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Metal effects on germination and seedling development in closely-related halophyte species inhabiting different elevations along the intertidal gradient

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ABSTRACT

Seed germination and seedling establishment are very sensitive plant stages to metal pollution. Many halophyte species colonizing salt marshes are able to germinate and establish in highly contaminated habitats and low marsh halophyte species seem to show higher tolerance to metals than high marsh species. We analyzed the effects of copper, zinc and nickel in concentrations up to 2000 μM on seed germination and seedling growth in two closely related species of *Sarcocornia*, *S. perennis*, a low marsh species, and *S. fruticosa*, a high marsh species. Germination of both halophytes was not affected by any metal concentration, and their seedling growth, mainly radicle length, was reduced by increasing metal concentrations. Seedlings of *S. perennis* showed higher tolerance to the three metals than those of *S. fruticosa*. Our results are useful for designing ecotoxicological bioassays and planning phytoremediation projects in salt marshes.

1. Introduction

Coastal marshes are usually exposed to high loads of contaminants such as metals (Gedan et al., 2009). This pollution, coming from industrial, mining, agricultural and transport activities, accumulates in intertidal sediments in concentrations that often exceed their toxicity threshold values (Sharifuzzaman et al., 2016). In this context, metal pollution is a major environmental problem in many estuaries due to their toxic nature, non-biodegradability and accumulative behaviors (Williams et al., 1994). Nevertheless, halophytes adapted to survive in brackish and salty environments frequently show high tolerance to metal pollution (Van Oosten and Maggio, 2015) since tolerances to salinity and metal stress share common mechanisms such as high levels of antioxidant defenses and vacuolar sequestration (Manousaki and Kalogerakis, 2011).

Seed germination and seedling establishment are the stages most sensitive to metal pollution in the plant life cycle (Munzuroglu and Geckil, 2002; Liu et al., 2005; Ahsan et al., 2007). Metals may provoke concentration-dependent reduction in germination and seedling growth (Kranner and Colville, 2011; Sethy and Ghosh, 2013; Asati et al., 2016) if they reach embryonic tissues across the seed coats, as well as the effect of the ions on germination metabolism (Ko et al., 2012). Defenses against metals during germination include reduction of metal uptake, chelation and the induction of antioxidant defenses (Kranner and Colville, 2011). Many halophyte species exhibit some of these adaptive mechanisms that enable them to germinate and survive under high metal concentrations (Thomas et al., 1998; Van Oosten and Maggio, 2015).

Plants colonizing salt marshes in the joint estuary of the Odiel and Tinto rivers (Gulf of Cadiz, southwest Iberian Peninsula) are excellent focus species for studying the effects of metal pollution on halophytes, since this estuary is one of the most metal-polluted in the world (Nelson and Lamothe, 1993; Sainz et al., 2004). The pollution in the Odiel Marshes results from industrial sources on the estuary itself, and chiefly

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mining upstream in the Iberian Pyrite Belt (Pérez-López et al., 2011; Muñoz-Vallés et al., 2017). The high metal concentrations recorded in the Odiel Marshes could have adverse effects on wildlife as they accumulate in halophyte species (Stenner and Nicless, 1975). Metal accumulation in halophytes in the Odiel Marshes decreases from low to high marshes, with its peak in those species inhabiting the lowest elevations along the intertidal gradient (Luque et al., 1999). This spatial pattern in metal accumulation may be related to the higher bioavailability of several metals under hypoxic and anoxic conditions at lower elevations in the intertidal gradient (O'Reilly Wiese et al., 1997; Reboreda and Caçador, 2007; Wang et al., 2012; Brito et al., 2021). Thus, germination and seedling growth of *Spartina maritima* (Curtis) Fernald, a native low-marsh species, were unaffected by Copper (Cu), Nickel (Ni) and Zinc (Zn) concentrations up to 2000 μM (Infante Izquierdo et al., 2020). In the same way, the final germination percentage and seedling development of *Salicornia ramosissima* J. Woods, an annual species sampled from low elevations in the Odiel Marshes, were not affected by increasing concentrations of Cu, Manganese (Mn) and Zn, but its final germination decreased c. 25% at Ni concentrations higher than 10 μM (Márquez-García et al., 2013). Furthermore, working with middle marsh populations of *S. densiflora* Brongn., an exotic invasive species in the Odiel Marshes, Infante Izquierdo et al. (2020) found that its final germination percentage was not affected by Cu, Ni and Zn concentrations up to 2000 μM, but its seedling growth, mostly radicle development, was reduced at metal concentrations higher than 100–250 μM. From the highest marsh elevations in the Odiel Marshes, germination of *Atriplex halimus* L. was not affected by increasing concentrations of Cu, Mn, Ni or Zn, but its seedling development was reduced at concentrations higher than c. 100 μM (Márquez-García et al., 2013). In view of these previous studies, it seems that low marsh species show higher tolerance to metals during seedling development than halophytes colonizing higher elevations in the intertidal gradient. However, the toxicity of metals in plants varies with plant species, soil characteristics, specific metals and their concentration and chemical forms (Nagajyoti et al., 2010; Asati et al., 2016).

