

## Chapter

# The Role of Mangroves and Nanomaterials in the Heavy Metals' Decontamination Process

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## Abstract

Green Chemistry aims to make processes more efficient and create products that reduce environmental pollution. This chapter presents the results of the combined use of *Rhizophora mangle* (*R. mangle*) and nano zero-valent iron (nZVI) for cadmium (Cd) remediation in contaminated areas. Here, the phytoremediation process using a 40-ppm cadmium solution in combination with *R. mangle* and nZVI for 3 days was evaluated. We used 20 *R. mangle* samples with and without nZVI to assess the efficiency of the phytoremediation process for the removal of cadmium by inductively coupled plasma (ICP) analytical measurements and confocal imaging. Translocation factors (TFs) were calculated.  $TF < 1$  indicates that *R. mangle* does not act as a hyperaccumulator but as an excluder of metallic Cd. The data obtained in the project suggests that the interaction of nZVI with *R. mangle* is an effective way to enhance the translocation of the metal by its aerial parts without affecting the stomatal opening.

**Keywords:** heavy metals, nanomaterials, mangroves, green synthesis, phytoremediation

## 1. Introduction

To maintain the concentration of essential metals within physiological limits and to minimize the harmful effects of nonessential metals, plants have developed complex mechanisms that serve to control the uptake, accumulation, handling, and detoxification of heavy metals. Some of these mechanisms include transport, chelation, compartmentalization, exclusion, and sequestration processes [1]. Of these, chelation constitutes one of the major detoxification mechanisms and involves the participation of high-affinity ligands such as phytochelatins (PCs) and metallothioneins (MTs), two of the best-characterized heavy metal-binding ligands in plant cells [2].

Several studies have reported the benefits of using plants to remove heavy metals from contaminated waters and soils. This process is known as phytoremediation. In the wetlands, plants that naturally thrive in these regions have been found to serve as important tools for heavy metal removal. They are preferred over other bio-agents because they not only uptake nutrients but are also able to adsorb and accumulate heavy metals. Wetland species differ in their ability to uptake and accumulate various metals in their tissues [3]. Some species have been found to have a great ability to accumulate metals in their biomass and are referred to as “hyperaccumulators” [4].

## 1.1 Mangroves and heavy metals

Mangrove forests are plant associations of great ecological service and economic importance [5, 6]. Mangroves are important wetland plants distributed in the transition zone between land and sea along intertidal coasts in the tropics and subtropics [7]. They form natural barriers with high productivity that protect coastal zones from tropical storms and hurricanes and function as a nursery for numerous species of terrestrial and aquatic fauna [8]. Mangroves have been considered a highly tolerant group of plants because they can survive stressful conditions in tropical and subtropical latitudes [9]. Many mangrove ecosystems located close to urban areas may be impacted by effluents from industrial sources and urban runoff that often contain high concentrations of toxic heavy metals such as cadmium, arsenic, lead, zinc, and nickel, among others [10].

*Avicennia* spp. and *Rhizophora mangle* are the most tolerant mangrove species. They have been reported to accumulate greater quantities of metals when compared to other mangrove species, even before any visible signs of toxicity appear. Many studies described these plant properties as a complex process of detoxification and tolerance mechanisms to heavy metals that consist of (a) binding to the cell wall and extracellular exudates, (b) reduced uptake or efflux pumping of metals at the plasmatic membrane, (c) detoxification of metals in the apoplasts and chelation of metals in the cytoplasm with various ligands, such as phytochelatin, metallothioneins, metal-binding proteins, and (d) sequestration of metals into the vacuole by tonoplast-located transporters [11, 12]. Nevertheless, these mechanisms in plants are not enough by themselves to substantially remove heavy metals from contaminated sites.

