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Abstract

Some tropical coastal areas that include mangroves are highly polluted by heavy metals, where lead (Pb) is prevalent. Few studies document how environment affects soil physicochemical characteristics and the availability of heavy metals. This study evaluated how different salt concentrations influenced the accumulation of Pb in plants of *Avicennia germinans* and *Laguncularia racemosa* and how salinity modified the substrate pH. Under unsalted conditions, Pb accumulation occurred continuously, mainly in *L. racemosa*, which was more tolerant to its toxic effects. Salt led to a decreased Pb uptake by the roots and an increase in the substrate pH. In addition, salinity also caused an increase in the translocation of Pb to the leaves. Because *L. racemosa* was tolerant to Pb, this species could be a better candidate for possible remediation and restoration programs in mangrove areas.

1. Introduction

Industrial, agricultural and mining activity, in addition to the rise of solid waste and oil spills, have considerably increased the environmental contamination by heavy metals (Du Laing *et al* 2009, He *et al* 2014). Moreover, because these pollutants cannot be chemically or biologically degraded, they become highly toxic when they accumulate in soil and water, causing poor development and death in living organisms (Páez-Osuna 2005).

Additionally, toxicity of heavy metals depends not only on their concentration but also on their degree of availability, which is determined by the chemical forms in which these elements are found. The speciation of metals depends on changes in the physicochemical parameters of soil and water such as pH, cation exchange capacity, organic matter content, redox conditions, dissolved oxygen concentrations, and lack or excess of nutrients, among others. At the same time, these parameters are influenced by environmental conditions such as hydroperiod, salinity, and temperature (Riba *et al* 2003, Du Laing *et al* 2009).

Metals with electropositive charges are attracted to surfaces with negative charges given by organic matter or clay, which determines the cation exchange capacity (CEC). A high CEC increases the retention of metal cations in the soil, reducing their metal mobility and availability (Du Laing *et al* 2009). In addition, pH is an important factor in the availability of metals in the soil. For instance, in acidic soils, where there is a greater release of hydrogen ions (H⁺), the competition of the metal cations with these H⁺ can cause the desorption of the metals, which increases their concentration and solubility in the soil solution and their possible absorption by the plant roots (García *et al* 2002).

In the soil, the fate of heavy metals is given by processes of adsorption/desorption, precipitation/dissolution, and reincorporation/mineralization, as well as by transport of solutes and extraction by plants (He *et al* 2005). In saline environments, the concentration of other cations that compete with metal ions for

sorption sites increases and metals can then form complexes of high stability and solubility with chloride. Therefore, CEC decreases and metals increase their mobility in the soil. Conversely, metals can also form complexes with other anions (e.g. sulfur), which causes low mobility and availability (Leonard *et al* 2011). However, the higher the salinity, the more alkaline the soil pH becomes, generating lower metal availability because they precipitate as insoluble hydroxides (Alloway 1995).

Salinity can affect metal ion absorption and transport to the roots. This is because Na^+ can be absorbed through different channels that compete against other ions (e.g. K^+) for entrance to cells. Conversely, internal transport of heavy metals is more limited and specific, so when competing for entrance to the same channel these are absorbed in lower concentration. This entrance is also influenced by the properties of transport and the concentration of ions in the soil (Li *et al* 2012, Mei *et al* 2014).

In Mexico, for several decades, the coast of the Gulf of Mexico has been the most impacted by heavy metals, where Pb has a major presence, in the range from 0.28 to 267 $\mu\text{g g}^{-1}$ dry weight of sediment (Villanueva and Botello 1992, Villanueva and Paéz-Osuna 1996, Botello *et al* 1999, Medina *et al* 2004, García 2006, Páez-Osuna and Osuna-Martínez 2011, Vazquez *et al* 2012, De la Cruz-Landero *et al* 2013, López 2015). Within these zones are mangroves, which are considered the most productive and diverse natural wetland systems (CONABIO 2013). However, few studies exist on the effect of these metal pollutants on mangrove species, despite that this coastal ecosystem helps to mitigate erosion and stabilize the coast by buffering floods and hurricanes, among other ecosystem services (Ezcurra *et al* 2009).

Mangrove tree species can accumulate various metals, mainly in their roots (Pulford and Watson 2003, MacFarlane *et al* 2007, Gabriel and Salmo III 2014, He *et al* 2014, Ochoa *et al* 2016). However, the influence of environmental conditions on the accumulation capacity of metals for plant species has been marginally studied (Riba *et al* 2003, Hao *et al* 2012). A previous study shows that plants of *Avicennia germinans* and *Laguncularia racemosa* have a greater capacity for accumulation and tolerance to lead than *Rhizophora mangle* under low salinity (Cabañas-Mendoza 2014).

Plants of *A. germinans* and *L. racemosa* can grow in a large spectrum of salinities, ranging from 0 to 100 ‰ and 0 to 90 ‰, respectively (Wang *et al* 2011). In addition, these species have specialized structures in the leaves such as glands (*A. germinans*) or small protuberances on the petioles (*L. racemosa*) to excrete saline solution, which, when water evaporates, forms crystals on the leaf surface that are wind-dispersed (Sobrado 2001 and 2004, Francisco *et al* 2009, Esteban *et al* 2013). Moreover, leaves accumulate salt as a function of the increase of salinity in the soil (Sobrado and Greaves 2000, Sobrado 2004) and, at maturity, they could accumulate salt even in the last stage before their abscission (Cram *et al* 2002).

The aim of this study was to evaluate how different salinity gradients can cause a change in the pH of the substrate and affect the accumulation of lead in the species *A. germinans* and *L. racemosa*. The information obtained can be used as a basis to understand some of the factors that regulate the mobility of heavy metals in saline conditions, such as those where mangroves grow, and identify the responses of the species to lead contamination.

2. Materials and methods

2.1. Plant material and growth conditions

Black (*Avicennia germinans*) and white (*Laguncularia racemosa*) mangrove propagules (about 400 of each species) were collected in the Ria Celestun Biosphere Reserve (the points of collection were 20.857874, -90.383454, and 20.856782, -90.376828; supplementary figure 1). This is a pristine preserve in Yucatan, Mexico, and our group has been working there for about 10 years and has the required collection permits. Propagules were transported in plastic bags with water from the lagoon to a greenhouse. From the pool of propagules, we selected 240 individuals per species, washed them with distilled water, and placed them individually in black plastic bags (20 × 10 cm) with silica sand 20/30 and agrolite (1:1). Bags with plants were then set into plastic boxes, so they had a shared medium of water and nutrients. Boxes were located in a greenhouse under an average temperature of 20 °C at night and 27 °C during the day, an average relative humidity ranging from 55% to 84%, and an average total daily photon flux density of 5.4 mol m⁻². The photon flux density was measured with a light meter (Photosynthetic Light PAR, Smart Sensor, S-LIA-M003, Onset, EEUU), air temperature and relative humidity with a Temp Smart Sensor (S-TMB-M003, Onset). All variables were recorded simultaneously every 10 s and 10-min averages were stored with a data acquisition system (HOBO U30-NRC Weather Station Starter Kit, Onset). Plants received water weekly with the commercial fertilizer Miracle-Gro (1 g l⁻¹, 200 ml of solution per plant).

2.2. Acclimatization of plants to salinity

After 4 months, plants were randomly assigned to different saline treatments, with salt concentrations similar to those where species commonly grow under natural conditions. Each week, 80 plants of each species were watered with 0, 7 and 15 mg l⁻¹ of commercial sea salt, during a period of two months; simultaneously, plants were irrigated with commercial fertilizer (Miracle-Gro; 1 g l⁻¹, 200 ml of solution per plant).

