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Accumulation and Effect of Heavy Metals on the Germination and Growth of *Salsola vermiculata* L. Seedlings

Israel Sanjosé [1], Francisco Navarro-Roldán [1,2,3,*], María Dolores Infante-Izquierdo [1], Gloria Martínez-Sagarra [4], Juan Antonio Devesa [4], Alejandro Polo [1], Sara Ramírez-Acosta [2,5], Enrique Sánchez-Gullón [1], Francisco Javier Jiménez-Nieva [1] and Adolfo Francisco Muñoz-Rodríguez [1,5]

[1] Department of Integrated Sciences, Faculty of Experimental Sciences, University of Huelva, 21004 Huelva, Spain; israel.sanjose@ono.com (I.S.); mariloli.infante@gmail.com (M.D.I.-I.); Alejandro.polo@hotmail.es (A.P.); enrique.sanchez.gullon@juntadeandalucia.es (E.S.-G.); jimenez@uhu.es (F.J.J.-N.); adolfo.munoz@dbasp.uhu.es (A.F.M.-R.)
[2] International Agrofood Campus of Excellence International ceiA3, University of Huelva, 21004 Huelva, Spain; sara.ramirez@dqcm.uhu.es
[3] International Campus of Excellence of the Sea—CEIMAR, Faculty of Experimental Sciences, University of Huelva, 21004 Huelva, Spain
[4] Department of Botany, Ecology and Plant Physiology, Faculty of Sciences, José Celestino Mutis Building, Campus de Rabanales, University of Córdoba, 14071 Córdoba, Spain; bv2masag@uco.es (G.M.-S.); bv1dealj@uco.es (J.A.D.)
[5] Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, 21004 Huelva, Spain
* Correspondence: fnavarro@uhu.es; Tel.: +34-959-219-880

Citation: Sanjosé, I.; Navarro-Roldán, F.; Infante-Izquierdo, M.D.; Martínez-Sagarra, G.; Devesa, J.A.; Polo, A.; Ramírez-Acosta, S.; Sánchez-Gullón, E.; Jiménez-Nieva, F.J.; Muñoz-Rodríguez, A.F. Accumulation and Effect of Heavy Metals on the Germination and Growth of *Salsola vermiculata* L. Seedlings. *Diversity* **2021**, *13*, 539. https://doi.org/10.3390/d13110539

Academic Editors: Orsolya Valkó, Wenzhi Liu and Jesús Cambrollé

Received: 8 September 2021
Accepted: 24 October 2021
Published: 27 October 2021

Abstract: The influence of different concentrations of heavy metals (Cu, Mn, Ni, Zn) was analyzed in the *Salsola vermiculata* germination pattern, seedling development, and accumulation in seedlings. The responses to different metals were dissimilar. Germination was only significantly reduced at Cu and Zn 4000 μM but Zn induced radicle growth at lower concentrations. Without damage, the species acted as a good accumulator and tolerant for Mn, Ni, and Cu. In seedlings, accumulation increased following two patterns: Mn and Ni, induced an arithmetic increase in content in tissue, to the point where the content reached a maximum; with Cu and Ni, the pattern was linear, in which the accumulation in tissue was directly related to the metal concentration in the medium. Compared to other *Chenopodiaceae* halophyte species, *S. vermiculata* seems to be more tolerant of metals and is proposed for the phytoremediation of soils contaminated by heavy metals.

Keywords: *Chenopodiaceae*; metal accumulation; metal tolerance; salt marshes

1. Introduction

Heavy metals, due to their high toxicity, persistence, and bioaccumulative behavior, could represent a threat to natural ecosystems, especially in estuary zones with salt marshes [1,2]. They can impact biochemical processes in plants, including nutrient homeostasis, gas exchange characteristics, enzyme and antioxidant production, protein mobilization, and photosynthesis [3].

Soil-restoration technologies are available such as chemical/physical remediation, animal remediation, phytoremediation, and microremediation with microbes [4]. Phytoremediation, or green remediation, uses plants that absorb the heavy metal by the roots, absorbing or removing environmental contaminants; this technology has gained in importance in the last two decades for being cost-efficient, environmentally friendly, producing fewer side-effects, and with no negative impact on landscaping methods [4–11]. In the phytoremediation processes, plants assume various roles in diminishing the effects of metals: phytoextraction by the harvest of above-ground organs where metals concentrate; phytovolatilization by water-soluble metals during transpiration; phytostabilization by immobilization through accumulation by roots or precipitation, or by changing their chemistry; phytodegradation by degradation into insoluble or non-toxic compounds; or phytoaccumulation by accumulation in plant biomass [12–16].

In green remediation strategies, it is necessary to analyze the different properties of species to determine tolerance to contaminants and accumulative behavior [10]; phytotolerance studies are required to determine metal tolerance in plant species and to understand the negative effect of metals on metabolism and the processes in those species [17]. In this regard, seed germination and the seedling stage are more vulnerable to metal stress than later vegetative stages; therefore, testing the effects of metal stress on these processes is useful for assessing a species' establishment potential in metal-contaminated soils [18,19]. The effects of heavy metals on seed germination have been widely studied [20,21], with some of the most common effects being germination rate reduction or damage to seedlings, including a reduction in the elongation and growth of roots, shoots, or leaves, which could kill the seedlings [22,23].

