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Instituto de Ciências Biológicas
Programa de Pós-Graduação em Biologia Vegetal

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MANGROVES AS PHYTOREMEDIATION AGENTS: a case study of young plant responses to cadmium toxicity

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Versão final

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"Mangroves as phytoremediation agents: A case study of young plant responses to cadmium toxicity"

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” Não nos esqueçamos de que a floresta se levantou de sementes quase invisíveis, de que o rio se forma das fontes pequeninas e de que a luz do Céu, em nós mesmos, começa de pequeninos raios de amor a se nos irradiarem do coração.” Meimei

(F.C.Xavier)

Resumo

Manguezais são ecossistemas de transição entre os ambientes terrestre e marinho, comuns em regiões tropicais e subtropicais. Eles possuem um papel importante na manutenção do equilíbrio ecológico da costa, além de oferecer diversos outros serviços ecossistêmicos importantíssimos, como proteger a linha costeira e promover o sequestro de dióxido de carbono (CO_2) e de contaminantes. Na primeira parte deste trabalho serão apresentados alguns exemplos de como é possível utilizar as plantas de mangue, com ou sem associação com outros elementos ou microrganismos para remediar solos e águas contaminados. Em seguida, um estudo das respostas morfofisiológicas de plantas jovens de três espécies de manguezal - *Avicennia schaueriana*, *Laguncularia racemosa* e *Rhizophora mangle* - expostas ao metal pesado cádmio (Cd), altamente tóxico e sem função biológica nas plantas. Foi observado que a espécie *A. schaueriana*, além de possuir maior tolerância a salinidade mais altas, também foi a espécie mais tolerante ao cádmio. Já a espécie *L. racemosa*, a menos tolerante ao sal, foi também a mais suscetível ao metal. No último capítulo, quando apresentamos a associação de cádmio com silício (Si), foi possível observar uma resposta positiva no desenvolvimento dos propágulos e das plantas da espécie *L. racemosa*. O silício vem sendo considerado um atenuador de estresses abióticos em diferentes espécies vegetais. Considera-se de grande importância conhecer as respostas morfofisiológicas das plantas jovens de mangue ao estresse induzido por metais tóxicos, uma vez que essas plantas poderão ser selecionadas para estratégias de restauração e descontaminação, bem como de manejo desse ecossistema.

Palavras-chave: Metal traço. Fitorremediação. Estresse abiótico. Toxicidade. Serviços ecossistêmicos.

Abstract

Mangroves are transitional ecosystems between land and sea, common in tropical and subtropical regions. They play a significant role in maintaining the ecological balance of the coast, besides offering several other important ecosystem services, such as protecting the coastline and promoting the sequestration of carbon dioxide (CO_2) and contaminants. In the first part of this work, some examples will be presented of how it is possible to use mangrove plants, with or without association with other elements or microorganisms to remedy contaminated soils and waters. Then, a study of the morphophysiological response of young plants of three mangrove species - *Avicennia schaueriana*, *Laguncularia racemosa*, and *Rhizophora mangle* - exposed to the heavy metal cadmium (Cd), which is highly toxic and has no biological function in plants. It was observed that the species *A. schaueriana*, besides having the highest tolerance to salinity, was also the most tolerant to Cadmium. On the other hand, the species *L. racemosa*, the least tolerant to salt, was also the most susceptible to the metal. In the last chapter, however, when we presented the association of cadmium and silicon (Si), it was possible to observe a positive response in the development of *L. racemosa* propagules and plants. Silicon has been considered an attenuator of abiotic stresses in different plant species. It is considered of great importance to know the morphophysiological responses of young mangrove plants to stress induced by toxic metals, since these plants may be selected for restoration and decontamination strategies, as well as for the management of this ecosystem.

Keywords: Trace metal. Phytoremediation. Abiotic stress. Toxicity. Ecosystem services.

Sumário

INTRODUÇÃO GERAL

Os manguezais desempenham um papel fundamental na manutenção da biodiversidade em ambiente estuarino, além de desempenhar uma função ecológica, social e econômica para as comunidades do entorno (Mitra, 2020; Aljahdali et al., 2020). Nas últimas décadas vem sendo observada uma significativa redução das áreas de manguezal em todo o globo e isso se deve principalmente ao desmatamento, contaminação do ecossistema por compostos orgânicos, como hidrocarbonetos e organoclorados, descarte incorreto de lixo e águas residuais de indústrias, e o uso indiscriminado de agroquímicos, contendo elementos tóxicos que acabam chegando até as águas oceânicas, e consequentemente ao mangue (Sandilyan & Kathiresan 2012; Li et al. 2015).

No Brasil, metais tóxicos são frequentemente identificados no sedimento dos manguezais (De Andrade & Patchineelam 2000; Santos et al. 2008; Oliveira et al. 2014). Na região da Bacia do Rio de Contas, Bahia, a presença de metais tóxicos, como chumbo (Pb), mercúrio (Hg) e cádmio (Cd), vem sendo observada não só no sedimento como também nos corpos de água (Paula et al. 2010). Na tabela 1 é possível observar o teor de Cd no solo de algumas regiões de manguezal do Brasil e do mundo mostrando a relevância deste elemento tóxico como contaminante neste ambiente.

O Cd é um elemento metálico de número atômico 48, de carga iônica Cd^{2+}, com elevada massa atômica (112.41 g mol^{-1}) e densidade (8.65 g cm^{-3}), e considerado um metal tóxico por não possuir função fisiológica conhecida em animais e plantas e é capaz de se acumular na cadeia alimentar (Duffus 2002; Arumugam et al., 2018). Pode ser disponibilizado na natureza por diferentes formas: naturalmente através do intemperismo de rochas; por atividades humanas como utilização indiscriminada de fertilizantes fosfatados na agricultura; através de pilhas e baterias descartadas incorretamente; além de processos de mineração, fundição e refinamento de metais não ferrosos, produção de ferro e aço, fabricação de plástico, fabricação de tintas e vernizes contribuem para a contaminação ambiental (Shanmugaraj et al., 2019).

Tabela 1 - Teor de Cd no solo de manguezal (mg kg^{-1}) no Brasil e no mundo.

Teor de Cd no solo (mg kg^{-1})	País	Região	Referência
0.3-4.9	Brasil	Sapetiba, Rio de Janeiro	Gomes et al, 2009
0.3-1.2	Brasil	Camocim, Ceará	Miola et al, 2016
1.1-3.7	Brasil	Bahia de Guanabara, Rio de Janeiro	Silveira et al., 2017
0.5-3.5	China	Futian	Shi et al., 2019
1.0 - 3.3	Iran	Azini Bay, Hormozgan	Ghasemi et al., 2018
0.2-23	Índia	Costa oeste Jam-Salaya	Kumar et al., 2015
0.05-0.5	Nova Zelândia	Mangawhai Harbour	Bastakoti et al, 2018

A entrada do Cd no sistema radicular das plantas ocorre principalmente através de transportadores de membrana, utilizando, por exemplo, os canais dos íons Ca^{2+}, Fe^{2+}, Mn^{2+} e Zn^{2+} devido à carga iônica, raio iônico e comportamento químico semelhantes (Ismael et al., 2018; Haider et al., 2021). O Cd, quando absorvido pelas plantas, é capaz de afetar o crescimento e o desenvolvimento de plântulas e indivíduos adultos (Benavides et al., 2005). O Cd interfere na germinação de sementes, no crescimento e desenvolvimento de plantas, na absorção de nutrientes, promove dano oxidativo, impacta o sistema fotossintético, a produção de aminoácidos e proteínas e afeta também as relações hídricas e nutricionais (Haider et al., 2021). O metal é capaz de induzir alterações morfológicas e anatômicas de raízes, caules e folhas, assim como deformações da ultraestrutura celular (Garcia et al, 2018) e alterações no metabolismo, como respiração celular, alterações genéticas e morte celular (He et al 2017).

Algumas espécies de mangue podem ser menos ou mais tolerantes aos metais pesados (Bernini et al., 2010). Os mecanismos de defesa à toxicidade do Cd em plantas podem ser diferenciados em duas estratégias, ou seja, a tolerância e a evitação ao metal (He et al 2017). A estratégia de evitação inclui limitar a absorção de Cd na planta. O mecanismo de tolerância em plantas inclui o armazenamento e o acúmulo de Cd ligando-o a peptídeos e aminoácidos (Haider et al., 2021). Os manguezais por apresentarem diversos mecanismos de tolerância a estresses abióticos são importantes elementos na retenção de contaminantes e poluentes (Mitra, 2020).

Segundo Paula et al. (2010) a Bacia Hidrográfica do Rio de Contas, no estado da Bahia, recebe 0,41 toneladas de Cd ao ano provenientes da agroindústria, do descarte de lixo e de esgoto entre outras atividades. O município de Itacaré está inserido nesta bacia e as espécies *Avicennia schaueriana*, *Rhizophora mangle* e *Laguncularia racemosa* são espécies abundantes nesta região (Delabie et al. 2006).

Ao longo dos anos pode ser observado um declínio na extensão das áreas de manguezal nas costas do globo (Bryam-Brow et al 2020). O Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, 2018) estimou que o Brasil possui cerca de 1.398.966,10 ha de manguezais e que o estado da Bahia possui aproximadamente 89.932,00 ha (Fig. 1), o sendo quarto estado brasileiro com a maior extensão deste bioma. Atribui-se ao desmatamento, à urbanização e à poluição e contaminação como sendo as principais causas de degradação deste ecossistema (Andrade & Patchineelam 2000; Oliveira et al. 2014; Bryam-Brow et al 2020).

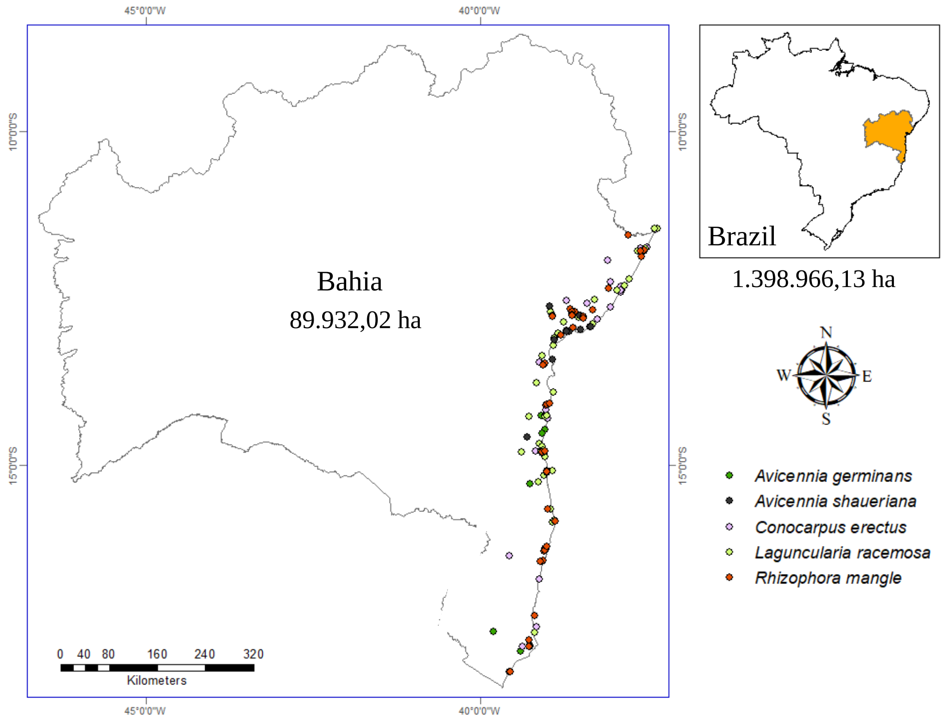

Figura 1 – Distribuição das espécies de manguezais ao longo do litoral do estado da Bahia.

A degradação do ecossistema compromete, significativamente, o estabelecimento e recrutamento de plantas jovens destas espécies neste ambiente (Aljahdali et al., 2020). Justificando-se a importância de fornecer ou melhorar as estratégias de restauração associadas ao ecossistema manguezal. Neste trabalho recompilamos da literatura informações sobre o uso de espécies de manguezais para remoção de contaminantes e propomos uma guia para a remediação de diferentes tipos de poluentes. Avaliamos a tolerância de três espécies de mangue ao Cd, sob a hipóteses que as espécies de mangue possuem tolerância diferencial à presença do Cd no meio, e que espécies mais tolerantes a salinidade serão mais tolerantes ao estresse pelo metal. Dentre estas três espécies selecionamos a mais susceptível para uma abordagem prática de atenuação do estresse propondo que o Cd acumula-se diferencialmente em propágulos, plântulas com cotilédone e plantas jovens da espécie *L. racemosa*, testamos a hipótese de que o silício é capaz de atenuar o estresse causado pelo cádmio nos estágios iniciais do desenvolvimento.

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CAPÍTULO 1: Mangrove Assisted Remediation and Ecosystem Services

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1. Mangrove Ecosystems

Mangroves are coastal ecosystems that encompass diverse gradients of latitude (30∘ N–37∘ S), tidal height (<1m to >4 m), geomorphology (oceanic islands for river systems), sedimentary environment (peat to alluvial), climate (hot temperate to arid and humid tropics) and availability of nutrients (oligotrophic to eutrophic). Mangrove ecosystems are also inhabited by large populations of aquatic and terrestrial animal and plant species, in addition to a wide variety of microorganisms [1]. More specifically, mangroves support a unique biological diversity, providing vital habitats, spawning areas, nurseries, and nutrients for various animals, including several endangered reptiles (e.g. crocodiles, iguanas, and snakes), birds (herons, herons, pelicans, and eagles) and mammals (e.g. monkeys) [2]. Mangrove sediments house a variety of bacteria and fungi that assist in the cycling of nutrients and immobilization of contaminants [3, 4].

A complex community structure is distributed vertically through supratidal, intertidal, and sub-tidal zones and horizontally through the land-sea ecotone, which has allowed for the emergence of species specializations, especially in the intertidal zone [2].

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The structure provided by the forest essentially defines mangrove communities and ecosystems, creating stable habitats and conditions, modifying abiotic environments, and modulating ecosystem processes [1].

Similar to terrestrial forests, mangrove forests are composed of a woody bole and a leafy canopy inhabited by ecological communities, not unlike those found in other tropical forests. However, unlike terrestrial plant species, the aerial root systems of mangroves also form an extensive above-ground structural framework, which dramatically increases the architectural complexity of these forests [1].

2. Mangrove Plants

The term ‘mangrove’ can also be used to describe trees or shrubs. Mangroves are a group of plants capable of growing in marine, estuarine, and, to some extent, freshwater. They occupy the shallow water margin between land and sea, in intertidal zones [4]. This very heterogeneous group of plants share several highly specialized adaptations that have allowed them to colonize and thrive in the intertidal zones. In particular, they developed special ways to deal with tidal fluctuation, anoxia, and high salt concentrations, which would kill or inhibit the growth of most other plants [5]. Mangrove plants display several adaptations that distinguish them from the vast majority of terrestrial plants, and these include the following: pneumatophores, lenticels, aerenchyma, aerial roots, succulence, wax coating on the leaves that limit the penetration of saltwater, salt-secreting glands in the leaves that allow the plant to get rid of excess salt, senescing of leaves containing concentrated salt, presence of a thicker Casparian strip that limits the entry of ions; and high salt concentrations in the sap and vacuole (Figure 1).

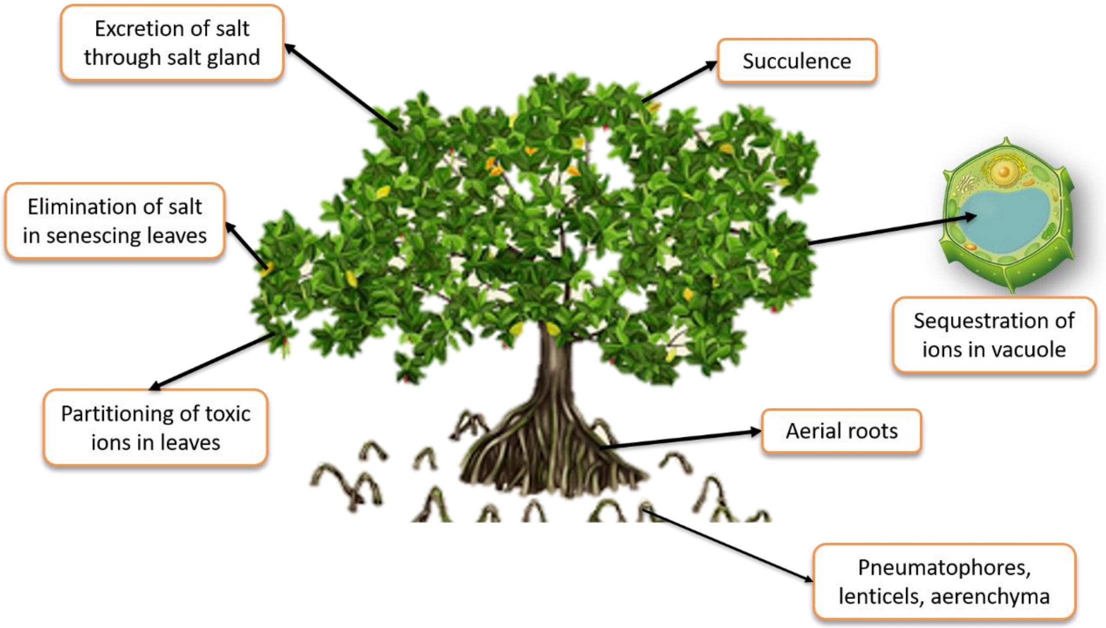

Figure 1 - Mangrove adaptations for saline and anoxic environment, (Garcia, J.).

Mangroves comprise variable combinations of 54 species, belonging to 20 genera and 16 families. These species fall into two groups: (i) True mangroves which occur in mangrove habitats only and whose existence is rare elsewhere (e.g. *Rhizophora*, *Kandelia*, *Ceriops*, *Bruguiera*, *Avicennia*, *Xylocarpus*, *Aegiceras*, *Sonneratia*, *Laguncularia*, *Lumnitzera*, *Nypa*), (ii) Mangrove associates which are non-exclusive mangrove species occurring in the landward margin of mangal and often non-mangal habitats such as rainforest, salt marsh, or lowland freshwater swamps (e.g. *Excoecaria*, *Camptostemon*, *Pemphis*, *Osbornia*, *Pelliciera*, *Aegialitis*, *Acrostichum*, *Scyphiphora*, *Heritiera*, etc.) [5].