2.3. Lead treatments

After the 2-month period, individual plants were assigned to lead treatments within each salt treatment. For each salt treatment, 20 individuals of each species were irrigated with deionized water prepared with 0, 40, 80 and 160 μM of PbNO₃. During the experiment, plant leaves were fertilized weekly with a nutritive commercial solution (Miracle-Gro; 1 g l⁻¹).

2.4. Concentration of lead in tissues

Root, stem and leaf samples were taken at 0, 2, 7, 15 and 30 days after exposure for the Pb quantification (n = 4). Samples were rinsed with distilled water to remove the external metal; only leaves of plants that were grown under salinity conditions were not washed to avoid the loss of the salt crystals. The complete tissue or, when appropriate, one gram of dry tissue was placed in crucibles inside a muffle furnace at 600 °C until obtaining white ash.

This ash was then diluted in 4 ml of 3 M hydrochloric acid; each sample was adjusted to 6 ml with 1% nitric acid v/v (Horwitz *et al* 1970, Perkin-Elmer Corporation 1996) to finally be centrifuged at 4000 rpm for 10 min. All samples were measured in an atomic absorption spectrometer (model 55 AA, Agilent Technologies, EE. UU.), using a wavelength of 217 nm and a calibration curve of 0, 1, 5, 25, 50 and 100 ppm of PbNO₃. Results were expressed based on 1 gram of dry weight; for the concentration of Pb in the plant, the total dry weight of each tissue was used.

2.5. Growth parameters

After exposure to Pb and for both species, fresh weight, dry weight, and total length of individuals from all treatment were obtained at 0, 2, 7, 15 and 30 days. Total plant length was measured with a measuring tape from the tip of the longest root to the highest leaf, which was generally above the apical meristem of the stem. Total fresh weight was obtained with an analytical balance; subsequently, tissues were placed in a tumble dryer at 50 °C for 3 days to obtain the total dry weight. During the period of exposure to Pb, photographic evidence was also taken to register any physical change in the plants.

2.6. Physicochemical parameters

Samples of substrate of each plant were taken at a depth of approximately 5 to 7 cm procuring the area of greatest interaction with the roots; substrate was then dried at room temperature before measurements. Electrical conductivity and pH were measured according to the methodologies proposed by USDA (1999) and by NOM-147-SEMARNAT/SSA1-2004 with some modifications. Fifty grams of substrate were placed into a beaker, 50 ml of distilled water were added and solution was stirred for 5 min; samples were then allowed to stand for one minute for the sedimentation of solids and supernatant was placed in plastic tubes for measurement. Parameter measurements were made using the Ysi Pro model 2030 (Professional Series, YSI Incorporated, EE. UU.) with the sensor calibrated for conductivity with a 10,000 μS cm⁻¹ calibration solution, and SM Titrino 702 (Metrohm AG, Switzerland) calibrated with pH solutions of 7 and 4. Measurements were made every 7 or 15 days, before and during the salt acclimation and at 0, 7, 15 and 30 days during lead treatment.

2.7. Statistical analysis

Factorial analyses of variance were carried out to compare the means across the factors Pb treatment, salinity treatment, time of exposure, and tissue of each species for the response variables Pb tissue concentration, total fresh weight, total dry weight, total plant length, and substrate pH. Tukey comparison tests were performed to identify treatment differences. In addition, simple linear regression analyses were applied to explore the relationship between soil physicochemical parameters as well as the relationship of Pb concentration with the other variables analyzed. All analyses were determined at a confidence level of 95% (p < 0.05) using the statistical package R version 3.5.1 for Windows.

Table 1. Concentration of endogenous lead ($\mu\text{g g}^{-1}$ dry weight) found in plants exposed to different concentrations of lead, grown in the absence of salt (0 mg l^{-1}) during a 30-day exposure period. Data are means \pm standard error.

	Day	<i>Avicennia germinans</i> A			<i>Laguncularia racemosa</i> B		
		40 μM	80 μM	160 μM	40 μM	80 μM	160 μM
Root	2 A A	8.14 \pm 1 a	26.9 \pm 4.4 a	58.7 \pm 8.3 b	42.8 \pm 9.3 a	63.5 \pm 12.4 a	155.9 \pm 26 b
	7 AB AB	17.5 \pm 1.8 a	63.2 \pm 5 b	89.1 \pm 5.5 c	48.2 \pm 11.9 a	138.1 \pm 20.3 b	293.5 \pm 21.2 c
	15 B AB	40.2 \pm 7.6 a	72.8 \pm 10.1 a	172.8 \pm 13.8 b	58.9 \pm 14.5 a	226.9 \pm 17.2 b	336.4 \pm 8.5 c
	30 B B	44.9 \pm 10 a	78 \pm 7.8 a	206.1 \pm 17 b	61.5 \pm 12.2 a	331.9 \pm 20.7 b	488.2 \pm 24.2 c
Stem	2 A A	5.8 \pm 1.3 a	11.8 \pm 2.4 ab	12.6 \pm 1.2 b	13.9 \pm 4.1 a	14.8 \pm 5.2 a	17.5 \pm 1.6 a
	7 A A	10.8 \pm 1.4 a	12.5 \pm 2.5 a	7.9 \pm 0.9 a	16.3 \pm 9 a	18.1 \pm 1.6 a	20.7 \pm 4.4 a
	15 A A	10.5 \pm 1.5 a	11.1 \pm 2.5 a	13.7 \pm 2.5 a	17.4 \pm 3.3 a	19.1 \pm 2.5 a	22.5 \pm 5.4 a
	30 A A	10.2 \pm 1.5 a	11.9 \pm 1.4 a	14.2 \pm 2.2 a	20.6 \pm 3.5 a	20.1 \pm 3.3 a	23 \pm 2.7 a
Leaf	2 A A	11.6 \pm 1 a	12.1 \pm 0.4 a	12.2 \pm 0.4 a	10 \pm 1.5 a	10.4 \pm 1.1 a	12.5 \pm 3.4 a
	7 A AB	10.4 \pm 1 a	12.5 \pm 0.7 a	12.3 \pm 1.4 a	12.3 \pm 3 a	18 \pm 3.4 a	21.2 \pm 4.2 a
	15 A AB	12.6 \pm 0.6 a	12.9 \pm 3.4 a	15.6 \pm 4 a	14.7 \pm 3.3 a	20.9 \pm 6.7 a	21 \pm 4.5 a
	30 A B	12.8 \pm 1 a	14.1 \pm 1.5 ab	18.5 \pm 1.6 b	14.7 \pm 1.5 a	22.7 \pm 5.3 ab	29 \pm 3.1 b

Different lowercase letters indicate significant differences among Pb treatments (columns) for each day of exposure, for each tissue and for each species ($n = 12$). Different capital letters indicate significant differences for comparisons among tissue/species (bold, $n = 288$), and among exposure days (rows) for each tissue and for each species (roman for *A. germinans* and italicized for *L. racemosa*, $n = 48$).

Table 2. Concentration of endogenous lead ($\mu\text{g g}^{-1}$ dry weight) found in plants exposed to different concentrations of lead, grown under 7 mg l^{-1} of salt during a 30-day exposure period. Data are means \pm standard error.