Although the seed coat can act as a barrier to limit the effects of heavy metals [24], most seeds and seedlings show a decline in germination and vigor in response to the presence of heavy metals; processes affected include imbibition [25], or the activity of certain enzymes involved in reserve mobilization, such as acid phosphatases, proteases, amylases, and proteolytic enzymes [26]. Heavy metals can also cause oxidative stress and damage the seedlings' photosynthetic systems [3].

In saline soils, some halophytic plants are now widely accepted as solutions for cleaning up coastal environments [12,27], acting as bioindicators or biomonitors to assess the extent of heavy metal contamination on sediments, due to the linear correlation coefficients between the concentration of metals in their tissue and concentration in the soil, or by contributing to the phytoremediation process by accumulating metals at higher concentrations [1,2,14,16,28].

The Halophytes' accumulative capacity could be the result of different mechanisms. The amount of salt in the soil affects the accumulation of metals in tissue [29,30], and its presence can alleviate the effects of metal toxicity [14,31]. Furthermore, halophytic plant species can reduce the effects of metals in other ways: by retaining the ion intake in structures used to accumulate salts, such as the cell wall, vacuole, or trichome; by using substances for metal chelation; or possessing antioxidant defense systems [14,16,32,33].

The *Chenopodiaceae* family contributes the largest number of halophyte species [34], and they are dominant in Mediterranean tidal marsh vegetation [35,36]. The *Chenopodiaceae* family is not included in the predominant families in heavy metal accumulation processes [11]; however, many halophyte species have been studied as potential accumulators of metals in saline soils, and they may be considered a valuable species for the phytoremediation of metal-polluted saline soils.

The most widely studied species are *Halimione portulacoides* Aellen, *Sarcocornia fruticosa* (L.) A.J. Scott, and *Ariplex halimus* L. [16]. *Halimione portulacoides* is considered a suitable accumulator for Hg, Cr, Cu, Cd, and Pb, contributing to their phytostabilization [37–42]; this species also uses surfactants that affect the mobility of metals in the rhizosphere and has stabilization potential for Cr and Cu [43]. *Sarcocornia fruticosa* accumulates As, Cd, Cu, Pb, and Zn in below-ground biomass in concentrations several times higher than concentrations of the metals in soil [40,44,45]. This has been demonstrated in phytoremediation of a metal-contaminated saline soil project, using liming to stimulate plant growth and enhance its capacity to stabilize metals [46]. *Ariplex halimus* has proved to be well-suited for the phytoextraction of Cd, Cu, and Zn found in saline soils [14,47–51].

Other species of *Chenopodiaceae* considered as accumulators for phytoaccumulation or phytostabilization are: *Arthrocnemum macrostachyum* (Moric.) K. Koch [42,52]; *Atriplex hortensis* L. and *A. rosea* L. [53]; *A. lentiformis* (Torr.) *S. Watson* and *A. undulata* (Moq.) D. Dietr. [54]; *A. atacamensis* Phil. [55]; *Hammada scoparia* Iljin [56]; *Salicornia europaea* L. [29]; *Salicornia ramosissima* J. Woods [57]; *Salsola fruticosa* Forssk. [42,58]; *S. glauca* M. Bieb. [59]; *S. passerina* Bunge [9]; *S. soda* L. [29]; *Sarcocornia perennis* (Mill.) A.J. Scott [37,40]; *Suaeda* *glauca* (Bunge) Bunge [60]; *S. maritima* (L.) Dumort. [61]; *S. salsa* (l.) Pall. [62]; *Salicornia arabica* L. [42]. *Salicornia bigelovii* Torr. has been tested for phytovolatilization of Se [63].

Salsola vermiculata is a shrub of wide distribution and ecological amplitude and is an important structural element in the vegetation of the arid and coastal zones of southern Europe, northern Africa, Macaronesia, and southwestern Asia [64]. This species disperses its seeds by wind during autumn and winter and is covered by a permanent calyx; its seeds have a high germination rate at low–medium salinities (to 0.3 M), and high recovery potential when exposed to fresh water following high salinity stress (0.6–0.9 M) [65]. In marshes in the southwestern Iberian Peninsula, it inhabits sandy sediments in high marsh areas only flooded during astronomic tides [36]. This species is widely distributed in the Odiel Natural Park Marshes (SW Spain) [35,66], which is one of the most heavily metal-polluted systems in the world, mainly as a result of the upstream presence of the Iberian Pyrite Belt (IPB), an important metal-rich sulfide deposit and, secondly, due to industrial activity on the estuary [67–69]. These findings would bolster its importance in restoration by phytoremediation in such habitats.

Salsola vermiculata was tested at mining sites in Morocco by Boularbah et al. [70], who analyzed its Cd, Cu, Pb, and Zn content in shoots and its toxicity. They concluded that the species is hypertolerant, accumulating 3.14, 69.5, 283.9, and 819 mg kg^{-1} DW of Cd, Cu, Pb, and Zn, respectively, with no toxic effects; thus, it can be used for phytostabilization in metal-contaminated sites.

For the first time, this work investigates the germination of *Salsola vermiculata* under exposure to metals, including Ni, in marsh environments. The analysis is compared to the effects of four heavy metals, Cu, Mn, Zn, and Ni, on the germination and initial seedling development of *S. vermiculata* to determine their accumulation in seedlings in order to evaluate its possible use in marsh ecosystem restoration by phytoremediation. The results are compared to those obtained for other *Chenopodiaceae* halophytes tested for heavy metal accumulation or tolerance.

Although the study of other metals such as Cd, Cr, and Co may be of great interest, the metals analyzed in this study are the most representative of those present in the Odiel marshes and, therefore, those which can have the greatest effect on its flora.