Our focus species in the Odiel Marshes for studying the germination and seedling development of halophytes inhabiting contrasted elevations along the intertidal gradient were *Sarcocornia perennis* (Mill.) A.J. Scott, a low marsh species (Davy et al., 2006), and *Sarcocornia fruticosa* (L.) A.J. Scott., a high marsh species (Contreras-Cruzado et al., 2017). These are closely-related species that often hybridize and play an important role in ecological succession (Figueroa et al., 2003). Moreover, they present similar germination responses to salinity characterized by germinating within a wide salinity range but diminishing at salt concentrations of over 0.3 M NaCl (Muñoz-Rodríguez et al., 2017). In addition, *S. perennis* shows great potential for phytoremediation of metal polluted salt marshes (Curado et al., 2014). We studied the germination and seedling responses of both *Sarcocornia* species collected from contrasted environments in the Odiel Marshes and exposed them to a wide concentration gradient (0–2000 μM) of Cu, Ni and Zn in controlled conditions. We hypothesized that *S. perennis* would be less sensitive to high metal concentrations than *S. fruticosa*, especially during seedling development, since this low marsh species would be exposed to higher bioavailable metal concentrations in its natural habitat. Our results are useful for designing ecotoxicological bioassays and planning phytoremediation projects in salt marshes.

2. Material and methods

2.1. Plant material sampling

Sarcocornia perennis and *S. fruticosa* fruits were collected at the Acebuchal area in the Odiel Marshes (37°12′29.71″ N, 6°57′32.60″ W; Gulf of Cadiz, Southwest Iberian Peninsula) in November 2020. *Sarcocornia perennis* fruits were collected from a low marsh area and *S. fruticosa* sampled from an adjacent high marsh area. We collected fresh ripe cymes from more than 10 individual plants of each species. Once in the laboratory, fruits were stored in paper bags at 20–25 °C for a week, then seeds were carefully extracted from the fruits. Plant species were identified following Castroviejo et al. (1990).

2.2. Soil sampling and analysis

Sediment samples were collected using stainless steel cores of 50 mm diameter and 50 mm height from the same low and high marsh areas where the fruits of both *Sarcocornia* species were sampled. Sediments were sampled to a depth of 50 mm, where most of the seeds of halophytes are accumulated in salt marshes (Zepeda et al., 2014). Sediment samples were placed in polyethylene bags that were hermetically sealed and stored at −20 °C until analysis. For soil electrical conductivity and pH measurements, 20 cc of soil and 20 ml of distilled water were deposited in a falcon tube (1:1), homogenized, and centrifuged at 3000*g* for 15 min. Electrical conductivity was measured in the supernatant using a conductivity meter (Horiba Laqua, Kyoto, Japan) and the pH using a pHmeter (Crison Basic 20+, Barcelona, Spain) ($n = 2$).

Sediment samples were pretreated for the quantification of bioavailable metals following Alan and Kara (2019). Samples ($n = 3$) were dried in an oven at +45 °C for two days and sifted using 100 μm sieve. Once sieved, 40 ml of 20 mM $CaCl_2$ was added to 1 g of sediment and were constantly stirred overnight at room temperature. Samples were subsequently centrifuged at 3000*g* for 15 min, recovering the supernatant fraction that was stored at +4 °C. Each supernatant was prepared by adding 5% of nitric acid (analytical reagent grade 65%) and 100 μg l^{-1} of Rh (100 ppb; Sigma-Aldrich, Steinheim, Germany) in the final volume as internal standard. This mix was diluted five-fold with ultrapure water and analyzed with an inductively coupled plasma mass spectrometer (ICP-MS) Thermo XSeries2 (Thermo Scientific, Bremen, Germany) equipped with a MicroMist nebulizer, Ni cones and Cetac ASX-500 autosampler (Agilent, Wilmington, DE, USA). All analyses were performed in triplicate.

