3	+/-0.06	1.02
Fe K	0.003	1.256	0.13	+/-0.04	0.03
Zn K	**0.008**	**1.329**	**0.42**	**+/-0.08**	**0.09**
Total			100		100

Fig. 39c. Representative EDX spectrum displaying relative elemental composition on the base region of the abaxial leaf surface of the 160 μgml^{-1} Zn^{2+} treatment. Spectrum indicates the presence of Zn^{2+}. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.

Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.947	1.573	80.48	+/-0.26	85.26
O K	0.027	11.835	17.53	+/-0.19	13.94
Na K	0.002	4.157	0.49	+/-0.01	0.27
P K	0.001	1.35	0.06	+/-0.00	0.03
S K	0.001	1.195	0.09	+/-0.00	0.04
Cl K	0.01	1.181	0.64	+/-0.01	0.23
K K	0.008	1.132	0.47	+/-0.01	0.15
Ca K	0.001	1.11	0.05	+/-0.00	0.01
Cu K	0.001	1.33	0.07	+/-0.01	0.01
Zn K	0.001	1.343	0.04	+/-0.01	0.01
Total			100		100
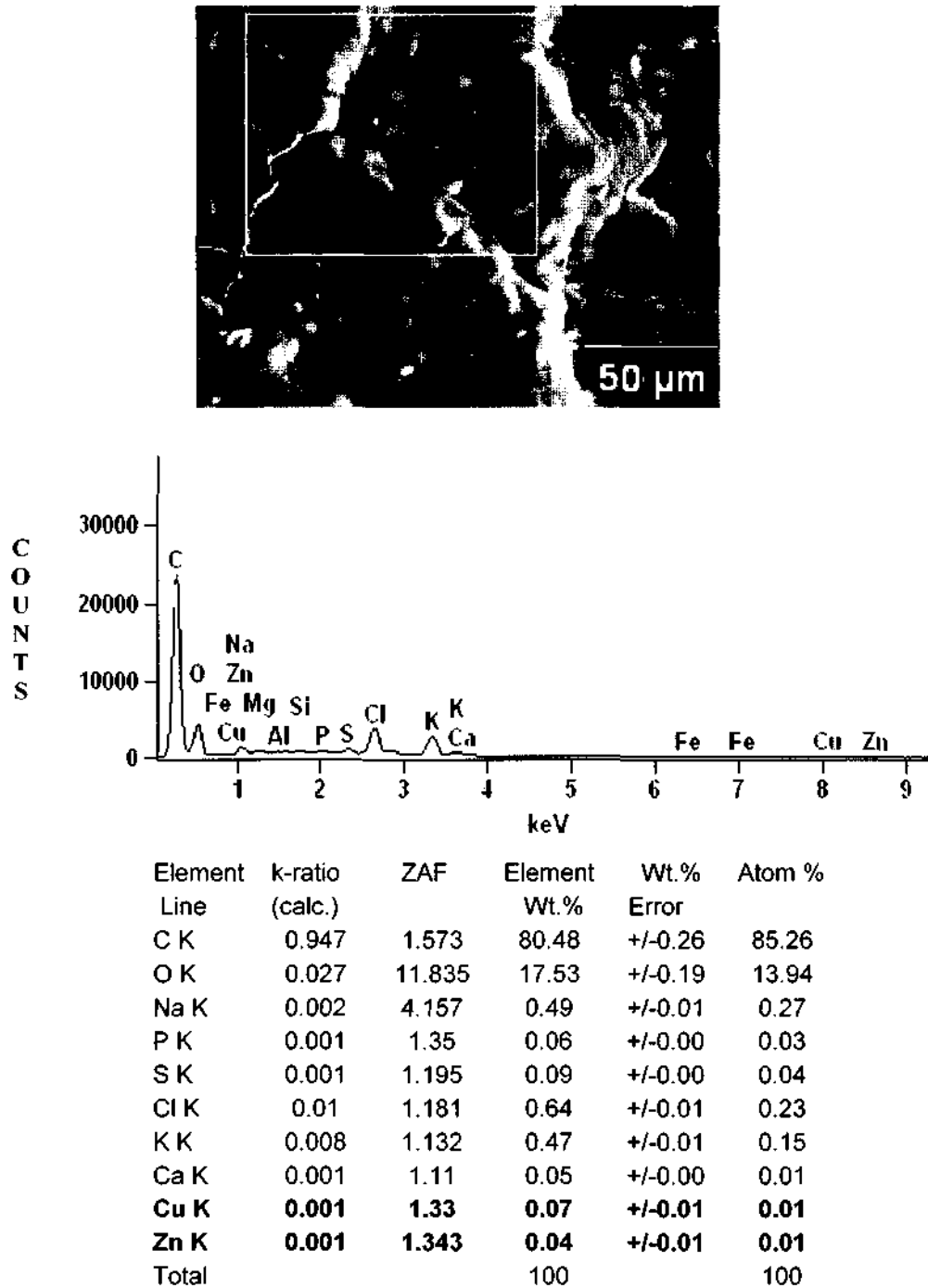
Fig. 40a.   Representative EDX spectrum displaying relative elemental composition on the basal region of the adaxial leaf surface of *A. marina* from the control treatment. Picture showing area of analysis on the adaxial leaf surface. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 μm and data collection time 60 s.