2. Materials and Methods

2.1. Plant Material

Seeds were collected from the Odiel Marshes (37°08′–37°20′ N, 6°45′–7°02′ W; Spain, southwestern Iberian Peninsula) on 5 October 2016, from more than 10 different individual plants randomly selected, cleaned under a magnifying glass and separated from the floral parts. Seeds from different plants were mixed and stored for 12 days in paper bags at 25 °C in dark conditions prior to the germination experiments.

2.2. Germination Experiments

The seeds were surface-sterilized by immersion in 5% (*v*/*v*) sodium hypochlorite for 10 min and rinsed three times in sterile water [65,71]. Next, the seeds were placed in Petri dishes (9 cm in diameter) with three layers of autoclaved filter paper, watered with 5 mL of different treatment solutions, and sealed with adhesive tape (Parafilm TM) to avoid desiccation. Three Petri dishes with 25 seeds per dish were used in each treatment. Although other methods exist, a seed germination test with a heavy metal solution in a Petri dish with moistened filter paper is the most common methodology for assessing metal phytotoxicity in plant species [72].

The seeds were exposed to eight different concentrations (10, 25, 50, 100, 250, 1000, 2000, 4000 µM) of Cu, Mn, Ni, and Zn added in the form of sulfates $CuSO_4$ $5H_2O$, $MnSO_4$ H_2O, $NiSO_4$ $6H_2O$, and $ZnSO_4$ $7H_2O$ and dissolved in deionized water. For the control treatment (0 µM) only deionized water was used. The sulfate form of the metals was selected according to the most abundant form found in the Odiel Natural Park Marshes [73] and references therein.

These metals are essential for plants that contribute to plant metabolic function, but excessive amounts are toxic and lead to growth inhibition [21,25]. They were chosen based on previous studies that describe the metal composition in the water and soils of the Odiel Natural Park Marshes [21] and references therein.

The seeds were germinated under controlled environmental conditions with 12/12 h of day/night at 24/20 °C, respectively; it has been demonstrated that such conditions are appropriate for stimulating high levels of germination in *Atriplex halimus* [71] and other *Chenopodiaceae*, including *Salsola vermiculata* [65]. Light was provided by fluorescent lamps that produced a photosynthetic photon flux density of 60 µmol m^{-2} s^{-1}. Germination was monitored for 30 days, with germination in each plate recorded daily in the first week and every 2 or 3 days thereafter. A seed was considered germinated when the radicle emerged.

The germination dynamic was analyzed by noting the final germination percentage after 30 days, and the number of days necessary to reach 50% of the final germination percentage (t_{50}) for each Petri dish.

2.3. Morphological Analysis of the Seedlings

To analyze the influence of the different treatments on seedling development, the length of the cotyledons, hypocotyls, and radicles were measured in 15-day-old seedlings, using 10 seedlings per dish. The measurements were taken under a magnifying glass [21,71,74].

To assess the tolerance of the seedlings to metals, the tolerance index was calculated according to Wilkins [75], applied to the length of the cotyledons, hypocotyls, and radicles (TI% = 100 × (mean organ length in the treatment/mean organ length in the control) [76,77].

2.4. Metal Content Analysis

To better understand whether the metals were taken up by the seedlings, whole 15-day-old seedlings (approximately 45 per metal and treatment) were carefully washed with ultrapure water, thoroughly dried, pulverized with mortar and pestle, and stored in hermetically sealed polypropylene tubes at 4 °C until analysis. Mass ratios between different parts could have changed as a result of treatments; therefore, accumulation in the whole seedling was only used as indicative of increased accumulation when the seedlings were exposed to increasing concentrations.

For the metal analysis, 50 mg of a powdered sample were mixed with 640 µL HNO_3 and 160 µL of H_2O_2 in polytetrafluoroethylene vessels and incubated for 10 min. Mineralization (CEM Matthews microwave oven, NC, USA, model MARS) was carried out at 800 W at room temperature, ramped to 180 °C for 10 min, and then maintained for 20 min at that temperature. Then, the solutions were prepared with up to 5 mL of ultrapure water, and the metals were analyzed with an inductively coupled plasma mass spectrometer (ICP-MS) Thermo XSeries2 (Thermo Scientific, Bremen, Germany) equipped with a MicroMist nebulizer, Ni cones, and a Cetac ASX-500 autosampler (Agilent, Wilmington, DE, USA). Rh was added as an internal standard (100 ppb) from Sigma-Aldrich (Steinheim, Germany). All analyses were performed in triplicate.

2.5. Statistical Analyzes

The statistical analyses of the data were performed using Statistica 8.0. The data were tested for normality and homogeneity of variance using the Kolmogorov–Smirnov and Levene tests, respectively. As data did not follow a normal distribution, Kruskal–Wallis and Mann–Whitney U tests were used to detect significant differences ($p < 0.05$).

The metal accumulations in seedlings at each metal concentration medium were fitted to polynomial curves type $y = ax^2 + bx + c$, and the threshold value of the model was based on R^2 and p values ($p < 0.05$).

3. Results

The final germination and the germination dynamics of *S. vermiculata* were minimally affected by the presence of metals in the germination media, with some differences depending on the metal (Table 1). Copper and zinc significantly reduced the final germination compared to the control when present at the highest concentration, 4000 µM. However, only zinc had a significant effect on the germination dynamics, with a considerable increase in the time-lapse to reach 50% of germination when present at 4000 µM, rising from 1.43 days in the control to 1.77 days at this concentration.