2.3. Germination assays

To prevent fungal contamination, seeds were surface-sterilized in 5% (v/v) sodium hypochlorite for 10 min then rinsed with distilled water (Infante Izquierdo et al., 2020). For each species, three replicates of 25 seeds were sown for each metal and concentration in Petri dishes (9 cm diameter) with two layers of autoclaved filter paper adding 5 ml of different treatments solutions: distilled water (control), solutions containing 100, 250, 500, 1000 and 2000 μM Cu, Zn or Ni in their sulphate forms ($CuSO_45H_2O$, $ZnSO_47H_2O$ and $NiSO_46H_2O$). These chemical forms were used because they are the most abundant in the Odiel Marshes (Barba-Brioso et al., 2010), and because the toxicity of other forms, such as chlorides, might have had inhibitory effects on seed germination (León et al., 2005). These metals and their concentrations were chosen based on the metal concentrations recorded previously in Odiel Marshes sediments (Achterberg et al., 2003; Borrego et al., 2002; Braungardt et al., 2003; Elbaz-Poulichet et al., 2001; Fernández-Caliani et al., 1997; Galán et al., 2003; González-Pérez et al., 2008). Metals were selected because they abound in the study area and due to the little information available on their effects on germination and establishment of halophytes (Márquez-García et al., 2013). Once sown, the Petri dishes were sealed with adhesive tape (Parafilm™) to avoid desiccation. Germination assays were carried out in freshwater conditions to maximize the germination percentages of both halophytes studied (Muñoz-Rodríguez et al., 2017). Germination assays were carried out under controlled-environmental conditions at +20–25 °C and during a 12 h/12 h photoperiod. Radiation was provided by fluorescent lamps that produced a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1}. Seeds were exposed to treatments for 30 days, and germination was recorded every 3–4 days (Keiffer and Ungar, 1997). A seed was recorded as germinated when the radicle emerged. The percentage of the 25 seeds that germinated and the number of days necessary to reach 50% of the final germination (T_{50}) were calculated for each Petri dish (Muñoz-Rodríguez et al., 2017). Seedlings are highly sensitive to the germination environment, therefore their growth is a useful indicator of environmental stresses such as high concentrations of metals (Mabrouk et al., 2019). For this reason, we measured, under a magnified glass, the length of the cotyledons, hypocotyl and radicle for 5 seedlings per Petri dish 15 days after their germination. Nutrients were not added during the germination assays since we aimed to test the response to metals during early establishment, when seedlings use nutrients reserves stored in the seeds.

2.4. *Statistical analyses*

Statistical analyses were carried out using STATISTICA 8.0 (StatSoft Inc., USA), applying a significance level (α) of 0.05. Deviations from the arithmetic mean were calculated as standard error (SE). Normality and homogeneity of variance of data series were tested using the Kolmogorov–Smirnov and the Levene tests, respectively. Sedimentary variables were compared between zones using the Student *t*-test or Mann-Whitney *U* test. To enable interspecific comparisons, we used the relative size of each seedling organ, calculated by dividing the data obtained in each species and organ series by the mean calculated in control conditions. Relative cotyledon size was compared between species, metals, and their concentrations and interactions using general linear models (GLM). Since normality or homogeneity of variance was not achieved, the final germination percentage, T_{50}, and relative hypocotyl and radicle size were analyzed using generalized linear models (GLZ) with the Wald Chi-square test (χ^2) (Ng and Cribbie, 2017). The effect of each metal on the seedling traits of each species was analyzed using one-way ANOVA and Tukey's honest significant difference (HSD) as post hoc test, or nonparametric Kruskal-Wallis and Mann-Whitney U for the same.

3. Results

3.1. *Sedimentary environment*

Low salt marshes colonized by *S. perennis* presented c. 20% lower electrical conductivity and higher total Ni (+55%) and Zn (+72%) concentration than high marshes colonized by *S. fruticosa* (Table 1).