Element Line	k-ratio (calc.)	ZAF	Element Wt.%	Wt.% Error	Atom %
C K	0.904	1.77	71.44	+/-0.25	77.75
O K	0.055	10.703	26.13	+/-0.20	21.35
Na K	0.001	4.533	0.27	+/-0.01	0.15
S K	0.001	1.22	0.05	+/-0.00	0.02
Cl K	0.017	1.196	0.92	+/-0.01	0.34
K K	0.017	1.141	0.85	+/-0.01	0.29
Ca K	0.002	1.122	0.12	+/-0.00	0.04
Cu K	0.001	1.324	0.08	+/-0.01	0.02
Zn K	0.001	1.337	0.05	+/-0.01	0.01
Total			100		100
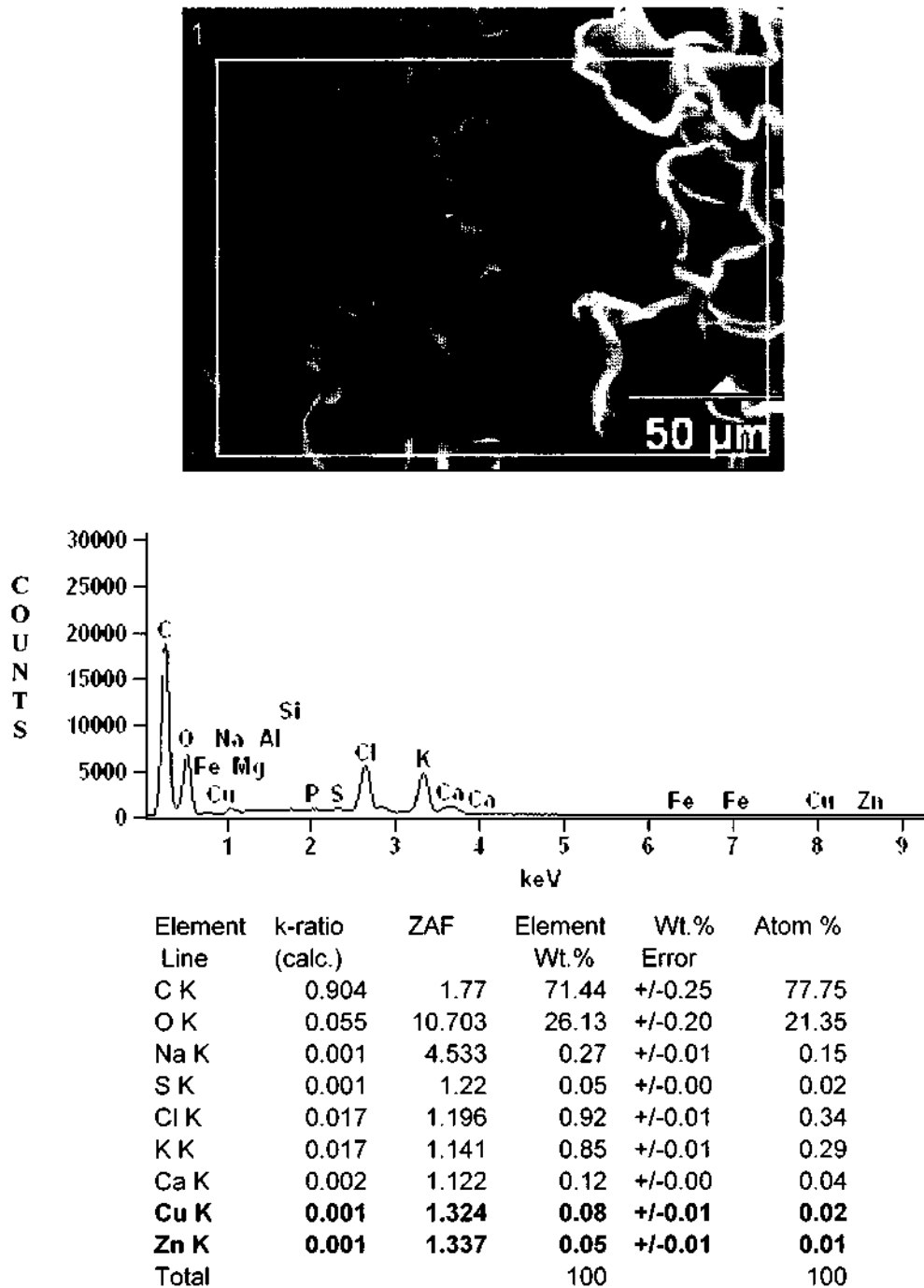
Fig. 40b. Representative EDX spectrum displaying relative elemental composition on the basal region of the abaxial leaf surface of *A. marina* from the control treatment. Picture showing area of analysis on the abaxial leaf surface. Acceleration voltage was 15 kV, working distance 15 mm, area diameter 50 µm and data collection time 60 s.
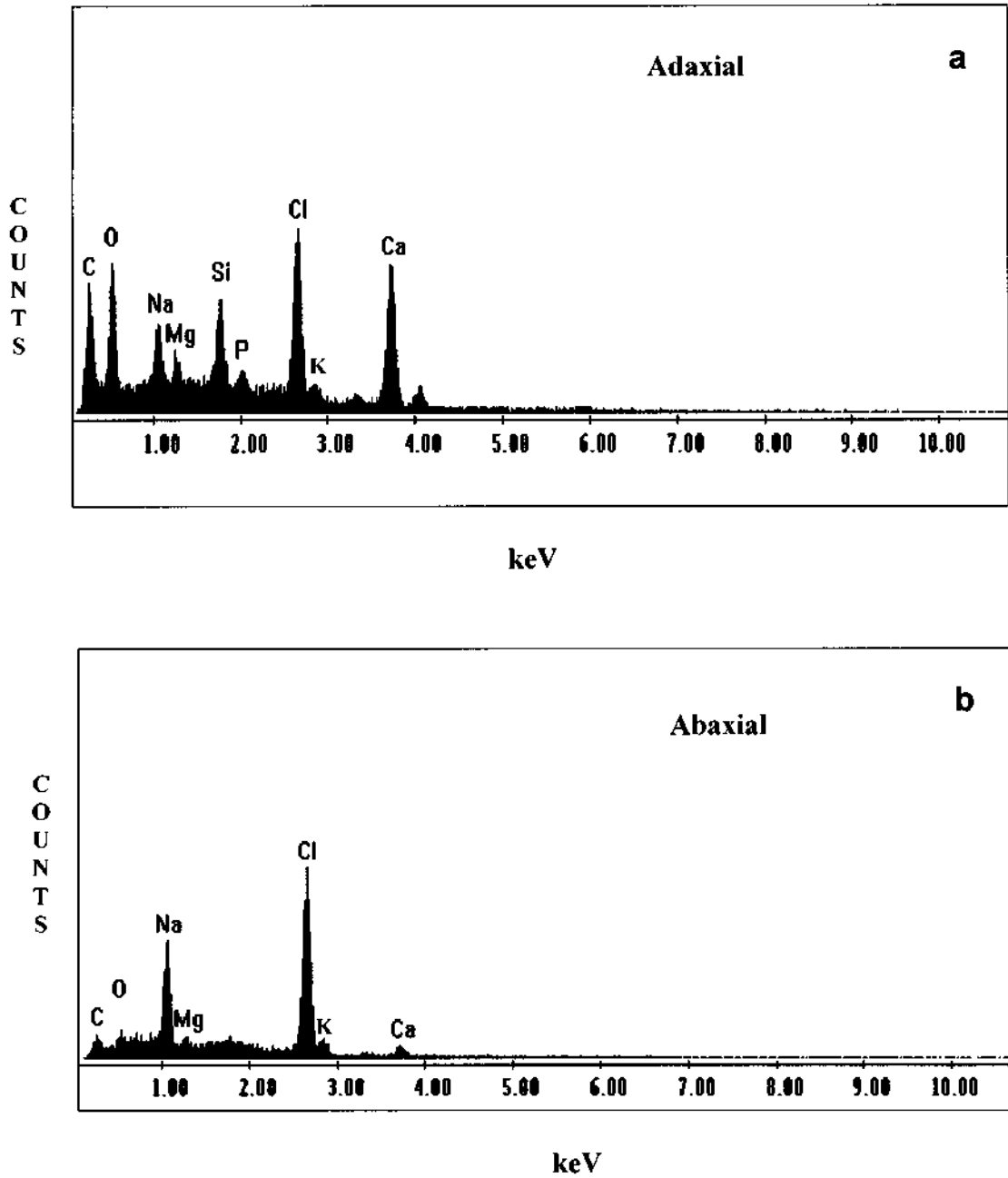
Fig. 41a – b. Representative EDX spectra displaying qualitative elemental presence in salt crystals on the adaxial and abaxial surface of *A. marina* from the control experiments. Spectra yields predominantly Na^+ and Cl^- with lower amounts of O, Si, Mg and Ca ions on the leaf surfaces in control plants.
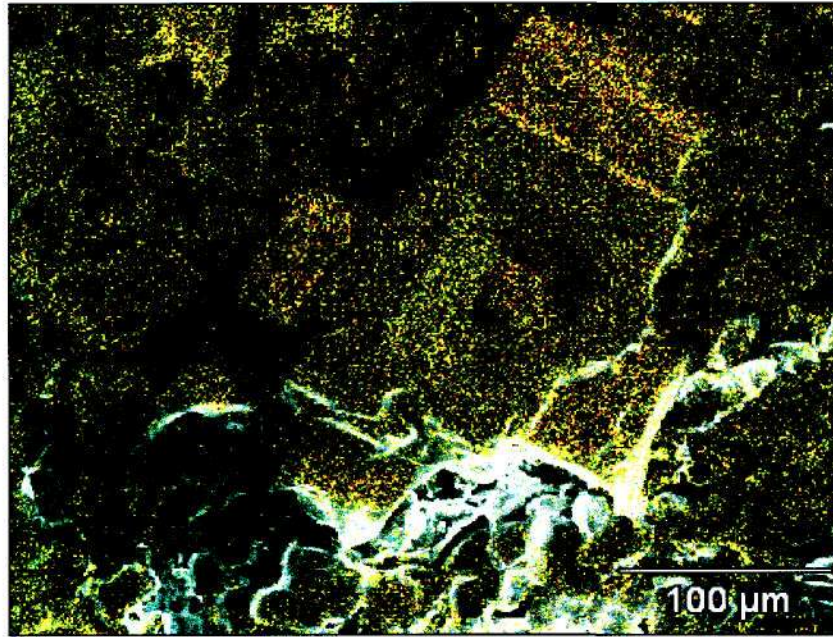
Fig. 42 X-ray map of prismatic crystals over trichomes in Cu²⁺ treated leaves from the 160 μgml⁻¹ Cu²⁺ treatment. Red and yellow colours indicate presence of NaCl, turquoise colour indicates presence of Cu²⁺.
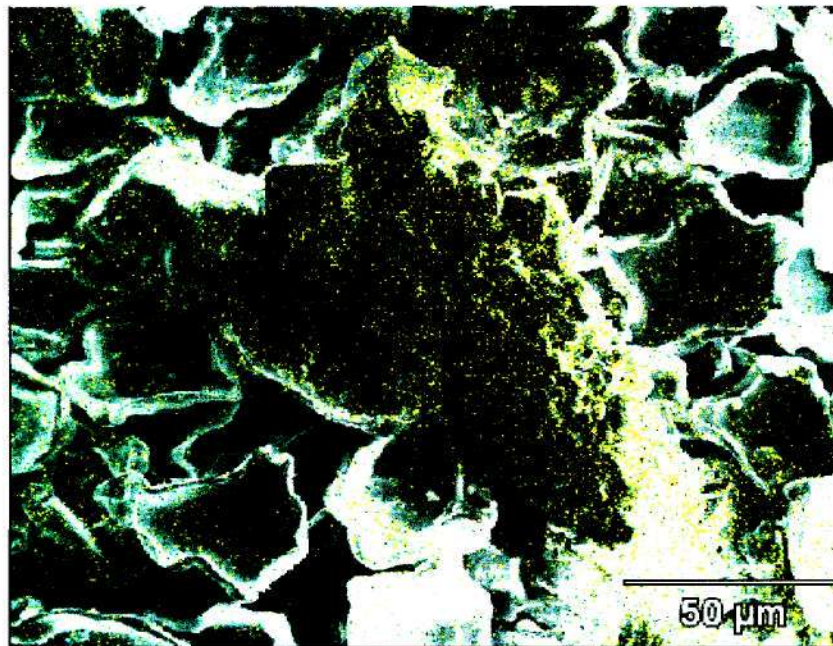
Fig. 43 X-ray map of prismatic crystals over trichomes in Zn²⁺ treated leaves from the 160 μgml⁻¹ Zn²⁺ treatment. Red and yellow colours indicate presence of NaCl, green colour indicates presence of Zn²⁺.
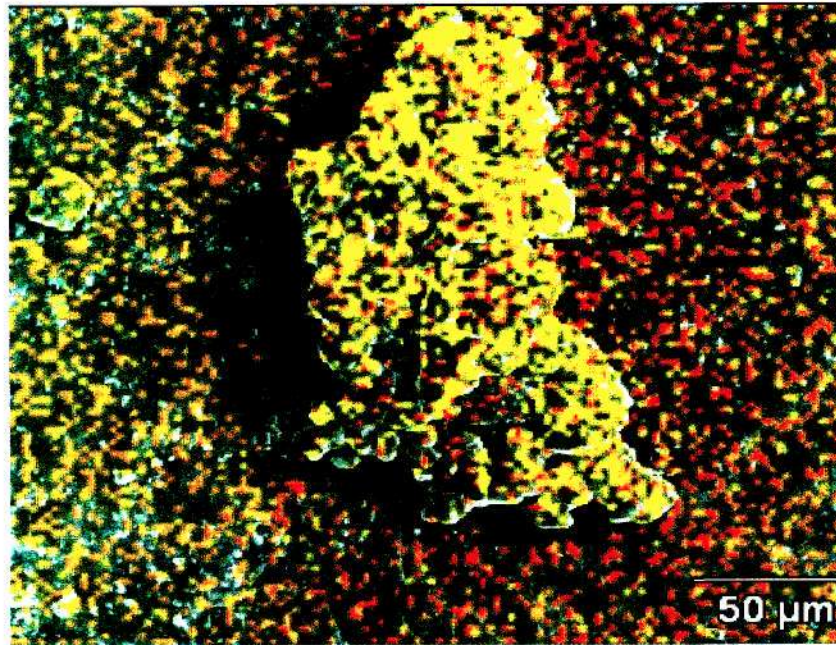
Fig. 44   X-ray map of salt gland partially occluded by a dense mass of crystals in 160 μgml⁻¹ Hg²⁺ treatment. Red and yellow colours indicate presence of NaCl⁻. Map indicates that Hg²⁺ (purple colour) is absent on leaf surface.

CHAPTER 5

DISCUSSION

5.1. PHYSIOLOGY

5.1.1 PLANT GROWTH

Significant decreases in total dry biomass accumulation in the Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} treatments at increasing metal concentrations have been demonstrated by this study. This has been attributed to ion toxicity if sequestration of ions into vacuoles or ion secretion is inadequate, resulting in a build-up of ions in the symplasm as it is also suggested by Storey and Wyn Jones (1979). At elevated concentrations, essential (Cu^{2+} and Zn^{2+}) and non-essential (Hg^{2+} and Pb^{2+}) metals are known to induce toxicity symptoms such as stunting and inhibition of root growth. These findings were also confirmed in the literature (Williams *et al.*, 2000; Wang, 2004). Reduced growth is probably mediated via inhibition of enzyme activity or damage to the protein structure as a result of metal toxicity within tissues. This contention was also supported by Van Assche and Clijsters (1990). Furthermore, the extent of inhibition of enzyme activities and protein damage were indirectly shown by the decrease of the physiological parameters such as CO_2 exchange, leaf conductance and photosystem II quantum yield in relation to photosynthesis and transpiration. These findings were supported by the literature as it was found that ribulose − 1, 5 − bisphosphate carboxylase (RuBPC), a key enzyme of photosynthesis, is highly sensitive to Zn^{2+} and Cu^{2+} toxicity in C_3 plants (Stiborova *et al.*, 1976; 1986). The other possible contributing factors in stunting and decrease in total dry biomass accumulation is due to possible effects of ion toxicity and interference during the plant growth process. Such ion toxicity and interference is assumed to include disruption of cell transport processes and oxidative damage which has already been reported in the literature (William *et al.*, 2000). Growth reduction may also be attributed to the relocation of energy resources from accumulative growth to maintenance processes such as ion exclusion, ion transport, ion compartmentation, salt

secretion and synthesis of osmoprotectants. In addition, apoplastic accumulation of ions in the cell walls is well-known that it reduces cell elongation and cell turgor (Flowers and Yeo, 1986). Therefore, decreases in turgor probably contributed to growth reductions at elevated metal concentrations. This contention is further supported by Chaoui *et al.* (1997) who have suggested that toxic metals are generally responsible for reduction of biomass.

Dry shoot biomass in all treatments was consistently higher than dry root biomass in all treatments suggesting that *A. marina* allocates more biomass to shoots than roots at the salinity used in the study (20 ‰). At higher metal concentrations reduced root biomass accumulation could be due to higher metal accumulation in the roots. Reduced growth and development are adaptive responses to function adequately under metal stress. Apart from biomass accumulation, plant height and number of leaves were also reduced significantly with increasing metal concentrations. This may be caused by callose deposits on sieve plates in the phloem as a result of toxic levels of Zn^{2+} and Cu^{2+} (Peterson and Rauser, 1979). These ions were reported to markedly restrict translocation of carbohydrates, leading to accumulation of sugars and starch in the leaves and reduced transport to growing parts of the plant (Rauser and Samarakoon, 1980). Furthermore, Samarakoon and Rauser (1979) showed that translocation of photoassimilates from the leaves to roots and expanding buds and leaves are seriously restricted by excess Zn^{2+}. It is also suggested that the same phenomenon has occurred in *A. marina* when it was subjected to toxic levels of Zn^{2+} and Cu^{2+}.

Despite growth reduction with increasing metal concentrations, *A. marina* seedlings were found to be highly tolerant to Cu^{2+} Pb^{2+}, Hg^{2+} and Zn^{2+} as no visible morphological differences or mortality of plants were observed amongst treatments. Similar findings by MacFarlane and Burchett (1999, 2000, 2001, 2002) support these observations.