Table 1. Germination percentage after 30 days, t_{50} and tolerance index for cotyledons, hypocotyls, and radicles.

Concentration (µM)	Germination (%)	t_{50} (Days)	IT Cotyledon	IT Hypocotyl	IT Roots
Cu (µM)					
0	94.65 ± 0.04 a	1.43 ± 0.12 a	100	100	100
10	94.66 ± 0.04 ab	1.35 ± 0.14 a	102	104	113
25	92.00 ± 0.08 ab	1.53 ± 0.14 a	94	91	97
50	96.00 ± 0.04 ab	1.47 ± 0.1 a	93	94	91
100	92.00 ± 0.06 ab	1.28 ± 0.24 a	101	100	113
250	93.33 ± 0.04 ab	1.30 ± 0.19 a	97	96	71 *
1000	98.66 ± 0.02 a	1.30 ± 0.05 a	95	88	38 *
2000	93.33 ± 0.08 a	1.43 ± 0.16 a	95	80	36 *
4000	80.00 ± 0.06 b	1.35 ± 0.10 a	73	46 *	28 *
Mn (µM)					
0	94.65 ± 0.04 a	1.43 ± 0.12 a	100	100	100
10	93.33 ± 0.02 a	1.33 ± 0.37 a	92	96	88
25	91.83 ± 0.04 a	1.44 ± 0.10 a	104	112	104
50	94.66 ± 0.02 a	1.35 ± 0.12 a	98	107	105
100	89.33 ± 0.06 a	1.36 ± 0.23 a	106	102	93
250	96.00 ± 0.04 a	1.55 ± 0.03 a	96	92	113
1000	89.11 ± 0.09 a	1.40 ± 0.09 a	103	106	113
2000	98.66 ± 0.02 a	1.40 ± 0.24 a	104	104	133
4000	90.50 ± 0.04 a	1.33 ± 0.18 a	95	100	116
Ni (µM)					
0	94.65 ± 0.04 a	1.43 ± 0.12 a	100	100	100
10	97.33 ± 0.02 a	1.38 ± 0.08 a	99	103	103
25	98.66 ± 0.02 a	1.41 ± 0.10 a	104	113	108
50	94.66 ± 0.06 a	1.30 ± 0.22 a	96	100	97
100	100.00 ± 0.00 a	1.31 ± 0.17 a	94	96	83
250	89.22 ± 0.04 a	1.36 ± 0.06 a	100	94	98
1000	94.55 ± 0.06 a	1.34 ± 0.08 a	111	96	78
2000	94.55 ± 0.02 a	1.53 ± 0.09 a	95	70 *	69 *
4000	90.66 ± 0.02 a	1.31 ± 0.23 a	85 *	74 *	31 *
Zn (µM)					
0	94.65 ± 0.04 a	1.43 ± 0.12 a	100	100	100
10	93.33 ± 0.02 ab	1.31 ± 0.16 a	96	103	101
25	97.33 ± 0.02 a	1.66 ± 0.32 ab	99	104	102
50	98.61 ± 0.02 a	1.48 ± 0.14 ab	101	103	130 *
100	92.00 ± 0 ab	1.67 ± 0.16 ab	102	107	95
250	96.00 ± 0.04 a	1.61 ± 0.12 ab	96	95	102
1000	96.00 ± 0.04 a	1.56 ± 0.06 ab	99	110	107
2000	94.66 ± 0.06 a	1.59 ± 0.04 ab	99	106	81
4000	79.83 ± 0.1 b	1.77 ± 0.08 b	96	100	49 *

The data show the mean ± standard deviation for 3 independent plates, with 25 seeds in each one. For each metal, different letters indicate significant differences ($p < 0.05$); IT data marked with * means significant differences with the control.

The initial development of the seedlings was affected by the presence of metals in different ways (Table 1, Figure 1). Copper did not affect the length of the cotyledons, but at 4000 μM it significantly reduced the length of the hypocotyls to 46% of the control, and the length of the radicle was significantly affected from 250 μM, with a reduction of 71% at 250 μM and 28% at 4000 μM (Table 1, Figure 1A). Manganese, at the concentrations tested, did not statistically affect the initial development of *S. vermiculata* seedlings (Table 1, Figure 1B). The presence of nickel significantly reduced the length of the cotyledon at 4000 μM to 85% of the control, and the length of the hypocotyl decreased from 2000 μM to around 70% of the control; the length of the radicle was 69% of the control at 2000 μM and 31% at 4000 μM (Table 1, Figure 1C). Zinc did not affect either the length of the cotyledons or the length of the hypocotyls at any of the concentrations tested; however, this metal induced the growth of the radicle at smaller concentrations (50 μM) reaching 130% of the control, but had a negative effect at 4000 μM, with a significant reduction in radicle length to 49% of the control (Table 1, Figure 1D).