## 1.2 Nanomaterials for environmental treatment

Nanomaterials iron-based have a great capacity to react, degrade, adsorb, or transform a wide range of contaminants in soils and water bodies. It is used in soil remediation, water treatment or conditioning, permeable reactive barriers, and other applications. Iron ions remain stable in a dry atmosphere and CO<sub>2</sub> free water. Under other conditions, iron oxidizes to Fe<sup>2+</sup> and Fe<sup>3+</sup>, forming FeO, Fe<sub>3</sub>O<sub>4</sub>, and  $\delta$ -Fe<sub>2</sub>O<sub>3</sub>, which are irreversible. The ferric form (Fe<sup>3+</sup>) is very prone to hydrolysis, giving rise to an insoluble iron hydroxide polymer. The predominant species in aqueous solution are Fe<sup>2+</sup> and Fe<sup>3+</sup> and the organic ferrous and ferric complexes.

Under aerobic conditions and neutral pH, inorganic iron (III) salts are the most stable species. The synthesis based on the chemical reduction of sodium borohydride for our studies with mangroves has not represented physiological effects on plants. Even so, it is necessary to consider the green synthesis of nanoparticles. Traditional chemical methods used for the synthesis of nano zero-valent iron (nZVI) still require toxicity studies for potentially hazardous by-products. Therefore, alternative methods of low-cost and green synthesis are urgently required. Plant extracts can be used as a highly efficient and environmentally sustainable alternative for the synthesis of nanoparticles.

## 1.3 Green synthesis of nZVI

Green synthesis for decontamination processes is essential to protect organisms present in ecosystems. On the other hand, the high cost of many techniques and syntheses greatly impedes the process of purification and environmental abatement.

Green synthesis to produce nZVI was first applied by VeruTEK and the US EPA. In this method, plant extracts (coffee, green tea, black tea, lemon, balm, sorghum, bran, grape, etc.) are heated in water to near boiling point to prepare a polyphenol solution [13]. The extract thus produced is separated from the plant debris and mixed with the  $\text{Fe}^{2+}$  solution. Iron ions in the presence of polyphenols were reduced to nZVI. It must be considered that the main problem when using plants for the synthesis of nanoparticles is the destruction of plants and plant parts. One way around this is to use agricultural residues, for example, Eucalyptus Leaf Fragments or extracts from various residues (peel, albedo, pulp) of lemons, tangerines, etc. Plant extracts reduce metal ions in a shorter time than microorganisms. Depending on the type of plant and the concentration of plant secondary substances, microbial-based nanoparticles are synthesized in minutes to hours [14].

Recently, *Ficus carica* dried fruit, citrus maxima aquatic peel, eucalyptus, and many *Avicennia marina* flower plants and leaves and green tea have been successfully used as green reducing agents for the synthesis of nZVI [15]. The leaf extract of *Cleistocalyx operculatus* has been used widely in medicine and beverages, containing a high proportion of reductants, including polyphenols. This substance can chemically convert Fe ions into iron with zero valency, as described in study [16]. In other studies, the walnut-green shell was used for the synthesis of spherical nZVI particles with a diameter of 18–72 nm [17]. Replacing chemical-reducing agents with plant extracts as reductants reduced operating costs, improved nZVI quality, and improved process performance. However, each plant produces specific extracts with different chemical properties and reducing power [18]. Moreover, the use of this green synthesis for ZVI nanoparticles is not yet widespread worldwide, as the quality of plant extracts depends on local conditions such as climate and soil factors. This chapter aims to show how the use of *R. mangle* and iron nanoparticles could be used as an alternative to the heavy metal decontamination process.

## 2. Experimental methods results

### 2.1 Materials

Iron (III) chloride hexahydrate (97%, ACS reagent), sodium borohydride (98.5%, reagent grade),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (F2877 Sigma Aldrich),  $\text{NaBH}_4$  (Alfa Aesar), and ethanol 200 proof, 99. All solutions were made using deionized water (18.2 M-cm, Nanopure Diamond, Barnstead).

### 2.2 Instrumentation and techniques

To analyze the size and morphology of the nanoparticles X-ray diffraction (XRD) and scanning electron microscope (SEM) were employed to determine iron compounds and their respective oxidation states. The phase composition and structures of the nZVI particles were determined by XRD patterns (PANalytical X'Pert Material Research Diffractometer) operating with a  $\text{Cu K}\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) source.