5.1.2 PHOTOSYNTHESIS

Gas exchange data indicated that decreases in CO_2 exchange, transpiration and leaf conductance with an application of heavy metals appeared to be directly or indirectly attributed to chlorophyll loss, inhibition of enzymes, changes in resistance of the stomata to CO_2 and diffusion of water. All these aspects in relation to interference of heavy metals in the transpiration and photosynthetic activities are highlighted in the literature in support of authors' findings (Bazzaz *et al.*, 1974; Stiborova *et al.*, 1976; Van Assche *et al.*, 1979, Porter and Sheridan, 1981, Bahlsberg-Pahlsson, 1989). The accumulation of Cu^{2+} and Zn^{2+} within the leaves suggests that the plants actively take up these elements as they are required for metabolic processes such as photosynthesis. A similar finding, which provided the necessity for the requirement of Cu^{2+} and Zn^{2+} to photosynthetic optimal activity, is also documented in the literature (Baker and Walker, 1990). However, when uptake exceeds metabolic requirements, a toxic impact may result. The data suggest that a build up of Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} in the roots and leaves may have disrupted nutrient transport and reduced hydraulic conductivity and water transport to the leaves. These heavy metals have been reported to cause changes in xylem tissues and blockages in the xylem elements, thus affecting water transport in the plant indirectly. In addition, other plant organelle functions such as mitochondrial reactions, enzyme systems related to photosystem II electron transport, respiration, carbohydrate metabolism and protein synthesis have been reported to be impaired as a result of reduced gas exchange (Verkleij and Schat, 1990; Williams *et al.*, 2000). Similarly, decreases in transpiration and leaf conductance with increasing heavy metal concentration might be attributed to accumulation in the leaves leading to stomatal closure. It has been suggested that prolonged transpiration, in combination with excess heavy metals, might also cause an increase in the accumulation of salt and other ions in the leaves and thus enhance senescence and negatively affect photosynthetic rates (Prisco and O'Leary, 1972; Rawson *et al.*, 1983).

Reduced plant growth may also be attributed to the effects of heavy metals on the biochemistry of photosynthesis. Decreases in quantum yield of PSII, ETR through PSII

and photochemical efficiency of PSII (Fv/Fm), in all heavy metal treatments, suggests the occurrence of photoinhibition, and thus damage to photosystem II (PSII) reaction centers. Simultaneous reductions in Fv/Fm and CO_2 uptake have been reported in other plant species (Valladares and Pearcy, 1997; Naidoo et al., 2002). Other studies showed that exposure to Cu^{2+}, Zn^{2+} and Pb^{2+} might cause a decrease in the level of chlorophyll a, chlorophyll b and carotenoids resulting in direct reductions in photosynthetic activity, and hence reduced carbon fixation and possibly deleterious effects at the whole plant level (Baker and Walker, 1990). Differential effects of heavy metals on various enzymes could also be the basis for reduced photosynthesis, respiration and hence plant growth (Nalewajko and Olaveson, 1995). In addition, the basic requirement for electron transport are enzymes of the Krebs Cycle such as malate dehydrogenase (MDH) and isocitrate dehydrogenase (ICDH) which are sensitive to excess Zn^{2+} (Agarwala et al., 1977, Malhotra and Khan, 1980). Other hydrolytic enzymes like phosphatase and ribonuclease might be similarly affected (Agarwala et al., 1977, Malhotra and Khan, 1980). Reduction in chlorophyll content with increasing metal concentration might also be attributed to changes in photosynthetic pigments in response to metal stress. This contention is also supported in the literature (Bahlsberg-Pahlsson, 1989). These findings are also supported by MacFarlane (2002) who reported that the increase in the chlorophyll a/b ratio indicated a greater depletion of the chlorophyll b pool at higher Zn^{2+} exposures in A. marina. A. marina also showed significant linear increases in peroxidase activity and decreases in photopigments when exposed to Cu^{2+} and Zn^{2+}. This relationship has also been established in laboratory-based studies with A. marina and in other mangrove species on exposure to metals (Chen et al., 1995; MacFarlane and Burchett, 2001). These findings further suggest that heavy metals reduce energy trapping efficiency of PSII, cause changes to electron transport and inhibit photosynthetic efficiency (Falkowski and Raven, 1997). Consequently, processes such as carbon assimilation, growth, survival and productivity are thereby negatively affected (Lagriffoul et al., 1998). Chlorophyll content in Avicennia species is also known to be sensitive to other stressors such as salinity (Ball, 1985; Sobrado and Ball, 1999). High salinities are known to inhibit enzymes involved in chlorophyll biosynthesis, which may confound possible relationships with metal loadings (De Filippis and Pallaghy, 1994).

Lead ions were shown to inhibit chlorophyll biosynthesis leading to lowered chlorophyll content (Bahlsberg-Pahlsson, 1989).

5.1.3 ION CONCENTRATION

The data on ion analyses in plant organs in the present study support the theory of sequestration of excess levels of essential (Cu^{2+}, Zn^{2+}) and non-essential (Hg^{2+}, Pb^{2+}) micronutrients in root tissues. Our findings revealed that accumulated Cu^{2+} and Zn^{2+} levels in leaf tissues (Cu^{2+} = 56.6 % and Zn^{2+} = 49.8 %) were significantly lower than those in the roots (Cu^{2+} = 73 % and Zn^{2+} = 69 %) and sediment metal concentrations, suggesting an exclusion or saturation mechanism to accumulation (MacFarlane and Burchett, 2002). According to McFarlane and Burchett (2000), the regulation of Cu^{2+}, Pb^{2+} and Zn^{2+} uptake in *A. marina* is achieved in part by the exclusion of acropetal transport initiated primarily at the root endodermal casparian strip. In addition, this study showed very conclusively that Cu^{2+} and Zn^{2+} concentrations in the roots were twofold higher than those in the leaves, and Hg^{2+} and Pb^{2+} concentrations in the roots were ten-fold higher than in the leaves. Other possible mechanisms, which are also suggested in the literature for transport and tolerance of metals, include cell wall adsorption at the root level or conveyance into the cytoplasm and compartmentation in vacuoles (Ernst *et al.*, 1992). Hence, *A. marina* may be classified and added to the list of well-known species in the literature as an indicator species for Zn^{2+} and possibly Cu^{2+} accumulation (Baker and Walker, 1990; MacFarlane and Burchett, 1999; 2000).

Generally, internal redistribution of ions takes place in all plants, but may be particularly important in the growing parts of halophytes (Adam, 1990). Non essential metals such as Hg^{2+}, Pb^{2+}, Cr^{2+} and Cd^{2+} have low solubility and translocation rates and are, therefore, more concentrated in the roots than in the stems or leaves (Alberts *et al.*, 1990). Our findings showed that significantly higher levels of heavy metals were also found in roots compared to leaves at the highest metal concentration. This investigation suggested that *A. marina* might actively sequester accumulated metals in root tissue or that these metals were precipitated onto root surfaces, particularly non-essential elements such as Hg^{2+}

and Pb^{2+}. Similar findings were reported for other mangrove species such as *Kandelia candel*, *Bruguiera gymnorrhiza*, *A. marina*, *R. mucronata*, *A. alba* and *Rhizophora mangle* (Walsh *et al.*, 1979; Thomas and Eong, 1984; MacFarlane and Burchett, 2002; Zhang *et al.*, 2007). Many studies have shown that plant roots accumulate Hg^{2+} when exposed to Hg^{2+}-contaminated soils (Lenka *et al.*, 1992; Coquery and Welbourne, 1994; Ribeyre and Boudou, 1994; Bersenyi *et al.*, 1999; Kalac and Svoboda, 2000;). Laboratory studies showed that plant roots absorbed Hg^{2+} from solution and roots accumulated much greater amounts of Hg^{2+} than shoots. Similar findings, which support these studies, are documented in the literature (Beauford *et al.*, 1977; Godbold and Hütterman, 1988; Cavallini *et al.*, 1999). The large difference between root and leaf concentrations of Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} appeared to indicate an important restriction of the internal transport of these metals from the roots to the leaves. This phenomenon was also confirmed by Kadukova and Kalogerakis (2007). Baker (1981), who reported that *A. marina* exhibited characteristics of a typical excluder species for Pb^{2+}, with a strong exclusion mechanism occurring at the root level. The primary location for Pb^{2+} exclusion in *A. marina* has been also reported to be in the root epidermal layer (Baker, 1981; MacFarlane and Burchett, 2000, 2001). Other possible mechanisms for the exclusion of Pb^{2+} and Hg^{2+} might occur through cytoplasmic responses such as active pumping of metal complexes into vacuoles and dictyosomal vesicles, specific metal binding proteins on the root surface, complexing by organic acids and immobilization along cell walls (Wozny and Krzeslowska, 1993). However, root accumulation might adversely affect metabolic processes, prohibiting normal plant functions such as plant growth. Soil samples from the control treatment revealed that Cu^{2+} and Zn^{2+} concentrations were within acceptable ranges whereas Pb^{2+} and Hg^{2+} were not within detectable limits.

Root tissues from mature *A. marina* trees growing naturally in the Bayhead Mangrove Heritage Site accumulated significantly higher concentrations of Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} in comparison to leaf tissues. These metals might be remobilized in the soil due to pollution from human activities such as mining and smelting of lead ores from industries in Durban Bay (Riedel and Sanders, 1988). Metal content in the roots of mature trees in the field was significantly higher than in the controlled study. However, leaf metal

concentrations were greater in the controlled study compared to the field. Among the four metals tested in the field, levels of Cu^{2+} and Zn^{2+} were higher in the roots and leaves. However, high concentrations of Pb^{2+} were only found in the roots. In the field, it is proposed that exposure of cells to metals in combination may have resulted in increased permeability of the plasma membrane, accompanied by increased cellular uptake and toxicity. A reduction in competition between metals, due to specific binding of one metal to active sites on the cell surface, appeared to remobilize the absorption of other metals within the cell wall or membrane region (Starodub and Wong, 1987).

In the field, regular tidal inundation, twice daily, might have remobilized heavy metals in the soil and made them available for absorption by the roots. Other heavy metal studies on angiosperms demonstrated that about 90 % of Pb^{2+} accumulated in the roots of plants from the Brassicaceae family (Kumar *et al.*, 1995). Sekhar *et al.*, (2005) also found that Pb^{2+} accumulated in roots of *Hemidesmus indicus* rather than in the shoots. Wozny (1995) reported that the roots of *Tamarix* plants accumulated 83.3 % to 94 % of the total Pb^{2+} accumulated by the plants. The differences in metal uptake between field and controlled conditions may be attributed to the different physiological state of plants, modification of the soil properties and environmental parameters under controlled conditions (Conesa *et al.*, 2007).

This study demonstrated that, in genera, *A. marina* is able to absorb a wide range of heavy metals (Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+}). Indicators of ion toxicity in *A. marina* included suppression of whole plant growth, i.e. reduction in biomass accumulation, number of leaves and plant height. Thus the physiological status of the plants was adversely affected by metal toxicity. Moreover, heavy metals reduced gas exchange, chlorophyll fluorescence and chlorophyll content. Reductions in photosynthesis might be attributed to inhibition of enzyme systems related to PSII electron transport, reduced water transport to the leaves and changes in photosynthetic pigments. *A. marina* is tolerant to Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} at concentrations of 0, 40 and 80 μgml^{-1}. Thus *A. marina* may be used to determine Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+} toxicity in aquatic ecosystems.

5.2 MICROSCOPY

5.2.1 LEAF MORPHOLOGY AND SEM MICROANALYSIS

Qualitative elemental analyses and X-ray mapping of the deposits over the glands and associated trichomes of *A. marina* yielded spectra showing predominantly Na^+ and Cl^- with trace amounts of Cu^{2+} and Zn^{2+} in control and metal treated plant leaves. Elemental weight data also confirmed the high levels of Na^+ and Cl^- on leaf surfaces. Other elements such as Mg^{2+}, Ca^{2+}, K^+, Si^{4+} and P were detected in the deposits, but at considerably lower concentrations than those of Na^+ and Cl^+. The high peaks of Na^+ and Cl^+ associated with the glands strongly suggested that the glands are the specific sites of secretion.