3. Factors responsible for mangrove degradation and destruction

Mangrove plants are exposed to many severe and unavoidable environmental stressors, which affect their development and their physiological, biochemical, morphological, and molecular functions. Abiotic stress in these plants arises from excesses or deficiencies in the physical or chemical environment, such as drought, salinity, high light, ultraviolet radiation, extreme temperatures (heat shock, cooling), heavy metals, xenobiotics (including herbicides), toxins, nutrient deprivation, and many others [1, 6]. They are also affected by biotic stresses through damage by living organisms such as pathogens, herbivores, and even other plants; the consequences of these interactions can be very complex [6]. Table 1 shows some examples of natural and anthropogenic factors responsible for mangrove stress, degradation, and damage.

Table 1- Natural and anthropogenic factors responsible for mangrove degradation and destruction. Adapted from Feller at al (2010) and Das et al (2015).

Natural factors	Anthropogenic factors
1. Cyclones	1. War
2. Tsunami	2. Invasion of alien species
3. Disease (virus, bacteria, fungi, nematodes)	3. Cattle grazing
4. Pest and parasites (herbivores, rodents)	4. Woodcutting
5. Flooding or anoxia	5. Overexploitation of fisheries resource
6. Heat (high temperature)	6. Urbanization
7. Radiation (high light, UV)	7. Pollution (heavy metal, herbicides, oil)
8. Wind (sand and dust particles in wind)	8. Agriculture/ aquaculture
	9. Mining
	10. Prevention of freshwater flow
	11. Sea level rise and global warming
	12. Increasing salinity
	13. Changes in the chemical nature of the soil
	14. Poor nutrient load
	15. Ecotourism

4. Ecosystem Services of Mangroves

Ecosystem services can be defined as the benefits that ecosystems provide to human populations and society. They are normally classified as regulating, provisioning, supporting, and cultural services, and can even be valued [7, 8]. Some examples of the ecosystem service provided by mangroves are listed below (also see Figure 2):

Regulating services: Climate regulation; coastal protection; erosion control; water and soil cleaning; pollination.

Provisioning services: Food and water provisioning; raw materials; medicinal, ornamental, and genetic resources; products as honey and seeds.

Supporting services: Maintenance of life cycles; soil formation; photosynthesis and nutrient production; safe nursery and habitat.

Cultural services: Recreation and ecotourism; aesthetic value; educational opportunities; spiritual and religious contributions.

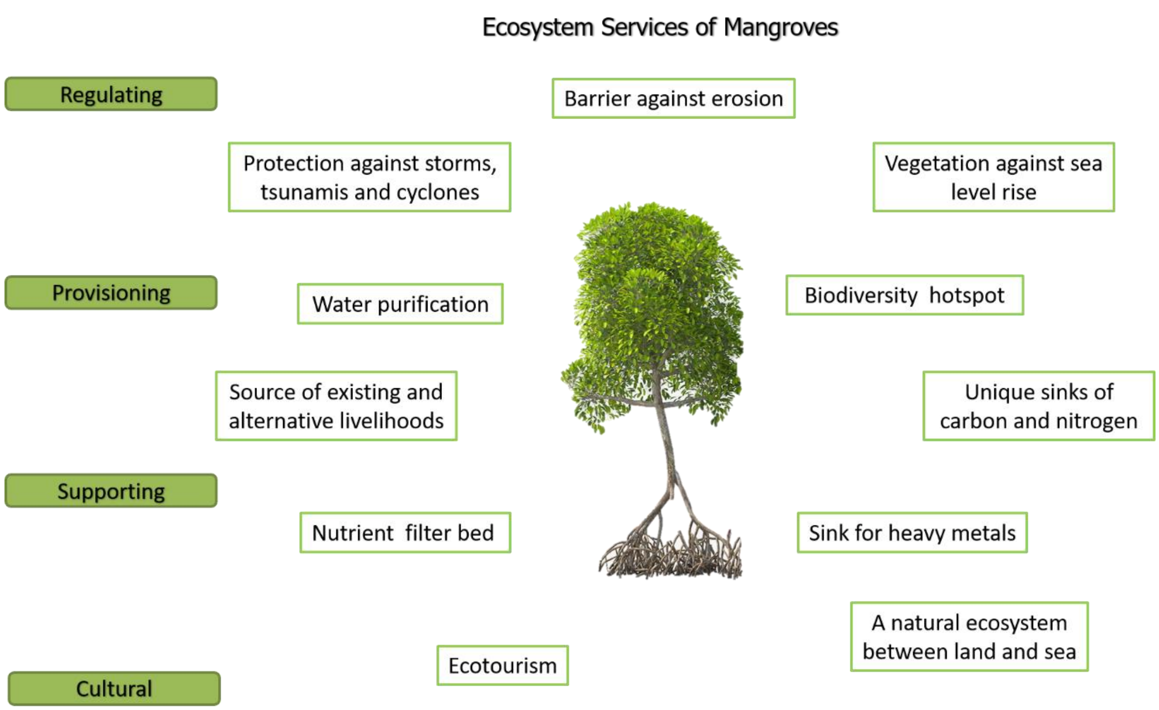

Figure 2 - Ecosystem services provided by mangroves, (Garcia, J.).

Mangroves provide several ecosystem services that improve human and environmental well-being. Mangroves are natural coastal habitats that protect coasts from erosion and flooding, providing important protection services. Other benefits include serving as nursery areas for fish species of commercial and recreational value, and a landing point for migratory birds. Mangroves are a sink of nitrogen and carbon and can filter sediment, absorbing nutrients, and pollutants. Another important service is the protection of the coastline against natural disasters such as floods and the protection of coastal human, animal, and plant communities against waves and extreme weather conditions. In addition, they provide cultural services such as ecotourism, spiritual, and aesthetic enrichment [8].

Given the wide range of services that mangroves provide, globally, the disappearance of these systems in many parts of the world is having a major impact on the vulnerability of coastal communities, particularly in developing countries. Of particular importance to the current contribution are the services offered by mangroves in the restoration of degraded environments and fixation of carbon (Figure 3), and immobilization of pollutants.

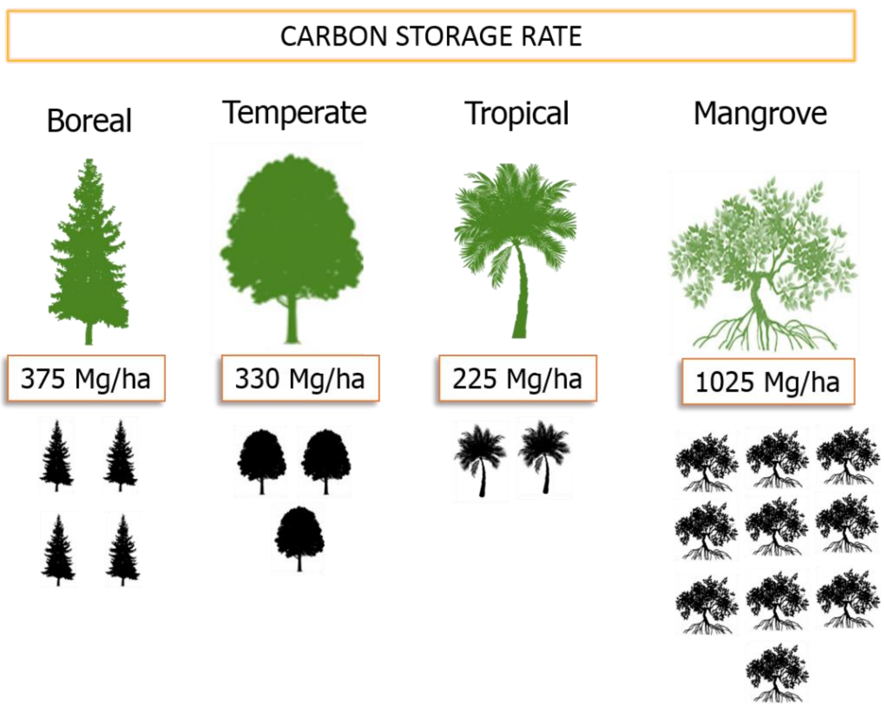

Figure 3 – Carbon storage by mangrove comparing to other forest, (Adapted from: https://sustainabletravel.org/what-is-blue-carbon/. Access 02 feb. 2022).

In this chapter we will review the role of mangroves in removing contaminants from soil, water, and air. Mangrove trees are used for the remediation of sites contaminated with heavy metals, hydrocarbons, and wastewater since they can withstand high concentrations of pollutants. As such, they can accumulate large amounts of contaminants in their tissues due to their large size, and the extensive coverage and depth of their root systems. They also have the ability to stabilize an area, prevent erosion, and minimize the spread of contaminants due to their perennial growth and presence. They can also be easily harvested and removed from the area in which they grow with minimal risk, effectively removing a large number of pollutants that were previously present in the soil.

4.1 Mangrove as a Sink of Pollutants

Polluted environments, wastewater from sewage, aquaculture, and drainage, can be treated, restored, and recovered using mangrove plants. Phytoremediation is a process that uses plants to decontaminate an environment by absorbing, accumulating, metabolizing, volatilizing and/or stabilizing inorganic and organic pollutants present in the soil, air, water, or sediments [9]. Based on the service provided we can classify phytoremediation into the following categories in (Figure 4) [10]:

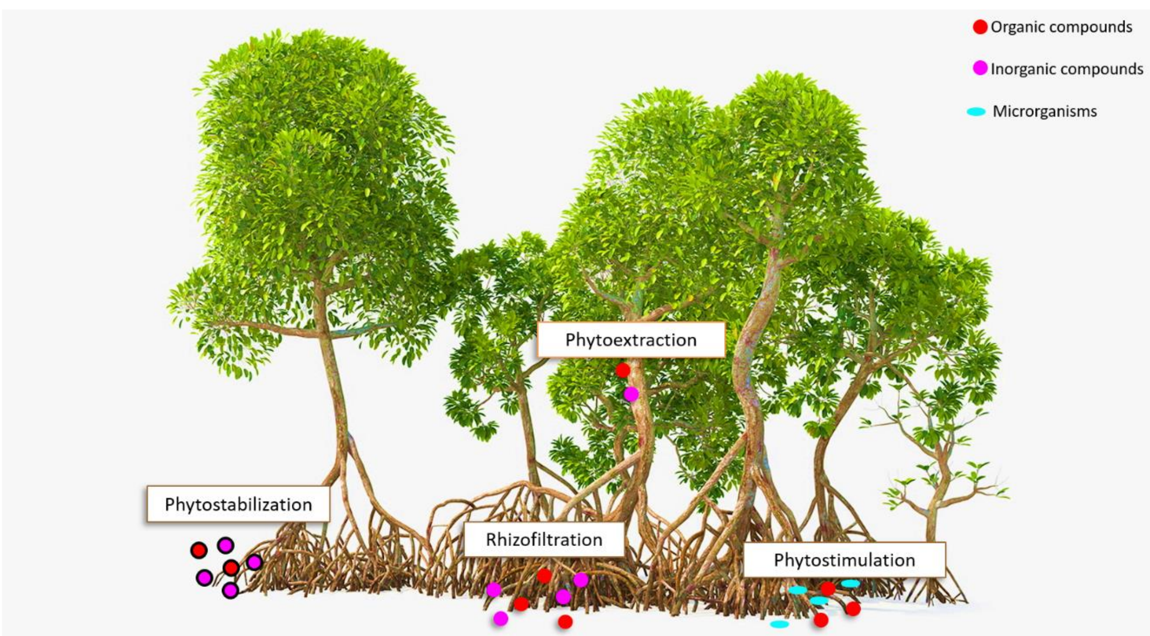

Figure 4 - The different mechanisms through which phytoremediation of inorganic and organic compounds can be achieved using mangroves, (Garcia, J.).

Phytoextraction: This process works through the absorption of soil contaminants by root. They can be used for contaminants such as metals (Cd, Ni, Cu, Zn, Pb) and can be used for inorganic (Na and Se) and organic compounds.

Phytostabilization: In this technique, pollutants are converted into a less toxic or bioavailable form by the continuous precipitation of the plant rhizosphere. In this case, organic and inorganic pollutants are incorporated into the lignin of the plant wall or soil humus.

Phytostimulation or Rhizofiltration: Growing roots promote the proliferation of microorganisms in the rhizosphere, which use the plant exudates such as carbohydrates and acids that stimulate microorganism activity and result in the biodegradation of the organic contaminants.

Phytodegradation: Contaminants and other nutrients are chemically modified through plant metabolism and render associated contaminants inactive in both plant root and shoot tissues. Organic pollutants are degraded or mineralized within plant cells by specific enzymes.

There is also phytovolatilization that removes the contaminant in the gaseous form. As previously described, phytoremediation can be used to remove or stabilize inorganic (heavy metals, nutrients, NO_3^-) or organic contaminants (polycyclic aromatic hydrocarbons [PAHs], xenobiotics). The following section will present some case studies where the mangrove was used to amend polluted water and soil.

4.1.1 Heavy Metals

Bioaccumulation of anthropogenic chemicals and non-essential nutrients through the food chain has recently become a matter of concern reported by several researchers [8]. Heavy metals like lead, cadmium, chromium, and mercury are highly toxic pollutants that could be significantly associated with bioaccumulation within many ecological systems because they cannot be biologically degraded and instead get concentrated within sediments. Globally, there has been widespread agreement that levels of heavy metals within mangrove sediments are increasing as a result of pollution and activities caused by developmental growth and urbanization [11, 12]. When measuring the level of metal contamination in an environment it is possible to use some established indices, which are described below.

4.1.2 Heavy Metal Indices

Some indices for studies with heavy metals and nutrients in the field or built systems are presented in the following section:

Translocation Factor (TLF) – The rate of translocation of heavy metals from roots to shoots.

TLF values > 1 represent metal accumulator species and TLF values < 1 represent metal avoiding species. The TLF can be calculated by Eq. (1) [12]:

$$TLF = \text{metal concentration in shoot} / \text{metal concentration in root} \quad (1)$$

Bioconcentration Factor – The ratio of the concentration of heavy metals in the shoot to their concentration in exchangeable form in soil. The BCF can be calculated by Eq. (2) [12]:

$$BCF = \text{metal concentration in shoot or root} / \text{metal concentration in sediment} \quad (2)$$

The Enrichment Factor (EF) in metals and Geoaccumulation Index (I_{geo}) are indices used to assess the presence and intensity of anthropogenic contaminant deposition on the surface soil.

Enrichment Factor - The ratio of heavy metals in a leaf to sediment is called enrichment coefficient of leaf (ECL), and the ratio of heavy metals in a root to sediment is called enrichment coefficient of root (ECR). The EF is a good way to discriminate between the natural levels and anthropogenic sources of pollution [3, 12]:

$$EF = (Me/Fe - \text{sample}) / (Me/Fe -– \text{background})$$

where, Me is the heavy metal to Fe concentration in the sample and the denominator is the natural background value for the ratio of heavy metal to Fe. The index of potential contamination is calculated by the normalization of one metal concentration in the topsoil relative to the concentration of a reference element. The classification of *Avicennia marina*, based on EF values is presented in Table 2 as an illustrative example.

Table 2 - Classification of *A. marina* according to Enrichment factor. Adapted from Ghasemi et al (2018).

Enrichment level	EF
No enrichment to low enrichment	≤ 2
Moderate enrichment	2–5
High enrichment	5–20
Very high enrichment	20–40
Extreme enrichment	≥ 40

Geoaccumulation Index (Igeo) - The I_{geo} has been widely used to quantify the level of contamination in mangrove sediments as interpreted in Table 3 [3]. Values were calculated using the following equation:

$I_{geo} = Log2\ (Cn/1.5Bn)$

where, Cn is the measured concentration of heavy metal in the mangrove sediment, Bn is the geochemical background value in average clastic sedimentary rock, and 1.5 is the background matrix correction factor due to lithogenic effects.

Table 3 - Level of contamination according to I_{geo} values adapted from Fernández-Cadena et al (2020):

I_{geo} values	Level of contamination
≤ 0	background concentration
$0 \leq I_{geo} \leq 1$	Unpolluted
$1 \leq I_{geo} \leq 2$	moderately polluted to unpolluted
$2 \leq I_{geo} \leq 3$	moderately polluted
$3 \leq I_{geo} \leq 4$	moderately to highly polluted
$4 \leq I_{geo} \leq 5$	highly polluted

> 5	very highly polluted

Protocols in many studies evaluating phytoremediation capacity involve collecting sediment from mangroves and plant tissues to assess the concentration of metals through chemical analysis. Some examples of how to apply heavy metal indices in field studies are described below.

The simplest methodology that can be applied is to assess the concentration of metals in plants and sediments in each location and then perform the index calculations and determine whether the plants in the location are capable of tolerating contamination levels. For example, in a study by Ghasemi et al. [12], samples of plants and sediments were collected from the protected area of Hara and Azini Bay, in the province of Hormozgan, in southern Iran. They estimated the accumulation of heavy metals in surface sediments, including the metals Zn, Cu, Pb, and Cd, and the distribution of these metals in grey mangroves, to establish whether local *A. marina* trees could be used for phytomanagement of heavy metals in this sensitive environment. For this, ecological-biological indices, such as TLF, BCF, and EF, were also used. In this study, the roots of *A. marina* showed higher EF than leaves for all metals, implying a greater potential for metal accumulation. On the other hand, lower BCF and TLF values were considered to be an exclusion strategy for the studied heavy metals. Finally, they established that *A. marina* trees could be used for potential phytomanagement of heavy metals in this vulnerable environment.

Analunddin et al. [11] evaluated the translocation and bioaccumulation capacity of *Rhizophora apiculata*, *Ceriops tagal*, *Bruguiera gymnorrhiza*, *Bruguiera parviflora*, *Lumnitzera racemosa*, *Xylocarpus granatum* and *Sonneratia alba* for Cu, Hg, Cd, Zn, and Pb. The study site was the mangrove forest in RAWN Park, in the ecoregion of the coral triangle, southeastern Southawesi, Indonesia. In this study, the BCF values of the metals Cu, Hg, Cd, Zn, and Pb in the mangroves were significantly different among species, which indicated the different capacities of mangrove species to absorb heavy metals from the environment. TLF trends suggest species differences in the physiological mechanism of mangroves to adapt to growth under heavy metal pollution.

Mangrove plants are known to be an important sink of toxic metals and some of these metals can be translocated to the leaves [8]. The concern is what happens when these leaves return to the ground. Almahasheer et al. [13] in a study on *A. marina* examined the capacity of the species to reabsorb heavy metals before leaves senescence in the Central Red Sea. Results showed that metal concentrations were independent of leaf age for most heavy metals, except for vanadium, Cd, and arsenic, which increased with leaf age, and Cu which decreased with leaf age. The role of leaf shedding as a source of metals to the environment should be taken into consideration when assessing the potential of mangroves as phytoremediation tools. They estimated that the metal remobilization with leaf shedding (i.e. transfer of deep-soil metal stocks into the soil surface) by mangroves in the Red Sea, totaled 120 tons of Fe, 91 tons of Al, 16 tons of Mn and Sr, 6 tons of V, 3 tons of Zn, 2 tons of Pb, 0.7 tons of As, 0.5 tons of Mo, 0.4 tons of Cu and Ni, 0.3 tons of Cr, and 0.05 tons of Cd.