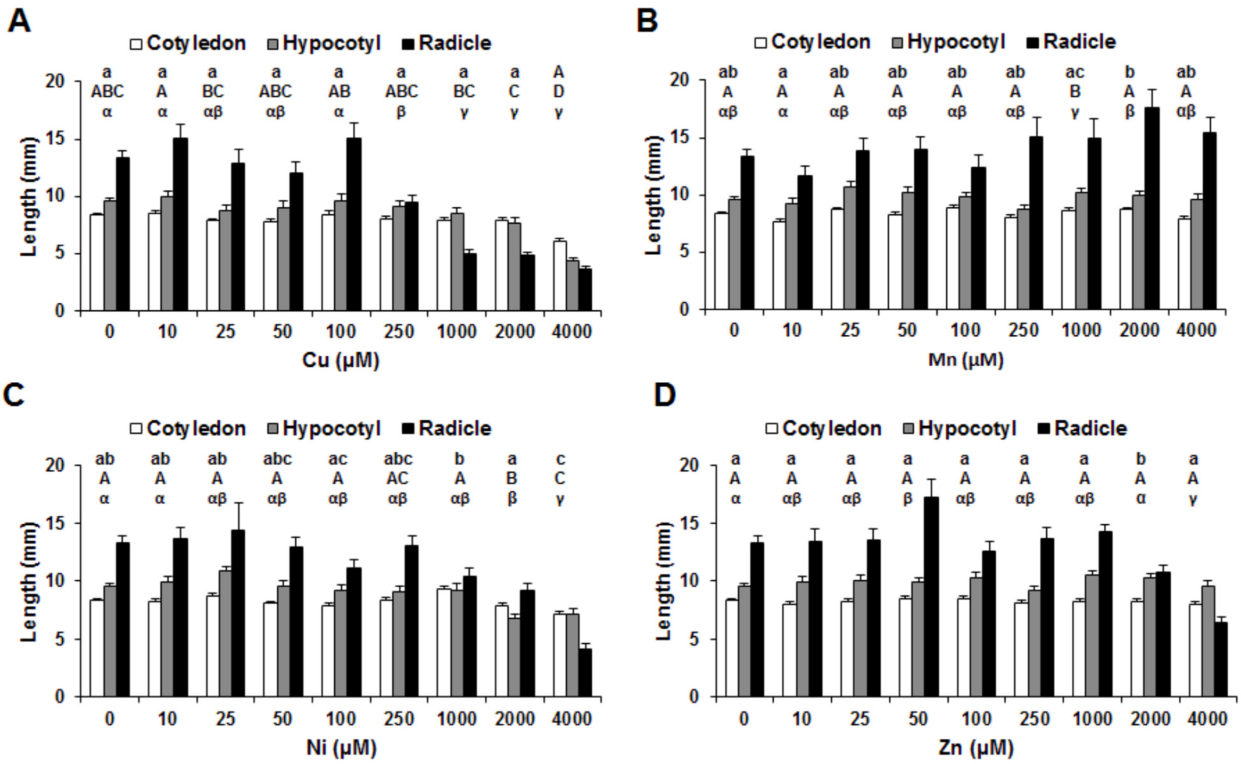

Figure 1. Effects of Cu (**A**), Mn (**B**), Ni (**C**), and Zn (**D**) on the initial development of the cotyledon, hypocotyl, and radicle of *Salsola vermiculata* seedlings. Figure 1 shows the mean and standard deviation. Different letters above the bars indicate significant differences ($p < 0.05$): lowercase Latin letters for cotyledons, uppercase Latin letters for hypocotyles, and Greek letters for radicles.

As shown in Figure 2, the metal content inside the seedlings rose significantly with the increase in metal in the germination media, reaching the following maximum mean values: 9186 mg kg^{-1} DW of Cu at 4000 μM; 4373 and 4676 mg kg^{-1} DW of Mn at 2000, and 4000 μM, respectively; 3990 and 3204 mg kg^{-1} DW of Zn at 2000 and 4000 μM, respectively; 7130 mg kg^{-1} DW of Ni at 4000 μM. However, the behavior is different for each metal studied.