Data revealed that Zn^{2+} and Cu^{2+} were present on the leaf surfaces. A substantial amount of Zn^{2+} and Cu^{2+} were translocated to the leaves, as evidenced by ion concentration data. However, little or no visible signs of morphological damage were observed in the leaves at increasing metal concentrations for all metals. Mercury and Pb^{2+} were not detected on the surface of the leaf and suggest that these metals were not secreted via the salt glands. Ion analyses revealed trace amounts of Hg^{2+} and Pb^{2+} in the leaves, suggesting that low levels of Hg^{2+} and Pb^{2+} were translocated to the leaves. However, the greater portion of Hg^{2+} and Pb^{2+} were chelated in the roots, possibly within the cell walls, or on root surfaces. These findings are also supported in the literature (MacFarlane and Burchett, 2000; Clemens *et al.*, 2002; Kadukova and Kalogerakis, 2007). This further reinforces the conclusion that Pb^{2+} and Hg^{2+} translocation from the roots to the leaves was strongly restricted. The low amounts of Pb^{2+} and Hg^{2+} translocated to the leaves were probably chelated within the leaf tissue and not available for secretion from the leaves.

A. marina roots appeared to possess two main barriers to translocation of ions to above-ground organs, i.e. the epidermal layers and the endodermis. This contention is also described in the literature (Kramer and Preston, 1978; Lawton *et al.*, 1981). These barriers probably limited excess Cu^{2+} and Zn^{2+} translocation to the shoots and severely reduced Pb^{2+} and Hg^{2+} transport to the leaves (McFarlane, and Burchett, 2000; Wang, 2004). The present study suggests that Cu^{2+} and Zn^{2+} concentrations in the roots were two-fold higher than in

the leaves while Hg^{2+} and Pb^{2+} concentrations in the roots were ten-fold higher than in the leaves. Thomas and Eong (1984) and MacFarlane and Burchett (2002) showed that these metals have differential solubility and are subjected to different levels of chelation at the roots.

SEM observations show that *A. marina* leaves seemed to possess numerous specialized secretory glands, which are actively involved in the elimination of mineral elements rich in Na^+, Cl^-, K^+ and Mg^{2+}. The secretion of excess salts through glands is thought to be a mechanism to maintain favourable osmotic gradients and ion balance in saline environments (Drennan and Pammenter, 1982; Drennan *et al.*, 1987; MacFarlane and Burchett, 1999). Salt glands on the adaxial surface appear to protrude from individual sunken crypts with salt exudates partially or completely occluding the glands (Fahn and Shimony, 1977). In this manner, *A. marina* salt glands were similar to those of *Tamarix* species which were shown to occur in epidermal crypts in which aqueous salt solutions are secreted from the glands and by evaporation (Kadukova and Kalogerakis, 2007). The crystallized salts from these salt glands accumulated in the densely distributed crypts. The present study further revealed that the glabrous upper surface of *A. marina* is covered by a thick, relatively smooth cuticle and lacks stomata. The relatively smooth surface probably appeared to allow for maximal light penetration for gas exchange.

Crystalline deposits composed primarily of Na^+ and Cl^- was observed on the regularly distributed salt glands on both leaf surfaces of *A. marina*. Earlier studies on the micromorphology of *A. marina* leaves further support these findings (Fahn and Shimony, 1977, Drennan and Berjak, 1982, Lipschitz and Waisel, 1982, Thomson *et al.*, 1988). It has been suggested that secretion occurred as the ions flow from the secretory cells to the subcuticular space, where it continued to collect until it ruptures the cuticle, releasing the secretion. Glands with ruptured cuticles appear depressed (Drennan and Berjak, 1979) as observed with the ESEM in the present study.

Energy dispersive X-ray microanalyses (EDX), used to determine distribution of elements in the leaves at the intercellular and intracellular level (Monni *et al.*, 2002), showed that

numerous specialized secretory glands on the adaxial and abaxial surfaces exuded salts rich in Na^+, Cl^- and K^+. The essential heavy metals, namely, Zn^{2+} and Cu^{2+}, were secreted from the salt glands on both surfaces. However Hg^{2+} and Pb^{2+} were undetected and it is, therefore, assumed that they were retained within the leaf tissues. Similarly, Cd^{2+} a heavy metal with low solubility, like Hg^{2+} and Pb^{2+}, was not detected in leaf secretions (Hagemayer and Waisel, 1988). Additionally, various studies have determined the distribution of heavy metals, particularly in aquatic and terrestrial angiosperms, as well as in algal tissues, by SEM and X-ray microanalysis (Spurr, 1980; Harvey, 1986; MacFarlane and Burchett, 1999, 2000).

Neumann *et al.* (1995) reported that *Armeria maritima*, emergent on copper rich soil, excreted alkaline ions as well as heavy metals such as Cu^{2+} and Zn^{2+} through multicellular salt secretory glands. *Tamarix aphylla*, a halophytic shrub, alleviates the effects of elevated concentrations of Cd^{2+} and Li^+ by excreting these ions through specialized salt glands (Hagemeyer and Waisel, 1988). Reports by Weis *et al.* (2002) on the C_4 plant *Spartina alterniflora*, revealed that the leaves contained 2-3 times more Hg^{2+}, Pb^{2+} and Cr^{2+} than *Phragmites australis* leaves. It was concluded that *S. alterniflora* leaves possessed salt glands that secreted twice the amount of metals into estuaries compared to *P. australis*, which lacks salt glands and that Hg^{2+} excretion correlated with Na^+ release (Weis *et al.*, 2002). This suggests that salt secreting species take up and secrete heavy metals. Similarly, this study demonstrated that *A. marina* is able to take up and secrete Cu^{2+} and Zn^{2+} in addition to Na^+ and Cl^-. However, Hg^{2+} and Pb^{2+} were not detected in leaf secretions.

Leaves of *A. marina* are hypostomatous and covered by a dense mat of overlapping, multicellular peltate trichomes which are hypothesized to limit light incident on photosynthetic tissues and salt spray damage to interior leaf tissues (Wagner, 1991; Naidoo and Chirkoot, 2004). Furthermore, this arrangement may afford greater protection to the glands and stomata, and probably represent an adaptive feature to minimize desiccation and evapotranspiration by shielding and trapping air over the stomata (Wagner, 1991). The trichomes or non-glandular hairs appear to form a saucer-like or open well for the collection of secretions exuded by concealed salt glands. The salt glands occur on the leaf surface and

are distributed among the trichomes. These morphological characteristics are also documented in the literature (Fahn and Shimony, 1977). Trichomes apparently play a major role in storage and detoxification of metals. Inside the cell, metals are chelated and excess metal sequestered by transport into the vacuole (Clemens *et al.*, 2002). From the roots, transition metals, i.e. Cu^{2+} and Zn^{2+}, are transported to the shoot via the xylem. Storage appears to occur preferentially in trichomes (Clemens *et al.*, 2002). However, the distribution pattern varies with plant species and element. This remarkable adaptation helps to regulate internal ionic concentrations by eliminating excess salts and ions before they reach toxic levels in the tissues (MacFarlane and Burchett, 1999). This is in agreement with the findings of the present study were Cu^{2+} and Zn^{2+}, as well as Hg^{2+} and Pb^{2+}, were translocated from the root to the shoots. Excess Zn^{2+} and Cu^{2+}, but not Hg^{2+} and Pb^{2+}, were secreted *via* the salt glands.

Qualitative elemental analyses and X-ray mapping of secretions over the glands and trichomes of *A. marina* showed that the secretion comprised predominantly of Na^+ and Cl^- with trace amounts of Zn^{2+}, Cu^{2+}, K^+, Mg^{2+}, Ca^{2+}, P and Si^{4+}. This study demonstrated that soluble heavy metals (Cu^{2+} and Zn^{2+}) are absorbed, translocated to shoots and secreted while those with lower solubility (Pb^{2+} and Hg^{2+}) are chelated primarily in roots with limited translocation to shoots. The absence of Pb^{2+} and Hg^{2+} in the secretion suggested that these metals are chelated within the leaves.

CHAPTER 6

CONCLUSION

Mangrove forests are an important buffer for adjacent marine ecosystems, trapping sediments and nutrients, as well as many anthropogenic chemical contaminants including heavy metals. Physical destruction of this ecosystem for building materials, fuel and land reclamation has increased greatly during this century, largely because of population pressures. Attempt has been made, in this study, to account for some possible factors which might contribute to environmental degradation in relation to ecophysiology and ecotoxicology of mangroves. *A. marina* was used as a model. This study has, therefore, demonstrated the effects of four heavy metals, namely Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}, on plant growth, gas exchange, chlorophyll fluorescence, chlorophyll content and ion relations in shoot and root tissues of *A. marina* under controlled conditions.

A. marina was found to be relatively tolerant to the metals applied. The author accepts the hypothesis that heavy metals are either chelated in the soil or roots or taken up by the plant. Generally, *A. marina* accumulated higher concentrations of Cu^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} in roots than the shoots probably due to an ion-exclusion mechanism (Peng *et al.*, 1997). The detection of Zn^{2+} and Cu^{2+} in the secretion and not Hg^{2+} and Pb^{2+} partially support the hypothesis that heavy metals transported into leaves are secreted *via* salt glands. Zinc and Cu^{2+} exhibited greater translocation to the leaves and were secreted *via* the salt glands on both leaf surfaces. In contrast, Pb^{2+} and Hg^{2+} exhibited low mobility with highest concentrations in roots. Decreases in biomass accumulation, number of leaves and plant height with increase in metal concentrations support the hypothesis that plant growth is adversely affected by heavy metal uptake. In addition, reduction in whole plant growth was negatively affected by decreases in gas exchange and chlorophyll fluorescence with increasing metal concentrations. The results of this study indicated that growth and photosynthetic performance of *Avicennia marina* decreased due to dose-dependent stress responses to all selected heavy metals. Additional research is needed to test and further elucidate these conclusions.

Previous investigations on metal toxicity in mangroves have examined sub-lethal effects on seedlings for short periods of exposure (MacFarlane and Burchett, 2001, 2002). These results are vital for establishing estuarine and marine sediment quality guidelines for plant toxicity data. There is also a lack of comprehensive information on the relative sensitivities of semi-aquatic angiosperms to heavy metals and the combined effects of metals on plants (Mohan and Hosetti, 1999). Exposure to heavy metal mixtures is more depictive of environmental situations and may change the biological responses of plants in quantitative ways relative to that of single toxicants (Boedeker *et al.*, 1993; MacFarlane and Burchett, 2002).

Metal translocation into shoots is very restricted in some wetland plants. This ensures that harvested plants will not be an effective source of metal removal in a wetland system (Deng *et al.*, 2004). *A. marina* falls under this category. Wetland plants play an important role in heavy metal removal from metal-polluted water by immobilizing metals in the roots and in the oxygenated rhizosphere (Ye *et al.*, 2001). This is also applicable to *A. marina*. Other wetland plant species are renowned for their ability to reduce metal translocation from roots to shoots. These plants are regarded as suitable phytostabilizers for revegetation of waterlogged mine tailings, and other waterlogged metal-contaminated lands (Ye *et al.*, 2001). Thus mangrove forests have the potential to act as a sink for pollutants (Harbison, 1986; De Lacerda *et al.*, 1993a; De Lacerda, 1998; Tam and Wong, 1993, 1995, 2000) or buffer and remove or immobilize heavy metals before entering nearby aquatic environments (MacFarlane and Burchett, 1999). In addition, these plants including *A. marina* have numerous mechanisms to detoxify heavy metals. In order to reduce the negative effect of heavy metals on plants, a further investigation has to be pursued to understand the other alternative biological mechanisms which might be possibly involved in heavy metal tolerance and ecotoxicology. This attempt would help in phytoremediation biotechnology. Such research would facilitate the selection and cultivation of appropriate plant species for the advancement of phytoremediation programmes (Cheng, 2003).

REFERENCES:

Adam, P., 1990. Saltmarsh Ecology. Cambridge University Press, Cambridge.

Agarwala, S.C., Bisht, S.S., Sharma, C.P., 1977. Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. Canadian Journal of Botany 55, 1299-1307.

Alberts, J.J., Price, M.T., Kania, M., 1990. Metal concentrations in tissues of *Spartina alterniflora* (Loisel) and sediment of Georgia salt marshes. Estuarine, Coastal and Shelf Science 30, 47-58.

Alves, da P.L.C.A., Magalhães, A.C.N., Barja, P.R., 2002. The phenomenon of photoinhibition of photosynthesis and its importance in reforestation. The Botanical Review 68 (2), 193-208.