It is also important to consider the effect of salinity and dry seasons on the use of mangrove plants for phytoremediation purposes. Despite the Pb levels found in the water of Batticaloa Lagoon, in Sri Lanka, heavy metals were not detected in the plants, and this was attributed to a possible interaction with Na^+ [14]. In addition to the influence of salinity, the efficiency of the ecosystem to absorb metals can also be associated with the interaction of metals with organic matter and with the redox properties of the soil [15].

Other studies are carried out under experimental conditions, to evaluate the phytoremediation capacity of plants. For example, young plants of *A. marina* and *Pluchea indica* were collected from the Pattani Baymangrove forest (Thailand) and cultivated in mangrove sediment for three months inside a greenhouse and in the field [16]. They analyzed heavy metals (Cd, Cr, Pb, Cu, Mn, Ni, Zn) and radionuclides (226Ra, 232Th, and 40K) concentrations. Heavy metals indices were also utilized and for the radioactivity concentrations, radionuclide indices were calculated using the IAEA [17] equation. Both *A. marina* and *P. indica* experienced a 100% survival rate with no toxicity symptoms during the respective experimental periods under greenhouse and field experiments. Based on BCF, TLC and radionuclide indices, *A. marina* and *P. indica* are considered to hold promise for contaminant phytomanagement.

4.1.3 Association with Other Elements

Another strategy to reduce the toxicity of heavy metals in a mangrove is to supplement the soil with other elements and to alter the soil organic matter. Exogenous application of Fe was used to evaluate whether Fe could alleviate the effects of cadmium phytotoxicity in *A. marina* [18]. In this study different concentrations of exogenous Fe were used to evaluate the mechanisms of Fe influence on Cd tolerance as follows: (i) plant physiological response in vivo, (ii) physicochemical impact on the environment of the root surface, and (iii) plant defense behavior. There was a significant positive correlation between low-dose and mid dose exogenous Fe supply and Cd tolerance of *A. marina*. The alleviatory effect of Fe on Cd stress was reflected in different ways. Moderate exogenous Fe directly contributed to more Fe oxidation and precipitation on the root surface, and more Cd immobilization was then discovered in Fe plaque. Exogenous Fe supply facilitated *A. marina* growth and increased chlorophyll concentration in leaves. Cd immobilization on the root surface significantly limited Cd absorption, transportation, and accumulation. Exogenous Fe increased Fe accumulation in *A. marina* tissues, improved Fe competition with Cd, and further alleviated Cd phytotoxicity.

In another study, silicon was used to alleviate the effects of Cd toxicity in *A. marina* through Cd compartmentation [19]. In this study, propagules of *A. marina* were collected in the estuary of the Jiulong River, in the province of Fujian, in China, and grown in a greenhouse under treatments of 0.5 mg of Cd L−1 with concentrations of 0.50 and 100 mg of L−1 Si. After eight weeks, the concentration of Cd in the leaves decreased by 23.0% (0.50 mg Si L−1) and 25.6% (100 mg Si L−1), but there was no significant difference between these two concentrations. This indicated that Si significantly inhibited the absorption and accumulation of Cd in *A. marina* seedlings. Silicon treatment did not change the distribution ratio of Cd between leaf compartments, but reduced Cd mobility and the concentration of biologically active Cd in the cell wall active space. Changes in the distribution of Cd between compartments in the root tips may also be an important mechanism for Si-induced Cd tolerance.

Exogenous phosphorus has also been shown to enhance Cd tolerance in *A. marina* and *Kandelia obovata* (S., L.). Young seedlings, differing in Cd accumulation. Mature and healthy propagules of *A. marina* and *K. obovata* were collected from the national mangrove reserve in Zhangzhou, Fujian Province, China [20] and exposed to 0, 0.5, and 5 mg Cd kg^{-1} ($CdCl_2 \cdot 2.5H_2O$), and 0, 30, 9, and 0 mg P kg^{-1} (KH_2PO_4). The objective of this study was to determine and compare pectin and hemicellulose concentrations, as well as PME activity levels, in seedlings of the two species, and to examine how the plant cell wall composition responds to Cd stress. Root elongation increased in both mangrove seedlings as P concentrations rose. For example, compared to the control, the elongation of roots in *A. marina* plants exposed to 5 mg Cd kg-1 with the addition of 90 mg P kg^{-1} increased by 18.6%. In *K. obovata*, the roots showed an increase of 41.86%. Under the treatment 5 mg Cd kg^{-1} the Cd accumulated in the root cell walls was 64%–30 mg P kg^{-1} and 88%–90 kg^{-1} of the total concentration of Cd in the roots of *A. marina*. The 5 mg Cd kg^{-1} treatment in *K. obovata* Cd accumulated in the root cell walls was 57 and 82% in the roots with 30 and 90 mg P kg^{-1}, respectively. Higher levels of pectin and pectin methyl esterase activity were also observed in both species and the authors suggested that this may lead to the production of freer carboxyl groups available for Cd binding in the root cell wall, thereby improving seedling Cd tolerance. A different approach was applied by Pittarello et al. [21] in Espírito Santo State, Brazil. They selected two sites with different levels of anthropic perturbation and tested the hypothesis of whether humic matter supply could modify the sediment retention potential of Cu, Pb, and Cd, or if the intrinsic characteristics of the sediments, particularly in terms of Fe, organic matter content, and texture, remain influential in heavy metal sequestration even at high pollutant concentrations. The artificial addition of humic substance (HS) in this work suggested a linear increase in sediment adsorption and complexation capacity of Cu and Cd, while Pb retention seemed to depend more on clay content. The Fe content, due to the great difference in concentration between the two sediments, may be considered another important factor due to its capacity to form spheroids linked to HS molecules on the clay surface and increase the adsorbing surface area.

4.1.4 Organic Compounds

Studies on the accumulation of organic contaminants in mangrove organisms are relatively less common as compared to metals. Table 4 presents some common organic contaminants and their abbreviations.

Table 4 - Common organic contaminants found in marine environments.

Organic contaminants	Abbreviation
Persistent Organic Pollutant	POP
Total petroleum hydrocarbons	TPH
Benzene, toluene, ethylbenzene, and xylene	BTEX
Polycyclic aromatic hydrocarbons	PAHs
Organo Chlorine Pesticides	OCP
Polychlorinated Biphenyls	PCB
Dichlorodiphenyltrichloroethane	DDT
Polybrominated Diphenyl Ether	PBDE

Mangroves are tidal wetlands and are considered to be the most highly susceptible marine environments to large scale and chronic oil spills [22]. A significant amount of crude oil is discharged into coastal environments and these mangroves are extremely responsive to contamination by oil and industrial waste. Once oil and marine tar residues are deposited on or around the mangroves, they adhere to plant surfaces, adsorbing to oleophilic surfaces. Oil coats breathing surfaces of roots, stems, seedlings, and it contaminates surrounding sediments and their sessile intertidal or burrowing fauna [23].

In the last two decades, the negative effects of aromatic hydrocarbons (AHs) on environmental integrity and human health have gained prominence in the scientific literature. The toxic, genotoxic, mutagenic, carcinogenic, and bioaccumulative properties of AHs can impair the balance of biological communities, increasing the mortality rates of several species [22]. The efficiency of the available remediation methods is dependent on the amount of oil spilled, the oil penetration depth into the soil, soil type, the plant age, and level of contamination.

In a study on *Rhizophora mangle* L., plants were submerged in sediments mixed with waste oil from the study area (Bay of Todos os Santos, Bahia, Brazil) [24]. In this laboratory simulation, the plants were planted in glass tubes, where the daily regimen was simulated with the tidal water of the mangrove and morphophysiological monitoring was conducted over 90 days. They also used bioattenuation monitoring with the degradation of hydrocarbons derived from petroleum hydrocarbon by bacteria present in the sediment. The results showed that the bioattenuation (Natural Attenuation Monitored) was able to remove 70% of total petroleum hydrocarbons (TPHs) individually, while the phytoremediation was able to remove approximately 87% of the TPH present in the contaminated sediment.

Other studies in the same area evaluated the use of *A. schaueriana* for phytoremediation when compared to intrinsic bioremediation in mangrove sediments contaminated by TPH [25]. The species were able to remove approximately 89% of TPH. The phytoremediation sing *A. schaueriana* was able to remove approximately 19% more TPH from the sediment than intrinsic bioremediation, with contaminant levels being reduced from 33.2 to 4.2 mg g^{-1}, whereas the intrinsic bioremediation only lowered the levels to 9.2 mg g^{-1} over three months. Mangrove species are therefore useful for the phytoremediation of TPH contaminated sediments, due to their filtering ability and strong interaction with the microbial community.

In southeast China, *K. obovata* seedlings were chosen as the model plant to evaluate the effects of flooding and aging on the phytoremediation of typical PAHs in mangrove sediments [26]. They investigated the PAHs dissipation rates; (i) in mangrove sediment under flooding conditions: (ii) the distribution of PAHs in root tissues was in situ under flooding or aging conditions using microscopic fluorescence spectra analysis method, finally, (iii) the bioavailability of PAHs after phytoremediation processes. Three typical PAHs, naphthalene (Nap), anthracene (Ant), and benzo [a] pyrene (B[a]P), were chosen as test molecules. The results showed that the PAHs decreased in the planted sediment, as well as in the unplanted control, but a more marked rate of PAHs disappearance was evident when the mangrove plants were present. The dissipation rates decreased to 50.05, 42.29, and 38.93% for Nap, Ant, and B[a]P in the non-rhizosphere zone under aged condition, respectively. These values were slightly lower than those in the rhizosphere zone, and senescence also decreased the levels of the bioavailable PAHs to 269.03 mg kg^{-1} for Nap, 135.95 mg kg^{-1} for Ant, and 16.49 mg kg^{-1} for B[a]P. That study showed the distribution of anthracene in roots using fluorescence microscopy and the results highlighted flooding and aging as an important factor in dissipation of PAHs.

The presence of PAHs in the mangrove ecosystem can interfere in the cycling of other nutrients such as nitrates (NO_3^-). One study investigated the influence of phenanthrene (PHE), a three-ring PAH compound, on nitrate (NO_3^-) reduction processes in mangrove sediments using *Aegiceras corniculatum* microcosms and three distinct sediment layers [27]. The results showed that after 10 days, around 15% of exogenous NO_3^- applied to mangrove sediments was removed, and the removal portion reached 50% after 50 days. After the 50-day incubation, the majority of PHE in the sediment was attenuated, although a decline of NO_3^- reduction capability in the mangrove substrate was detected and related to contamination level and with presence/absence of interaction between sediments and mangrove roots. Generally, PAHs play an inhibitory role, slowing NO_3^- turnover rates, which warrant attention from coastal managers.

4.1.5 Wastewater

The presence of aquaculture activities in mangroves is becoming increasingly common and widespread. Shrimp breeding grounds, for example, are a great source of N, which, in the long run, could result in eutrophication. Therefore, action is needed to remove excess nitrogen from the ecosystem. Gautier [28], for example, evaluated the use of a mangrove wetland as a biofilter to treat shrimp pond effluents in Colombia. The shrimp farm pumped 345 600 m^3 of renewal water per day, to exchange about 8 cm of water per day in each pond. That water flowed through the biofilter, controlled by levees and concrete structures. The biofilter was composed of mangrove trees of *R. mangle* (46%), *Laguncularia racemosa* L. (11%), *Avicennia germinans* L. (1%) and *Conocarpus erectus* L. (1%), and the fern *Acrostichum aureum* L. (41%). All nutrient concentrations except nitrite increased in the biofilter after 12 weeks, demonstrating the system to be very efficient at removing suspended solids. Additionally, a significant decrease in dissolved oxygen and pH was observed in the biofilter. Constructed wetlands may be easier to control, but the difficulties are finding the space to build them and their cost. Another closed system was proposed for the removal of nutrients (i.e. NH_4^+, NO_2^-, NO_3^-, and PO_4^{-3}) by seedlings of *A. germinans*, *L. racemosa*, and *R. mangle* in a closed silverfish system with a constant population of *Dormitator latifrons* [29]. The system was designed with a vertical flow of water and presented a hydroponic mangrove arrangement suspended over the surface by a wooden structure. The results demonstrated that *A. germinans*, *L. racemosa*, and *R. mangle* have the potential to remove an average of 63% of NH_4^+ and NO_2^-. Contrastingly, the average removal efficiency of NO_3^- and PO_4^{-3} was 50% under the same treatments. The N cycle in mangroves is complex and depends on several environmental conditions. However, N transformation in mangroves occurs by five principal biological processes: ammonification, nitrification, denitrification, nitrogen fixation, and nitrogen assimilation. In this study, the growth of mangroves played a key role in the removal of inorganic forms of N.

A mangrove tide-tank system consisting of 12 PVC tanks was constructed in Hong Kong, China tidal + glucose, (iii) Short tidal and (iv) Short tidal + glucose [30]. Each PVC tank with a dimension to evaluate the capability of *K. candel* to remove N under four treatments: (i) Long tidal, (ii) Long of 1 m (L) × 0.5 m (W) × 0.3 m (D) was filled with 100 kg fresh surface sediment collected from a typical mangrove swamp and had 15–20 individuals of *K. candel* (2 years-old) transplanted from the same mangrove swamp. The sediment was not pre-treated before being added to the tank to simulate the conditions found in the field. At the beginning of the experiment, more than 90% nitrogen was accumulated in sediment, while less than 1.7% N was assimilated in plants, and macroalgae were not detected during the acclimation year before the experiment, probably due to nutrient limitations as only artificial seawater (without nutrients) was irrigated. With the addition of ammonium, N uptake by mangrove plants and macroalgae increased significantly (up to 10% N was assimilated); on the other hand, the relative proportion of nitrogen stored in sediment decreased to less than 75%. Around 15 and 30% of nitrogen from the inputs were lost as nitrogen gas (N2) via the nitrification–denitrification process from the mangrove tide-tanks with and without glucose addition, respectively. Also, the population sizes of nitrifiers and denitrifiers increased significantly during the experiment, and up to 30% of total N inputs were lost to the atmosphere as N_2 gas via nitrification and denitrification processes. Tidal regime and glucose addition had significant effects on the concentrations of ammonium and nitrate in sediment.

Mangroves have a huge demand for nutrients because of rapid growth, and high primary productivity, metabolism, and turnover [31]. One study in Futian, in Shenzhen, China evaluated the potential use of mangroves as a constructed wetland for municipal sewage treatment. They used

Aegiceras corniculatum, *Kandelia candel* and *Sonneratia caseolaris* in a constructed system (Figure 4) built in the experimental zone of the reserve and consisting of three belts. Each belt was 33 m (long) 3 m (wide) 0.5 m (deep) and filled with stone (bottom, 20 cm deep), gravel (20 cm deep), and mangrove sand (surface, 10 cm deep). The belt was divided into two parts, part I and part II, which were connected by a transition zone (1 m wide). Part II was 20 cm lower than the part I and wastewater flowed from part I to part II by gravity. After plants adapted to the system the results were very promising approximately 70% of the organic matter, 50% of TN, 60% of NH_3^-N, 60% of the TP, and 90% of coliforms were removed from the influent by the constructed mangrove wetlands indicating a high efficiency of this system in sewage treatment. The treatment efficiency of *S. caseolaris* and *A. corniculatum* was higher than that of *K. candel*. Faster plant growth was obtained for *S. caseolaris*, however, this an exotic plant and its use can have implications on alien management. Mangroves are perennial plants and continue to grow for many years even when reaching 100% coverage; therefore, it is not necessary to harvest or replace them regularly in systems such as this.

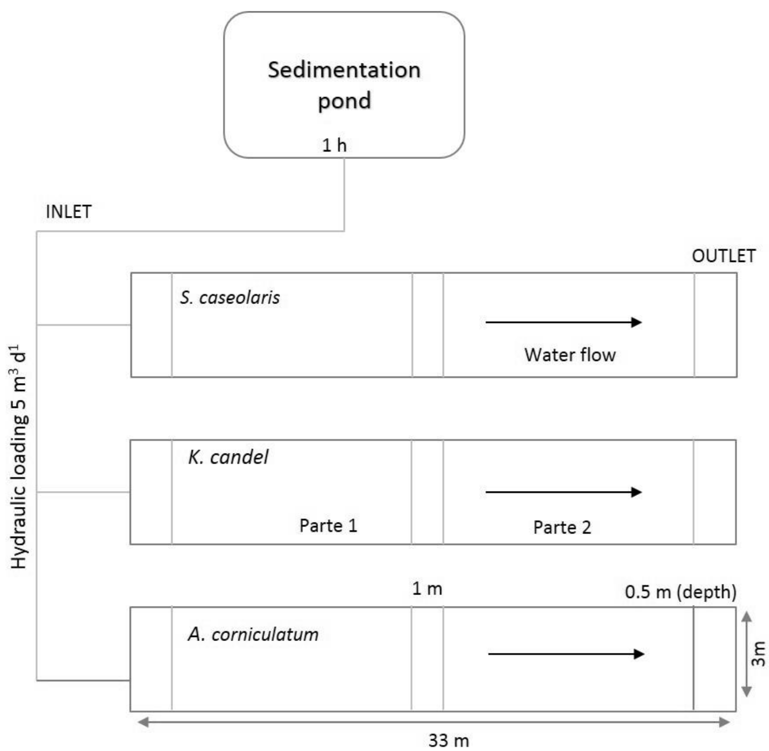

Figure 5 - Schematic diagram of the constructed mangrove wetlands. Source: adapted from [71].

Greenhouse experiments were conducted in China by growing the Sonneratia apetala Buch-Ham species in ceramic pots that were filled with the mangrove wetland soil and irrigated with different concentrations of artificial wastewaters (Table 4). After four months of treatment, root activity, and growth of the *S. apetala* species were not retarded by the wastewater [32]. The total biomass of the *S. apetala* species increased with wastewater pollution levels and this increase was more significant as time elapsed. No significant differences in leaf catalase activity were observed among the wastewater pollution levels. In general, the leaf chlorophyll content increased with wastewater pollution levels.

The species selected for this study could be used in constructed wetlands for wastewater treatment, although the use of this exotic species may have implications on alien management.

4.1.6 Microorganism Association and Isolation

Bioremediation can be applied both *in situ* and *ex situ* using different strategies, such as bioattenuation, bioaugmentation, and biostimulation, defined by Machado et al. [33] as follows:

Bioattenuation – also known as monitored natural attenuation (MNA), is an *in situ* technique that consists of contaminant degradation by natural physical, chemical, and biological processes without human intervention, through volatilization and degradation of the toxic compounds by the autochthonous microbiota.

Bioaugmentation – consists of applying microorganisms grown in the laboratory, autochthonous from the contaminated site, with known ability to degrade the target contaminant. It can be used in situ and *ex situ*, being particularly important for in situ treatments, when the autochthonous microbiota is not presented in sufficient amount to degrade the contaminant efficiently.