To determine the effect of the nZVI nanoparticles in the number of Cd ions in each system and plant after the treatment period time, all the samples were processed and analyzed using ICP-AES Model Perkin Elmer 4300 DV with a detection limit of 0.002 ppm. ICP-AES is one of the most common techniques for elemental analysis. Its high specificity, multi-element capability, and good detection limits result in the

use of the technique in a large variety of applications. Three *R. mangle* individuals were selected that were exposed to a system contaminated with 10 ppm cadmium, and another three were exposed to a 1 g/L solution of nZVI only. Translocation Factor (TF) Technique was used to measure the Cd translocation from shoot to root, which is given below:

$$TF = C_{\text{shoot}} / C_{\text{root}} \quad (1)$$

where  $C_{\text{shoot}}$  and  $C_{\text{root}}$  are metals concentration in the shoot ( $\text{mg}\cdot\text{kg}^{-1}$ ) and root of the plant ( $\text{mg}\cdot\text{kg}^{-1}$ ), respectively. A TF value larger than 1 represents the effective translocation of metals to the shoot from the root. To confirm the effect on stomata, a Nikon Eclipse Ti-E inverted fluorescence microscope confocal microscope images were obtained using *R. mangle* leaves from the cadmium solution system and the nZVI and cadmium solution system.

### 2.3 Sampling and acclimation

The seedlings of *R. mangle* were obtained from Pinones State Forest through direct harvesting during January 2023 (**Figure 1**). This area is the most extensive natural system of mangroves in Puerto Rico. Mangrove plants were first kept in a nursery station under shaded cover using the same substrate of marsh to maintain their natural conditions.

The growth and maintenance of these plants once brought from the wetland was performed in a greenhouse chamber adapted by construed four small chambers that were prepared to carry out different experiments. This chamber from Carolina Biological Company allows to manipulation of environmental conditions to investigate plant growth and development. This strong, long-lasting chamber is made of galvanized, powder-coated steel. Dimensions: 26" L × 26" W × 37" H. Around 4600 lumens of light are provided by four compact fluorescent lamps (CFLs). Greenhouse conditions were 25 degrees Celsius and 60–70% humidity. After an acclimation period of 28 to 30 days, surviving plants are considered fully adapted to their new environmental conditions in the phytonanoremediation system, as seen in **Figure 2**.

The phytonanoremediation system shown in **Figure 2** was adapted using a conceptual design of direct injection in situ. All the systems have water and soil with the highest concentration of Cd to simulate contaminated sites. The purpose of this



**Figure 1.** Puerto Rico map (A), sampling zones and mangrove areas at State Forest Pinones marsh (B).

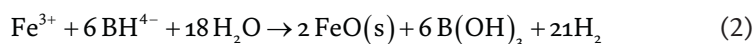


**Figure 2.**  
 Images of *R. mangle* obtained from State Forest Pinones and transplanted to a new system for phytonanoremediation system. The experiment employed a total of 20 seedlings. Each one's height and chlorophyll concentration in the leaves were measured.

phytonanoremediation system is to evaluate if the interaction of *R. mangle* with the nZVI in soil and water collectively mitigates and removes heavy metals.

## 2.4 Synthesis of nZVI

nZVI particles were produced by adding  $\text{NaBH}_4$  as a reducing agent to a solution of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . The resulting reductive reaction may be given as:



To obtain approximately 1.00 g of nZVI, a 0.6 M solution of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (molar mass 270.30 g/mol) was prepared in 30 mL of ethanol (83% v/v) in nanopure water. The solution was purged with  $\text{N}_2$  for 30 minutes to remove oxygen before the reaction to avoid the rapid oxidation of iron. Then, the solution was titrated by adding a total of 100 mL of 0.8 M aqueous solution of  $\text{NaBH}_4$  under a nitrogen atmosphere. After 30 minutes of stirring with a magnetic bar at 600 rpm, the solution was filtered using a 0.22  $\mu\text{m}$  filter paper (Millipore) under a vacuum at 25°C. The sample was rinsed three times with 99.5% absolute ethanol. Various filtered samples, prepared as mentioned, were placed immediately in a vacuum desiccator. Each synthesis of nZVI was prepared to use all the nanoparticles in each treatment.