Andrade, L.R., Farina, M., Filho, A.M.G., 2004. Effects of copper on *Enteromorpha flexuosa* (Chlorophyta) in vitro. Ecotoxicology and Environmental Safety 58, 117-125.

Anzecc and Armcnz, 1999. Australian water quality guidelines for fresh and marine waters. Australian and New Zealand environment and conservation council and agriculture and agriculture and resource management council of Australia and New Zealand, Canberra, Australia.

Aust, S.D., Marehouse, C.E., Thomas, C.E., 1985. Role of metals in oxygen radical reactions. Journal of Free Radical and Biological Medicine 1, 3-25.

Bahlsberg-Pahlsson, A.M., 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air and Soil Pollution. 47, 287-319.

Baker, A.J.M., 1981. Accumulators and excluders strategies in the response of plants to heavy metals. Journal of Plant Nutrition 3, 643-654.

Baker, A. and Walker, P., 1990. Ecophysiology of metal uptake by tolerant plants. In: Shaw, J. (Ed.), Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Florida, pp. 155-178.

Ball, M.C., 1985. Photosynthesis in mangroves. Wetlands (Aust) 6(1), 12-22.

Ball, M.C. and Anderson, J.M. 1986. Sensitivity of photosystem II to NaCl in relation to salinity tolerance: Comparative studies with thylakoids of the salt-tolerant mangrove, *Avicennia marina*, and the salt-sensitive pea, *Pisum sativum*. Australian Journal of Plant Physiology 13, 689-698.

Ball, M.C. 1996. Comparative ecophysiology of mangrove forest and tropical lowland and moist rainforest. In: Mulkey, S.S., Chazdon, R.L., Smith, A.P. (Eds.), Tropical forest ecophysiology. Chapman and Hall, New York, pp. 461-496.

Banks, R., 2003. Mangroves: The best halophytes ever.

Banus, M.D., Valiela, I., Tel, J.M., 1975. Lead, zinc and cadmium budgets in experimentally enriched salt marsh ecosystems. Estuarine, Coastal and Marine Science 3, 421-430.

Barber, J., Beauford, W., Shieh, Y.J., 1973. Some aspects of mercury uptake by plant, algal and bacterial systems in relation to its biotransformation and volatilization. In: Miller, M.W., Clarkson, T.W. (Eds.), Mercury, mercurials, and mercaptans. Charles D Thomas Publisher, Illinois, USA, pp. 325-345.

Basak, U.C., Das, A.B., Das, P., 1996. Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangroves. Bulletin of Marine Science 58(3), 654-659.

Battaglia, A., Calace, N., Nardi, E., Petronio, B.M., Pietroletti, M., 2007. Reduction of Pb and Zn bioavailable forms in metal polluted soils due to paper mill sludge addition. Effects on Pb and Zn transferability to barley. Bioresource Technology 16, 2993-2999.

Bawa, K.S. and Hadley, M., 1990. Reproductive Ecology of Tropical Forest Plants. (Eds.), Volume 4. Boston; UNESCO and the Parthenon Publishing Group.

Bazzaz, F.A., Carlson, R.W., Rolfe, G.L., 1974. The effect of heavy metals on plants: Part I. Inhibition of gas exchange in sunflower by Pb, Cd, Ni and Ti. Environmental Pollution 7, 241-242.

Beachwood Mangrove Nature Reserve Management Plan, 1986. Part I: Information section. Natal Parks, Game and Fish Preservation Board, Pietermaritzburg, p. 45.

Beauford, W., Barber, J., Barringer, A.R., 1977. Uptake and distribution of mercury within higher plants. Physiologia Plantarum 39, 261-265.

Beckett, P.H.T. and Davis, R.D., 1977. Upper critical levels of toxic elements in plants. New Phytologist 79(1), 95-106.

Berjak, P., Campbell, G.K., Huckett, B.I. and Pammenter, N.W., 1977. In the mangroves of southern Africa. A wildlife handbook Natal branch of the wildlife society of southern Africa. p. 73

Bersenyi, A., Fekete, S., Hullar, I., Kadar, I., Szilagyi, M., Glavits, R., Kulcsar, M., Mezes, M., Zoldag, L., 1999. Study of the soil-plant (carrot)-animal cycle of nutritive and hazardous minerals in a rabbit model. Acta Veterinaria Hungarica 47(2), 181-190.

Boedeker, W., Drescher, K., AltenBurger, R., Faust, M., Grimme, L.H. 1993. Combined effects of toxicants: the need and soundness of assessment approaches in ecotoxicology. The Science of the World Environment, Supplement, pp. 931-939.

Bowman, H.H.M., 1917. Ecology and physiology of the red mangrove. American Philosophical Society Proceedings 56, 589-672.

Brown, M.T. and Newman, J.E., 2003. Physiological responses of *Graciliariopsis longissima* (S.G. Gmelin) Steentoft, L.M. Irvine and Farnham (Rhodophyceae) to sub-lethal copper concentration. Aquatic Toxicology 64, 201-213.

Burton, K.W., Morgan, E., Roig, A., 1983. The influence of heavy metals upon the growth of Sitka spruce in South Wales forests. I. Upper critical and foliar concentrations. Plant and Soil 73, 327-336.

Burton, K.W., Morgan, E., Roig, A., 1986. Interactive effect of cadmium, copper and nickel on the growth of Sitka spruce and studies of metal uptake from nutrient solutions. New Phytologist 103 (3), 549-557.

Bussler, W., 1981. Copper in soils and plants. In: Loneragan, J.F., Robson, A.D., Graham, R.D. (Eds.), The Golden Jubilee International Meeting, Australia. Academy Press. Australia, p. 213.

Cavallini, A., Natali, L., Durante, M., Maserti, B., 1999. Mercury uptake, distribution and DNA affinity in durum wheat (*Triticum durum* Desf.) plants. The Science of the Total Environment 243/244, 119-127.

Chaoui, A., Mazhoudi, S., Ghorbal, M.N., Ferjani, E.E., 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzymes activities in bean (*Phaseolus vulgaris* L.). Plant Science 127, 139-147.

Chapman, V.J., 1975. Mangrove biogeography. In: Proceedings of the international symposium on the biology and management of mangroves. Honolulu (Eds.), Walsh, G.D., Snedaker, S.C. and Teas, H.J.I., University of Florida, Gainesville, pp. 3-22.

Chen, G.Z., Miao, S.Y., Tam, N.F.Y., Wong, Y.S., Li, S.H., Lan, C.Y., 1995. Effect of synthetic wastewater on young *Kandelia candel* plants growing under greenhouse conditions. Hydrobiologia 295, 263-273.

Cheng, S., 2003. Heavy metal pollution in China: origin, pattern and control. Environmental Science and Pollution Research 10(3), 192-198.

Chesworth, W., 1991. Geochemistry of micronutrients. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M., USA: Soil Science Society of America, pp. 1-30.

Chigbo, F.E., Smith, R.W., Shore, F.S., 1982. Uptake of arsenic, cadmium, lead and mercury from polluted water by the water hyacinth *Eichhornia crassipes*. Environmental Pollution Series A, Ecological and Biological 27, 31-36.

Chiu, C.Y. Hsiu, F.S., Chen, S.S., Chou, C.H., 1995. Reduced toxicity of Cu and Zn to mangrove seedlings in saline environments. Botanical Bulletin Acad. Sin. 36, 19-24.

Cid, A., Herrero, C., Torres, E., Abalde, J., 1995. Copper toxicity on the marine microalga *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. Aquatic Toxicology 31, 165-174.

Clarkson, T. W., 1972. The pharmacology of mercury compounds. Annual Review of Pharmacology and Toxicology 12, 375-406.

Clarkson, D.T. and Hanson, J.B., 1980. The mineral nutrition in higher plants. Annual Review of Plant Physiology 31, 239-298.

Clemens, S., 2001. Molecular mechanisms of plant metal homeostasis and tolerance. Planta 212, 476-486.

Clemens, S., Palmgren, M.G., Krämer, U., 2002. A long way ahead: understanding and engineering plant metal accumulation. Trends in Plant Science 7(7), 309-315.

Clijsters, H. and Van Assche, F., 1985. Inhibition of photosynthesis by heavy metals. Photosynthesis Research 7, 31-40.

Collins, J.C., 1981. Effect of heavy metal pollution on plants. In: Lepp, N.W. (Eds.), Vol.1. Applied Science Publishers, London and New Jersey, p. 145.

Conesa, H.M., Schulin, R., 2007. A laboratory study on revegetation and metal uptake in native plant species from neutral mine tailings. Water Air Soil Pollution 183, 201-212.

Coquery, M. and Welbourne, P.M., 1994. Mercury uptake from contaminated water and sediment by the rooted and submerged aquatic macrophyte *Eriocaulon septangulare*. Archives of Environmental Contamination and Toxicology 26(3), 335-341.

Davis, R.D. and Beckett, P.H.T., 1978. Upper critical levels of toxic elements in plants. Ii. Critical levels of copper in young barley, wheat, rape, lettuce and ryegrass, and of nickel and zinc in young barley and ryegrass. New Phytologist 80(1), 23-32.

Dawes, C., 1981. Journal of Marine Botany. New York: A Wiley-Interscience publication.

De Filippis, L.F. and Pallaghy, C.K., 1994. Heavy metals: sources and biological effects. In: L.C. Rai, J.P. Gaur and C.J. Soeder (Eds.), Algae and water pollution. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp. 31-37.

De Lacerda, L., Conde, J.E., Bacon, P.R., Alarcon, C., D'Croz, L., Kjerfve, B., Polania, J., Vannucci, M., 1993a. Mangrove ecosystems of Latin America and the Caribbean: a summary. In: Conservation and sustainable utilization of mangrove forests in Latin America and Africa regions. Part I - Latin America. Lacerda, L.D. (Ed.), ITTO/International Society for Mangrove Ecosystems, Okinawa, Japan, pp. 1-42.

De Lacerda, L., Carvalho, C.E.V., Tanizaki, K.F., Ovalle, A.R.C., Rezende, C.E., 1993b. The biogeochemistry and trace metals distribution of mangrove rhizospheres. BTROA 25, 252-257.

De Lacerda, L., Ittekot, V., Patchineelam, S.M., 1995. Biogeochemistry of mangrove soil organic matter: a comparison between *Rhizophora* and *Avicennia* soils in southeastern Brazil. Estuarine, Coastal and Shelf Science 40, 713-720.

De Lacerda, L., 1998. Trace metals in mangrove plants: why such low concentrations? In: Lacerda, L.D., Diop, H.S. (Eds.), Mangrove ecosystem studies in Latin America and Africa. UNESCO, pp. 171-178.

Deng, H., Ye, Z.H., Wong, M.H. 2004. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environmental Pollution 132(1), 29-40.

Dewez, D., Geoffroy, L., Vernet, G., Popovic, R., 2005. Determination of photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliiquus*. Aquatic Toxicology 74, 150-159.

Dietz, K-J., Baier, M., Krämer, U., 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad, M.N.V, Hagemeyer, J. (Eds.), Heavy metal stress in plants: from molecules to ecosystems. Berlin. Springer-Verlag, 73-97.

Dos Santos Utmazian, M.N. and Wenzel, W.W., 2007. Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. Journal of Plant Nutrition and Soil Science 170, 265-272.

Drennan P. and Berjak, P., 1979. Degeneration of foliar glands correlated with the shift too abaxial salt excretion in *Avicennia marina* (Forssk.) Vierh. Proceeding of Electron Microscopy Society of Southern Africa 10, 55-56.

Drennan, P. M. and Berjak, P., 1982. Degeneration of the salt glands accompanying foliar maturation in *Avicennia marina* (Forsk.) Vierh. New Phytologist 90, 165-176.

Drennan, P. M. and Pammenter, N.W., 1982. Physiology of the salt excretion in the mangrove *Avicennia marina* (Forsk.) Vierh. New Phytologist 91, 597-606.

Drennan, P. M., Berjak, P., Lawton, J.R. and Pammenter, N.W., 1987. Ultrastructure of the salt glands of the mangrove, *Avicennia marina* (Forsk.) Vierh., as indicated by the use of selective membrane staining. Planta 172, 172-183.