	Day	<i>Avicennia germinans</i> A			<i>Laguncularia racemosa</i> B		
		40 μM	80 μM	160 μM	40 μM	80 μM	160 μM
Root	2 A A	12 \pm 3.3 a	15.5 \pm 2.9 a	35.6 \pm 7 b	12.3 \pm 0.9 a	24.3 \pm 6.4 a	48.8 \pm 8 b
	7 AB A	24.7 \pm 0.5 a	27.4 \pm 1 a	38.7 \pm 4.6 b	22.8 \pm 1 a	35.7 \pm 8 a	65.1 \pm 8.9 b
	15 B A	29 \pm 6.6 a	31 \pm 1.7 a	49.3 \pm 8.2 a	24.9 \pm 2.4 a	50.7 \pm 16.5 ab	65.2 \pm 7.5 b
	30 A A	12.3 \pm 4.4 a	22 \pm 3.7 ab	28 \pm 3.8 b	29.4 \pm 3.7 b	51.3 \pm 8.4 a	50.5 \pm 4.8 a
Stem	2 A A	6.2 \pm 1 a	10.5 \pm 2.5 a	11.6 \pm 4.4 a	8.8 \pm 1.3 a	9.7 \pm 1.2 a	11.9 \pm 2.8 a
	7 A AB	11.5 \pm 3.1 a	11.9 \pm 1.9 a	12.1 \pm 2.7 a	15.9 \pm 3.5 a	16.8 \pm 3.2 a	18.3 \pm 6.5 a
	15 A B	11.8 \pm 2.2 a	12.5 \pm 1.8 a	17.4 \pm 4.8 a	18.1 \pm 2 a	18.7 \pm 5 a	19.7 \pm 4.6 a
	30 A B	6.9 \pm 0.8 a	16.2 \pm 3 a	19.1 \pm 6.5 a	19.4 \pm 3.7 a	19.9 \pm 2.1 a	22.8 \pm 3.4 a
Leaf	2 A A	8.6 \pm 0.7 a	13.3 \pm 2.9 a	13.8 \pm 1.6 a	10.3 \pm 1.7 a	12.5 \pm 2.1 a	16.1 \pm 0.6 a
	7 AB AB	16.9 \pm 4.7 a	17.3 \pm 4 a	23.2 \pm 3.8 a	17.1 \pm 4.9 a	21.8 \pm 4.8 a	24.2 \pm 2.8 a
	15 AB AB	17.4 \pm 3.3 a	16.9 \pm 3.2 a	27.5 \pm 6 a	18.2 \pm 3.9 a	22.1 \pm 3.6 a	22.8 \pm 4.7 a
	30 B B	16.8 \pm 1.6 a	28.1 \pm 5.3 a	27.2 \pm 6.6 a	20.2 \pm 4 a	25.1 \pm 1.5 ab	35.4 \pm 6 b

Different lowercase letters indicate significant differences among Pb treatments (columns) for each day of exposure, for each tissue and for each species ($n = 12$). Different capital letters indicate significant differences for comparisons among tissue/species (bold, $n = 288$), and among exposure days (rows) for each tissue and for each species (roman for *A. germinans* and italicized for *L. racemosa*, $n = 48$).

3. Results

3.1. Concentration of lead in tissues

Tissues of *L. racemosa* had the highest Pb concentrations in all salt treatment, compared to tissues of *A. germinans* ($p < 0.05$; tables 1–3, Table S1 is available online at stacks.iop.org/ERC/2/061004/mmedia). For both species, roots were the tissues that accumulated the highest Pb concentrations.

In the absence of salt (table 1), in both *A. germinans* and *L. racemosa* Pb uptake by the roots increased significantly with time, as the Pb concentration in the substrate increased. Lead translocated to the leaf was accumulated in proportions similar to those found in the stem, but leaf showed significant increases and the highest Pb concentration at the end of the experiment in both species.

Salinity significantly affected the Pb uptake capacity of both species. Accumulation of Pb by roots decreased and translocation of Pb to the leaves increased (highest values in *A. germinans* at 7 mg l^{-1} of salt and in *L. racemosa* at 15 mg l^{-1} of salt); the translocation to the stem was similar in all treatments (tables 2 and 3).

During the first 15 days, the Pb concentration in the roots for plants of *A. germinans* and *L. racemosa* growing under 7 mg l^{-1} of salt increased as Pb concentration in the medium increased (table 2); however, at the end of the experiment

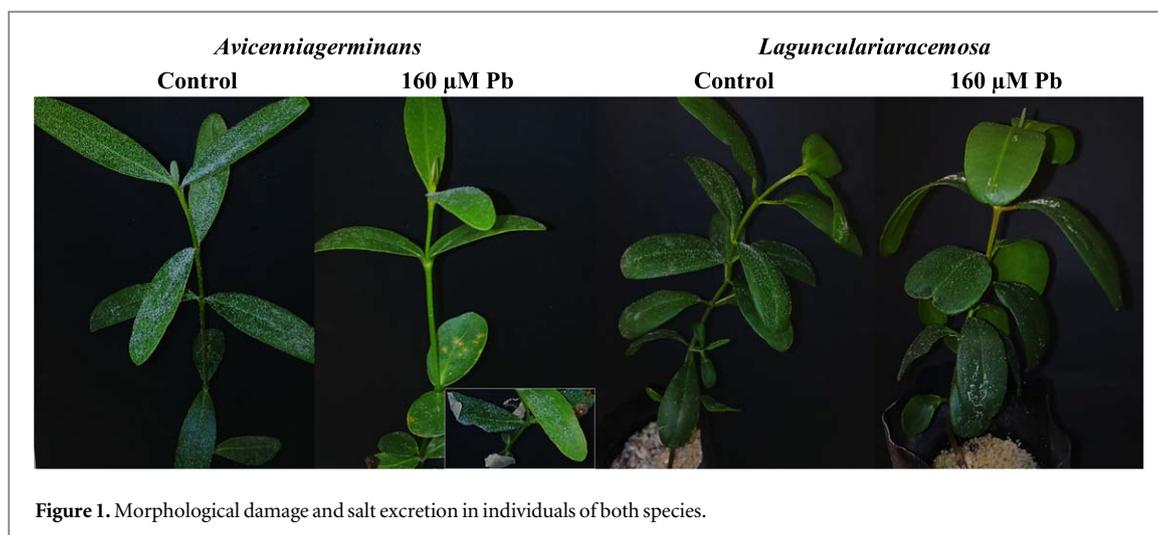


Figure 1. Morphological damage and salt excretion in individuals of both species.

Table 3. Concentration of endogenous lead ($\mu\text{g g}^{-1}$ dry weight) found in plants exposed to different concentrations of lead, grown under 15 mg l^{-1} of salt during a 30-day exposure period. Data are means \pm standard error.