## 2.5 Preparation of contaminated solution

Cd solutions were prepared using  $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  due to their high solubility in water and ethanol. To represent different samples of wastewater contaminated with Cd solutions of 40 ppm were prepared. The solutions of Cd were treated with 1.0 g/L of nZVI particles for a period of 1 hour. Concentration units were presented as

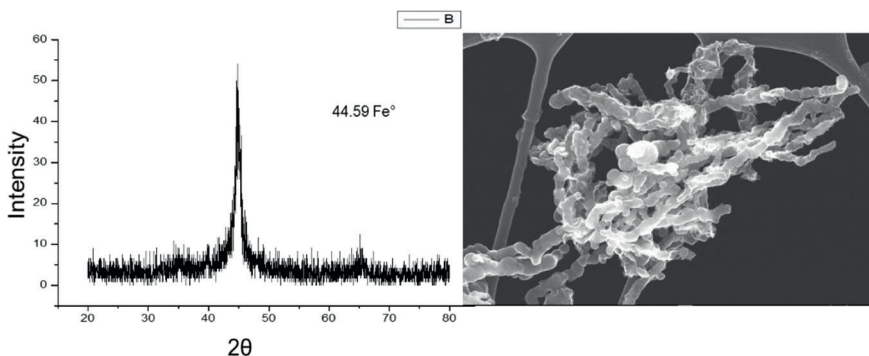
“ppm” keeping with toxicity nomenclature ( $\text{ppm} = \text{mg}\cdot\text{L}^{-1}$ ). After 1 hour of stirring with a magnetic bar at 600 rpm, the solution was filtered using a  $0.22\ \mu\text{m}$  filter paper (Millipore) under vacuum conditions at  $25^\circ\text{C}$ . The filtered samples were placed immediately in a desiccator to be analyzed.

In the phytoremediation system, two treatments were carried out: the first involved immersing *R. mangle* seedlings in a 40-ppm cadmium solution, and the second involved immersing *R. mangle* seedlings in a 1 g/L nZVI solution near the roots. Chlorophyll content changes if a plant is stressed. A SPAD meter can be used for a rapid determination of the chlorophyll content of individual leaves. An important indicator of plant health is its concentration of chlorophyll, which will be measured using a chlorophyll meter model SPAD (Konica Minolta company) on each plant for a period of 3 days. The chlorophyll meter measures the light transmittance in two wavelength ranges (600 to 700 and 400 to 500 nm) to determine the relative amount of chlorophyll in the leaves. The three treatments included 40- ppm cadmium solution, 1 g/L nZVI solution, and a control group with only water. The chlorophyll levels were measured on three leaves per individual and averaged out in triplicate, resulting in a total of nine leaves analyzed for each treatment.

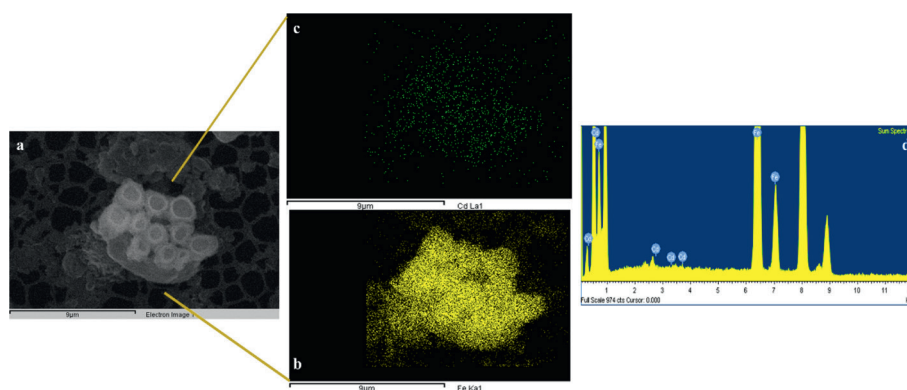
## 2.6 Characterization of nZVI

Nanoscale zero-valent iron particles were produced by a chemical reduction technique of  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  using  $\text{NaBH}_4$  to complete  $\text{Cd}^{2+}$  fixation from water [19]. In **Figure 3**, X-ray diffraction of nZVI shows a characteristic peak of  $\text{Fe}^0$ , usually appearing at  $2\theta = 44.59$ . The SEM image shows nano spherical iron particles that form chains like aggregates due to their magnetic properties. The diameter of the iron nanoparticles was in the range of 20–100 nm.