Duke, J.A., 1983. Handbook of Energy Crops.

Duke, N.C., 1992. Mangrove floristics and biogeography. In: Robertson, A.I. and Alongi, D.M. (Eds.), Coastal and estuarine studies. Tropical mangrove ecosystems. Washington, D.C.: American Geophysical Union, pp. 63-100.

Egler, F.E., 1948. The dispersal and establishment of red mangrove, *Rhizophora*, in Florida. Caribbean Forestry 9, 299-310.

Erenoglu, B., Nikolic, M., Römheld, V., Cakmak, I., 2002. Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency. Plant and Soil 241, 251-257.

Ernst, W.H.O., 1972. Schwermetallvegetation der Erde. Fischer, Stuttgart.

Ernst, W.H.O., Verkleij, J.A.C., Schat, H., 1992. Metal tolerance in plants. Acta Botanica Neerlandica 41, 229-248.

Ernst, W.H.O. and Nelissen, H.J.M. 2000. Life-cycle phases of a zinc- and cadmium-resistant ecotype of *Silene vulgaris* in risk assessment of polymetallic mine soils. Environmental Pollution 107, 329-338.

Ewel, K.C., Twilley, R.R., Ong, J.E., 1998. Different kinds of mangrove forests provide different goods and services. Global Ecology and Biogeography Letters 7, 83-94.

Fahn, A. and Shimony, C., 1977. Development of the glandular and non-glandular leaf hairs of *Avicennia marina* (Forsk.) Vierh. Botanical Journal of the Linnean Society 74, 37-46.

Falkowski, P.G. and Raven, J.A. 1997. Aquatic photosynthesis. Blackwell Science, Oxford.

Fernandes, J.C. and Henriques, F.S., 1991. Biochemical, physiological, and structural effects of excess copper in plants. The Botanical Review 57(3), 246-273.

Fiskesjö, G., 1988. The *Allium* test-an alternative in environmental studies: the relative toxicity of metal ions. Mutation Research 197, 243-260.

Flowers, T.J. and Yeo, A.R., 1986. Ion relation of plants under drought and salinity. Australian Journal of Plant Physiology 13, 75-91.

Folsom, B. L., Jr., Lee, C.R., Bates, D.J., 1981. Influence of disposal environment on availability and plant uptake of heavy metals in dredged material. Technical report EL-81-12, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Mississippi, p. 158.

Godbold, D.L., Tischner, R. and Hüttermann, A., 1987. Effects of heavy metals and aluminium on the root physiology of spruce (*Picea abies* Karst.) seedlings. In: Lindberg, S.E. and Hutchinson, T.C. (Eds.), Heavy metals in the environment, vol. II. International Conference, New Orleans, p. 253.

Godbold, D.L. and Hütterman, A., 1988. Inhibition of photosynthesis and transpiration in relation to mercury-induced root damage in spruce seedlings. Physiologica Plantarum 74, 270-275.

Godbold, D.L., 1991. Mercury induced root damage in spruce seedlings. Water Air and Soil Pollution 56, 823-831.

Godbold, D.L., 1994. Mercury in forest ecosystems: Risk and research need. In: Watras, C.J., and Huckabee, J.W., (Eds.), Mercury pollution-integration and synthesis. Lewis Publishers, Boca Raton, pp. 295-303.

González, H. and Ramírez, M., 1995. The effect of nickel mining and metallurgical activities on the distribution of heavy metals in Levisa Bay, Cuba. Journal of Geochemical Exploration 52, 183-192.

Grant, C.A., Buckley, W.T., Bailey, L.D., Selles, F., 1998. Cadmium accumulation in crops. Canadian Journal of Plant Science 78, 1-17.

Grill, E. Winnacker, E.L., Zenk, M.H., 1987. Phytochelatins, a class of heavy metal-binding peptides from plants, are functionally analogous to metallothioneins. Proceedings of the National Academy of Science U.S.A 84, 439-443.

Grill, E., 1989. Phytochelatins in plants. In: Hamer, D.H. and Winge, D.R. (Eds.), Metal ion homeostatis: molecular biology and chemistry. Alan R. Liss, New York, pp. 283-300.

Guo, D., 1994. Environmental sources of Pb and Cd and their toxicity to man and animals. Advances in Environmental Science 2(3), 71-76.

Gupta, U.C., 1979. Copper in agricultural crops. In: Nriagu, J. O. (Eds.), Copper in the environment. Part I: Ecological cycling. John Wiley and Sons, New York, pp. 255-288.

Hagemeyer, J. and Waisel, Y., 1988. Excretion of ions (Cd^{2+}, Li^+, Na^+, and Cl^-) by *Tamarix aphylla*. Physiologia Plantarum 73, 541-546.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53(366), 1-11.

Hampp, R. and Lendzian, K., 1974. Effect of lead ions on chlorophyll synthesis. Die Natuurwissenschaften 61, 218-219.

Harbison, P., 1986. Mangrove muds – a sink and a source for trace metals. Marine Pollution Bulletin 17, 246-250.

Harvey, D.M.R., 1986. Applications of X-ray microanalysis in botanical research. Scanning Electron Microscopy 3, 953-973.

Haslett, B.S., Reid, R.J., Rengel, Z., 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Annals of Botany 87, 379-386.

Haughey, M.A., Anderson, M. A., Whitney, R.D., Taylos, W.D., Losee, R.F., 2000. Forms and fate of Cu in a source drinking water reservoir following $CuSO_4$ treatment. Water Resourses 34, 3440-3452.

Heale, E.L. and Ormrod, D.P., 1982. Effects of nickel and copper on *Acer rubrum*, *Cornus stolonifera*, *Lonicera tatarica* and *Pinus resinosa*. Canadian Journal of Botany 60(12), 2674-2681.

Herren, T. and Feller, U., 1994. Transfer of zinc from xylem to phloem in the peduncle of wheat. Journal of Plant Nutrition 17, 1587-1598.

Herren, T. and Feller, U., 1996. Effect of locally increased zinc contents on zinc transport from the flag leaf lamina to the maturing grains of wheat. Journal of Plant Nutrition 19, 379-387.

Hoagland, D.R. and Arnon, D.I., 1950. The water culture method for growing plants without soil. California Agriculture Exp. St. Circular 347, 1-32.

Hogarth, P. J., 1999. The Biology of Mangroves. Oxford University Press, New York.

Howeler, R. H., 1983. Análisis del tejido vegetal en el diagnóstico de problemas nutricionales algunos cultivos tropicales. Centro Internacional de Agricultura Tropical. California, Colombia, p. 28.

Hsiao, T.C., 1973. Plant responses to water stress. Annual Review of Plant Physiology 24, 519-570.

Hutchings, P. and Saenger, P., 1987. The ecology of mangroves. University of Queensland Press, St. Lucia, Queensland, Australia.

Japenga, J., Zschuppe, K.H., DeGroot, A.J., Salomons, W., 1990. Heavy metals and organic micropollutants in flood plain of the River Waal, a distributary of the River Rhine, 1958-1981. Netherlands Journal of Agricultural Sciences 38, 381-397.

Jordan, M. J., 1975. Effects of zinc smelter emissions and fire on a chestnut-oak woodland. Ecology 56, 78-91.

Kabata- Pendias, A. and Pendias, H., 1984. Trace elements in soils and plants. CRC Press, Florida.

Kadukova, J. and Kalogerakis, N., 2007. Lead accumulation from non-saline and saline environment by *Tamarix smyrnensis* Bunge European journal of Soil Biology 43, 216-223.

Kahle, H., 1993. Response of root of trees to heavy metals. Environmental and Experimental Botany 33(1), 99-119.

Kalac, P. and Svoboda, L., 2000. A review of trace element concentrations in edible mushrooms. Food Chemistry 69(3), 273-281.

Kastori, R., Plesnicar, M., Sakac, D., Pankovic, D., Arsenihjevic-Maksimovic, I., 1998. Effect of excess lead on sunflower growth and photosynthesis. Journal of Plant Nutrition 21(1), 75-85.

Katz, S. and Santilli, V., 1962. The reversible reaction of tobacco mosaic virus ribonucleic acid and mercury chloride. International Journal of Biochemistry, Biophysics and Molecular Biology 55, 621-626.

Kawade, Y., 1963. The interaction of mercuric chloride with ribonucleic acids and polyribonucleotides. Biochemical and Biophysical Research Communication 10, 204-208.

Koeppe, D.E., 1981. Effect of heavy metal pollution on plants. Vol. 1. In: Lepp, N.W. (Ed.), Effects of trace metals on plant function. Applied Science Publishers Ltd., London, UK, p. 55.

Kramer, D. and Preston, J., 1978. A modified method of X-ray microanalysis of bulk-frozen plant tissue and its application to the problem of salt exclusion to mangrove roots. Microscopica Acta 2, 193-200.

Krupa, Z., Baranowska, M., Orzol, D., 1996. Can anthocyanins be considered as heavy metal indicator in higher plants? Acta Physiologica Plantarum 18(2), 147-151.

Kuenzler, E.J., 1974. Mangrove swamp systems. In: Coastal ecological systems of the United States, (Eds.), Odum, H. T., Copeland, B. J. and McMahen, E. A. Conservation Foundation, Washington D.C., pp. 346-371.

Kumar, N.P.B.A., Dushenkov, V., Motto, H., Raskin, I., 1995. Photoextraction: The use of plants to remove heavy metals from soils. Environmental Science and Technology 29, 1232-1238.

Lagriffoul, A., Mocquot, B., Mench, M., Vangronsveld, J., 1998. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plant (*Zea mays* L.). 200(2), 241-250.

Lawton, J.R., Todd, A., Naidoo, D.K., 1981. Preliminary investigations into the structure of the roots of the mangroves, *Avicennia marina* and *Bruguiera gymnorrhiza*, in relation to ion uptake. New Phytologist 88, 713-722.

Lee, K. C., Cunningham, B.A., Chung, K.H., Paulsen, G.M., Liang, G.H., 1976. Lead effect on several enzymes and nitrogenous compounds in soybean leaf. Journal of Environmental Quality 5 (4), 357-359.

Lenka, M., Panda, K.K., Panda, B.B., 1992. Monitoring and assessment of mercury pollution in the vicinity of a chloralkali plant. 4. Bioconcentration of mercury in in-situ aquatic and terrestrial plants at Ganjam, India. Archives of Environmental Contamination and Toxicology 22(2), 195-202.

Liao, Z., 1993. The environmental chemistry and biological effects of microelement. Beijing, China Environmental Science Press.

Liphschitz, N. and Waisel, Y., 1982. Adaptations of plants to saline environments: salt excretion and glandular structure. In: D.N. Sen and K.S. Rajpurohit (Editors), W. Junk: The Hague. Tasks for Vegetation Sciences 2, 197-214.

Little, E.L., Jr., 1983. Common Fuelwood Crops: A handbook for their identification. Parsons, W.V., McClain Printing Company.

Long, S.P., Humphries, S. Falkowski, P.G., 1994. Photoinhibition of photosynthesis in nature. Annual Review of Plant Physiology and Plant Molecular Biology 45, 633-662.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19(1), 81-97.

Lugo A.E. and Snedaker, S. C., 1974. The ecology of mangroves. Annual Review of Ecology and Systematics 5, 39-64.

Lugo, A.E., Brown, S., Brinson, M. M., 1990. Concepts in wetland ecology. In: A. E. Lugo, M. Brinson and S. Brown (Eds.), Ecosystems of the World. 15. Forested Wetlands. Amsterdam: Elservier Science, pp. 53-85.

Lugon-Moulin, N., Zhang, M., Gadani, F., Rossi, L., Koller, D., Krauss, M., Wagner, G.J., 2004. Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana tabacum* L.) and other plants. Advances in Agronomy 83, 111-180.

Ma, M., Zhu, W., Wang, Z., Witkamp, G., 2003. Accumulation, assimilation and growth inhibition of copper on freshwater alga (*Scenedesmus subspicatus* 86.81 SAG) in the presence of EDTA and fulvic acid. Aquatic Toxicology 63, 221-228.