3.2. *Effects of metals on germination*

Final germination percentage ranged from 41.4 ± 11.0% to 65.1 ± 5.0% for *S. perennis*, and from 72.2 ± 3.7% to 95.8 ± 2.4% for *S. fruticosa* (Table 2). Final germination percentage did not change significantly between species, metals, concentrations or their interactions (Table S1). T_{50} was higher for *S. perennis* (8.6 ± 0.4 days) than for *S. fruticosa* (4.9 ± 0.2 days) (Table 2), with no differences between the metals and their concentrations (Table S1).

Table 1
Electrical conductivity (mS cm^{-1}), pH and bioavailable content of metals (mg kg^{-1}) for salt marsh sediments colonized by *Sarcocornia perennis*, a low marsh species, and *Sarcocornia fruticosa*, a high marsh species, in the Odiel Marshes (Southwest Iberian Peninsula). Values are mean ± SE ($n = 2$–3). Different letters indicate significant differences between species habitats (Student *t*-test or Mann-Whitney *U* test, $p < 0.05$).

	Conductivity	pH	Cu	Ni	Zn
S. perennis low marsh	28.8 ± 0.1^a	5.7 ± 0.3^a	194.7 ± 4.4^a	11.5 ± 0.2^a	157.7 ± 32.9^a
S. fruticosa high marsh	36.5 ± 0.3^b	6.2 ± 0.1^a	201.6 ± 6.2^a	5.2 ± 0.2^b	44.8 ± 1.8^b

Table 2
Final germination percentage and number of days necessary to reach 50% of final germination (T_{50}) for *Sarcocornia perennis* and *S. fruticosa* at different concentrations of Copper (Cu), Nickel (Ni) and Zinc (Zn). Values are mean ± SE ($n = 3$).

	Concentration (µM)	*S. perennis*		*S. fruticosa*	
		Germination (%)	T_{50} (days)	Germination (%)	T_{50} (days)
Control	0	49.1 ± 6.4	4.5 ± 0.2	94.0 ± 3.8	6.4 ± 0.4
Cu	100	58.2 ± 1.9	5.3 ± 0.9	90.4 ± 1.5	6.8 ± 0.6
	250	46.7 ± 4.4	4.1 ± 0.2	81.6 ± 1.7	1.4 ± 2.0
	500	52.3 ± 2.5	4.3 ± 0.2	75.8 ± 0.8	8.1 ± 0.7
	1000	61.6 ± 0.4	5.9 ± 0.8	80.1 ± 3.6	1.2 ± 1.5
	2000	56.4 ± 1.8	4.9 ± 0.8	72.2 ± 3.7	5.2 ± 0.9
Ni	100	65.1 ± 5.0	4.6 ± 0.8	79.6 ± 4.5	6.5 ± 0.3
	250	41.4 ± 11.0	5.5 ± 1.3	78.1 ± 1.1	9.1 ± 0.9
	500	53.4 ± 13.5	6.6 ± 1.4	69.7 ± 7.2	10.0 ± 1.9
	1000	57.6 ± 3.1	4.8 ± 0.9	84.9 ± 1.6	7.6 ± 1.5
	2000	49.9 ± 5.9	4.4 ± 0.2	76.9 ± 3.8	10.6 ± 1.1
Zn	100	64.0 ± 5.1	3.9 ± 0.1	95.8 ± 2.4	9.7 ± 1.2
	250	47.4 ± 4.2	4.5 ± 0.1	80.7 ± 5.1	9.7 ± 1.5
	500	55.6 ± 6.3	4.9 ± 0.6	83.2 ± 5.1	13.3 ± 1.3
	1000	49.2 ± 4.0	5.1 ± 0.7	89.1 ± 3.7	7.7 ± 0.8
	2000	50. 7 ± 2.9	5.4 ± 0.8	82.4 ± 1.6	9.2 ± 0.4

3.3. *Effects of metals on seedlings*

The relative size of cotyledons, hypocotyl and radicle changed between species, metal concentrations, and the interaction between species and metal concentrations. Additionally, the relative size of cotyledons and radicle also changed in the metals tested, and relative cotyledon size was also affected by the interactions between species and metals, and metals and their concentrations (Table S2).

Increasing Cu concentration did not affect cotyledon and hypocotyl size of *S. perennis*, but its radicle was c. 80% shorter at concentrations higher than 250 µM Cu than under control conditions (Fig. 1A). Cotyledons, hypocotyl and radicle length of *S. fruticosa* decreased gradually and significantly at concentrations higher than 100 µM Cu than under control conditions (Fig. 1B) (Table S3).