	Day	<i>Avicennia germinans</i> A			<i>Laguncularia racemosa</i> B		
		40 μM	80 μM	160 μM	40 μM	80 μM	160 μM
Root	2 AA	14.5 \pm 2.5 a	20.7 \pm 1.6 a	53.9 \pm 5 b	39.2 \pm 6 a	44.9 \pm 5.7 a	130.7 \pm 7.6 b
	7 ABAB	31 \pm 4.2 a	43.4 \pm 1.4 b	80.2 \pm 3.4 c	47 \pm 5.6 a	65.6 \pm 12.8 a	186.9 \pm 27.8 b
	15 BAB	35.6 \pm 7.1 a	67.8 \pm 6.4 a	157.2 \pm 17.9 b	49 \pm 9.9 b	169.3 \pm 33.6 a	208.6 \pm 16.3 a
	30 A B	33.4 \pm 4.9 a	34.7 \pm 3.5 a	50.6 \pm 14.6 a	58.7 \pm 18.5 a	179.9 \pm 14.2 b	266 \pm 5.2 c
Stem	2 AA	6.8 \pm 0.5 a	7.2 \pm 0.8 a	9.5 \pm 2 a	15.5 \pm 1.2 a	17.1 \pm 1.2 a	22.2 \pm 0.9 b
	7 BA	10.3 \pm 1.1 a	11.5 \pm 1.9 a	11.8 \pm 1.6 a	16.1 \pm 2 a	18.5 \pm 3.4 a	23.9 \pm 3.9 a
	15 BA	11.5 \pm 1.6 a	12.2 \pm 1.7 a	12.5 \pm 1.7 a	16.9 \pm 3.7 a	18.5 \pm 6.3 a	35.6 \pm 2.8 b
	30 BA	13.9 \pm 1.5 a	14.3 \pm 2.2 a	15.2 \pm 2.3 a	21.3 \pm 3.2 a	21.2 \pm 4.8 a	24 \pm 4.6 a
Leaf	2 AA	9.4 \pm 1.6 a	13.1 \pm 1.8 a	14.2 \pm 2 a	12.3 \pm 4.1 a	19.7 \pm 3.8 ab	26 \pm 2.5 b
	7 AA	12.2 \pm 0.5 a	14.1 \pm 2.3 a	19.4 \pm 5.9 a	16.2 \pm 1.8 a	20.4 \pm 5.4 ab	46 \pm 12.2 b
	15 AA	14.9 \pm 2.4 a	15.4 \pm 3.4 a	18 \pm 1.9 a	18.8 \pm 5.5 a	21.3 \pm 6.7 a	51.1 \pm 10.2 b
	30 AA	28.7 \pm 16.8 a	17.6 \pm 2.3 a	18.5 \pm 2.2 a	18.9 \pm 4.9 a	28.6 \pm 7.9 ab	50.1 \pm 9.6 b

Different lowercase letters indicate significant differences among Pb treatments (columns) for each day of exposure, for each tissue and for each species ($n = 12$). Different capital letters indicate significant differences for comparisons among tissue/species (bold, $n = 288$), and among exposure days (rows) for each tissue and for each species (roman for *A. germinans* and italicized for *L. racemosa*, $n = 48$).

the tissue concentration was maintained or even decreased. For both species, the translocation of Pb to the stem was similar among the different treatments and days of exposure. The Pb accumulation in leaves for *A. germinans* was significantly greater as the time of exposure passed, but it was similar among Pb treatments. At the end of the experiment, in *L. racemosa* the leaf Pb concentration values increased significantly.

Plants of *A. germinans* and *L. racemosa* under 15 mg l^{-1} of salt had a greater root Pb accumulation than at low salinity (table 3). For *L. racemosa*, Pb accumulation increased significantly as Pb treatment concentration increased. For *A. germinans* this also occurred, but after day 15 Pb root concentration decreased. Only *L. racemosa* had the highest Pb concentrations in the leaf, which was greater than those found in the stem.

3.2. Effect of Pb accumulation on plant morphology

Although *L. racemosa* showed the highest Pb accumulation in all treatments, there were no visible symptoms of toxicity in any of the individuals analyzed even under high concentrations of Pb and at the end of the experiment (figure 1). Only some individuals of *A. germinans* that grew with or without salt showed leaf yellowing and wilting from day 7, but most damage occurred at 15 and 30 days of exposure, and at the highest Pb concentration (figure 1). In both species, salt crystals in leaves were observed in plants under halophytic conditions because of the mechanism of excretion characteristic of these species.

3.3. Effect of Pb and salinity on plant growth

The species with the highest weight and length was *A. germinans*, but *L. racemosa* values varied less with salinity than those of *A. germinans* at the end of the experiment (table 4). In both species, although some plants exposed

Table 4. Growth parameters during 30 days of Pb treatments in plants of *A. germinans* and *L. racemosa* grown under different salinity conditions. Data are means \pm standard error.

Species	Salt treatment (mg/L)	Pb treatment (μ M)	Fresh weight (g FW)	Dry weight (g DW)	DW/FW ratio (%)	Plant length (cm)
<i>Avicennia germinans</i>	<u>A</u>	0	15.7 \pm 1.5 a	4.2 \pm 0.4 a	27.3 \pm 0.6 a	72.3 \pm 2.1 a
	A	40	13 \pm 1.3 ab	3.8 \pm 0.3 a	30.1 \pm 1.6 a	67.8 \pm 2.2 a
	A	80	9.1 \pm 1.3 b	2.5 \pm 0.3 b	28.4 \pm 1.4 a	55 \pm 2.7 b
		160	9.1 \pm 0.8 b	2.5 \pm 0.2 b	28.5 \pm 1.3 a	56.5 \pm 2.4 b
		0	18.7 \pm 1.9 a	4.6 \pm 0.5 a	24.1 \pm 0.3 a	70.2 \pm 3.1 a
		40	15.1 \pm 1.5 ab	3.6 \pm 0.4 ab	23.8 \pm 0.4 a	64.8 \pm 3.6 a
	A	80	16.3 \pm 1.5 ab	3.9 \pm 0.3 ab		67.1 \pm 4.4 a
		160	12.6 \pm 1.3 b	3.1 \pm 0.3 b	24.8 24.6 \pm 0.5 a \pm 0.5 a	61.9 \pm 3.4 a
		0	13.7 \pm 0.8 a	3.5 \pm 0.2 ab	25.8 \pm 0.4 a	64.6 \pm 2.3 a
		40	15.9 \pm 1.4 ab	3.9 \pm 0.3 ab	24.7 \pm 0.4 ab	62.5 \pm 2.7 a
	A	80	18.5 \pm 1.4 b	4.5 \pm 0.4 a	24.2 \pm 0.3 b	68.7 \pm 1.9 a
		160	13 \pm 1.1 a	3.2 \pm 0.2 b	25.1 \pm 0.3 ab	62.6 \pm 2.6 a
<i>Laguncularia racemosa</i>	<u>A</u>	0	9.9 \pm 1.5 a	2.1 \pm 0.3 a	20.4 \pm 0.7 a	49.9 \pm 2.7 a
	AB	40	12.1 \pm 1.7 a	2.6 \pm 0.4 a	19.9 \pm 0.7 a	49.2 \pm 3.2 a
	A	80	10.9 \pm 1.5 a	2.4 \pm 0.4 a	21 \pm 0.8 a	49.5 \pm 2.8 a
		160	9.7 \pm 1.3 a	2.1 \pm 0.3 a	20.6 \pm 0.8 a	47.5 \pm 2.7 a
		0	13.3 \pm 1.6 a	2.9 \pm 0.4 a	21.7 \pm 0.4 a	54.2 \pm 2.6 a
		40	12 \pm 1.1 a	2.6 \pm 0.2 a	21.6 \pm 0.3 a	53.5 \pm 2.1 a
	A	80	11.3 \pm 1.3 a	2.5 \pm 0.3 a	22.9 \pm 0.7 a	49.5 \pm 2.7 a
		160	10.3 \pm 1.1 a	2.3 \pm 0.2 a	24 \pm 1.4 a	50.3 \pm 2.9 a
		0	9.9 \pm 2.2 a	2.2 \pm 0.5 a	21.8 \pm 1 a	43.2 \pm 3.3 a
	<u>B</u>	40	7.4 \pm 1.2 a	1.7 \pm 0.3 a	21.6 \pm 0.7 a	41.3 \pm 2.3 a
	B	80	7.8 \pm 1.1 a	1.7 \pm 0.3 a	23.4 \pm 1.7 a	38.9 \pm 2.4 a
		160	8.2 \pm 1.2 a	1.8 \pm 0.3 a	21.5 \pm 0.9 a	43.2 \pm 2.3 a

Different lowercase letters indicate significant differences for comparisons among Pb treatments for each parameter within each salinity and each species ($n = 80$). Different capital letters indicate significant differences for comparison among salinity treatments (underlined for fresh weight, italicized for dry weight and bold for plant length) for each species ($n = 240$).

to 15 mg l⁻¹ of salt had a lower weight and length, there was no significant correlation between the growth variables studied and the electrical conductivity values of the substrate ($R^2 < 0.1$), so that salinity did not influence growth.