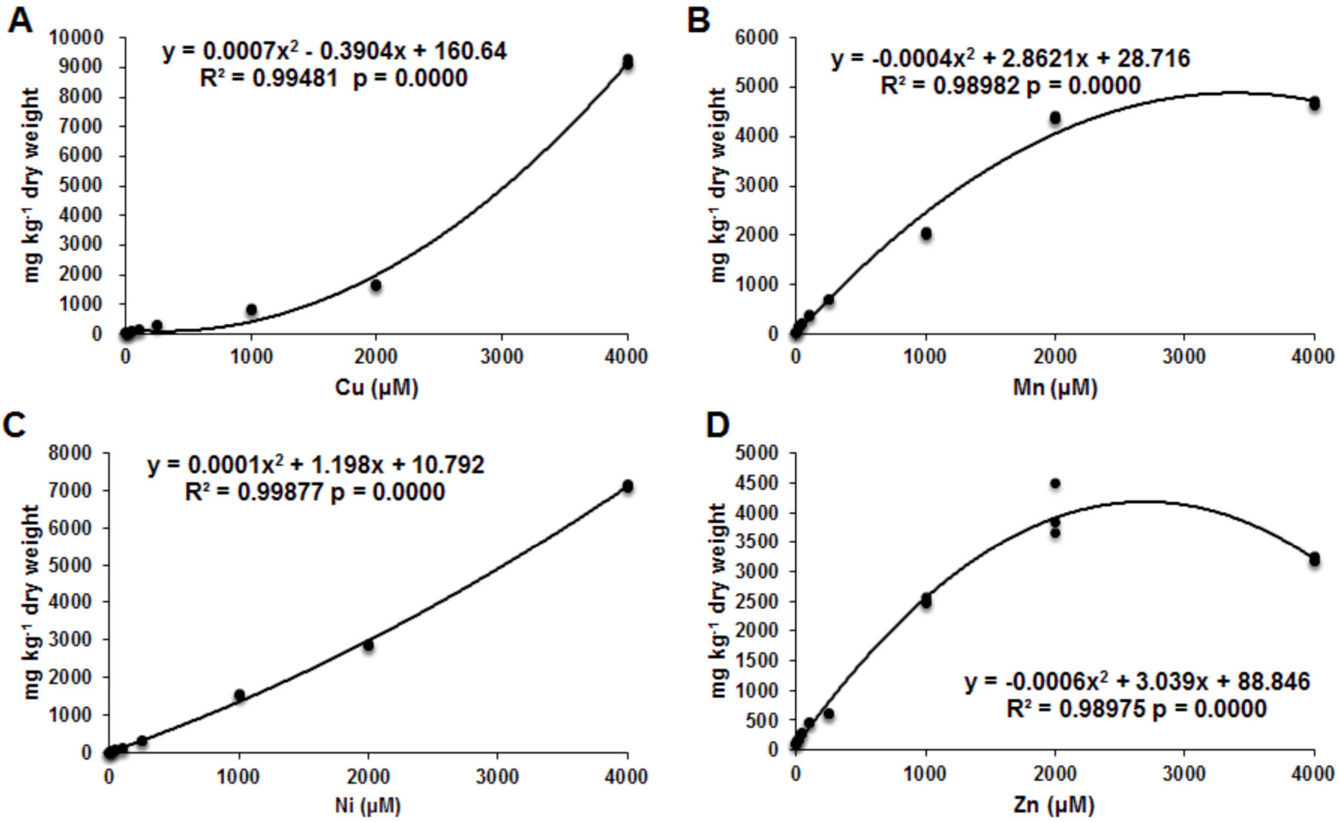

Figure 2. Levels of Cu (**A**), Mn (**B**), Ni, (**C**) and Zn (**D**) (mg kg^{-1} or ppm) accumulated in the seedlings germinated at different concentrations of metals, and quadratic equations for curves with their determination coefficient (R^2) and *p* value.

Accumulation curves can fit significantly ($p < 0.05$) to second-degree polynomials. The equation presents a high positive value for component "a" in the case of copper; therefore, the accumulation increases exponentially with the increase in copper in the medium. However, nickel registers a very low value for component "a"; therefore, the increase in accumulated metal rises arithmetically as its concentration in the culture medium increases. On the other hand, manganese and zinc behave similarly, with a negative "a" component, which means that when a concentration value is reached, the accumulation of metal in the tissue begins to decrease.

4. Discussion

4.1. Copper

In our study, copper reduced the final germination percentage at 4000 µM but had no effect at concentrations of 2000 µM or lower and did not affect the speed of germination (t_{50}). These results are the same for other *Chenopodiaceae* plants from the Odiel marshes, such as *Atriplex halimus* and *Salicornia ramosissima*, studied by Márquez-García et al. [21].

In addition, copper concentration affected seedling development in hypocotyl growth at 4000 µM and reduced the length of radicles at concentrations up to 250 µM, with significant reductions over 1000 µM, falling below 40% of the control length. *Salsola vermiculata* exhibited a greater tolerance than *Atriplex halimus* and *Salicornia ramosissima*, in which cotyledon and hypocotyl development was affected from 1000 and 2000 µM, respectively. Radicle development was affected in the same way, being reduced from 250 and 1000 µM in both species, respectively [21]. The biggest effect of Cu on the root was due to its accumulation mainly in this organ, with little translocation to the shoots [50,78].

In sensitive plants, Cu can become toxic when it accumulates in plant tissue at levels exceeding 20 mg kg^{-1} dry weight; these data differ according to plant species and growth conditions [79]. Our results showed that copper in *Salsola vermiculata* reached levels of 1664 and 9186 mg kg^{-1} DW, grown in solutions of 2000 and 4000 µM, respectively. However, it presented the first negative effects on the radicle at concentrations of 250 µM, the plants remained unaffected when cultivated in 100 µM solution, accumulating 154 mg kg^{-1} DW. These data match those of Boularbah et al. [70], who found Cu content of 69.5 mg kg^{-1} DW in mining sites in Morocco, with no toxicity symptoms.

The accumulator capacity of *Salsola vermiculata* seems to be higher than that of *Atriplex halimus*, which Mateos-Naranjo et al. [50] studied in the Odiel marshes, where they detected clear phytotoxicity symptoms at tissue concentrations greater than 38 mg kg^{-1} DW.

The maximum concentrations of this metal registered in the Odiel Marshes Natural Park ranged from 500 to 1000 µM. In halophytes in this estuary, Luque et al. [80] found Cu accumulations that ranged from 12.3 mg kg^{-1} DW in *Halimione portulacoides* to 878 mg kg^{-1} DW in *Zostera noltii* Hornem., and at the same location Park, Stenner, and Nikless [81] found in the latter species accumulations in tissue of 1350 mg kg^{-1} DW. These levels are far superior to the data collected by other authors for these species in other estuaries around the world [1,2], which clearly demonstrates the high level of contamination in the Odiel Marshes Natural Park.

As described previously, the Cu accumulation curve equation presents a high positive value for component "a"; therefore, the accumulation rises exponentially with the increase in the metal in the medium, which matches Kabata-Pendias and Pendias [79], who established that Cu concentration rises exponentially when concentrations in the medium increase. This also fits with observations by Mateos-Naranjo et al. [50] in *Atriplex halimus* and with the accumulation in *Arctium tomentosum* Mill. observed by Al Harbawee et al. [77], and in roots, shoots, and leaves of *Sesuvium portulacastrum* (L.) L. studied by Feng et al. [82]. This exponential accumulation could be linked to damage in the roots, which impacts negatively on the root transport system. This could be explained by the fact that at lower concentrations the relation between the concentration of the metal in the medium and tissue is arithmetic [9,83].