In *S. perennis*, cotyledons were 22% shorter at 2000 µM Ni than under control conditions. The longest hypocotyls were recorded at 250 µM Ni, decreasing c. 40% at concentrations higher than 500 µM Ni. Radicle length decreased more than 80% at concentrations higher than 250 µM Ni compared to control conditions (Fig. 1C). In *S. fruticosa*, cotyledon length decreased gradually at higher Ni concentrations, whereas hypocotyl and radicle length decreased more than 57 and 93%, respectively, at concentrations higher than 100 µM Ni than under control conditions (Fig. 1D) (Table S3).

Increasing Zn concentrations did not affect cotyledon and hypocotyl size in *S. perennis*, but its radicle was 66% shorter at 2000 µM Zn than under control conditions (Fig. 1E). In *S. fruticosa*, cotyledons were c. 35% shorter at concentrations higher than 500 µM Zn than under control conditions, whereas hypocotyl and radicle growth reduction was recorded at concentrations higher than 100 µM Zn (Fig. 1F) (Table S3).

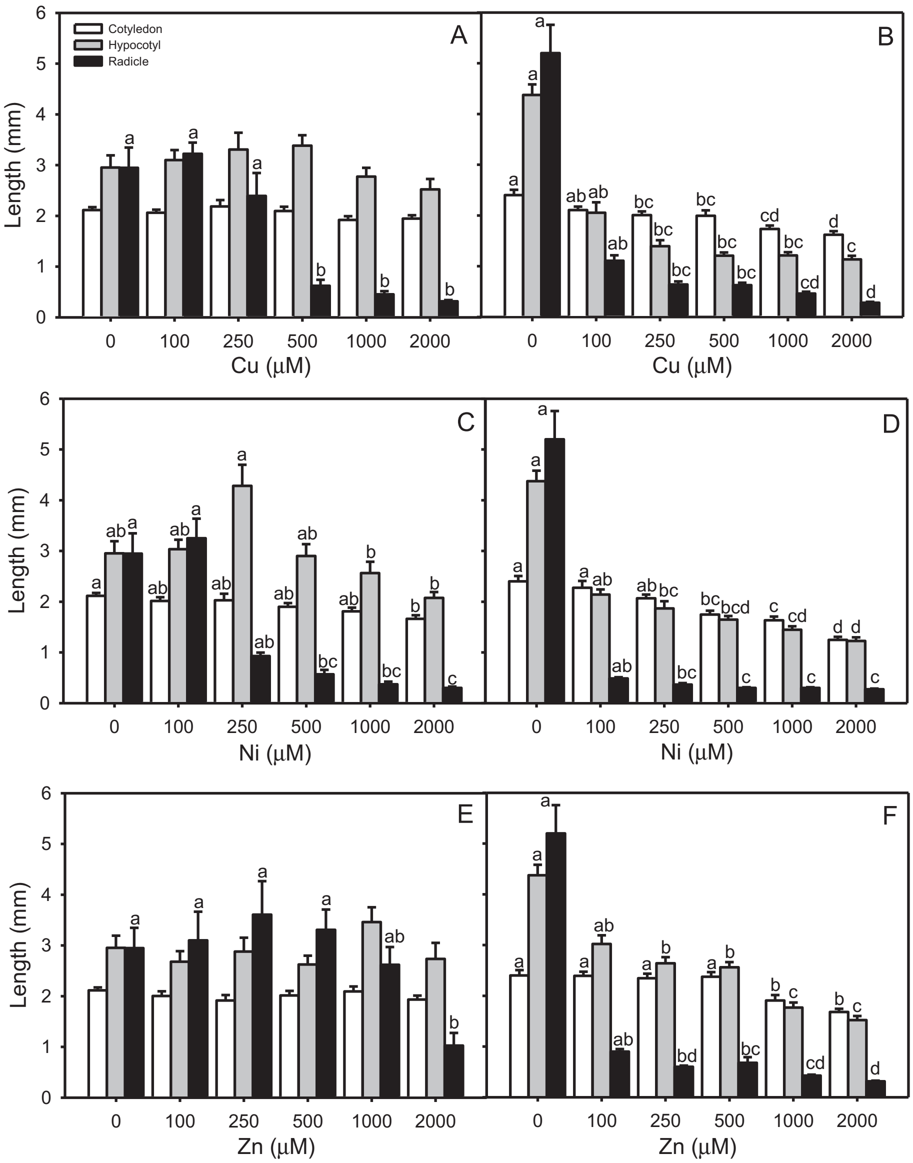


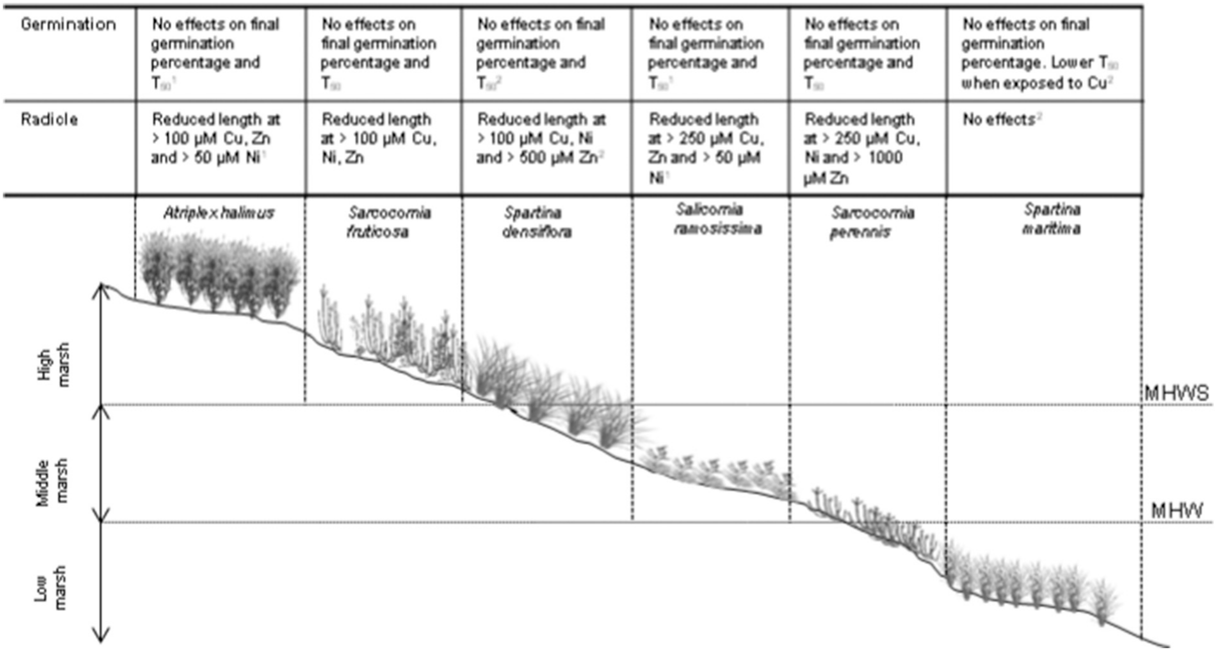
Fig. 1. Size (mm) of cotyledons (white bars), hypocotyl (grey bars) and radicle (black bars) for *Sarcocornia perennis* (A, C, E) and *S. fruticosa* (B, D, F) seedlings under different (A, B) Copper (Cu), (C, D) Nickel (Ni) and (E, F) Zinc (Zn) concentrations. Different letters indicate significant differences between treatments for a given organ (Tukey HSD test or Mann-Whitney test). Values are mean ± SE ($n = 3$).

4. Discussion

Our results show that the germination of both halophyte species studied was not affected by concentrations up to 2000 µM Cu, Ni or Zn. Nevertheless, their seedling growth was reduced by increasing metal concentrations. In accordance with to our hypothesis, seedlings of *S. perennis*, a low marsh species, showed higher tolerance to the three metals tested than those of *S. fruticosa*, a high marsh halophyte.