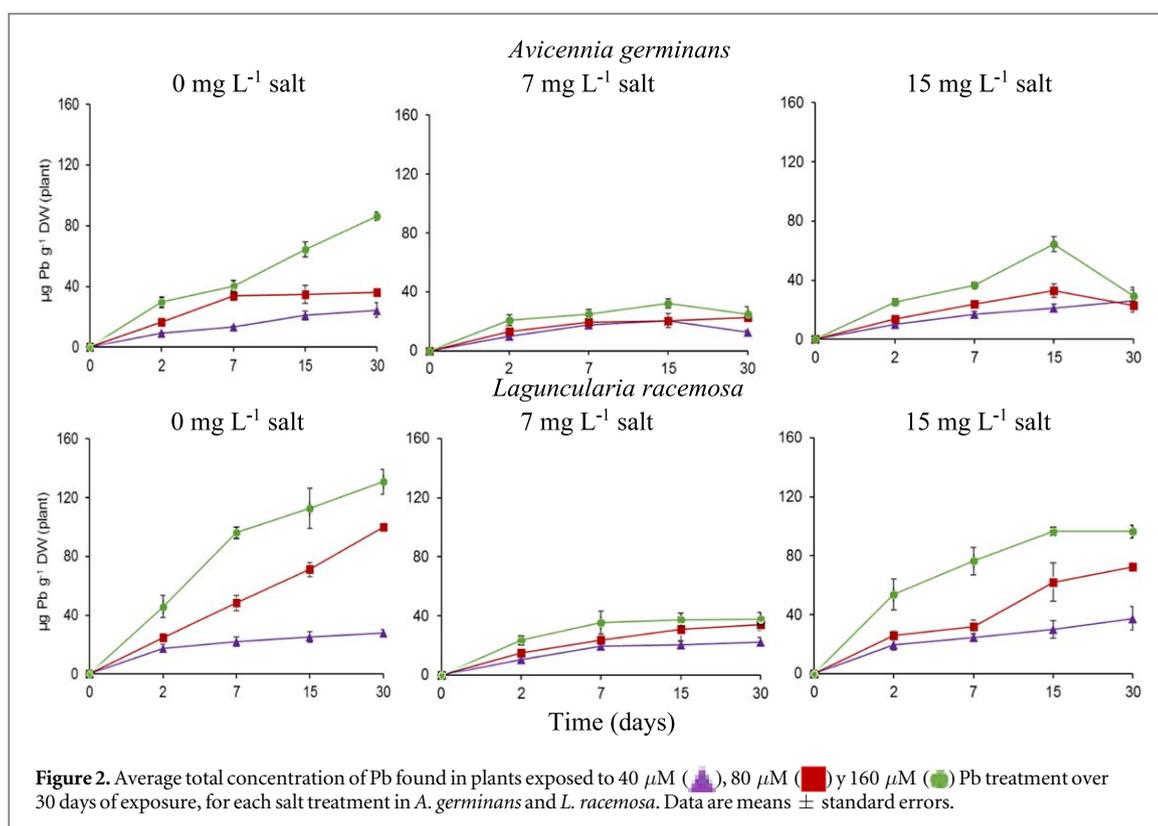
For *A. germinans*, the higher the Pb concentration the lower the biomass and length for the plants. In all salt treatments, the ratio of length and dry weight with respect to the total accumulation of Pb in the plant was negative (figures 2 and 3(a)–(f)); but, only in plants that were kept under 7 mg l⁻¹ of salt was the correlation significant. In addition, some decreasing tendencies in the growth values were observed for *L. racemosa*, but none was significant.

In addition, for the treatments of 0 and 15 mg l⁻¹ of salt in *L. racemosa*, most of the obtained correlations between Pb tissue concentration and plant biomass or length were positive, but not significant. Larger plants of this species accumulated higher Pb concentrations but toxic effect in this species was not visible (figures 2 and 3). However, in both species, it appears that small-sized plants tend to accumulate lower Pb concentrations (~ 10 – 40 μ g g⁻¹ in treatments with 40 and 80 μ M of Pb).

3.4. Effect of salinity on pH and its relation to Pb uptake

In both species, as expected, electrical conductivity (EC) was proportional to the amount of salt in the substrate. Plants grown without salt presented the lowest EC values, which decreased significantly in the last weeks of the experiment (figures 4(a) and (c)). With 7 and 15 mg l⁻¹ of salt, EC increased drastically and gradually; when irrigating with salt and Pb, EC values first decreased and later gradually increased again. In both species and for both treatments with salt, EC values of all weeks of measurement differed statistically.

Substrate pH for plants of *A. germinans* growing without salt maintained an average range of 4.9 to 5.8, being the lowest among all treatments (figure 4(b), table S1). For the salt treatments, pH increased gradually and significantly four weeks after starting the salt irrigation and stabilizing at the end of the experiment. Plants of *L. racemosa* that grew without salt initially had a higher pH (≥ 6) than plants of *A. germinans*, but gradually decreased until the end of the experiment (average range of 5.4 to 6.1, figure 4(d)). Under salinity conditions, the



former species grew in a less acidic environment compared to *A. germinans*, since some plants showed point values of pH ≥ 7 when the plants were acclimated to 15 mg l⁻¹ of salt.

In both species, the relationship between EC and pH was significant showing that an increase in the salinity of the substrate can increase the pH; although, the correlation was low ($R^2 = 0.26$ for *A. germinans* and $R^2 = 0.21$ for *L. racemosa*; figure 5). Also, EC and pH were related to the roots Pb concentration as well as the EC with the leaf Pb concentration (figures 6 and 7).

For *A. germinans*, an increase in EC and pH, as well as a longer exposure time to the metal, leads to a lower accumulation of Pb in the roots. Also, *L. racemosa* had a negative relationship between EC and pH, which increase the first 15 days of exposure. However, correlation values were lower compared to those of *A. germinans*. Furthermore, for both species, the higher the salinity and longer the time of exposure, the higher accumulation of Pb in the leaf was, especially for *L. racemosa* at day 30.