MacFarlane, G.R. and Burchett, M.D., 1999. Zinc distribution and excretion in the leaves of the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Environmental and Experimental Botany 41, 167-175.

MacFarlane, G.R. and Burchett, M.D., 2000. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stresss in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin 42(3), 233-240.

MacFarlane, G.R. and Burchett, M.D., 2001. Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Aquatic Botany 68, 45-59.

MacFarlane, G.R. and Burchett, M.D., 2002. Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove Avicennia marina (Forsk.) Vierh. Marine Environmental research 54, 65-84.

MacFarlane, G.R., 2002. Leaf biochemical parameters in *Avicennia marina* (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. Marine Pollution Bulletin 44, 244-256.

MacNicol, R.D. and Beckett, P.H.T., 1985. Critical tissue concentrations of potential toxic elements. Plant and Soil 85, 107-129.

Malhotra, S.S. and Khan, A. A., 1980. Effects of sulphur dioxide and other air pollutants on acid phosphatase activity in Pine seedlings. Biochemie und Physiologie der Pflanzen 175, 228-236.

Mallick, N. and Mohn, F.H., 2003. Use of chlorophyll fluoresecence in metal-stress research: a case study with the green microalga *Scenedesmus*. Ecotoxicology and Environmental Safety 55, 64-69.

Marschner, H., 1995. Mineral nutrition in higher plants, 2nd Edn., Academic Press, London. pp. 333-347.

Mohan, B.S. and Hosetti, B.B. 1999. Aquatic plants for toxicity assessment. Environmental Research 81, 259-274.

Monni, S., Bücking, H., Kottke, I., 2002. Ultrastructural element localization by EDXS in *Empetrum nigrum*. Micron 33, 339-351.

Nag, P., Paul, A.K., Mukherji, S., 1980. Effects of mercury, copper and zinc on the growth, cell division, GA-induced alpha-amylase synthesis and membrane permeability of plant tissues. Indian Journal of Experimental Biology 18(8), 822-827.

Naidoo, G., Tuffers, A.V. and Von Willert, D.J., 2002. Changes in gas exchange and chlorophyll fluorescence characteristics of two mangroves and a mangrove associate in response to salinity in the natural environment. Trees 16, 140-146.

Naidoo, G. and Chirkoot, D., 2004. The effects of coal dust on photosynthetic performance of a mangrove *Avicennia marina* in Richards Bay, South Africa. Environmental Pollution 127, 359-366.

Naidoo, G. and Naidoo, Y., 2005. Coal dust pollution effects on wetland tree species in Richards Bay, South Africa. Wetlands Ecology and Management 13, 509-515.

Naidoo, G., 2006. Factors contributing to dwarfing in the mangrove, *Avicennia marina*. Annals of Botany 97, 1095-1101.

Nalewajko, C. and Olaveson, M..M., 1995. Differential responses of growth, photosynthesis, respiration and phosphate uptake to copper in copper-tolerant and copper-intolerant strains of *Scenedesmus acutus* (Chlorophyceae). Canadian Journal of Botany 73, 1295-1303.

Neales, T.F. and Sharkey, P.J., 1981. Effect of salinity on growth and mineral and organic constituents of the halophyte *Disphyma australe* (Soland.), J.M. Black. Australian Journal of Plant Physiology 8: 165-179.

Neumann, D., Nieden, U., Lichtenberger, O., Leopold, I., 1995. How does *Armeria maritima* tolerate high heavy metal concentrations? Journal of Plant Physiology, 146, 704-717.

Nriagu, J.O., 1979. The global copper cycle. In: Nriagu, J.O. (Eds.), Copper in the environment. Part I: Ecological cycling. John Wiley and Sons, New York, p. 1-17.

Nriagu, J.O. and Pacyna, J.M., 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333, 134-139.

Oberländer, H. E. and Roth, K., 1978. Effect of the heavy metals chromium, nickel, copper, zinc, cadmium, mercury and lead on uptake and translocation of K and P by young barley plants. Z. Pflanzenernaehr Bodenk 141, 107-116.

Odum, W.E., McIvor, C.C. and Smith, J., 1982. The ecology of the mangroves of South Florida: A community profile. Biological Services Programme, FWS/OBS-81/24. Bureau of Land management, Fish and Wildlife Service. U.S.A. Department of the Interior, p. 154

Osmond, C.B., 1994. What is Photoinhibition? Some implications for the energetics of photosynthesis. Biochimica et Biophysica Acta 639, 982-988.

Page, V. and Feller, U., 2005. Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants. Annals of Botany 96, 425-434.

Page, V., Weisskopf, L., Feller, U., 2006. Heavy metals in white lupin: uptake, root-to-shoot transfer and redistribution within the plant. New Phytologist 171, 329-341.

Päivöke, A., 1983. The long-term of lead and arsenate on the growth and development, chlorophyll content and nitrogen fixation of the garden pea. Annales Botanici-Fennici 20(3), 297-306.

Palmer, E. and Pitman, N., 1973. Trees of Southern Africa covering all known Indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho and Swaziland. Dr. L.E.W. Codd and Botanical Research Institute, Volume 3. A.A. Balkema, Cape Town, pp. 1947; 1971-1975.

Patra, M. and Sharma, A., 2000. Mercury toxicity in plants. The Botanical Review 66(3), 379-422.

Pearson, J.N., Rengel, Z., Jenner, C.F., Graham, R.D., 1995. Transport of zinc and manganese to developing wheat grains. Physiologia Plantarum 95, 449-455.

Peng, L., Wenjian, Z., Zhenji, L. 1997. Distribution and accumulation of heavy metals in *Avicennia marina* community in Shenzhen, China. Journal of Environmental Science 9(4), 472-479.

Perales-Vela, H.V., González-Moreno, S., Montes-Horcasitas, C., Cañizares-Villanueva, R.O., 2007. Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae). Chemosphere 67, 2274-2281.

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H., and Power, E.A., 1997. Ecotoxicology of tropical marine ecosystems. Environmental Toxicology and Chemistry 16, 12-40.

Peterson C. A. and Rauser, W. E., 1979. Callose deposition and photoassimilate export in *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiology 63, 1170-1174.

Pichtel, J., Kuroiwa, K., Sawyerr, H.T., 2000. Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. Environmental Pollution 110, 171-178.

Porter, J.R. and Sheridan, R.P., 1981. Inhibition of nitrogen fixation in alfalfa by arsenate, heavy metals, fluoride, and simulated acid rain. Plant Physiology 68: 143-148.

Potts, M., 1984. Nitrogen fixation in mangrove forests. In: Por, F.D. and Dor, I. (Eds.), Hydrobiology of the Mangal. Dr W. Junk Publishers, The Hague, pp. 155-162.

Powles, S.B., 1984. Photoinhibition of photosynthesis induced by visible light. Annual Review of Plant Physiology 35, 15-44.

Prasad, M.N.V. and Strzalka, K., 1999. Impact of heavy metals on photosynthesis. In: Heavy metal stress in plants: from molecules to ecosystems (Eds.), M.N.V. Prasad and J. Hagemeyer, pp. 117-138.

Prisco, J.T. and O'Leary, J.W., 1972. Enhancement of intact bean leaf senescence by NaCl salinity. Physiologia Plantarum 27, 95-100.

Przywara, G. and Stepniewski, W., 1999. The influence of waterlogging at different temperatures on penetration depth and porosity of roots and on stomatal diffusive resistance of pea and maize seedlings. Acta Physiologiae Plantarum 21: 405-411.

Quarto, A., 2000. The mangrove forest: background paper. The Ramsar Convention on Wetlands.

Rajesh, K.M., Jose, M.V., Ramana, K., Rizwana, A., Thomas, C.H., Priti, M., 1996. Optical spectroscopic and reerse-phase HPLC analyses of Hg (II) binding to phytochelatins. Biochemical Journal 314, 73-82.

Rauser, W.E. and Samarakoon, A.B., 1980. Vein loadings in seedlings of *Phaseolus vulgaris* exposed to cobalt, nickel and zinc. Plant Physiology 65, 578-583.

Rawson, H.M., Hindmarsh, J.H., Fischer, R.A., Stockman, Y.M., 1983. Changes in leaf photosynthesis with plnt ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. Australian Journal of Plant Physiology 10, 503-514.

Rey, J.R., 1999. Mangroves. Florida Medical Entomology On-line Publications, University of Florida, I.F.A.S.

Ribeyre, F. and Boudou, A., 1994. Experimental study of inorganic and methylmercury bioaccumulation by four species of freshwater rooted macrophytes from water and sediment contamination sources. Ecotoxicology and Environmental Safety 28, 270-286.

Riedel, G.F. and Sanders, J.G., 1988. Factors affecting the bioavailability of toxic trace elements to estuarine organisms. In: Lynch, M.P., Krome, E.C. (Eds.), Proceedings on understanding the estuary: Advances in Chesapeake Bay Research, Baltimore, MD, USA. pp. 121-131.

Riesen, O. and Feller, U., 2005. Redistribution of nickel, cobalt, manganese, zinc and cadmium via the phloem in young and in maturing whet. Journal of Plant Nutrition 28, 421-430.

Robertson, A.I., 1992. Concluding remarks: research and mangrove conservation. In: Robertson, A.I. and Alongi, D.M. (Eds.), Tropical Mangrove Ecosystems, American Geophysical Union, Washington, DC.

Römer, W., Kang, D.K., Egle, K., Gerke, J., Keller, H., 2000. The acquisition of cadmium by *Lupinus albus* L., *Lupinus angustifolius* L. and *Lolium multiflorum* Lam. Journal of Plant Nutrition and Soil Science 163, 623-628.

Römer, W., Egle, K., Kang, D.K., Keller, H., Gerke, J., 2002. L'absorption de Cd par différentes variétés de lupins (*Lupinus albus* et *Lupinus angustifolius*) comparée à celled u ray-grass (*Lolium multiflorum*) en function de la fertilization phosphatée. Agronomie 22, 431-442.

Rutzler, K. and Feller, I.C., 1996. Caribbean mangrove swamps. Scientific American: March, pp. 94-99.

Saenger, P., Hegerl, E.J. and Davie, J.D.S., 1983. Global status of mangrove ecosystems. Environmentalist 3, 1-88.

Samarakoon, A.B. and Rauser, W.E., 1979. Carbohydrate levels and photoassimilate export from leaves of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiology 63, 1165-1169.

Samecka-Cymerman, A. and Kempers, A.J., 2007. Heavy metals in aquatic macrophytes from two small rivers polluted by urban, agricultural and textile industry sewages SW Poland. Architectural Environmental Contamination and Toxicology 53, 198-206.

Sanità di Toppi, L. and Gabbrielli, R., 1999. Response to cadmium in higher plants. Environmental and Experimental Botany 41, 105-130.

Sauvé, S., Norvell, W.A., McBride, M., Hendershot, W., 2000. Speciation and complexation of cadmium in extracted soil solutions. Environmental Science and Technology 34, 291-296.

Scholander, P. F., Hammel, H.T., Hemmingson, E., Garey, W., 1962. Salt balance in mangroves. Plant Physiology 37, 722-729.

Scholander, P.F., 1968. How mangroves desalinate sea water. Plant Physiology 21, 251-261.

Schützendübel, A. and Polle, A. 2002. Plant responses to abiotic stress: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany. 53(372), 1351-1365.

Seaward, M.R.D. and Richardson, D.H.S., 1990. Atmospheric sources of metal pollution and effects on vegetation. In: Shaw, A.J. (Ed.), Heavy metal tolerance in plants: Evolutionary aspects. Boca Raton, FL, USA: CRC Press, pp. 75-92.

Sekerka, V. and Bobak, M., 1974. Influence of lead upon the plant cell. Acta Fac. Rerum Nat. Universitatis Comenianae Physiologia Plantarum 9, 1-12.

Sekhar, K. C., Kamala, C.T., Chary, N.S., Balaram, V., Garcia, G., 2005. Potential of *Hemidesmus indicus* for phytoextraction of lead from industrially contaminated soils. Chemosphere 58(4), 507-514.