Biostimulation – the process by which an intentional stimulation of autochthonous microbial communities takes place through the provision of nutrients and electron acceptors/donors, pH and temperature adjustment, and aeration of the substrate. This technique can be performed in situ and ex situ, but it depends on the existence of autochthonous microbiota capable of degrading the target contaminant.

Bacillus pumilus has been isolated from mangrove plants and used for bioremediation purposes. The bacteria have been tested for Pb, Cd, Ba, Cr, Fe, Cu, and F toxicity and was found to be resistant against all. Studies performed with this multi-metal tolerant halophilic microbe in the Karnataka mangrove region [34–36] have evaluated its use for fluoride (F^-), lead (Pb) and chromium (Cr) bioremediation. *Bacillus pumilus* can tolerate up to 1000 ppm of fluoride and its bioremediation efficiency has been shown to increase from 40 to 55% at pH 7 to 60–78% at pH 6 (34, 35, 36). In terms of heavy metals, *B. pumilus* was satisfactory in adsorption and absorption of Pb and Cr and the pH was again impor pH 7 to >99% at pH 6 and 8, and for Cr increased from 86% at pH 7 to 96% at pH 8. The mangrove tant in improving the performance of the microbe: efficiency for Pb has increased from 96.8% at environment can be used to isolate many other microbes, which can be effective in bioremediation potential.

Some bacteria in polluted sediments can be used as sentinel species [3]. The bacterial community can differ between polluted and non-polluted mangrove sediments. The amount of *Sulfurovum lithotrophicum*, *Leptolinea tardivitalis*, *Desulfococcus multivorans*, and *Aminobacterium colombiense* increases, when metal concentrations rise. Contrastingly, the amount of *Bacillus stamsii*, *Nioella nitrareducens*, and *Clostridiisalibacter paucivorans* increased, when metal levels were reduced [3].

One study performed in Rio de Janeiro, Brazil selected and assembled an oil-degrading bacterial consortium containing local mangrove native aerobic and facultative anaerobic bacteria and developed an innovative *in situ* mesocosm system in the mangrove [33]. This innovative mesocosm system was both safe against environmental contamination and dynamic to generate a realistic system that included tidal influence. After a local and controlled oil spill simulation in the mesocosms, they evaluated the following: (i) the presence of different hydrocarbon degradation genes during the oil contamination and bioremediation processes, (ii) oil degradation in response to different bioremediation processes, (iii) differences in bacterial diversity patterns along the sediment depth at this site. Generally, hydrocarbon consumption is higher and faster in the presence of oxygen than in its absence. Four different bioremediation techniques were tested to mitigate the effects of the MF-380 oil spill simulation: bioattenuation (BT), bioaugmentation (BA), biostimulation (BS), and bioaugmentation + biostimulation (BB). The community composition at the phylum and order levels did not differ among the treatments, whereas differences were detected between the two sediment layers in all the treatments. For all the treatments and depths, the most abundant phyla were Proteobacteria, Firmicutes, and Bacteroidetes, with Gammaproteobacteria, Flavobacteriales, and Clostridiales being the most common orders.

One study conducted in Rio de Janeiro, Brazil, highlights the importance of mangroves as degradation [37]. Xylanase and α-amylase enzymes participate in the degradation of organic an enzyme source and shows that bacterial groups can be used for starch and hemicellulose matter, acting in hemicellulose and starch mineralization, respectively, and are in high demand for industrial use. The study aimed at bioprospecting xylanases and amylases from mangrove soil using cultivation-dependent and cultivation-independent methods. The culture-dependent method associated with molecular analyses enabled the authors to show that *Bacillus* and *Paenibacillus* possess xylanolytic capacity, supporting the participation of these bacteria in xylan degradation. This method was more effective to obtain the initial target enzyme, as it allowed the direct isolation of microorganisms that produce xylanases. Through the culture-independent encodes an amylase, the 1,4-α-glucan branching enzyme. The amylase accessed through the method, the gene that encodes xylanase was not obtained, but this method isolated a gene that independent approach is produced by Planctomycetes, a trait newly described for this bacterium in mangroves, indicating its participation in starch degradation.

The petroleum hydrocarbon degrading rhizobacteria from *Rhizophora* sp. was isolated from the rhizospheric soil samples by inoculating them into Bushnell-Haas Minimal Salt Broth Medium (BHMS) with the addition of 0.01% crude oil [38]. The results showed that the TPH decreased 98.72% in TPH levels after 10 days of incubation. Therefore, it could be said that the rhizosphere of mangrove plants has a great potential for obtaining novelty bacterial isolates that can degrade petroleum hydrocarbons.

In Table 5 we list other examples of the use of mangroves for the remediation of environments polluted by different types of contaminants.

Table 5. Bibliographical suggestions for the use of mangroves in the removal of contaminants and ecorestoration.

Title	**Reference**
Plant-assisted remediation of hydrocarbons in water and soil: Application, mechanisms, challenges and opportunities.	Abdullah et al 2020
Determination of linear alkylbenzenes (LABs) in mangrove ecosystems using the oyster *Crassostrea belcheri* as a biosensor.	Alkhadher et al 2020
Electro-bioremediation: an advanced remediation technology for the treatment and management of contaminated soil.	Annamalai & Sundaram M 2020
Effectiveness of remediation of metal-contaminated mangrove sediments (Sydney estuary, Australia).	Birch et al 2015
Comparison of chemical sediment analyses and field oiling observations from the Shoreline Cleanup Assessment Technique (SCAT) in heavily oiled areas of former mangrove in Bodo, eastern Niger Delta.	Bonte et al 2020
A case study of in situ oil contamination in a mangrove swamp (Rio De Janeiro, Brazil).	Brito et al 2009
Biodegradation of polybrominated diphenyl ethers in mangrove sediments.	Chen et al 2020
Biodegradation of artisanally refined diesel and the influence of organic wastes on oil-polluted soil remediation.	Chikere et al 2020
Exploring the genetic potential of a fosmid metagenomic library from an oil-impacted mangrove sediment for metabolism of aromatic compounds.	de Sousa et al 2020
Biodegradation ability and dioxgenasedioxygenase genes of PAH-degrading *Sphingomonas* and *Mycobacterium* strains isolated from mangrove sediments.	Guo et al 2010

Mangroves-a potential phyto-remediator and useful bio-indicator against heavy metal toxicity.	Gupta & Chakrabarti 2013
Polybrominated diphenyl ethers and alternative halogenated flame retardants in mangrove plants from Futian National Nature Reserve of Shenzhen City, South China	Hu et al 2020
Removal of tetrabromobisphenol A from contaminated sediments	Jiang et al 2020
Potential contribution of multifunctional mangroves	Kumari et al 2020
Approaches for remediation of sites contaminated with total petroleum hydrocarbons.	Kuppusamy et al 2020
Kandelia obovata improve removal of BDE-209 in mangrove soils	Li et al 2020
Rhizodegradation gradients of phenanthrene and pyrene in sediment of mangrove (*Kandelia candel* (L.) Druce).	Lu et al 2011
Bioremediation of a crude oil polluted tropical mangrove environment.	Odokuma LO, Dickson AA 2003
Biodegradation of polycyclic aromatic hydrocarbons by a bacterial consortium enriched from mangrove sediments	Oghadam et al 2014
Biosurfactant-enhanced remediation of hydrocarbon contaminated mangrove swamp	Okoro 2010
Application of seawater microbial inocula for the remediation of hydrocarbon polluted mangrove swamp in the Nigerian oil rich Niger Delta	Okoro 2009
Degradation of BDE-47 in mangrove sediments with amendment of extra carbon sources.	Pan et al 2020
Biotechnological utilization of mangrove resources.	Patra et al 2020
Phytoremediation by mangrove trees: experimental studies and model development.	Richter et al 2020
Industrial applications of enzymes derived from Indian mangroves.	Sengupta et al 2020

Halotolerant and halophilic bacteria from mangrove ecosystems and their remediation potential.	Thatoi et al 2020
Changes of substrate microbial biomass and community composition in a constructed mangrove wetland for municipal wastewater treatment during 10-years operation.	Tian al 2020
Environmental technologies for remediation of contaminated lands in the Niger Delta region of Nigeria: Opportunities for ecosystem services to host communities.	Ngene & Tota-Maharaj 2019
Approaches for degradation and decolorization of dye by mangrove plants	Vaish & Pathak 2020
Interactions of soil metals with glomalin-related soil protein as soil pollution bioindicators in mangrove wetland ecosystems	Wang et al 2020
Organic acids (low molecular-weight) and dehydrogenase activity in rhizosphere sediments of mangrove plants on phytoremediation of polycyclic aromatic hydrocarbons.	Wang et al 2014
Mangrove reforestation may influence the quantity and quality of long-term carbon sequestration and storage	Wu et al 2020
Assessing impacts of metallic contamination along the tidal gradient of a riverine mangrove: multi-metal bioaccumulation and biomagnification of filter-feeding bivalves.	Yam et al 2020
The distribution and characteristics of microplastics in coastal beaches and mangrove wetlands.	Zhou et al 2020

5. Methodologies to Use Mangroves for Remediation

Based on the examples covered in this chapter and the literature in general, a practical guide (Figure 6) has been developed to assist researchers and practitioners in projects aimed at rehabilitation, restoration, and decontamination of environments. The causes of mangrove pollution can be diverse, and according to chemical properties and characteristics, they can be inorganic or organic, however, they are often associated (Figure 6).

As presented in this chapter, mangrove species belong to different groups of plants and, consequently, have differential tolerance to contaminants. Some species, such as *A. marina*, have already been extensively explored to verify their ability to survive in polluted environments. The vast majority of studies are focused on heavy metals compared to persistent organic pollutants.

When faced with the problem of contamination, it is important to carry out preliminary analyses of sediment, surface water, and, if possible, interstitial water, as pollutants are likely to have contact with the roots. It is recommended to use the most sensitive techniques for detecting trace metals (Cd, Pb, Hg, Cr) because even in small quantities they are capable of causing great damage to sensitive species and are likely to bioaccumulate in the food chain. Atomic absorption spectrophotometry inductively coupled plasma-optical emission spectrometry (ICP-OES) or associated with a mass spectrometer (ICP-MS), etc. are suggested for characterization. Gas chromatography is normally used for the analysis of organic contaminants; however, organic compounds are complex, and the analysis is often expensive. It is recommended to carry out a detailed history of the site and ascertain whether the estuary receives pharmaceutical or industrial waste, or is close to agricultural areas, or petrochemical stations.

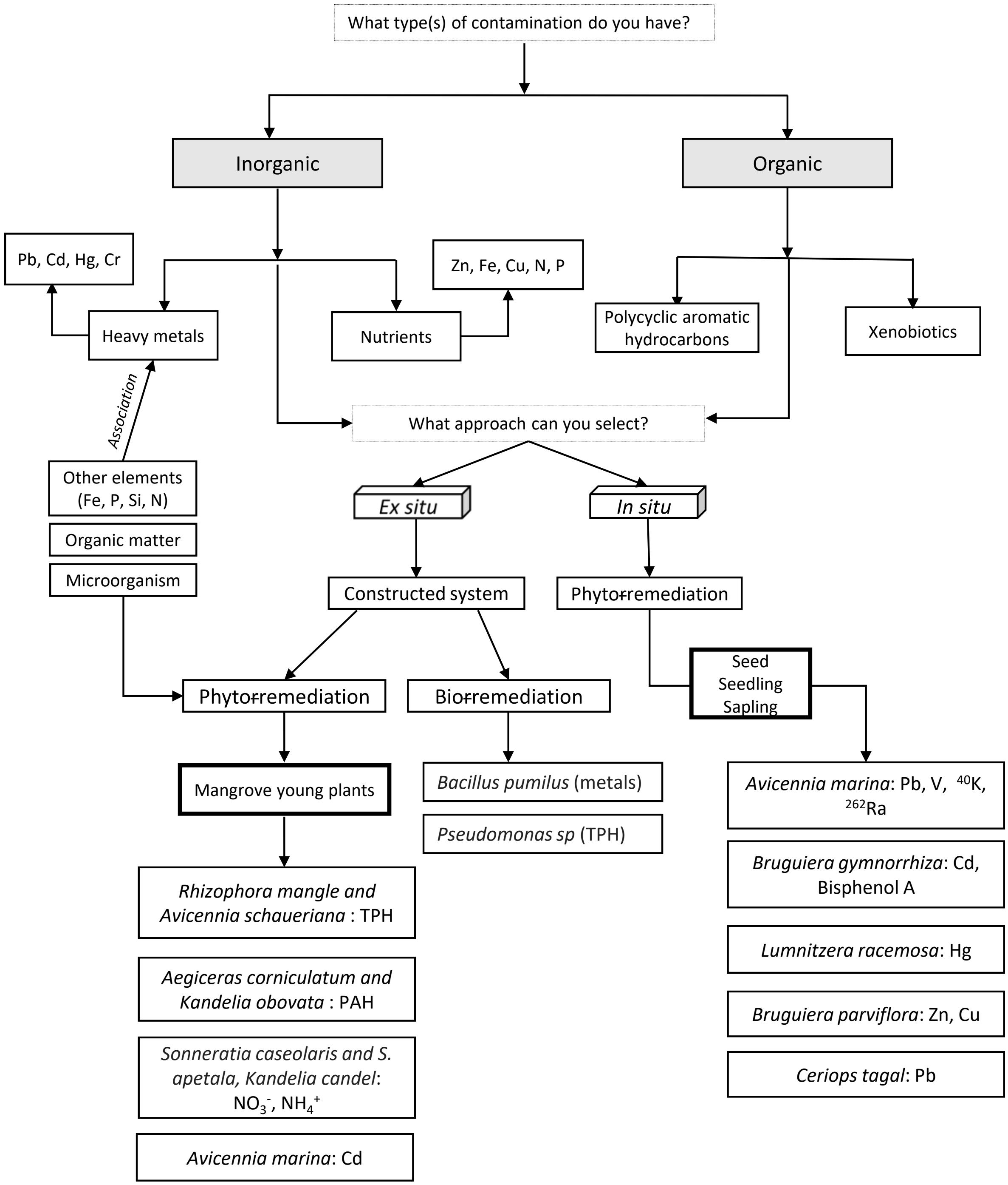

Figure 6 - Mangrove suggestions for environmental decontamination.

6. Final Comments

The importance of mangroves for coastal resilience and humans has been well documented throughout the tropics. In a broad sense, the importance of mangrove forest systems is related to ecological sustainability (e.g. sediment control, retaining pollutant and nutrient, and organic matter cycling), environmental security (mitigating the effects of the tsunami, cyclones, floods, and greenhouse gas), and economic prosperity (in supporting fish catch).

Trees are ideal for the remediation of heavy metal polluted mangroves because they can withstand higher concentrations of pollutants. As a consequence of their large biomass, they can accumulate large amounts of contaminants, and they can do this over huge areas and to great depths, because of their extensive root systems. Furthermore, they can stabilize an area, prevent erosion, and minimize the spread of contaminants because of their perennial presence. They can also be easily harvested and removed from the area with minimal risk, effectively taking with them a large quantity of the pollutants that were once present in the soil.

Mangroves can be used at different stages of life and different species combinations to remove organic or inorganic contaminants. Therefore, a careful choice of species based on the type of contamination, is necessary to ensure the optimal performance of the system.

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CAPITULO 2: A comparison of cadmium removal capability and Cd-tolerance of selected mangrove young plant

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ABSTRACT

The arboreal species *Avicennia schaueriana*, *Laguncularia racemosa* and *Rhizophora mangle* are abundant in mangrove sites, and in recent decades, environmental cadmium (Cd) sources have increased in this ecosystem. We evaluated the Cd toxicity in seedlings after being collected in the mangrove and previously acclimated in a hydroponic solution for 2 weeks until 4-6 leaves developed. After this period, seedlings were subjected to 0, 0.5 and 5 mg Cd L^{-1} for 21 days. Results clearly indicated that Cd induced a decrease in leaf area, CO_2 assimilation, stomatal conductance

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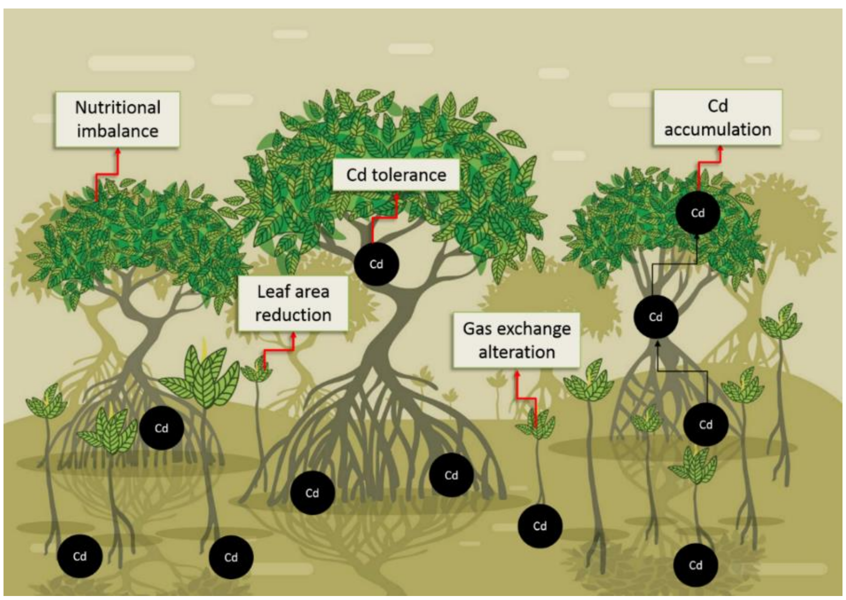
(*gs*), transpiration (*E*), relative water content, osmotic potential and Ca, Mg, K, Cu and Zn uptake in *L. racemosa*. Seedlings of *R. mangle* and *A. schaueriana* decreased *gs* and *E* values in response to Cd toxicity. The highest Cd accumulation was detected in roots of *L. racemosa* (56.8 mg g^{-1} ± 9.2), followed by *R. mangle* (41.7g ± 1.9) and *A. schaueriana* (14.8g ± 0.9). In terms of physiological responses, the results suggest that *A. schaueriana* and to a lesser extent *R. mangle* were the most tolerant to Cd toxicity, therefore, most suitable for rehabilitation and reintroduction of species in mangroves subject to Cd contamination.

Keywords Gas exchange • Heavy metal • Pollution • Toxicity

Highlights

- Seedlings of three mangrove species differed in terms of responses to cadmium (Cd) stress.
- Cd uptake affected leaf area, gas exchange, water relations and elemental composition.
- Sensitivity to Cd stress was lowest in *Avicennia schaueriana* and highest in *Laguncularia racemosa*.