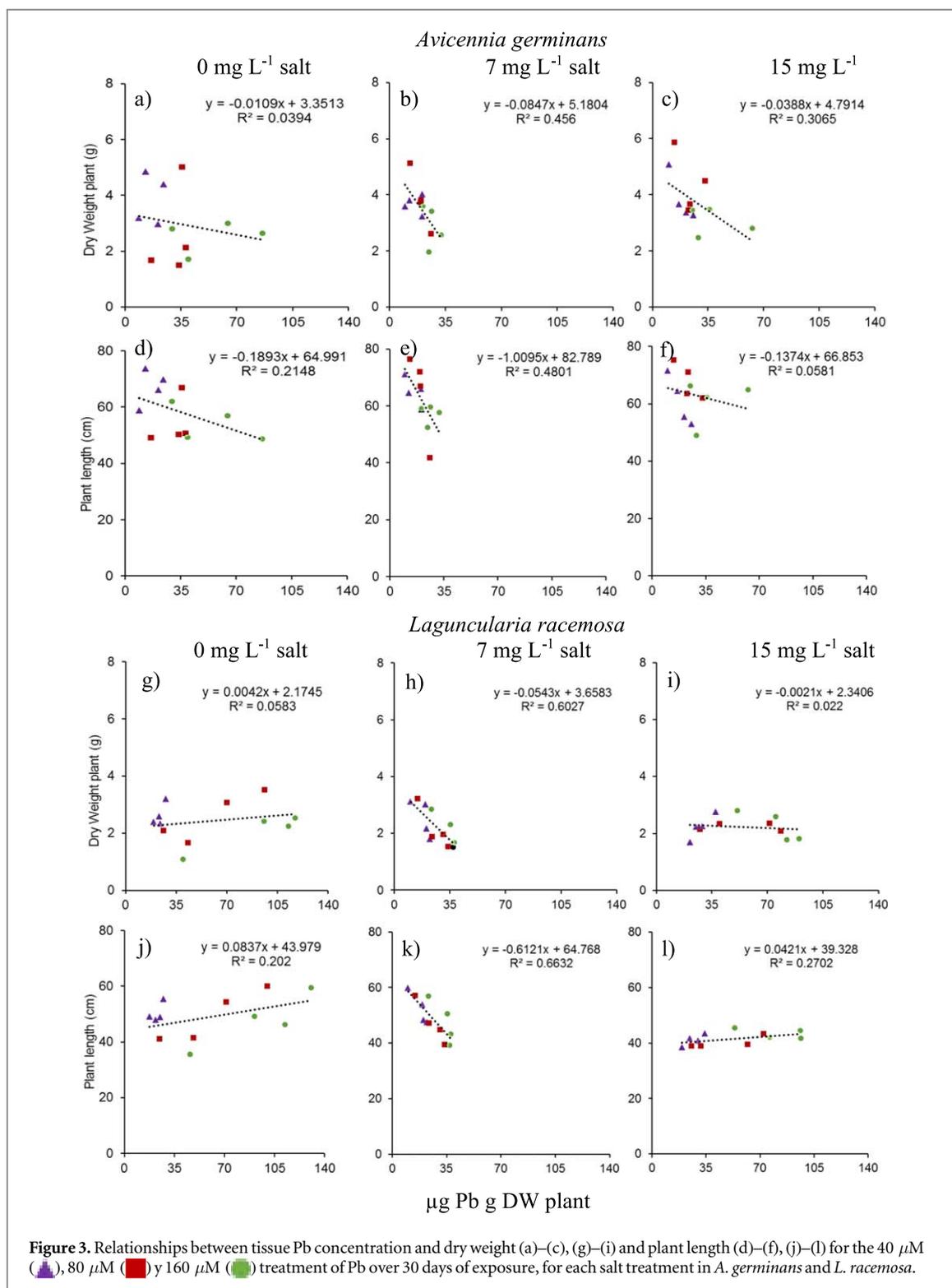
4. Discussion

We found that *Avicennia germinans* and *Laguncularia racemosa* accumulated Pb, mainly in the roots, and translocate it in low concentrations towards the aerial parts (phyto-stabilization), which has already been reported for other mangrove species (Nirmal Kumar *et al* 2011, Wang *et al* 2013, Cabañas-Mendoza 2014 Pérez-Sirvent *et al* 2017, Qiu and Qiu 2017). This mechanism is common in tree species because it allows them to be more tolerant to the toxic effects (Diez 2008, Trejo-Calzada *et al* 2015, Salas-Luévano *et al* 2017). Under no salinity conditions, Pb accumulation tends to be higher when the level of contamination and the time of exposure to the Pb is higher.

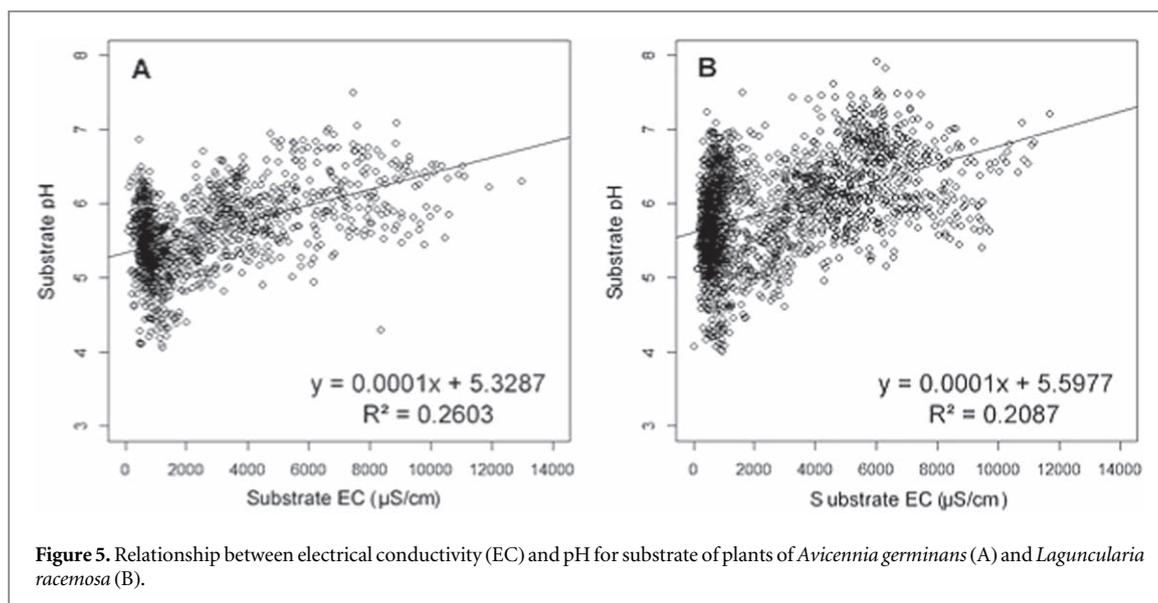
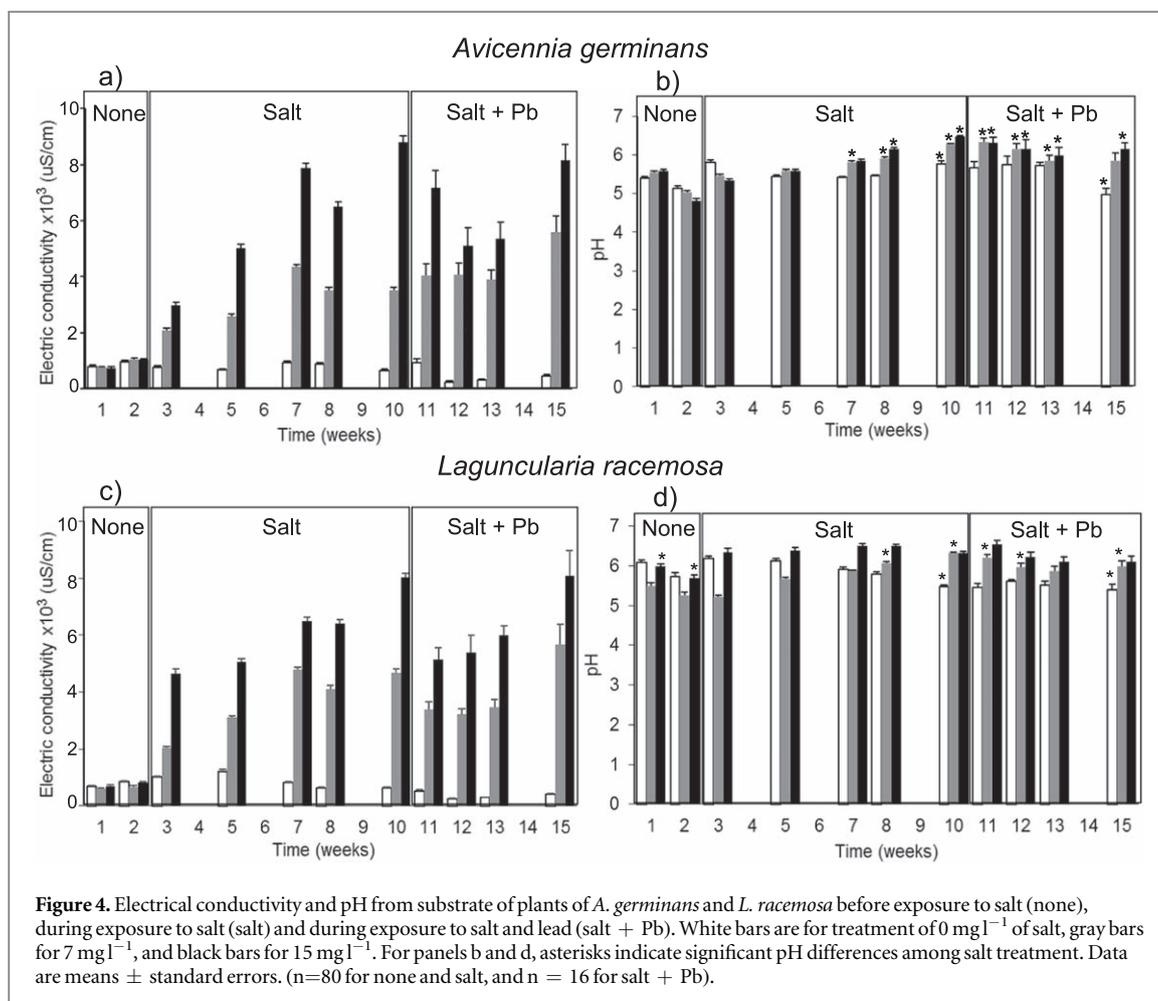
In all Pb treatments, *L. racemosa* not only had the highest accumulation capacity but also the highest tolerance without showing any visible damage; both biomass and length did not change for this species, even though some plants accumulated high Pb concentrations. Our results showed that *A. germinans* could have a greater vulnerability to the toxic effects of Pb as time of exposure passed.