It is generally assumed that high metal concentrations inhibit germination (Kranner and Colville, 2011) and that halophytes show higher tolerance to metals than glycophytes (Thomas et al., 1998; Van Oosten and Maggio, 2015). Mrozek and Funicelli (1982) recorded no inhibitory effects of Zn on seed germination in the low marsh halophyte *Spartina alterniflora* Loisel., and recent studies have recorded a gradual reduction in the germination percentage of different halophytes from non-inundated areas under increasing metal concentrations (Jiang et al., 2020; Zhang et al., 2020; Yao et al., 2021). Nevertheless, our results and previous studies in the joint estuary of the Odiel and Tinto rivers, one of the most metal-polluted estuaries in the world, recorded no effects on the germination of halophytes from all along the entire tidal gradient under metal loads of up to 2000 µM (Mateos-Naranjo et al., 2011; Márquez-García et al., 2013; Infante Izquierdo et al., 2020) (Fig. 2). In fact, the germination of the invasive halophyte *S. densiflora* is reduced in the Odiel-Tinto estuary only when very high metal loads are combined with highly acidic sediments (pH < 4.5) (Curado et al., 2010) and germination of high marsh shrub *Salsola vermiculata* L. from Odiel Marshes was only reduced at concentrations as high as 4000 µM Cu and Zn (Sanjosé et al., 2021). Infante Izquierdo et al. (2020) recorded that *S. densiflora* seedlings emerging from seeds from highly polluted sediments in the Tinto Estuary showed higher tolerance to metals than those from adjacent less polluted estuaries. In this context, the high metal tolerance recorded in our study for the germination of seeds of both *Sarcocornia* species coming from polluted sediments in the Odiel Marshes may reflect local adaptation.

Regarding early seedling growth, the detrimental effects of metals on both halophytes were more evident in their radicle than in cotyledons or hypocotyl. In this sense, Cu, Ni and Zn mainly accumulate in root tissue in many species (Sheldon and Menzies, 2005; Varhammar et al., 2019;



Germination	No effects on final germination percentage and T_{50}[1]	No effects on final germination percentage and T_{50}	No effects on final germination percentage and T_{50}[2]	No effects on final germination percentage and T_{50}[1]	No effects on final germination percentage and T_{50}	No effects on final germination percentage. Lower T_{50} when exposed to Cu[2]
Radicle	Reduced length at > 100 μM Cu, Zn and > 50 μM Ni[1]	Reduced length at > 100 μM Cu, Ni, Zn	Reduced length at > 100 μM Cu, Ni and > 500 μM Zn[2]	Reduced length at > 250 μM Cu, Zn and > 50 μM Ni[1]	Reduced length at > 250 μM Cu, Ni and > 1000 μM Zn	No effects[2]

Fig. 2. Main effects of metals on germination and radicle growth for six halophyte species distributed along the intertidal gradient in the Odiel Marshes (Southwest Iberian Peninsula). Metals: Cu, Copper; Ni, Nickel; Zn, Zinc. Tidal levels: MHWS, mean high water spring; MHW, mean high water. T_{50}, number of days necessary to reach 50% of final germination. References: 1, Márquez-García et al., 2013; 2, Infante Izquierdo et al., 2020.