Graphical abstract



1. Introduction

Mangroves are intertidal marine ecosystems composed of plants capable of withstanding the most adverse conditions: anoxia, eutrophication, siltation, and significant changes in soil temperature, flooding and high salt concentrations (Kathiresan and Bingham 2001). These complex ecosystems located in tropical and subtropical regions across the world play a key role in maintaining coastal ecological balance and supporting a wide variety of species (Feller et al. 2010). Reports on the effects of pollution from urbanization and industrialization on mangroves date back decades, but currently researchers have become concerned with the threats posed by specific pollutants such as metals. In this regard, heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg) and chromium (Cr) represent some of the most reported and threatening pollutants within mangrove ecosystems, due to their toxicity, persistence and bioaccumulation potential in plants, animals and humans (MacFarlane et al. 2003; Li et al. 2015; Miola et al. 2015; Liu et al. 2018).

Cadmium is a trace element with no established function in plant metabolism (Benavides et al. 2005). It is commonly reported to occur in soil and water owing to natural and anthropogenic sources (Khan et al. 2017; Rizwan et al. 2017; Suhl et al. 2018; Liu et al. 2018). Cadmium enrichment in the environment is a consequence of variable combinations of uncontrolled and improper waste disposal practices, mining, smelting, wastewater irrigation, industrial and vehicular emissions, manufacturing, and runoff from phosphate fertilizer on cropland (Khan et al. 2017).

In mangrove plants, cadmium phytotoxicity induces morphological changes in roots, sprouts, and leaves, and can adversely affect seed development and germination of *Kandelia candel* young plants, for example (Rahman et al. 2011). This metal-induced declines in net photosynthetic rate and water use efficiency of *A. germinans* (Gonzalez-Mendoza et al. 2007) and *A. schaueriana* seedlings (Garcia et al., 2018).

Cadmium uptake by plants can interfere with physiological processes such as altered uptake, transport and assimilation of nutrients, decreased photosynthesis and water uptake, and can promote morphological and ultrastructural abnormalities (Rizwan et al. 2017). Its effects in mangrove species specifically include the induction of water stress by decreasing stomatal conductance, transpiration rate, and relative leaf water content (e.g. *A. schaueriana* Garcia et al.

2018) and negative impacts on nutrient uptake and transport, and assimilation (Souza et al. 2014). While some mangrove species absorb Cd and accumulate it in roots, others translocate and subsequently accumulate the metal in leaves (MacFarlane et al. 2003). Bioaccumulated metals, including Cd, can return to the environment through plant senescence and mortality events and enter the trophic chain.

Several studies have investigated the accumulation of Cd in soil and in mangrove trees (Analuddin et al. 2017; Ghasemi et al. 2018), but inter-species comparisons, particularly at the seedling stage are limited. This motivated the present study, which compared seedling physiological and root and shoot elemental composition responses to Cd exposure across three mangrove species, viz. *Avicennia schaueriana* Stapf & Leechm. ex Moldenke, *Laguncularia racemosa* (L.) C. F. Gaertn. and *Rhizophora mangle* L. The study addressed the following research questions: (1) Are mangrove seedlings negatively affected in terms of gas exchange, plant water relations and elemental composition by Cd exposure?; (2) Does Cd accumulate in roots and/or leaves in mangrove seedlings?; (3) Are the effects of Cd exposure and/or species dependent? The results of this study will inform species selection for rehabilitation and species reintroduction in mangroves that are susceptible to Cd pollution.

2. Materials and methods

2.1. Experimental set-up

A controlled experiment was conducted under hydroponic conditions within a greenhouse (3 × 3 × 5 m), 26.7 ± 1.2ºC at the Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil (14°47'47.4"S 39°10'22.7"W). Seedlings of *A. schaueriana*, *L. racemosa* and *R. mangle* from Itacaré, Bahia, Brazil (14°16'28.7"S and 38°59'52.7"W) were collected (ensuring that the roots were undamaged) and acclimatized in Hoagland solution (1/:10; Hoagland and Arnon 1950) supplemented with NaCl (3 g L^{-1}), for two weeks until 4-6 leaves developed.

2.2. Cadmium treatment

After acclimatization, seedlings were treated with Cd at three concentrations: 0, 0.5 and 5.0 mg cadmium chloride hemi (pentahydrate) ($CdCl_2$ 2.5 H_2O L^{-1}) in quarter-strength Hoagland solution supplemented with NaCl (3g L^{-1}), pH 5-6, for 21 days. Cadmium concentrations were based on experimental previous studies (Garcia et al. 2018; Dai et al. 2018). The experiment used a factorial design (3x3) with four plants in each of four pots per treatment and the control. Solutions were replaced once a week, pH and conductivity were measured every 24 hours and, whenever necessary adjusted with HCl (1M) and NaOH (1M). After 21 days of exposure to Cd, physiological parameters, and growth (biomass and leaf area) were assessed, and plants were harvested and measured for leaf area (using ImageJ software) and oven-dried to determine the biomass of shoots and roots for individual seedlings.

2.3. Leaf gas exchange and chlorophyll index

On day 21 of treatment, we randomly selected four seedlings for each treatment and the control of each species for gas exchange measurements. Using young leaves, located at the second or third node, CO_2 assimilation (*A*), stomatal conductance (*gs*), transpiration rate (*E*), were analyzed using an infrared gas analyzer (IRGA, 6400XT, LI-COR, USA), fixed CO_2 at ± 390 ppm and photon flux density of 1000 µmol s^{-1} m^{-2}, the temperature of 28ºC (82,4ºF) and relative humidity of 70% (Garcia et al., 2018). All measurements were performed between 9:00 am and 11:00 am. These data were also used to calculate water use efficiency (WUE) and C*i*/C*a* (ratio of intercellular to atmospheric CO_2).

Falker chlorophyll content indexes (FCI) were obtained through a portable chlorophyll meter (ClorofiLog, Falker, Brazil). Measurements were performed on a single leaf located at the second or third node, for each of four replicates per treatment and for the control.

2.4. Macro- and micro-nutrient and Cd analysis

After 21 days, shoot and root samples were collected for chemical analysis. Macro and micronutrients concentration, as well as the relative Cd content, were quantified. A pool of four plants per pot, from each of four pots for each treatment and the control were sampled for these analyses. Samples were dried at 75ºC in a forced-circulation oven for five days due to the high-water content of the tissues. The plant samples were ground into fine particles in a tissue mill. The ground samples were then dried at 105ºC for four hours, placed in silica crucibles, weighed and then ashed in a muffle furnace at 500ºC for four hours. The ashed samples were digested in 2 mL of concentrated HCl (37%) diluted in water (1:1) on a heating hot plate set at 100°C. After digestion, sample volumes were adjusted to 25 or 10 mL, according to sample mass. Sample free blanks were produced along with laboratory reference material: using 1g oven-dried *Eucalyptus grandis* leaf material per 50 mL 16% HCl. Samples were analyzed on an Agilent 4100 MP-AES (Agilent, USA). Standard curves were used to calibrate the element analysis. Elemental total carbon, nitrogen and sulphur percentages were quantified using 0.1 g of dried plant material for each sample, on a Leco Trumac CNS (Leco, USA). These analyses were performed by the Analytical and Research Laboratory at the Institute for Commercial Forestry Research (ICFR), Pietermaritzburg, South Africa, in collaboration with University of Kwazulu-Natal.

2.5. Water relations

To measure osmotic potential ($\Psi\pi$) five leaf discs with 0.5 cm diameter were collected from four individual plants, from each species treatment combination after 21 days of treatment. Five leaf discs were measured individually using a thermocouple psychometer (C-52, Wescor), in combination with a dew point microvoltmeter (Psy-PRO, Wescor, Logan, USA). For relative water content (RWC %), five leaf discs with equal diameter were used. The fresh mass of each disc was measured at the end of treatment, after 24 hours in distilled water and dry mass was measured after 48 h in an oven at 75ºC. These data were then used to calculate RWC, according to the following equation: (RWC = (fresh mass – dry mass) / (turgid mass – dry mass) × 100).

2.6. Statistics analysis

Data were analyzed using the Generalized Linear Models (GLM) and comparisons of means were performed by contrast analysis at 5% probability. When at least two species showed significant differences for the same parameter, the relative impact of Cd on each species was calculated using the formula Mc - Mt / Mc x 100%, where Mc is the arithmetic mean of the control and Mt is the measure of the treatment. The percentages were analyzed by GLM and the means were compared by contrast analysis at 5% probability. All statistical analyses were performed using R version 3.0.0.

3. Results

3.1. Growth and Chlorophyll Index

A significant reduction in leaf area was observed for *L. racemosa* and *R. mangle* seedlings subjected to 0.5 and 5 mg Cd L^{-1}; however, leaf area was not significantly affected by Cd in *A. schaueriana*. There were no significant changes in shoot and root dry mass with exposure to Cd in all three species (Table 1).

Apart from a slight increase related to the control in chlorophyll *a* and *b* values at 0.5 mg Cd L^{-1} in *A. schaueriana* and *L. racemosa* and at 5 mg Cd L^{-1} in *R. mangle*, Cd exposure did not result in any significant changes in chlorophyll indices across the three species (Table 1).

3.2. Leaf gas exchange

Stomatal conductance and CO_2 assimilation were significantly lower in *L. racemosa* seedlings exposed to 5 mg L^{-1} Cd compared to the other treatment (0.5 mg L^{-1}) and control. This decrease in *gs* was equivalent to 22%, while the decrease in *A* was 66%. Seedlings of *A. schaueriana* and *R. mangle* also exhibited a 48% and 63% decrease in *gs*, respectively, when exposed to 5 mg L^{-1} of Cd but no significant changes in *A* were observed (Fig. 1A). In terms of C*i*/C*a* ratio, a 32% decline relative to the control was observed for *R. mangle* seedlings exposed to 5 mg Cd L^{-1} only (Fig. 1D).

Exposure to the highest Cd concentration induced a decrease in *E* in the three species evaluated, with decreases of 42%, 71% and 54% relative to the control being observed in *A. schaueriana*, *L. racemosa* and *R. mangle* seedlings, respectively (Fig. 1C). Water Use Efficiency (WUE) in control *R. mangle* seedlings was 57%, lower, relative to seedlings exposed to 5 mg Cd L^{-1}. Exposure to Cd did not change WUE in the other two species.

3.3. Cadmium, macro and micronutrient content

In terms of macronutrients, Cd affected the absorption of potassium (K) in two species: *A. schaueriana* seedlings showed a reduction in root K content, with values in seedlings exposed to 5 mg Cd L^{-1} being nearly 50% lower than in the control; in *L. racemosa* there was a 50% decrease, relative to control for 5 mg Cd Kg^{-1} and 17% for 0.5 mg Cd Kg^{-1} in root K levels (Table 2). On the other hand, there was an increase in K concentration in the shoots of *R. mangle*, when grown at 5 mg Cd L^{-1}. As for Ca content, significant changes were only observed for *L. racemosa* shoots, which displayed a 60% decrease in levels of this nutrient relative to the control, when exposed to 5 mg Cd L^{-1} and a 40% decrease at the lowest Cd treatment. The levels of Mg decreased by about 27% and 39% in *A. schaueriana* shoots after exposure to 0.5 and 5 mg Cd L^{-1}, compared to the control. In *R. mangle* shoots, a 70% increase in sulphur (S) was also detected when 5.0 mg Cd L^{-1} exposed seedlings were compared to the control. Concerning micronutrient concentrations, Cd induced changes in the accumulation of boron (B), manganese (Mn), zinc (Zn), copper (Cu), and sodium (Na) were observed (Table 3). The shoots of *A. schaueriana* displayed reduced levels of B, compared to the control, regardless of the Cd concentration. Contrastingly, the B content in *R. mangle* shoots increased by 61%, in relation to the control in seedlings treated with the highest Cd concentration.

Avicennia schaueriana demonstrated higher Cu accumulation in shoots, compared to the control when cultivated at 0.5 mg Cd L^{-1} (13 mg of Cu Kg^{-1}). Additionally, roots of *A. schaueriana* seedlings treated with Cd exhibited Mn levels 65% lower than those control seedlings. This species also displayed a 20% decrease in Na when exposed to both Cd concentrations. Conversely, shoot Cu levels in *L. racemosa* decreased if compared to the control in both treatments. On the other hand, Na concentration in *L. racemosa* roots increased 42-29% with Cd presence. The exposure of *L. racemosa* to Cd also affected the levels of Zn in the shoots, with reductions in the range of 30-40%. For all three species, tissue analyses revealed shoot Cd levels to be higher than in the control. Shoots of control seedlings displayed Cd levels that were below the detectable limit (<0.01 mg Cd Kg^{-1}).

The presence of Cd in root and shoot tissues of the treatment seedlings also suggested root uptake and effective translocation. However, Cd levels were higher in roots than in shoots and there were significantly higher Cd concentrations in the organs of plants exposed to the highest Cd concentration (Table 3). Across the three species, *L. racemosa* had the highest Cd absorption capacity: 89% higher for roots and 11% higher for shoots. *R. mangle* plants also exhibited higher Cd concentrations than *A. schaueriana* at the highest treatment concentration. The total Cd level in *L. racemosa* tissues of roots and shoot combined was 289% higher than *A. schaueriana* and 35% more than *R. mangle* (data not shown).

3.5. Water relations

Despite changes in the element concentrations brought about by Cd exposure described above, exposure to the metal-induced did not affect RWC of *L. racemosa* exposed to 5 mg L^{-1} Cd only. However, there was a significant reduction (-2.9 MPa) in the osmotic potential ($\Psi\pi$) compared to the control plants, and the *L. racemosa* seedlings treated with 5 mg L^{-1} Cd. Cadmium exposure did not affect water relations in *A. schaueriana* and *R. mangle* (Fig. 2).

4. Discussion

Mangroves around the world have been shown to exhibit high levels of heavy metals, particularly Cd, in sediments accumulated in the soil (Kumar et al. 2015; Silveira et al. 2017; Ghasemi et al. 2018; Shi et al. 2019). Cadmium accumulation in plants can alter uptake, transport and assimilation of macro and micronutrients, decrease photosynthesis, growth and water uptake, and induce morphological abnormalities. In the present study, Cd exposure affected leaf expansion in *L. racemosa* and *R. mangle,* where these species exhibited reduced leaf area, when exposed to all Cd concentrations used. However, root, shoot, and total biomass were not significantly affected, contrary to the literature on the effects of Cd on plants (Benavides et al. 2015) and mangroves species in particular (Dai et al. 2018; Jiang et al. 2019). Cadmium tolerance can be observed by plant growth under Cd treatment (He et al. 2017; Lira et al., 2018). It was also encouraging to note that even though leaf area was reduced in two, *L. racemosa* and *R. mangle*, of the three species investigated, biomass allocation to roots and shoots and total biomass was not significantly affected by Cd. Other studies involving heavy metal toxicity in plants have shown that some plants can reduce leaf area and specific leaf area whilst not compromising biomass accumulation (Maksymiec

& Baszyński 1996; Lira et al. 2018). The effects on leaf area observed in this study were probably a consequence of the fact that the seedlings were in the intensive leaf growth stage.

Cadmium is an efficient inhibitor of photosynthesis through its negative effects on photochemical efficiency and chlorophyll content, and these parameters are regarded as useful indicators of Cd stress (Chen et al. 2008). In this study, the effects of Cd exposure on chlorophyll *a* and *b* contents were not significant, despite contrary reports on the effects of heavy metals on leaf chlorophyll content in mangrove species *A. marina* (Jian et al. 2019) and other plants (Rizwan et al. 2017). There are also reports of leaf chlorophyll contents not being affected significantly at the low Cd levels (8.9 µM), but decreasing significantly at higher levels (44.5, 89 and 178 µM Cd) (e.g. *Conocarpus erectus* [Rehman et al. 2019]). Even though chlorophyll contents were not affected, CO_2 assimilation was reduced in *L. racemose*, when seedlings were exposed to 5 mg Cd L^{-1} and this was probably a consequence of the accompanying reduction in stomatal conductance. Cadmium has been shown to reduce photosynthetic rates in plants (He et al. 2017), including mangrove and aquatic species (Gonzalez- Mendonza et al. 2007; Souza et al. 2009). Garcia et al. (2018), working on *A. schaueriana*, have suggested that CO_2 assimilation (*A*) can be used as an indicator of mangrove plant toxicity to Cd, and reduced *A* (Prasad 1995) can inhibit photosystem II and various stages of the Calvin cycle. There is a well-established correlative relationship between stomatal conductance and photosynthetic rate, and it should be noted that the quantum yield, which has a direct influence on photosynthetic rate, was compromised in *L. racemose,* as a result of the reduced leaf area. The reduction in CO_2 assimilation under Cd stress in this species was most likely due to stomatal limitation and this is supported by the fact that transpiration rate was reduced in *L. racemosa* only under Cd exposure, specifically, 5 mg Cd L^{-1}. However, it is worth noting that the reduction in photosynthetic rate in *L. racemosa* was not severe enough to decrease biomass accumulation in this species. Like *A. schaueriana* and *R. mangle* other species (Perfus-Barbeoch et al. 2002; He et al. 2017), have also been shown to maintain photosynthetic rates under Cd stress. In the case of *R. mangle* this maintenance of growth and gas exchange status under Cd stress is particularly interesting, since this species exhibited a large increase in WUE and reduction in Ci/Ca, when exposed to the highest Cd concentration. When a reduction in Ci/Ca is followed by an increase in WUE, it can be inferred that there is no stomatal limitation for the entry of CO_2 (Ellsworth and Cousins 2016), however, biochemical processes may be affected.

In terms of leaf water status, osmotic potential was only affected in *L. racemosa*, where seedlings showed a significant reduction in $\Psi\pi$ related to control plants; however, this did not affect RWC, which remained unchanged by Cd in all species investigated. Cadmium can affect membrane permeability, causing a reduction in tissue water content (Gallego et al. 2012), and can pass through calcium channels disturbing plant water status (Perfus-Barbeoch et al. 2002). The changes in $\Psi\pi$ in *L. racemosa* may have been a consequence of an increase in Na and decrease in Ca and K uptake but can also be related to a decrease in stomatal conductance and transpiration rates induced by Cd in this species.