We corroborated that pH increased in the substrate at higher salinities, but the relationship between these two variables was low. Few studies have shown the influence of salinity on pH (Saraswat *et al* 2015). More experiments with longer residence times and higher salt concentrations in the soil are required to show an evident relationship. Besides, other environmental variables can also influence changes in pH, such as temperature which, when increases, causes a decrease in pH (Dotro *et al* 1994).

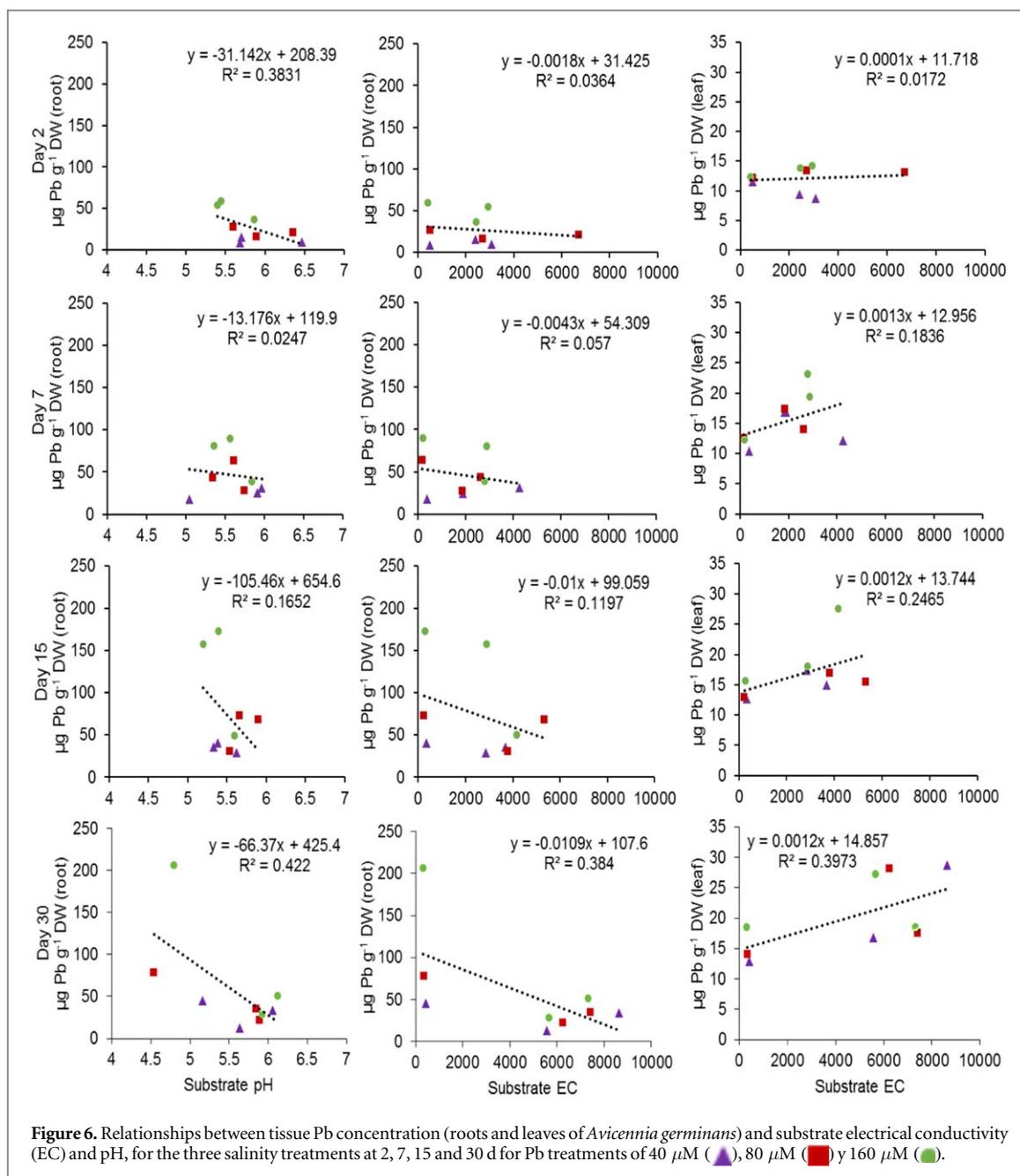
At higher salt concentrations there is an increase in the uptake of Cd, Zn and Pb in some plants, due to competition with other cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) for sorption sites. This could increase the formation of



complexes with chloride anions, causing desorption of metals and their mobility (Fritioff *et al* 2005, Manousaki and Kalogerakis 2009, Manousaki *et al* 2009, Gharaibeh *et al* 2015). Additionally, some studies on sediments and different plant species show that higher salinity leads to a decrease in the mobilization and accumulation of Ni, Cu, Zn, Cd and Pb in plant tissues (Smolders and McLaughlin 1996, Riba *et al* 2003, Fritioff *et al* 2005, Manousaki and Kalogerakis 2009, Leblebici *et al* 2011). These last studies support our results, because we found that salinity caused a lower accumulation of Pb in the root. In addition, in this study, the greater the exposure period, a decrease in Pb availability of the metal occurred, mainly in *A. germinans*. This can occur because metals can also bind to other anions forming less soluble complexes that can limit the absorption of the metal through coagulation, precipitation and flocculation (Smolders and McLaughlin 1996).