Yusuf et al., 2011). Moreover, *S. perennis* was more tolerant to increased metal concentrations than *S. fruticosa*, which was reflected in reductions in seedling growth recorded at higher metal concentrations that did not affected to hypocotyl and cotyledons, except for Ni concentration higher than 500 μM. This result corroborates our hypothesis and may reflect that *S. perennis* was exposed to higher bioavailable concentrations of Ni and Zn in low marshes than *S. fruticosa* in high marshes. Sediment redox potential in low marshes colonized by *S. perennis* (c. +100 mV) is lower than that recorded in high marshes colonized by *S. fruticosa* (between +70 and +270 mV) (Gallego-Tévar et al., 2018; Castillo et al., 2021), which would increase metal bioavailability at lower elevations under hypoxic conditions (Alhdad et al., 2015; Wang et al., 2012; Brito et al., 2021). In this context, *S. perennis* exhibited similar tolerance to Cu, Ni and Zn, recorded as metal concentration thresholds at which significant reductions in seedling growth were found, than low marsh *Salicornia ramosissima* and lower tolerance than the primary colonizer *Spartina maritima* (Márquez-García et al., 2013; Infante Izquierdo et al., 2020) (Fig. 2). The sensitivity levels to Cu, Ni and Zn recorded for these three low marsh halophytes are in accordance with their position in the tidal frame and the accumulation of metals in their tissues. Thus, *Spartina maritima* presents the lowest sensitivity to metals, colonizes the lowest elevations and shows the highest metal accumulation, whereas *S. perennis* presents the highest sensitivity to metals, occupies the highest elevations and accumulates the least amount of metals (Luque et al., 1999; Gallego-Tévar et al., 2018; Infante Izquierdo et al., 2020). Looking at halophytes from high salt marshes, our results show that *S. fruticosa* seedlings present lower metal tolerance than those of the low marsh species and similar levels to those of middle-high marsh species *Spartina densiflora* and *Atriplex halimus* (Márquez-García et al., 2013; Infante Izquierdo et al., 2020) (Fig. 2). In this sense, previous studies showed that metal exposure led to marked reductions in seedling growth in different high marsh and salt desert halophytes (Thomas et al., 1998; Zhang et al., 2020; Duarte et al., 2021; Yao et al., 2021). According to these data, the tolerance to metals of halophyte seedlings colonizing tidal marshes increases at lower elevation in the intertidal gradient. Nevertheless, the seedlings of high marsh *Suaeda vermiculata* showed higher tolerance to Ni and Zn (radicle growth decreasing at >1000 μM Ni and 2000 μM Zn) (Sanjosé et al., 2021) than other halophytes colonizing lower elevations in the tidal gradient (Fig. 2). *Suaeda vermiculata* colonizes alkaline sandy soils with low salinities that are only flooded during astronomical tides and exposed to severe drought conditions (Contreras-Cruzado et al., 2017). The high tolerance to metals, including Zn and Ni, recorded for *S. vermiculata* (Sanjosé et al., 2021) would be related to its high tolerance to drought (Al-Masri, 2007), since some plant adaptations to deal with drought also increase their metal tolerance (Zhang et al., 2007). In this context, the trend of halophyte seedlings to show high metal tolerance when coming from low elevations in the tidal gradient could be mediated by their tolerance to several environmental stress factors, such as flooding, hypoxia and high metal loads. Previous studies in adult plants have reported potential molecular evidence of the great metal tolerance of low marsh species, including *Spartina maritima* and *Sarcocornia perennis* (Negrin et al., 2017; Wu et al., 2022). Even, the growth of low marsh *S. alterniflora* was promoted at Zn concentrations between 100 and 500 $\mu g\ g^{-1}$ (Pan et al., 2016). Despite the recorded inter-specific differences in metal tolerance for halophylic seedlings, patterns of metal accumulation in adult halophytes are broadly similar among plant type, plant form and habitat in salt marshes (Alam et al., 2021).

Plants can be introduced as different life stages, from seeds to adult plants, in phytoremediation projects (Prasad and Freitas, 2003). In this context, adult plants of *S. perennis* and *S. fruticosa* accumulate high metal loads so they may be used as biotools for salt marsh phytoremediation (Cacador et al., 2009; Curado et al., 2014). This study increase our knowledge about the tolerance of *S. perennis* and *S. fruticosa* to metals during germination and early seedling growth, enabling the identification of those metal concentrations at which these halophyte species may be seeded into metal-polluted soils. In view of our results, the concentrations recorded in solution in the Odiel Marshes for Cu (0.07–745 μM) and Zn (61 to 4220 μM) (Achterberg et al., 2003; Borrego et al., 2002; Braungardt et al., 2003; Elbaz-Poulichet et al., 2001; Fernández-Caliani et al., 1997; Galán et al., 2003; González-Pérez et al., 2008) may reduce the early development of both *Sarcocornia* species studied, which could affect their establishment mainly by diminishing their radicle growth.

5. Conclusions

Based on our results, the growth of *S. fruticosa* seedling is a good candidate for ecotoxicological bioassays in controlled laboratory conditions, providing a good set of metal pollution morphological biomarkers that are easy to record, such as radicle length. Additionally, our results enable identification of metal toxicity thresholds for sowing *S. perennis* and *S. fruticosa* in phytoremediation projects in salt marshes.

CRediT authorship contribution statement

ISJ, AFMR, FR, FN, ESG, FJJN, AP, MDII carried out the germination experiments. ISJ, AFMR and JMC lead data analyses and paper writing. All authors contributed to the interpretation of the results and the drafts reviews.

Sampling data and analysis were performed by ISJ, AFMR, FR, FN, ESG, FJJN, AP and MDI. ISJ, AFMR and JMC contributed to the interpretation of the results and lead the paper writing. All authors reviewed the drafts reviews.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113375.

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