4.2. Manganese

In our work, manganese did not affect the final germination percentage or the germination dynamics of *Salsola vermiculata*, which coincides with data on *Atriplex halimus* and *Salicornia ramosissima*, other *Chenopodiaceae* plants from the Odiel marshes presented by Márquez-García et al. [21]. Neither did it affect *Salsola vermiculata* seedling development in concentrations up to 4000 µM. Márquez-García et al. [21] found that manganese had no effect on the cotyledons or hypocotyls of *Atriplex halimus* and *Salicornia ramosissima* in concentrations up to 2000 µM, but there was an increase in radicle length at 10 µM and a reduction in concentrations over 250 µM in *Atriplex halimus*, and over 2000 µM in *Salicornia ramosissima*.

Normal Mn content differs greatly between species (30–500 mg kg^{-1} DW) [84]. The threshold of damage caused by Mn depends on the plant species and cultivars or genotypes within a species [85,86]. In general, plants are negatively affected by Mn concentrations above 500 ppm, although concentrations over 1000 ppm have been described in tolerant species [79].

Salsola vermiculata reached levels of up to 4675 mg kg^{-1} DW, grown in solutions up to 4000 µM, without presenting any negative effects. By contrast, this metal significantly curtailed the growth of *Suaeda glauca* when accumulation in tissue reached approximately 1000 mg kg^{-1} DW [60], revealing, for the first time, that *Salsola vermiculata* is a better accumulator for this metal.

Mn is present in the Odiel marshes at maximum concentrations of between 50 and 500 µM, occupying third place in metal concentration in plant tissue in these marshes, behind Fe and Zn, and its concentrations range from 25.4 mg kg^{-1} DW in *Arthrocnemum macrostachyum* plants to 1960 mg kg^{-1} DW in *Zostera noltii* [80].

Accumulation of Mn in seedlings increased to 4373 mg kg^{-1} DW when exposed to 2000 µM, and maintained this level in higher concentrations, reaching 4676 mg kg^{-1}

DW at 4000 μM Mn. This behavior contrasts with that established by Kabata-Pendias and Pendias [79], who determined that Mn concentration in plants is proportional to its presence in soil, and with observations by Zhang et al. [60], who found arithmetic increases in Zn content in *Suaeda glauca* up to 2812 mg kg^{-1} DW. However, Lidon and Teixeira [87] observed, in *Oryza sativa*, a stabilization in accumulation when it reached 2000 mg kg^{-1} DW in the culture medium with concentrations that exceeded 8 mg L^{-1}.

4.3. Nickel

In many plant species, increasing concentrations of Ni inhibit and delay seed germination and seedling growth, generally due to the suppression of amylase and protease activity [88–94]. However, this metal does not affect the final percentage of *Salsola vermiculata* germination, or germination dynamics, as was the case in *Atriplex halimus* and *Salicornia ramosissima*, other *Chenopodiaceae* plants from the Odiel marshes studied by Márquez-García et al. [21]. In some species, Ni improves both the rate and percentage of seed germination [22,88], but we did not observe this effect, perhaps due to the high percentage of germination in the control.

On the other hand, some authors state that this metal retards germination in crop plants [95], but this toxic effect was not observed in this study, in *Salsola vermiculata*, *Atriplex halimus*, or *Salicornia ramosissima* [21].

There are many examples of the toxic effects of Ni on seedlings that reduce their growth [93,96]. In the case of *Salsola vermiculata*, nickel reduces cotyledons at 4000 μM, diminishes hypocotyls at 2000 μM, and affects radicles from 2000 μM, causing a drastic reduction at 4000 μM. The threshold concentrations for damage observed in this species are higher than those observed in *Atriplex halimus* and *Salicornia ramosissima*, studied in the same location, with both species registering damage at 1000, 250, and 100 μM for cotyledons, hypocotyls, and radicle, respectively.

As we have described, the concentration of nickel required for normal growth in most plants is very low; from 0.05 to 0.1 mg kg^{-1} DW to 5 mg kg^{-1} DW, according to Kabata-Pendias and Pendias [79], from 10 mg kg^{-1} DW, in Ain et al. [90], or from 20–30 mg kg^{-1} DW, as described by White and Brown [97]. In most plants studied, Ni in tissue is toxic in concentrations from 10–100 mg kg^{-1} DW [79,91,98].

In our study, *Salsola vermiculata* seedlings reached levels of 7130 mg kg^{-1} DW when grown in 4000 μM of Ni medium, but the highest concentration at which toxic effects on seedlings were not observed was 1000 μM, accumulating in seedlings up to 1537 mg kg^{-1} DW.

In the Odiel estuary, this metal has been found in concentrations below 50 μM, and its accumulation in the plants studied ranged from 13.0 mg kg^{-1} DW in *Salicornia ramosissima* to 45.7 mg kg^{-1} DW in *Spartina maritima* (Curtis) Fernald [80].