All species showed signs of absorbing and translocating Cd. *In situ* studies have shown mangrove species to exhibit different partitioning and uptake capabilities for heavy metals such as Cd (Analuddin et al. 2017; Ghasemi et al. 2018). When these metals accumulate in plants they can return to the environment after senescence and threaten other organisms through trophic magnification (Costa et al. 2017; Almahasheer et al. 2018: Carvalho-Neta et al. 2019). Cadmium toxicity is dependent on its biosorption, bioaccumulation and ion-exchange mechanisms (Haider et al., 2021). Cadmium generally compromises the uptake of macro and micronutrients (Li et al. 2018), leading to nutritional imbalances in bivalent cations such as Zn, Ca, Fe, Mg and Mn, since they compete with Cd for ion-exchange sites in soil, consequently affecting their uptake (He et al. 2017). This appears to have been the case in *A. schaueriana* in this study, where Cd exposure altered levels of Zn, Mn and Mg decreased significantly compared with the control plants. Another example is the reduced levels of Ca in Cd-treated *L. racemosa*, which was higher than *A. schaueriana* and *R. mangle* under similar Cd treatments. Higher accumulation of Cd in *L. racemosa* may be due to its ion exchange with Ca^{2+} followed by adsorption/bioaccumulation, Cd can replace Ca at the cation exchange sites and compete for the same Ca^{2+} channels in shoots and roots, which may explain the reduction in Ca content in Cd exposed *L. racemosa* plants (Perfus-Barbeoch et al. 2002). Rehman et al. (2019) observed that in the mangrove *C. erectus* Cd exposure increased the concentrations of K in roots and decreased concentrations in shoots. Those authors also showed that Na has an important role in modulating Cd toxicity in mangroves. The elements Ca, K, and Na have an important function in water regulation in plants (López-Portillo et al. 2014; Reef and Lovelock 2015), and the imbalance in their absorption could explain alterations in RWC and $\Psi\pi$ observed in *L. racemosa.* In addition, Cd can negatively affect the absorption, transport and utilization of Mg, Zn and Mn and water relations, jeopardizing general plant metabolism, enzyme activation, the concentration of ATP, chlorophyll and oxygen production (Das et al. 1997). Magnesium also interferes in Ca and K uptake, as observed in *L. racemosa* and in *Citrus maxima* considering that such nutrients are strongly antagonistic (Nguyen et al. 2017).

Interestingly, the effects of Cd exposure on shoot Cu concentration differed across species. While Cu levels were decreased in *L. racemosa* leaves under Cd stress, in *A. schaueriana* leaves Cu concentrations increased. Copper is present in chloroplasts and plays an important function in the efficiency of PSII (Bishop, 1964). In plants, Cu levels can influence cellular respiration, leaf transpiration rate, photosynthesis and growth (He et al. 2017). There is no fixed Cu response pattern for plants exposed to Cd; levels of this micronutrient can increase or decrease under Cd stress. In this sense, Kabata-Pendias (2011) concluded that the interaction between Cd and Cu can be antagonistic or synergistic. *Avicennia schaueriana* also exhibited a decrease in B content, and Chen et al. (2018) suggest that B might have the ability to form a complex with Cd or induce Cd to deposit in the cell walls, thus lowering Cd toxicity in rice plants. That could be an explanation for Cd responses in this specie.

While all three species investigated exhibited the ability to take up and translocate exogenously applied Cd, the effects of Cd on growth, physiology and elemental composition were species-specific in some cases. *Laguncularia racemosa* was negatively affected in terms of leaf area, gas exchange, plant water relations and elemental composition. Importantly, the Cd-induced decline in *A* in this species appears to have been a consequence of stomatal limitation, rather than an altered chlorophyll index. *Rhizhophora mangle* was less severely affected, showing no alterations in *A* under Cd stress, but a decline in WUE, Ci/Ca, leaf area and altered K and B levels related to the control plants were observed. Despite a decrease in leaf area, reduction in *E* and alterations in Cu, B, K and Na uptake, *A. schaueriana* seedlings appear to have been the most tolerant to Cd exposure, maintaining the physiological status in terms of photosynthesis, WUE and water relations under Cd stress. *Avicennia* has been reported to exhibit strategies for Cd tolerance, such as retention in roots, immobilization in cell walls, and complexation by phytochelatins and metal-binding proteins (Dai et al. 2018; Garcia et al. 2018; Rahman et al. 2019). Cadmium accumulated in roots and shoots in all three species and the greater susceptibility of *L. racemosa* to Cd stress appears to be a consequence of the fact that seedlings of this species contained 300% more Cd than *A. schaueriana* and 40% more *R. mangle*.

5. Conclusions

Seedlings of three mangrove species differed in terms of responses to Cd stress. Even at low concentration, *A. schaueriana*, *L. racemosa* , and *R. mangle* exhibited the ability to uptake and translocate Cd. Cadmium affected growth, physiology, and elemental composition and those responses were species-specific in some cases. The highest salt tolerance in *A. schaueriana* confers the lowest sensitivity to Cd stress, the opposite was observed in *L. racemosa*. The experimental approach adopted in this study is important to understand the physiology of these species, looking for strategies that promote the establishment of young plants in natural environments contaminated with Cd. Collectively, results suggest that *A. schaueriana* and, to a lesser extent, *R. mangle* are suitable for rehabilitation and species reintroduction programs in mangroves subject to Cd pollution. The experimental approach and bio-indicators adopted in this study may be useful in selecting species for such programs.

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Figures and captions

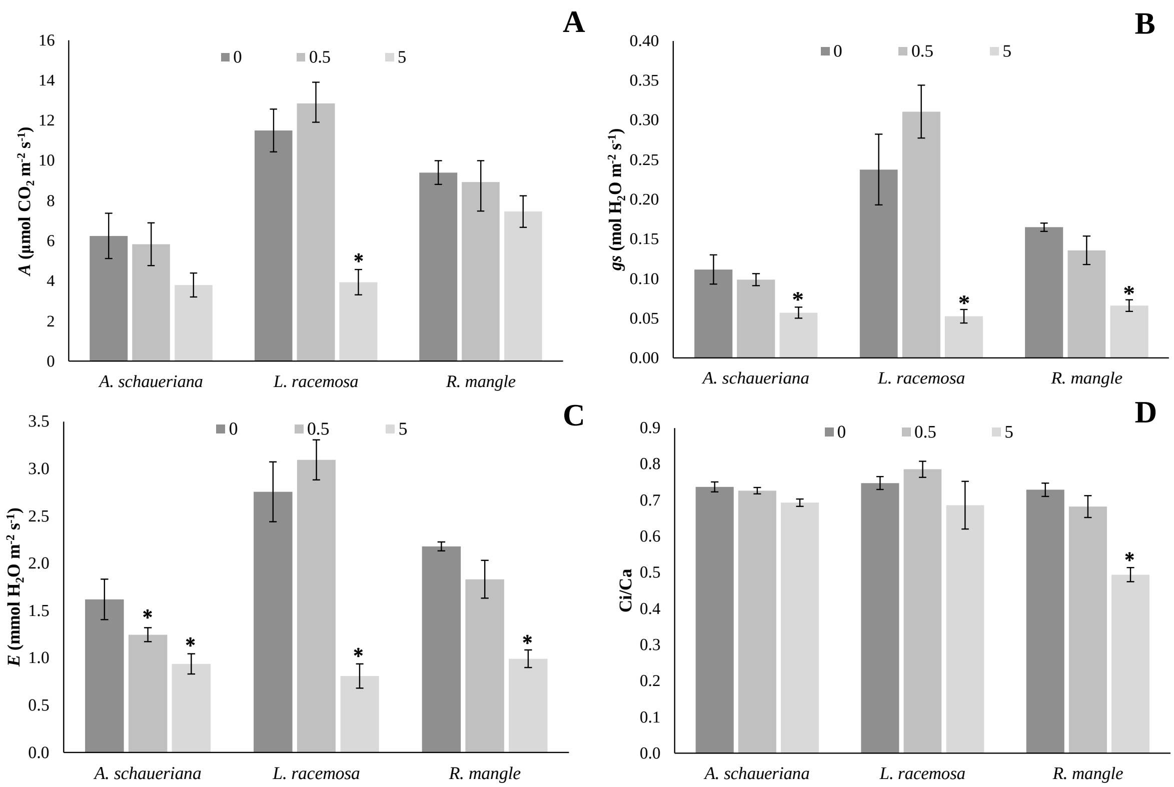

Figure 1. Leaf gas exchange in *A. schaueriana*, *L. racemosa* and *R. mangle* seedlings exposed to three $CdCl_2$ concentrations (0, 0.5 and 5 mg L^{-1}) for 21 days. A - CO_2 assimilation (*A*, µmol CO_2 m^{-2} s^{-1}); B - stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}); C - leaf transpiration (*E*, mmol H_2O m^{-2} s^{-1}) and D - Internal/atmospheric CO_2. Columns represent mean ± SE (n=4) and when labeled with an asterisk indicate treatments where the parameter measured, differ significantly of the control for the respective species according to Analysis of Deviance followed by a post hoc contrast test ($p < 0.05$). Mean ± standard error (n=3).

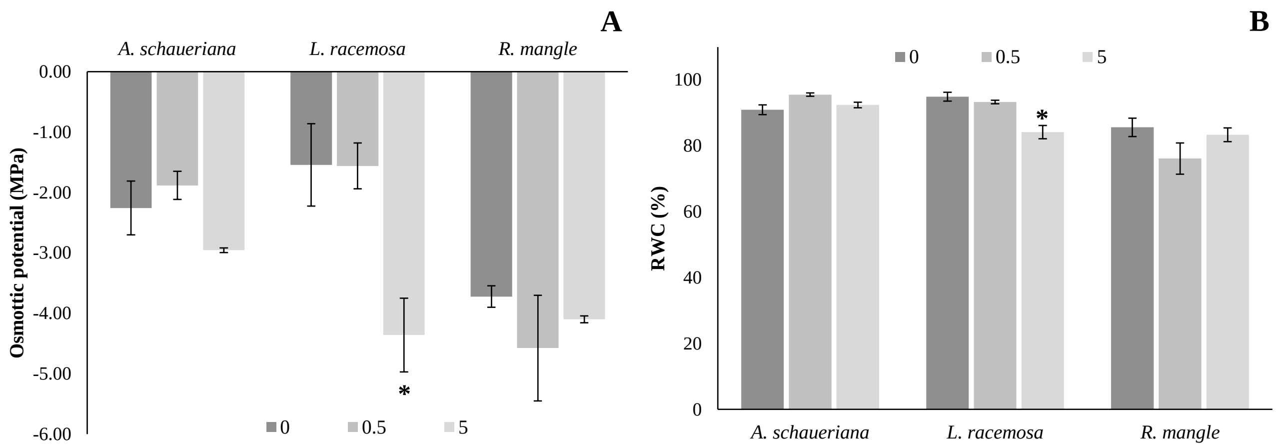

Figure 2. Cadmium effect on hydric status of *A. schaueriana*, *L. racemosa* and *R. mangle* mangrove species after 21 days of exposure. A - Osmotic potential (MPa) and B - Relative water content (RWC, %). Columns represent mean ± SE (n=3) and when labeled with an asterisk indicate treatments, where the parameter measured, differ significantly of the control for the respective species according to Analysis of Deviance followed by a post hoc contrast test ($p < 0.05$).

Table 1 – Morphophysiological parameters of three mangrove species (*A. schaueriana*, *L. racemosa*, and *R. mangle*) after 21 days of growth in nutrient solution supplemented with different concentrations of $CdCl_2$ (0, 0.5, 5 mg L^{-1}).

Parameters	***Avicennia schaueriana*** Cd (mg L^{-1})			***Laguncularia racemosa*** Cd (mg L^{-1})			***Rhizophora mangle*** Cd (mg L^{-1})		
	0	**0.5**	**5**	**0**	**0.5**	**5**	**0**	**0.5**	**5**
Leaf area (cm^2)	3.42 ± 0.53	2.62 ± 0.22	2.21 ± 0.19	6.58 ± 0.15	**4.45 ± 0.39**	**3.46 ± 0.35**	33.17 ± 8.30	**18.02 ± 2.20**	**16.83 ± 2.07**
Shoot Biomass (g)	1.78 ± 0.52	1.24 ± 0.23	1.56 ± 0.17	1.29 ± 0.22	1.46 ± 0.12	1.24 ± 0.12	9.42 ± 0.72	9.09 ± 1.51	8.68 ± 1.20
Root Biomass (g)	0.84 ± 0.16	0.70 ± 0.09	0.70 ± 0.03	0.76 ± 0.09	0.70 ± 0.03	0.55 ± 0.04	1.95 ± 0.15	2.18 ± 0.46	2.27 ± 0.41
Total Biomass (g)	2.62 ± 0.67	1.94 ± 0.31	2.27 ± 0.17	2.06 ± 0.30	2.15 ± 0.15	1.79 ± 0.15	11.37 ± 0.86	11.27 ± 1.97	10.99 ± 1.61
Chlorophyll *a*	28.70 ± 0.88	30.72 ± 1.57	29.62 ± 0.66	32.05 ± 0.94	32.57 ± 0.37	29.47 ± 1.04	34.80 ± 0.35	34.57 ± 0.90	35.67 ± 0.49
Chlorophyll *b*	13.85 ± 1.02	19.35 ± 2.58	12.92 ± 1.35	19.90 ± 4.54	21.82 ± 0.43	16.15 ± 1.62	30.37 ± 0.80	30.30 ± 2.46	33.45 ± 1.80
Total Chlorophyll	42.55 ± 1.75	50.07 ± 4.15	42.55 ± 0.86	51.95 ± 5.46	54.40 ± 0.74	45.62 ± 2.64	65.17 ± 1.04	64.87 ± 2.99	69.12 ± 2.25

Values in bold indicate treatments where the parameter measured, differ significantly of the control for the respective species according to Analysis of Deviance followed by a post hoc contrast test ($p < 0.05$). Mean ± standard error (n=4).

Table 2 – Macronutrient concentration in the shoot and roots of three mangrove species (*A. schaueriana*, *L. racemosa*, and *R. mangle*) after 21 days growing in nutrient solution with different concentrations of $CdCl_2$ (0, 0.5, 5 mg L^{-1}).

Species		**Cd (mg L^{-1})**	**C**	**N**	**S**	**P**	**Ca**	**Mg**	**K**
				%		**g Kg^{-1}**	**g Kg^{-1}**	**g Kg^{-1}**	**g Kg^{-1}**
A. schaueriana	Shoot	0	41.31	2.13	0.30	1.16 ± 0.08	2.92 ± 0.33	3.11 ± 0.13	19.54 ± 2.33
		0.5	41.42	2.12	0.19	1.53 ± 0.23	2.26 ± 0.77	**2.26 ± 0.04**	15.85 ± 1.24
		5	42.17	2.03	0.14	1.34 ± 0.14	1.28 ± 0.36	**1.90 ± 0.11**	12.51 ± 0.37
	Root	0	37.28	1.51	0.32	1.89 ± 0.22	3.07 ± 0.09	3.39 ± 0.30	28.31 ± 1.59
		0.5	39.37	1.32	0.20	1.84 ± 0.08	3.58 ± 0.90	3.39 ± 0.37	**19.71 ± 0.85**
		5	39.18	1.43	0.15	1.48 ± 0.15	1.62 ± 0.38	2.53 ± 0.59	**13.04 ± 3.12**
L. racemosa	Shoot	0	44.35	1.82	0.26	1.05 ± 0.28	11.59 ± 0.84	3.00 ± 0.17	15.17 ± 0.35
		0.5	42.78	1.52	0.26	1.02 ± 0.05	**7.15 ± 1.12**	**2.26 ± 0.27**	12.09 ± 1.62
		5	43.76	1.30	0.21	1.11 ± 0.14	**4.65 ± 1.12**	**1.65 ± 0.35**	9.31 ± 1.98
	Root	0	43.03	1.40	0.26	1.25 ± 0.21	3.48 ± 0.28	0.77 ± 0.06	17.08 ± 2.08
		0.5	43.72	1.75	0.27	1.37 ± 0.24	5.31 ± 0.74	1.00 ± 0.10	14.14 ± 0.40
		5	44.19	1.60	0.21	1.12 ± 0.09	4.05 ± 0.33	0.84 ± 0.10	**8.75 ± 0.61**
R. mangle	Shoot	0	46.32	0.83	0.13	1.54 ± 0.03	2.64 ± 0.66	1.34 ± 0.10	3.40 ± 0.12
		0.5	45.96	0.84	0.18	1.11 ± 0.14	2.11 ± 0.37	1.39 ± 0.04	3.61 ± 0.13
		5	45.56	0.98	**0.22**	1.05 ± 0.23	2.81 ± 0.36	1.70 ± 0.17	**4.56 ± 0.07**
	Root	0	43.99	0.74	1.04	1.45 ± 0.24	3.42 ± 0.51	2.05 ± 0.23	4.30 ± 0.36
		0.5	44.41	0.82	0.85	1.61 ± 0.18	3.26 ± 0.20	2.41 ± 0.09	4.99 ± 0.23
		5	42.20	0.77	0.68	1.05 ± 0.23	2.50 ± 0.11	2.01 ± 0.157	4.08 ± 0.32

Values in bold indicate treatments where the parameter measured, differ significantly of the control for the respective species according to Analysis of Deviance followed by a post hoc contrast test ($p < 0.05$). Mean ± standard error (n=3).

Table 3 – Micronutrients and non-essential elements concentration in the shoot and roots of three mangrove species (*A. schaueriana*, *L. racemosa*, and *R. mangle*) after 21 days growing in nutrient solution with different concentrations of $CdCl_2$ (0, 0.5, 5 mg L^{-1}).