The nZVI particles (1 g/L) interact with cadmium ions in the solution for 1 contact hour. Energy dispersive X-ray spectroscopy (EDS) was used to qualitatively determine the morphology, size, and composition of the samples. **Figure 4** shows images of the surface of the ZVI nanoparticles that were taken at different extensions. Simultaneously, the EDS spectrum was obtained in selected areas on the nZVI surface to give information on the chemical composition and surface atomic distributions of

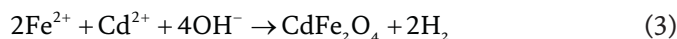


**Figure 3.** X-ray diffraction (XRD) pattern and scanning electron microscope (SEM) image of freshly prepared nZVI particles.



**Figure 4.** SEM image of nZVI particles in cadmium solution (a) image of the selected area of nZVI in Cd solution (yellow dots represent Fe and green dots Cd atoms, (b and c respectively), (d) EDS spectrum of nZVI particles in the Cd solution.

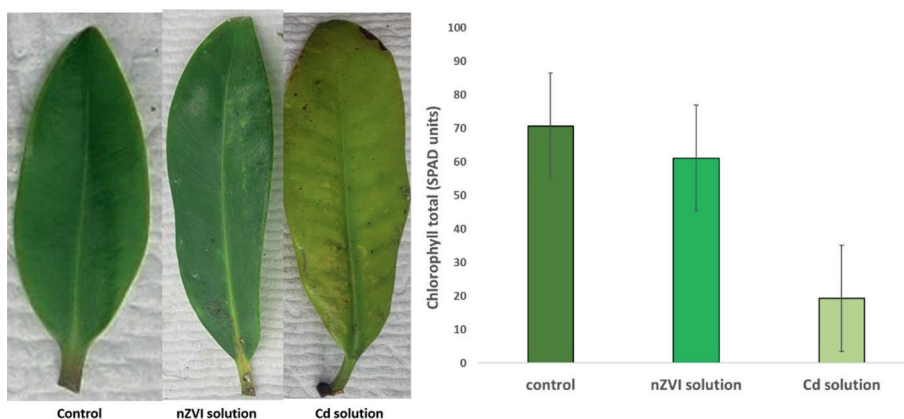
iron and cadmium. The concentration of  $\text{Cd}^{2+}$  used was 40 ppm, which is within the range of values found in Cd-contaminated areas of Puerto Rico. These results on the distribution of Cd and Fe ions are consistent with our previous studies [20]. When removing cadmium ions, Fe nanoparticles oxidized as FeO and other iron oxides (such as  $\text{Fe}(\text{OH})_2$ ) form favorable interactions as cadmium ferrites by the following equation.



Our studies showed that using *R. mangle* plants with nZVI exposed to cadmium accumulated high concentrations of metals in the roots and aerial organs. The TF was  $1.223 \times 10^{-4}$  for plants exposed for three days to 40 ppm Cd solutions for ICP analysis in the roots, stem, and leaves, and it was  $1.420 \times 10^{-2}$  for plants exposed to nZVI and cadmium solution. Even though these differences are not statistically significant, the TF of *R. mangle* seedlings exposed to nZVI and cadmium exhibits higher translocation mobility. The longer the nanoparticles are exposed, the more efficient the phytoremediation process will be.