Sela, M., Garty, J., Tel-Or, E., 1989. The accumulation and the effect of heavy metals on the water fern, *Azolla filiculoidees*. New Phytologist 112, 7-12.

Shenyu, M. and Chen, G., 1998. Allocation and migration of lead in simulated wetland system of *Kandelia candel*. China Environmental Science 18(1), 48-51.

Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q., Wong, M.H., 2002. Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. Environmental Pollution 120, 445-453.

Silva, C.A., Lacerda, L.D., Rezendde, C.E., 1990. Metal reservoirs in a red mangrove forest. Biotropica 22, 339-345.

Singh, O.P. and Sharma, A., 1980. Effects of certain metallic pollutants on genetic system. The Nucleus. 23, 15-29.

Smith III, T.J., 1987. Effects of light and intertidal position on seedling survival and growth in tropical tidal forests. Journal of Marine Biology and Ecology 110, 133-146.

Sobrado, M.C. and Ball, M.C., 1999. Light use in relation to carbon gain in the mangrove, Avicennia marina, under hypersaline conditions. Australian Journal of Plant Physiology 26, 245-251.

Spurr, A.R., 1980. Applications of X-ray microanalysis in botany. Scanning Electron Microscopy 11, 535-564.

Starodub, M.E. and Wong, P.T.S. 1987. Short term and long term studies on individual and combined toxicities of Cu, Zn and Pb to *Scenedesmus quadricauda*. The Science of the Total Environment 63, 101-110.

Steinke, T.D., 1995. A general review of the mangroves of South Africa. (Ed.), G.I. Cowan. Wetlands of South Africa.

Stevenson, F.J., 1986. Cycles of soil – Carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and Sons, New York, p. 380.

Stiborova, M. and Leblova, S., 1976. Purification and properties of rape alcohol dehydrogenase. Physiologia Plantarum 38(3), 176-180.

Stiborova, M., Doubravova, M., Leblova, S., 1986a. A comparative study of the effect of heavy metal ions on ribulose-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase. Biochemistry and Physiology Pflanz 181, 373-379.

Stiborova, M., Hromadkova, R., Leblova, S., 1986b. Effects of ions of heavy metals on the photosynthetic characteristics of maize (*Zea mays* L.). Biologica (Bratislava) 41, 1221-1228.

Stoltz, E. and Greger, M., 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environmental and Experimental Botany 47, 271-280.

Storey, R. and Wyn Jones, R.G. 1979. Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. Plant Physiology. 63(1), 156-162.

Su, N., Zhang, J., Wang, Y., 1994. The pollution band evaluation of Cd in soil in Hujian Province. Acta Hujian-Agriculture University 23(4), 434-439.

Takemura, T., Hanagata, N., Karube, I., Dubinsky, Z., 2000. Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. Aquatic Botany 68, 15-28.

Tam, N.F.Y. and Wong, Y.S., 1993. Retention of nutrients and heavy metals in mangrove sediments receiving wastewater of different strengths. Environmental Technology 14, 719-729.

Tam, N.F.Y. and Wong, Y.S., 1995. Retention and distribution of heavy metals in mangrove soils receiving wastewater. Environmental Pollution 94, 283-291.

Tam, N.F. Y. and Wong, Y.S., 2000. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. Environmental Pollution 110, 195-205.

Teas, H.J., 1979. Silviculture with saline water. In: The biosaline concept. Plenum Publishing Corporation, pp. 117-161.

Thomas, C. and Eong, O.J., 1984. Effects of the heavy metals Zn and Pb on *R. mucronata* and *A. alba* seedlings. In: Soepadmo, E., Rao, A.M., MacIntosh, M.D. (Eds.), Proceedings of the Asian Symposium on Mangroves and Environment Research and Management, University of Malaysia, ISME, pp. 568-574.

Thompson,W.W., 1975. The structure and function of salt glands. In: Poljakoff-Mayber, A., Gale, J. (Eds.), Plants in Saline Environments. Springer, New York, pp. 118-146.

Thomson, W.W., Faraday, C.D. and Oross, J.W., 1988. Salt glands. In: D.A. Baker and J.L. Hall (Editors), Solute transport in plant cells and tissues, Longman Scientific and Technical, Essex, pp. 498-537.

Tomlinson, P.B., 1986. The botany of mangroves. Cambridge University Press, London, p. 413.

Twilley, R.R., 1998. Mangrove wetlands. In: M.G. Messina and W.H. Conner (Eds.), Southern forested wetlands and management. Boca Raton, Florida: Lewis Publishers, pp. 445-473.

USEPA, 1995 Clean Water Act. Guidance is in the water quality standards handbook, Second Edition, pp-1-9.

Valladeres, F. and Pearcy, R.W., 1997. Interactions between water stress, sunshade acclimation, heat tolerance and Photoinhibition in the sclerophyll *Heteromeles arbutifolia*. Plant, Cell and Environment 20, 25-36.

Van Assche, F., Clijsters, H., Marcelle, R., 1979. Photosynthesis and plant development. In: Marcelle, R. (Eds.), W. Junk Publishers, The Hague, p. 175.

Van Assche, F. and Clijsters, H., 1986. Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentrations of zinc: effects on electron transport and photophorylation. Physiologia Plantarum 66(4), 717-721.

Van Assche, F. and Clijsters, H., 1990. Effects of metals on enzyme activity in plants. Plant, Cell and Environment 13, 195-206.

Vangronsveld, J. and Clijsters, H., 1994. Toxic effects of metals. In: Farago, M.E. (Ed.), Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity. VCH, New York, pp. 149-177.

Vannucci, M., 2000. What is so special about mangroves? Brazilian Journal of Biology, 61(4), 599-603

Verkleij, J.A.C. and Schat, H., 1990. Mechanisms of metal tolerance in plants. In: Heavy metal Tolerance in Plants; Evolutionary Aspects (Ed.), A.J. Shaw. CRC Press, Boca Raton, FL, pp. 179-193.

Verma, S. and Dubey, R.S., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science 164, 645-655.

Wagner, G.J., 1991. Secreting glandular trichomes: more than just hairs. Plant Physiology 96, 675-679.

Wainwright, S.J. and Woolhouse, H.W., 1977. Some physiological aspects pf copper and zinc tolerance in *Agrostis tenuis* Sibth.: cell elongation and membrane damage. Journal of Experimental Botany 28, 1029-1036.

Waisel, Y., 1972. Biology of halophytes. Academic Press, New York.

Waisel, Y., Eshel, A. and Agami, M., 1986. Salt balance of leaves of the mangrove *Avicennia marina*. Physiologia Plantarum 61, 67-72.

Wallace, A., 1984. Effect of phosphorus deficiency and copper excess on vegetation growth of bush bean plants in solution culture at two different solution pH levels (toxicity). Journal of Plant Nutrition 7(1/5): 603-608.

Walsh, G.E., Ainsworth, K.A., Rigby, R., 1979. Resistance of the red mangrove (*Rhizophora mangle* L.) seedlings to Pb, Cd and Hg. Biotropica 11(1), 22-27.

Wang, S., Li, J., Shi, S., 2001. Geological disease caused by ecological environment: An example of a cancer-inflicted village in Shanxi Province. Environmental Protection 5, 42-43, 46.

Wang, Y., 2004. Phytoremediation of mercury by terrestrial plant. Department of Botany Stockholm University. Wales, UK. pp. 1-43.

Ward, C.J. and Steinke, T.D., 1982. A note on the distribution and approximate areas of mangroves in South Africa. South African Journal of Botany 1, 51-53.

Weis, P., Windham, L., Burke, D.J., Weis, J.S., 2002. Release into environment of metals by two vascular salt marsh plants. Marine Environmental Research 54, 325-329.

Welch, R.M., 1995. Micronutrient nutrition of plants. Critical Reviews in Plant Science 14, 49-82.

Williams, L. E., Pittman, J.K., Hall, J.L., 2000. Emerging mechanisms for heavy metal transport in plants. Biochimica et Biophysica Acta 1465, 104-126.

Wittig, R., 1993. General aspects of bio-monitoring heavy metals by plants. In: Market, B. (Ed.), Plants as bio-monitors. Indicators for heavy metals in the terrestrial environment. VCH Press, Weinheim, pp. 3-27.

Wong, Y.S., Tam, N.F.Y., Chen, G.Z., Ma, H., 1997. Response of *Aegiceras corniculatum* to synthetic sewage under simulated tidal conditions. Hydrobiologia 352, 89-96.

Woolhouse, H.M. and Walker, S., 1981. The physiological basis of copper toxicity and copper tolerance in higher plants. In: J.F. Loneragan, A.D. Robson, and R.D. Graham (Eds.), Encyclopedia of plant physiology: new series, vol. 12C, Berin: Springer, pp.246-299.

Woolhouse, H.M., 1983. Toxicity and tolerance in the responses of plants to metals. In: O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (Eds.), Encyclopedia of plant physiology: new series, vol. 12C, Berlin: Springer, pp. 246-299.

Wozny, A. and Krzeslowska, M., 1993. Plant cell response to Pb. Acta Societatis Botanicorum Poloniae 62, 101-105.

Wozny, A., 1995. Lead in Plant Cells. Sorus, Poland.

Wu, Y., Chen, T., Zhang, X., 1989. Ecological study on the Cd pollution in Zhangshi irrigation at Shenyang. Acta Ecologica Sinica 9(1), 21-26.

Ximénez-Embún, P., Rodríguez-Sanz, B., Madrid-Albarrán, Y., Cámara, C., 2002. Uptake of heavy metals by lupin plants in artificially contaminated sand: preliminary results. International Journal of Environmental Analytical Chemistry 82, 805-813.

Yañez-Arancibia, A.A.L., Lara-Domínguez, J.L., Rojas-Galaviz, P., Sánchez-Gil, J.W., Day and Madden, C.J., 1988. Seasonal biomass and diversity of estuarine fishes coupled with tropical habitat heterogeneity (southern Gulf of Mexico). Journal of Fisheries Biology 33, 191-200.

Ye, Z.H., Whiting, S.N., Lin, Z.Q., Lytle, C.M., Qian, J.H., Terry, N. 2001. Removal and distribution of iron, manganese, cobalt and nickel within a Pennsylvania constructed wetland treating coal combustion by-product leachate. Journal of Environmental Quality 30, 1464-1473.

Ye, Y., Tam, N.F.Y., Wong, Y.S., Lu, C.Y., 2003. Growth and physiological responses of two mangrove species (*Bruguiera gymnorrhiza* and *Kandelia candel*) to waterlogging. Environmental and Experimental Botany 49, 209-221.

Yim, M.W. and Tam, N.F.Y., 1999. Effects of wastewater-borne heavy metals on mangrove plants and soil microbial activities. Marine Pollution Bulletin 39, 179-186.

Zenk, M.H., 1996. Heavy metal detoxification in higher plants-A review. Gene 179(1), 21-30.

Zhang, N., 1999. Advance of the research on heavy metals in soil-plant system. Advances in Environmental Science 7(4), 30-33.

Zhang, F-Q., Wang, Y-S., Lou, Z-P., Dong, J-D., 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of the two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere 67, 44-50.

Zheng, W.J. and Chen, X.Y., 1997. Accumulation and biological cycling of heavy metal elements in *Rhizophora stylosa* mangroves in Yingluo Bay, China. Marine Ecology and Programming Series 159, 293-301.

Zimdahl, R. L. and Arvik, J.H., 1973. Lead in soils and plants: A literature review. CRC. Critical Reviews in Environmental Control 3(2), 213-224.

Zimdahl, R. L., 1976. Entry and movement in vegetation of lead derived from air and soil sources. Journal of Air Pollution Control Association 26, 655-660.

Zornoza, P., Vázquez, S., Esteban, E., Fernández-Pascual, M., Carpena, R., 2002. Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiology and Biochemistry 40, 1003-1009.

Websites

http://www.epa.qld.gov.au/nature_conservation/habitats/mangroves_and_wetlands/mangrov es/ Accessed 31 March 2004.

http://forest.and.nic.in/frst-mangroves1.htm Accessed 6 September 2004.

http://www.enhg.org/b/b32/32_02.htm Accessed 27 April 2006.