Species		**Cd (mg L^{-1})**	**Fe**	**Cu**	**Mn**	**Zn**	**B**	**Na**	**Cd**
			g Kg^{-1}	**mg Kg^{-1}**	**mg Kg^{-1}**	**mg Kg^{-1}**	**mg Kg^{-1}**	**g Kg^{-1}**	**mg Kg^{-1}**
A. schaueriana	Shoot	0	0.81 ± 0.02	8.7 ± 0.6	19.7 ± 1.8	14.0 ± 2.5	18.0 ± 0.7	30.22 ± 0.88	<0.01
		0.5	0.81 ± 0.09	**13.0 ± 1.1**	30.9 ± 16.4	12.6 ± 3.1	**8.6 ± 0.4**	**24.84 ± 19.31**	**0.1 ± 0.07**
		5	0.93 ± 0.16	8.9 ± 1.2	15.1 ± 3.8	11.5 ± 0.8	**4.2 ± 0.1**	**23.59 ± 0.99**	**1.6 ± 0.2**
	Root	0	5.68 ± 1.00	17.0 ± 2.2	134.5 ± 21.7	31.6 ± 4.1	11.2 ± 1.8	17.46 ± 0.67	0.05 ± 0.02
		0.5	5.62 ± 1.43	15.7 ± 1.7	**51.3 ± 15.3**	**13.8 ± 2.5**	6.0 ± 2.7	16.21 ± 1.54	**6.2 ± 0.4**
		5	4.92 ± 0.97	16.3 ± 4.5	**43.3 ± 17.4**	**11.4 ± 1.7**	5.2 ± 2.2	11.92 ± 2.40	**14.8 ± 0.9**
L. racemosa	Shoot	0	0.84 ± 0.09	18.9 ± 1.4	43.0 ± 3.9	20.7 ± 2.7	23.1 ± 1.8	14.95 ± 0.18	<0.01
		0.5	0.95 ± 0.04	**11.5 ± 1.7**	20.4 ± 1.9	**14.2 ± 0.5**	17.5 ± 3.6	14.68 ± 1.24	2.0 ± 0.6
		5	1.14 ± 0.18	**10.5 ± 1.7**	31.0 ± 5.9	**12.4 ± 0.5**	21.0 ± 3.6	13.42 ± 3.12	**7.0 ± 1.2**
	Root	0	4.35 ± 0.65	22.3 ± 3.4	45.6 ± 17.3	58.6 ± 17.1	22.6 ± 2.8	9.51 ± 0.67	1.0 ± 0.04
		0.5	6.86 ± 1.09	17.7 ± 1.5	43.4 ± 13.5	29.8 ± 7.6	28.1 ± 4.9	**13.58 ± 0.79**	**24.5 ± 6.2**
		5	5.51 ± 1.04	14.1 ± 1.6	100.8 ± 58.8	25.5 ± 2.7	20.4 ± 2.6	**12.28 ± 0.91**	**56.8 ± 9.2**
R. mangle	Shoot	0	0.38 ± 0.10	7.5 ± 0.8	25.2 ± 6.0	3.9 ± 0.8	7.0 ± 0.5	15.78 ± 0.51	<0.01
		0.5	0.51 ± 0.09	7.4 ± 0.8	31.5 ± 3.7	2.3 ± 0.2	7.7 ± 0.6	17.78 ± 1.25	**0.48 ± 0.21**
		5	0.46 ± 0.06	11.4 ± 1.8	37.0 ± 7.1	2.5 ± 0.7	**11.4 ± 0.4**	17.67 ± 0.29	**5.6 ± 0.25**
	Root	0	1.82 ± 0.61	14.2 ± 3.1	36.4 ± 1.7	11.9 ± 5.0	6.2 ± 0.5	17.86 ± 1.36	0.32 ± 0.10
		0.5	2.25 ± 0.48	15.5 ± 2.4	41.8 ± 17.6	7.1 ± 2.3	7.1 ± 0.6	19.38 ± 0.31	**6.5 ± 0.9**
		5	2.33 ± 0.30	15.2 ± 1.6	29.4 ± 9.4	33.9 ± 28.7	8.6 ± 0.7	17.09 ± 1.08	**41.7 ± 1.9**

Values in bold indicate treatments where the parameter measured, differ significantly of the control for the respective species according to Analysis of Deviance followed by a post hoc contrast test ($p < 0.05$). Mean ± standard error (n=3).

CAPITULO 3: Silicon alleviation of cadmium toxicity in the early development of white mangrove (*Laguncularia racemosa*) (L.) C. F. Gaertn.

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Abstract

Cadmium (Cd) induced toxicity in most plants can be mitigated by interactions with other chemical elements such as silicon (Si). Silicon is a chemical element widely known for its attenuative effect on biotic and abiotic stresses. However, the mechanisms involved in the initial stages of development in mangroves are still only partially understood. Based on this premise, this study aims to evaluate the effect of sodium silicate in the attenuation effects of Cd toxicity in white mangroves (*Laguncularia racemosa*). Propagules, seedlings with cotyledon, and young plants of *L. racemosa* were exposed to treatments: (1) control (nutrient solution); (2) 0.1% sodium silicate (Diatom®); (3) Cd at a concentration of 5 mg L^{-1} and (4) Cd + Si association. Silicon increased lignin deposition and reduced necrosis in the apex of hypocotyl, and also decreased the Cd accumulation in propagules and young plant roots. A photosynthetic maximum quantum yield (F_v /F_m) analysis showed similar values in plants subjected to Cd + Si and 5 mg Cd L^{-1} in a nutrient solution, indicating that Si could be involved in the reduction of photoinhibition. However, no

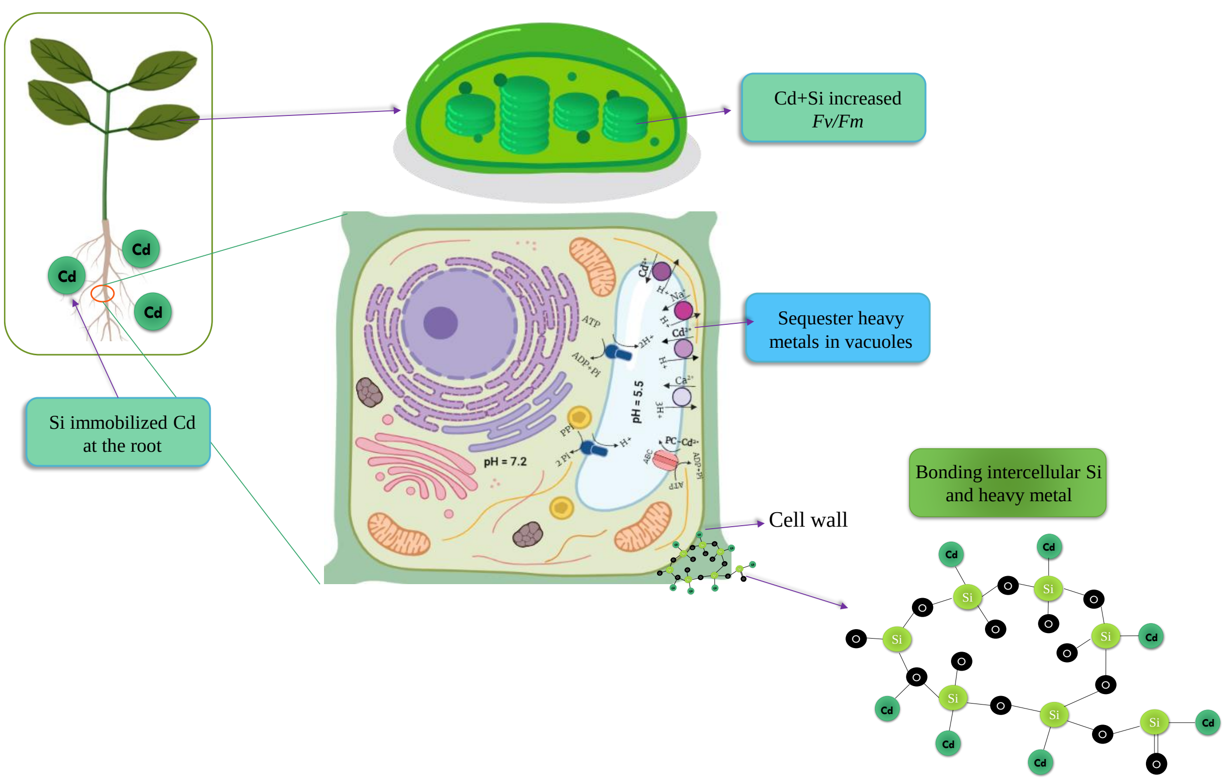
differences were observed in leaf gas exchange in plants subjected to Cd and Cd + Si. Application of Si could reduce Cd toxicity in *L. racemosa* metabolism, especially in propagules.

Keywords**:** Phytoremediation, heavy metals, pollution, coastal ecosystem.

Highlights

- Silicon (Si) attenuates cadmium (Cd) stress on the early development of *L. racemosa*.
- Cd + Si affects hypocotyl development and xylem vessel diameter size.
- Si mitigates the negative effects of Cd on photosynthetic maximum quantum yield.
- Cd content in propagules exposed to Cd + Si was reduced by almost 50%

Graphical abstract



1. Introduction

Mangroves are one of the most highly dynamic ecosystems, with high sensitivity to environmental disturbances (Bernini et al., 2010). Mangroves and their sediments have an important ecological value because they can act as natural sinks for heavy metals due to their high capacity to sequester such metals from tidal waters, estuaries, and rivers (Mitra, 2020). Mangrove plants may alleviate the toxicity of heavy metals through cell regulation mechanisms, including metal accumulation in the cell wall, metal compartmentation in the vacuole, and complexation with cellular ligands (Zhang et al., 2013; Garcia et al., 2018). Tolerance to heavy metals is a trait that often co-occurs with salt-tolerant mechanisms in some mangrove plants (Sari & Din, 2012; Kumari et al, 2019), for example, in *Avicennia schaueriana*, cadmium (Cd) can be excreted through salt glands on the adaxial surface of leaf tissue (Mizushima et al, 2019).

In recent years, the increase in Cd concentration has been detected at high levels in various ecosystems, including mangroves (Ghasemi et al., 2018; Zhao et al., 2018; Mahmood et al., 2019;). Cadmium enters ecosystems via numerous anthropogenic activities, reaching the soil principally by way of phosphate fertilizers (Haider et al., 2021). Cd-induced stress can lead to photosynthetic deficiency, leaf chlorosis, nutrient reduction, compromised water absorption, stomatal closure, increased reactive oxygen species (ROS) production, and can also induce morphological, anatomical, and ultrastructural changes in plants (Rizwan et al., 2017; Garcia et al., 2018, Mizushima et al, 2019; Haider et al., 2021).

There is increasing evidence that silicon (Si) has many beneficial effects on plant growth and development; in certain plant species, Si can improve resistance to environmental stresses such as drought, high temperature, ultraviolet radiation, loading, freezing, as well as chemical stress, including salinity, nutrient imbalance, and metal toxicity (Sahebi et al., 2014). The addition of Si to a soil solution can reduce metal toxicity by increasing soil pH, complexing with heavy metal in soil, and altering the speciation of metals in the soil solution. In roots, Si reduces heavy metal uptake by immobilizing metals in the root apoplast, reducing oxidative stress, increasing root hair frequency, and consequently increasing root biomass (Sahebi et al., 2014; Emamverdian et al., 2018). In stems, Si increased xylem sap, decreases metal concentration in sap, and promotes metal immobilization in the stem (Adrees et al., 2015). In leaves, Si can increase antioxidant compound levels and photosynthetic pigments, modulate genes that protect the photosynthetic machinery, increase the stomatal frequency and biomass, promote the co-precipitation of Si with metals, and chelation of metals with ligands (Rastogi et al., 2021).

Silicon can alleviate Cd toxicity in many plants (Wu et al., 2019), including mangrove species such as *A. marina* (Zhang et al., 2014) and *Kandelia obovata* (Ye et al., 2012). Silicon can decrease Cd toxicity caused by increased pH due to detoxification processes (Sahebi et al., 2014). Deposition of Si around the endodermis provides the potential to control Cd apoplastic transport by physically obstructing the apoplast bypass flow in the root (Zhang et al., 2013). Si immobilizes metals in the cell walls of the root and inhibits their transport to the cytosol (Zhang et al., 2014).

Laguncularia racemosa can tolerate medium amounts of salinity and usually grows in zones of relatively lower saline concentration (Reef & Lovelock, 2014); this species can be very sensitive to pollution (Rocha et al., 2009; Bernini et al., 2010; Souza et al., 2014). There is no information in the literature about the effect of Si to attenuate Cd stress in *L. racemosa*. Hence, in this work, we investigate the response of propagules, seedlings, and young plants to the association of Cd and Si. The present study proposes that Cd accumulates differentially in the early stage of development of the species *L. racemosa*, compromising vital establishment and development processes. Also, this work proposes that the presence of Si can reduce plant organ heavy metal content, in turn reducing damage caused to photosynthetic metabolism and anatomy.

2. Material and methods

2.1. Plant material, experimental treatments and propagules, seedlings, and young plant growth

Propagules, seedlings, and young plants of *L. racemosa* were collected from the Contas River Estuary (40°45′36″N and 73°59′2.4″W) in Itacaré, Bahia, Brazil. The experiments were performed at the Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil (14°47'47.4"S and 39°10'22.7"W). Four experimental solutions were tested: (1) Hoagland and Arnon (1950) nutrient solution with 1/4 of ionic strength containing NaCl 0.3%, pH = 5.5, control (2) Si 0.1%, nutrient solution with sodium silicate (Diatom®), (3) Cd 5 mg L^{-1} nutrient solution with Cd $(NO_3)_2$, (4) Cd + Si, nutrient solution with Cd 5 mg L^{-1} + Si 0.1%.

For the first experiment, propagules of *L. racemosa* were placed in plastic containers (n = 15) with 200 mL of experimental solutions for 13 days. The plastic containers with 15 propagules for each experimental solution were placed in the greenhouse at 25°C for 13 days. Solutions were replaced twice a week. The fresh and dry plant weights (g) and hypocotyl lengths (cm) were measured at the end of the experiment. Propagules were rinsed with distilled water three times, weighed, and oven-dried at 75°C until reaching a constant dry weight. Visual aspects were also recorded.

In the second experiment, seedlings with cotyledons were planted in plastic cups (n = 5) with experimental solutions for seven days. Finally, in the third experiment, three young plants with three to four pairs of leaves were placed in plastic cups (n = 6) with experimental solutions also for seven days.

2.2. Effect of Cd + Si on root anatomy of propagule hypocotyl

After 13 days of treatment, the hypocotyls of the propagules were sectioned 1 cm from the root apex and freehand cross-sections were obtained and stained with 2% phloroglucinol (Johansen, 1940) to mark the lignin in red. Root area (mm), vascular cylinder area (mm), number of vascular bundles, and vessel diameter (µm) were measured using ImageJ.

2.3. Cd content in propagules, seedlings, and young plants

Plants were divided into roots and shoots. Length and width of the second pair of fully developed leaves were measured and leaf area was calculated as an ellipse using ImageJ. All plant materials were thoroughly rinsed with distilled water three times. Samples were oven-dried at 75°C until constant dry weight was reached. Dry samples were ground in a mortar, and resultant plant powders (about 0.1g) were digested with a mixture of ultrapure concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (1:1, v/v) in a digestion block at a temperature of 140°C. After digestion, the Cd concentrations were determined by inductively coupled plasma-mass spectroscopy (ICP-MS), using the ELAN DRC-e (Perkin Elmer, UK).

2.4. Leaf gas exchange and maximum quantum yield of PSII

After seven days of growth, six young plants subjected to each treatment solution were randomly selected to measure photosynthesis through leaf gas exchange, CO_2 assimilation (A), stomatal conductance (g_s), transpiration (E), and the instantaneous CO_2 carboxylation efficiency (A/Ci) with a portable system for gas exchange analysis (LI-6400XT, LI-COR, USA).

Measurements were taken under saturated light conditions (1000μmol quanta m^{-2} s^{-1}), with reference CO_2 (400 μmol CO_2 mol^{-1}) at 26°C ± 2°C and 80% relative humidity. The maximum quantum yield of PSII (F_v/F_m) was measured with a fluorometer MINI-PAM (miniaturized pulse amplitude–modulated photosynthesis yield analyzer; Walz, GmbH, Effeltrich, Germany). Leaf clips were placed for 30 min for darkness adaptation prior to measurement.

2.5. Statistical analysis

The Shapiro Wilk test was used to evaluate normality. A Levene's test was used to evaluate homoscedasticity, and a one-way ANOVA and Tukey's post hoc test at $P \leq 0.05$ were applied to test for significant differences between groups, using the PAST program.

3. Results

3.1 Propagules response to Cd + Si

After thirteen days of treatment, propagules grown in the presence of Cd or Si presented very contrasting visual aspects. Hypocotyls that grew in the presence of only Si were more elongated and greener, as observed in the recorded lengths of hypocotyls (Table 1). Despite being smaller compared to Cd, the Cd + Si treatment showed a healthy root apex at the end of the experiment, while the propagules treated with only Cd showed visible necrosis in the root apex (Fig. 1). Plants subjected to 0.1% Si were significantly different from treatments with Cd 5 mg L^{-1} and Cd + Si, which were also different from each other. Plants exposed to treatment with Si had the highest fresh and dry weight compared to plants of other treatments, though statistical significance was not observed. No differences were observed between treatments concerning root area (mm), vascular cylinder area (mm), and number of vascular bundles (Table 1). Only the vessel diameter (mm) was different, where the presence of Cd with the added Si caused a significant reduction in the vessel lumen and a lignin deposition was observed (Fig. 2).

3.2. Cd content in propagules, seedlings, and young plants

Cadmium was detected in all vegetative stages of all *L. racemosa*. The association of Cd + Si reduced nearly half of Cd contents present in propagules. In cotyledons, the concentration of Cd was not different across treatments. Young plant shoots did not present differences in Cd content between Cd and Cd + Si treatments. In the roots, Cd + Si reduced almost 28% of the Cd content compared with Cd treatment (Fig. 3).

3.3 Leaf gas exchange and maximum quantum yield of PSII (F_v/F_m) in response to Cd + Si of young plants

Regarding gas exchange, Cd significantly reduced CO_2 assimilation, stomatal conductance, and transpiration, and the association of Cd + Si results was not different from Cd 5 mg L^{-1}. The *A*/Ci ratio was also reduced in the presence of Cd (Fig. 4). However, when the chlorophyll fluorescence was evaluated, we observed that the Cd + Si treatment had similar values to the control and the treatment with Si alone. As expected, Cd significantly reduced F_v/F_m values (Fig. 5). However, in association with Si, the effect of Cd was attenuated, and the values of maximum quantum yield of PSII were comparable to control and Si treatments.

4. Discussion

The beneficial effects of Si on plant growth maintenance under Cd toxicity have been reported in earlier studies with mangroves (Ye et al., 2012; Zhang et al., 2014). Here, positive effects of Si in terms of hypocotyl growth in propagules could be detected after short-term Cd exposure in *L. racemosa* plants (Fig. 1). A positive effect of silicon on seed germination has been observed under water stress (Shi et al., 2014), and Si seed priming has been shown to attenuate Cd toxicity in lettuce seedlings (Pereira et al., 2021), nonetheless, these studies are about crop plants, and our work shows for the first time the effects of Cd + Si association in mangrove propagules.

Silicon displays a crucial role in the intercellular and extracellular parts of plants cells. The extracellular activities of Si can limit the penetration of heavy metals into the cytoplasm depending on its concentration (Zhang et al., 2014). Sequestering of heavy metals in vacuoles is the intercellular activity that occurs in the cytoplasm (Sahebi et al., 2014

To avoid Cd translocation, hypocotyls showed xylem vessel wall thickening with a visible increase in lignin deposition. (Fig. 2). Si can covalently bind with heavy metals and form an unstable silicate form, which subsequently suppresses the toxicity of the metals and is easily degraded to silicon dioxide ($SiOp_2$). Silicon deposition near the endodermis decreases the porosity of the Casparian strip and reduces the apoplastic transport of Cd (Rizwan et al., 2016). Ye et al. (2012) reported that Si enhanced the binding of Cd to the cell walls and restricted the apoplastic transport of Cd in root tips of *K. obovata* seedling and the same was observed in *A. marina* (Zhang et al., 2013; Zhang et al., 2014).

By analyzing the effect of the different treatments in propagules, cotyledons, and young plant shoots and roots, we found significant differences in metal accumulation, with the highest Cd contents, were found in propagules subjected to 5 mg L^{-1}. Treatment Cd + Si was reduced to almost half the concentration of Cd in the propagules, and the roots of young plants were reduced by almost 28%. The role of Si in the attenuation of Cd effects during crop plant germination can be associated with its capacity to reduce oxidative stress and consequently promote root development (Sahebi et al., 2014; Howladar et al., 2018; Liu et al., 2019). Regarding young plants, treatment with Si did not change the proportion of Cd distribution among the shoots, but it may reduce Cd mobility and available Cd concentration by immobilizing it in the cell wall (Zhang et al., 2014).