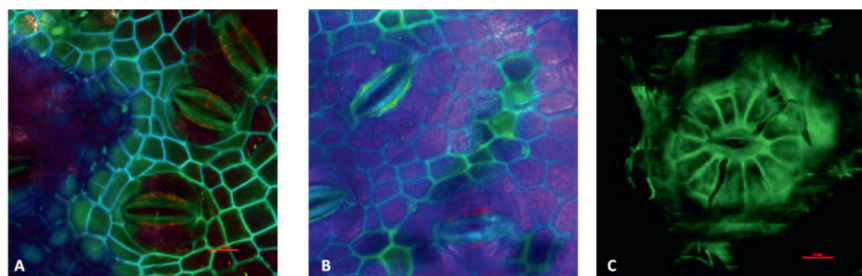
Results of chlorophyll analyses were measured in *R. mangle* to compare the possible effect of nZVI in plants. Results indicate that the concentration of chlorophyll content of systems does not change (see **Figure 5**). These results confirm that the presence of nZVI can interact with different mangrove species to remove heavy metals without affecting the physiology of plants.

In **Figure 6**, we can show the interaction of nZVI and Cd in the stomata of red mangroves. Images A and B show the stomata open, but image C with the presence of cadmium, directly affects the structure and morphology of the stomata. Confocal microscopy images, taken at low and high magnification, of guard cells after one week of interaction with cadmium and nZVI solutions. The average stomatal openings of control (a), nZVI exposure (b), and Cd solution (c). The stomatal openings in plants exposed to nZVI allow the gas exchange of carbon dioxide and oxygen for



**Figure 5.**

Variation of chlorophyll content in control, treated with 1 g/L of nZVI and treated with 40 ppm of Cd solution during an exposure period of 3 days. Means  $\pm$  SD ( $n = 9$  per treatment). When exposed to Cd, the green color of seedling leaves changes due to the decrease in chlorophyll concentration.



**Figure 6.**

Confocal microscopy images shows the interaction of nZVI and Cd in the stomata of *R. mangle*. Images A and B show the stomata open, but image C with the presence of cadmium, directly affects the structure and morphology of the stomata. Red scale bars: 20  $\mu$ m.

efficient photosynthesis. Our results indicate that nZVI can promote plant growth by increasing their photosynthesis and nutrient accumulation.

### 3. Role of green chemistry and plants in the removal of heavy metals

One of the main objectives of green chemistry is to develop and produce chemical products and processes that reduce pollution. There are many processes and techniques for the removal of heavy metals, including reverse osmosis, chemical precipitation, ion exchange, adsorption, and solvent extraction [21]. However, these techniques require significant maintenance capacity and cost and are generally not sustainable. For a process to comply with the principles of green chemistry, the degradation of active products must be considered because many chemicals do not fully break down into carbon dioxide, water, and inorganic salts.

Chemical generation may be resistant to environmental biodegradation including microorganisms present. In the environmental area, the use of decontaminants is a critical point of concern since many current chemicals can bioaccumulate and cause toxic effects in organisms. For a compound to be considered biodegradable,

its structure, size, and application must be evaluated. Environmental conditions in which they can react, etc. Therefore, the products considered in green chemistry must be biodegradable and nontoxic [22]. The use of plants is an economical and alternative method to synthesize decontamination products for medicines. And it offers one of the greenest ways to overcome costs and toxic effects.

#### **4. Conclusions**

The data obtained in these studies suggest that the interaction of nZVI with *R. mangle* is an important way to improve the bioavailability of  $\text{Cd}^{2+}$  and help mangroves to better translocate them in harvestable portions. The presence of nZVI in *R. mangle* facilitates the entry of ions. The tolerance of *R. mangle* seedlings to high concentrations of Cd may be due to the formation of nontoxic sulfides in the roots or on their surfaces, tissue detoxification, and ion exclusion mechanisms within the roots or inside. These preliminary results, therefore, suggest that *Rhizophora* may act better as phytoremediators against heavy metals in contaminated areas due to their ability to accumulate at low concentrations in aged leaves. By encapsulating pollutants instead of venting them to the outside, they prevent further pollution of the surrounding ecosystem. The use of red mangroves is important because they are highly resilient ecosystems to the effects of climate change. The approach provides a real alternative to remove human carcinogens such as Cd, As, Pb, and Hg by using affordably synthesized particles at the nanoscale and plant levels.

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#### **Conflict of interest**

The authors declare no conflict of interest.

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