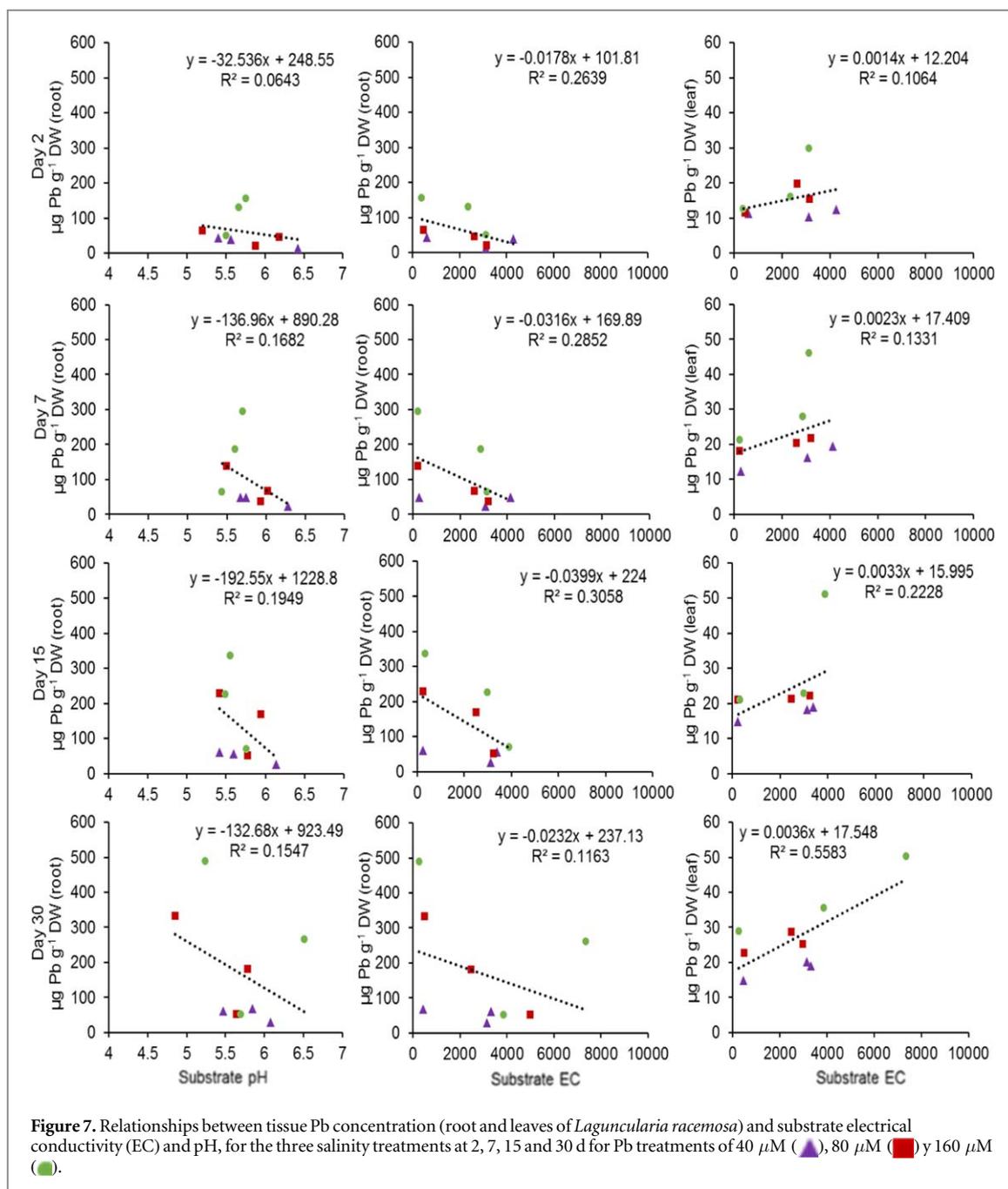


Few studies mention the influence of environmental variables on the uptake of metals for mangrove species (Dai *et al* 2017, Pittarello *et al* 2018). For instance, Cheng *et al* (2012) reported that an increase in salinity reduces the loss of radial oxygen, which stimulates the lignification of the exodermis, and alters the permeability of the root, influencing a lesser accumulation of metals (Pb, Zn and Cu) at low salinity. Our results agreed with these findings, since at low salinity in the substrate there was the lowest Pb tissue accumulation.



Nevertheless, some studies show that the transformation of free ion metals to complexes, and their low availability, is not the only reason why metal accumulation in plants decreases in the presence of salt. Mei *et al* (2014) show that a blockage in the Ca^{2+} channels significantly decreases the absorption of Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cd^{2+} by the root of amaranth plants grown under different concentrations of NaCl . This study also reports that a blockage of the K^+ channels diminishes the uptake of Na^+ , and K^+ but not of Ca^{2+} , Mg^{2+} , and Cd^{2+} , which suggests competition between the Na^+ and Cd^{2+} for passage through the channels of Ca^{2+} . This affinity of Na^+ for the sites of sorption in the walls of the root cells displaces other ions and, consequently, results in a low accumulation of the metal in the roots. These findings can help explain the decrease of metal in mangrove roots, which can lead to more studies on the specificity of the transporters in the accumulation of metals in mangroves.

Our results showed that the accumulation of Pb in roots was associated with pH changes; and that the decrease in the availability of Pb in the roots is more evident at longer time of exposure to the metal. A study also reports that the accumulation of metals in plants is more effective when the pH is low, since acidification releases metals associated with sediments (Riba *et al* 2003). But further studies are needed to know how pH increase of the substrate affects Pb availability.



An interesting finding in our study was that salinity also influences a greater translocation of Pb to the leaves. This can occur because these species excrete salt through their leaves. MacFarlane and Burchett (1999, 2000) show that the mangrove species *Avicennia marina*, treated with Zn and Cu, has an increase in these metals in the leaf and in the salt crystals on leaf surfaces, suggesting a strategy of the plant to deal with excess micronutrients by eliminating them through the glandular trichomes of the epidermis. This could help explain the lowest root Pb concentration values at the end of the experiment for mangrove plants in the low salinity treatment.

Soils in mangrove ecosystems, in addition to having a wide salinity gradient, are also flooded either temporarily or permanently and are rich in organic matter, which influences changes in pH and other physicochemical variables, such as processes of oxide reduction (Lugo and Medina 2014, Naidoo 2016). Plants of *A. germinans* and *L. racemosa* can co-exist in basins or semi-flooded areas, but when salinity is high, *A. germinans* dominates, whereas when salinity is low the dominant species can be *L. racemosa* (Reese 2009, Zaldivar-Jiménez *et al* 2010). Both species are distributed within bands or plots along the Gulf of Mexico and the Pacific coast (López-Portillo and Ezcurra 2002). Based on our findings, *L. racemosa* has several advantages that would allow it to be a better candidate for the remediation of Pb in these ecosystems. However, more studies under higher salinities need to be done to show the potential of *A. germinans*.

5. Conclusion

Salinity, and the changes caused in pH, influenced the availability, accumulation and translocation of Pb in *A. germinans* and *L. racemosa*, which was more evident under prolonged periods of time. Nevertheless, *L. racemosa* not only had a greater capacity for Pb accumulation and tolerance, but this species also occurs in conditions where the availability of Pb discharges may be greater, which makes it more efficient for phytoremediation. Additionally, *A. germinans* can be more efficient for phytoremediation in places with low pollution. The contamination by heavy metals in coastal lagoons of Mexico is worrisome so it is urgent to carry out more studies on how these species, and other mangrove species, can coexist and cope with these pollutants and if this could somehow help heavy metal pollution remediation.

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