An important mechanism of salt tolerance in *L. racemosa* is the translocation of NaCl to mature leaves and subsequent elimination (Reef and Lovelock, 2014). Despite the small amount of Cd absorbed by the cotyledons and shoots, it is important to highlight that both cotyledons and mature leaves containing Cd will be eliminated and returned to the soil. For this reason, the association Cd + Si stands out; the addition causes heavy metal. For the Si concentration and the period of exposure to Cd tested in this experiment, young plants of *L. racemosa* did not present recovery in gas exchange parameters with the Cd + Si association, however, the effects of Cd were evident (Fig. 4). Reduction of CO_2 assimilation (*A*), transpiration rate (*E*), stomatal conductance (*gs*) has been also reported in treatments involving Cd +Si (Howladar et al., 2018) however, in this study, Cd + Si association in wheat increased values of gas exchange as compared to Cd. Considering that Cd can promote ultrastructural changes in chloroplasts, especially in thylakoids (Garcia et al., 2018), it is possible to infer that the effect of Si is more expressive in the photochemical processes of photosynthesis as compared to CO_2 assimilation.

Cadmium toxicity inhibits photo-activation in photosystem II (PSII) due to electron transfer inhibition (Farooq et al., 2016). The association of Cd + Si increased F_v/F_m compared to Cd 5 mg L^{-1} indicating that Si could enhance photosynthesis by maintaining the integrity of photosynthetic structures (Zhu et al., 2019). This positive effect of Si on photosynthetic machinery may be due to the restriction of Cd uptake, which could reduce the damage to photosynthetic machinery by ROS, increase antioxidant mechanisms of the plant defense system, and possibly alter the anatomy and ultrastructure of photosynthetic apparatus (Adrees et al., 2015; Emamverdian et al., 2018). In addition, several photosynthesis-related genes were also regulated by Si supplementation (Rastogi et al., 2021).

Silicon application under Cd stresses protected the white mangrove (*L. racemosa*) in the initial stages of development which might be due to compartmentation in xylem, coprecipitation, and/or chelation of heavy metals in different plant parts. As a result, free metal ion concentration decreased. These results indicate that the addition of Si significantly alleviated the inhibition of growth under Cd toxicity and decreased the Cd uptake by *L. racemosa*. The effect of Si on the hypocotyl anatomy of *L. racemosa* propagules may be one of the most important mechanisms of the reduction of Cd uptake. The Si-mediated reduction of Cd effect in maximum quanta yield in the leaves may be responsible for the alleviation of Cd toxicity in *L. racemosa* young plants. In the early stages of development, the effect of Cd in the white mangrove can compromise the establishment of the species in its habitat, however, Si addition can help to sustain propagule development and later be fixed in roots, preventing translocation to cotyledons and shoot.

5. Conclusion

Silicon addition can be beneficial in the early stages of development of the white mangrove (*L. racemosa*) as it increases the deposition of lignin in the xylem, with a consequent reduction in the absorption of the metal, especially in propagules hypocotyl and young plants roots. It increases the efficiency of photochemical processes and reduces hypocotyl oxidation. It is indicated as an auxiliary treatment in the cultivation of plants in environments contaminated by Cd.

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Figures and captions

Table

Table 1. Growth measures of propagules after 13 days of treatment. Values represent mean ± SD, different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey test, n=4-15).

Growth measures of propagules	Control	Si 0.1%	Cd 5 mg L^{-1}	Cd+Si
Fresh weight (g)	0.68 ± 0.14^{ab}	0.78 ± 0.15^{a}	0.64 ± 0.09^{b}	0.58 ± 0.08^{b}
Dry weight (g)	0.21 ± 0.05^{a}	0.27 ± 0.07^{b}	0.21 ± 0.04^{a}	0.20 ± 0.04^{a}
Hypocotyl length (cm)	5.21 ± 0.46^{a}	5.17 ± 0.38^{a}	3.98 ± 0.52^{b}	3.27 ± 0.24^{c}
Root cross section area (mm)	3.27 ± 0.23^{a}	3.23 ± 0.11^{a}	3.30 ± 0.10^{a}	2.99 ± 0.37^{a}
Vascular bundle area (mm)	0.56 ± 0.05^{a}	0.56 ± 0.03^{a}	0.60 ± 0.02^{a}	0.52 ± 0.09^{a}
Number of vascular bundles	8 ± 1^{a}	7 ± 1.5^{a}	8 ± 1^{a}	7 ± 0.0^{a}
Diameter of vessels (µm)	6.67 ± 1.04^{a}	6.67 ± 1.13^{a}	3.41 ± 0.61^{b}	3.8 ± 0.93^{b}

Figures

Fig 1. Response of *L. racemosa* propagules after 13 days of treatment nutrient solution (Control), silicon 0.1% (Si), cadmium 5mg L^{-1} (Cd), and solution Cd 5mg L^{-1} + Si 0.1% (Cd + Si).

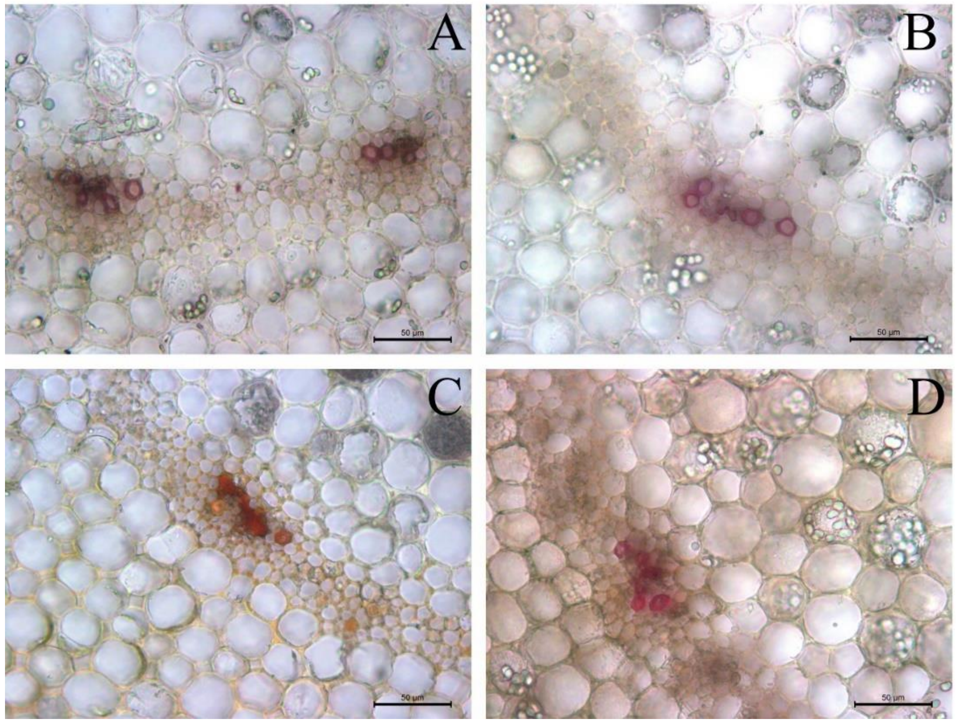


Fig 2. Cross-section of *L. racemosa* hypocotyl after 13 days of growth in nutrient solution (A), in silicon 0.1% (B), in Cd 5 mg L^{-1} (C), and Cd + Si (D). Histochemistry analysis indicates lignin deposition (50 μm).

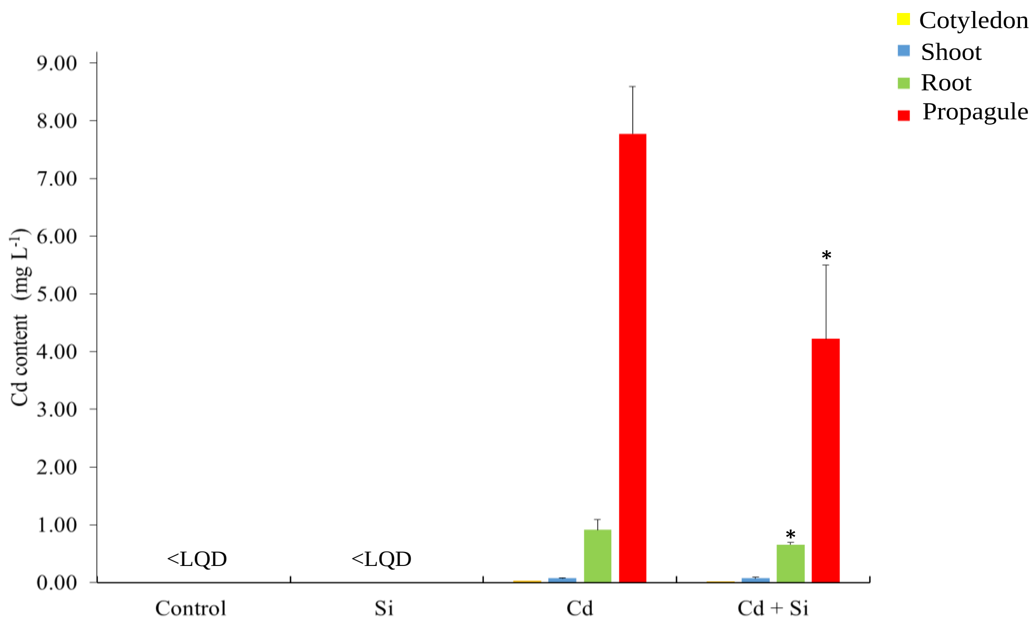


Fig 3. Cadmium content in *L. racemosa* propagules, cotyledon, shoot and root after treatments with nutrient solution (Control), silicon 0.1% (Si), cadmium 5mg L^{-1} (Cd), and solution Cd 5mg L^{-1} + Si 0.1% (Cd + Si). Values of Control and Si were <0.02. Asterisk (*) indicates significant differences between Cd and Cd + Si at $P \leq 0.05$ (ANOVA, Tukey test, n=3).

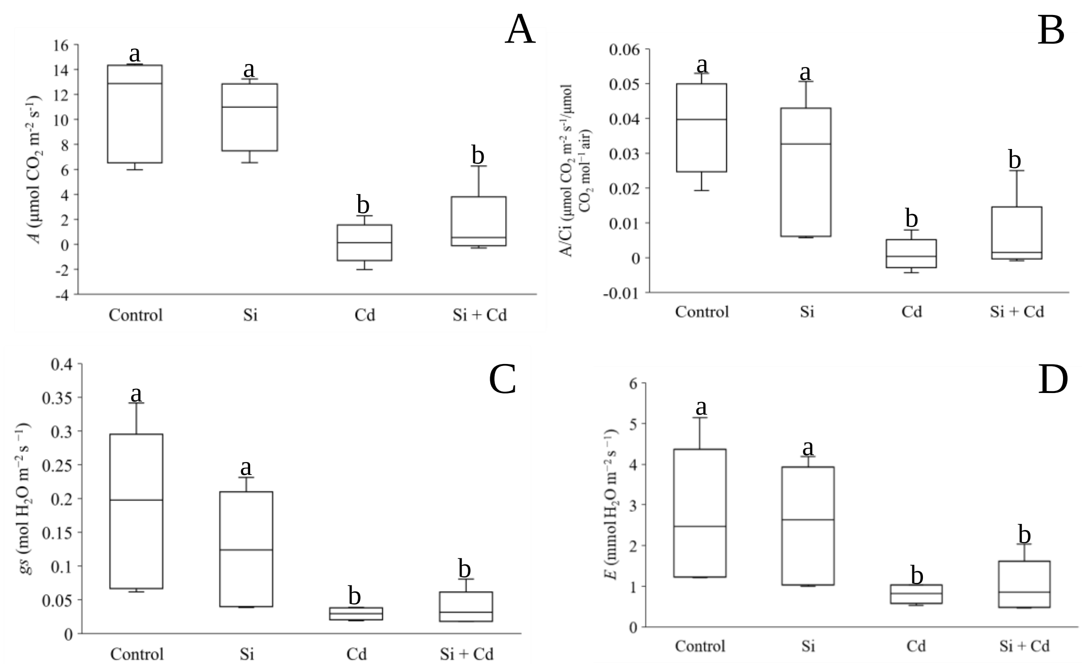


Fig 4. Leaf gas exchange of young *L. racemosa* plants at the end of a seven-day treatment period for treatments with nutrient solution (Control), silicon 0.1% (Si), cadmium 5mg L^{-1} (Cd), and solution Cd 5mg L^{-1} + Si 0.1% (Cd + Si). (A) CO_2 assimilation (*A*), (B) instantaneous CO_2 carboxylation efficiency (*A*/Ci), (C) stomatal conductance (*gs*), (D) transpiration (*E*). Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey test, n=6).

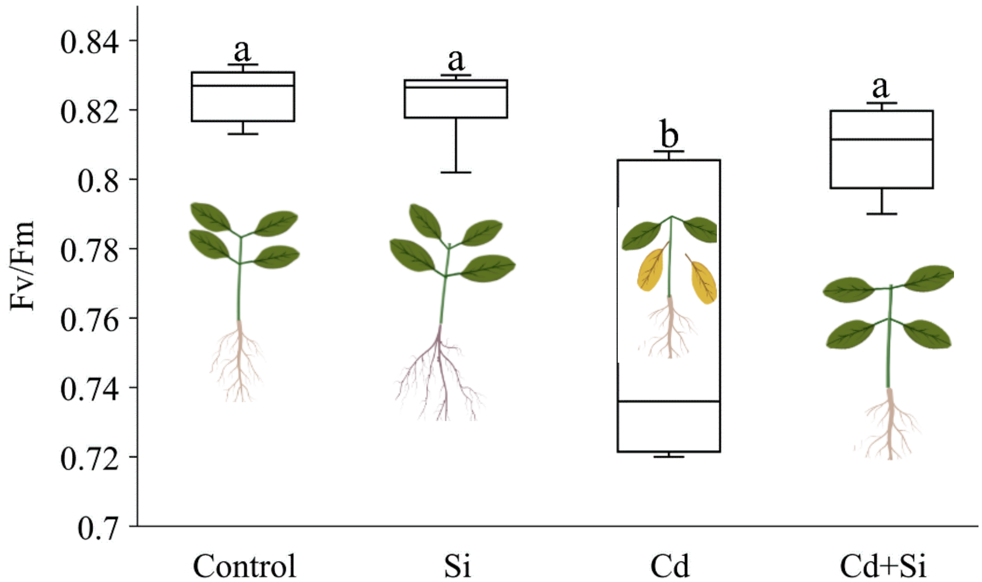
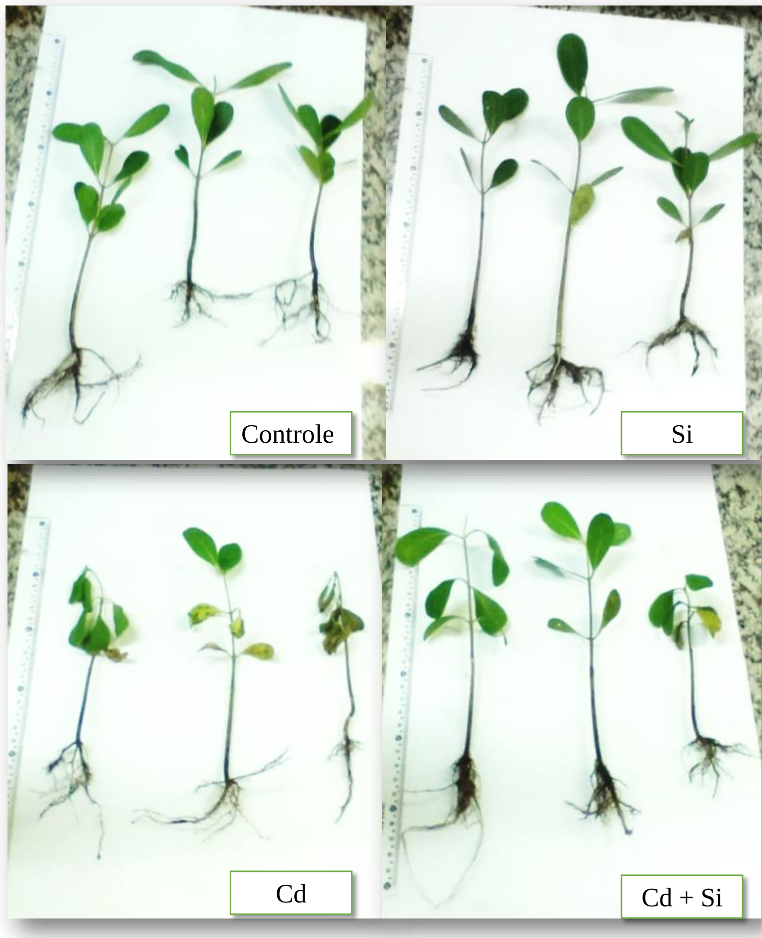


Fig 5. The maximum quantum yield of PSII (F_v/F_m) in young *L. racemosa* plants after 7 days of growth. Treatments: nutrient solution (Control), silicon 0.1% (Si), cadmium 5mg L^{-1} (Cd), and solution Cd 5mg L^{-1} + Si 0.1% (Cd + Si). Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Tukey test, n=6).

Supplementary material – *L. racemosa* after 7 days of the experiment.



CONSIDERAÇÕES FINAIS

O ecossistema manguezal tem um valor inestimável para a manutenção da vida marinha e do equilíbrio de diversos processos biogeoquímicos. A conservação deste ecossistema deveria ter caráter prioritário nas agendas governamentais, porém, cada ano sua extensão diminui e a poluição aumenta. Apesar da grande resiliência deste ecossistema, em absorver poluentes e suportar os impactos mecânicos de grandes magnitudes como furacões e tsunamis, em todo o globo vem sendo reportados perdas significativas de áreas de manguezais. Neste projeto foi possível demonstrar que a espécie *A. schaueriana* é uma espécie tolerante ao aumento da deposição de Cd e na literatura está reportado que este gênero é o mais tolerante aos estresses abióticos. Também pôde ser comprovado que a aplicação de silício nos estágios iniciais de desenvolvimento de *L. racemosa*, espécie mais sensível ao Cd, foi possível reduzir o teor do metal pesado nos órgãos vegetativos, principalmente nos propágulos. Iniciativas de restauração com base em seleção de plantas mais tolerantes aos diversos tipos de contaminantes como o Cd, ou a combinação de outros elementos como Zn, Ca e Si que são competidores pelos sítios de ligação do metal nos estágios juvenis das plantas, poderiam auxiliar no sucesso de projetos de preservação, recuperação e manutenção desse ecossistema.