At the concentration levels tested, accumulation of this metal increased arithmetically as concentrations in the medium rose, which is consistent with Lu et al. [9], who established that Ni concentrations in many plants positively correlated to concentrations in the medium. But this is only true until the medium concentration reaches a certain level [79]; this threshold varies in different species, for example, in *Lepidium ruderale* L. the threshold concentration in the medium was 20 μM, while it reached 30 μM in *Capsella bursa-pastoris* Moench [99]; it was 100 μM in *Arctium tomentosum* [77]; in *Odontarrhena bracteata* (Boiss and Buhse) Spaniel and *O. inflata* (Nyár.) D.A. German, the threshold was approximately 150 μM [100]; and in varieties of Brassica juncea (L.) Czern, it was 400 μM [96]. The persistence of Ni intake until the concentration in the medium of 4000 μM could indicate the non-existence of mechanisms involved in the control of Ni intake.

4.4. Zinc

Salsola vermiculata germination fell to 79.83% and was delayed at 4000 μM, but no effects were observed at lower concentrations, which coincides with figures for *Atriplex halimus* and *Salicornia ramosissima* reported by Márquez-García et al. [21], who did not observe any reduction at 2000 μM of Zn.

The Zn concentrations tested did not affect cotyledon or hypocotyl size, but at 50 µM the radicles of *Salsola vermiculata* seedlings were, significantly, 30% longer than the control; the effects of hormesis have also been observed in *Medicago sativa* L. [22]. Nevertheless, at 4000 µM a reduction of almost 50% was observed in the radicles. The study by Márquez-García et al. [21] of *Atriplex halimus* noted a reduction in cotyledons and hypocotyls at 2000 µM, and in radicles from 250 µM. They also described a reduction in the radicles of *Salicornia ramosissima* seedlings from 1000 µM, which demonstrates higher tolerance in *Salsola vermiculata*.

Most plants presented critical toxicity levels for this metal in their tissue from 100–500 mg kg^{-1} DW [79]. In *Salsola vermiculata*, Zn reached maximum accumulations of 3990 mg kg^{-1} DW when cultivated at 2000 µM, showing no seedling damage at this concentration. Our results are consistent with Boularbah et al. [70], who found Zn content of 819 mg kg^{-1} DW in mining areas in Morocco, with no toxicity symptoms.

The maximum concentrations of this metal recorded at the Odiel marshes ranged from 500 µM to 1000 µM, occupying second place in metal concentration in plant tissue in these marshes. In the plants studied, Zn accumulation ranged from 62.9 mg kg^{-1} DW in *Arthrocnemum macrostachyum* to 2440 mg kg^{-1} DW in *Zostera noltii* [80,81].

Accumulation of Zn in seedlings increased to 3990 mg kg^{-1} DW of Zn at 2000 µM, and maintained this level in higher concentrations, accumulating to 3204 mg kg^{-1} DW at 4000 µM, showing similar behavior to that observed for Mn. As in the case of Mn, Kabata-Pendias and Pendias [79] determined that Zn concentration in plants is proportional to its presence in the soil, but Al Harbawee et al. [77] observed similar dynamics to those we observed in *Arctium tomentosum*: that when concentrations in the medium increased to 1000 uM in the plate, they registered an arithmetic increase in accumulation, and the accumulation did not increase at the same rate when the concentration was higher. This was also observed by Pandey [101] in *Raphanus sativus* L. and *Spinacia oleracea* L., and by Kozhevnikova et al. [99] in *Lepidium ruderale* and *Capsella bursa-pastoris*. Nevertheless, Ivanov et al. [102], observed in *Pinus sylvestris* L. seedlings, that while Zn accumulation in the radicles increased proportionally to the concentration in the nutrient solution to 300 µM, the Zn content in leaves stabilized at a concentration of 150 µM.

Although plant metal intake behavior depends on the range of concentrations in the medium analyzed [103], some authors [104] have described three patterns for accumulations of heavy metals: as the concentration in the medium increases, (1) accumulator plants show an arithmetic increase in content in tissue to the point where content reaches a maximum level; (2) non-accumulators show a low level of accumulation in tissue until the concentration in the medium reaches a point where the restricting mechanism breaks down and there is unlimited accumulation, resulting in plant death; (3) a linear pattern in which the accumulation in tissue is directly related to the metal concentration in the medium. Observations in our study resemble the first pattern for Mn and Zn, the second pattern for Cu, and the third pattern in the case of Ni.

5. Conclusions

Under control group conditions, *Salsola vermiculata* reached germination levels of above 90%, in line with data previously published by Muñoz-Rodríguez et al. [65], with seeds from the same location and under the same conditions. In that study, *S. vermiculata* demonstrated its adaptation to soil salinity, germinating at above 90% in salinities of up to 0.2 M when planted without the calyx, and at over 80% with the calyx; even after exposure to 0.6 M salinities, they germinated in distilled water in a proportion higher than 80%.

Regarding the toxic effects of the metals studied, the roots are the primary target of the metals, and their growth is usually more severely affected than that of the aerial parts, probably since the roots are the first contact point with toxic elements and provide an entrance to the cellular structure inside the plant. This is confirmed by the results in our work, as the roots were affected at lower concentrations in the culture medium than the hypocotyls or cotyledons for all the metals tested.

Our results clearly show how *Salsola vermiculata* can tolerate the presence of metals and successfully germinate. With no toxic effects, its seedlings accumulate Cu up to 299 mg kg^{-1} DW, Mn up to 4675 mg kg^{-1} DW, Ni up to 1537 mg kg^{-1} DW, and Zn up to 2507 mg kg^{-1} DW.

Salsola vermiculata exhibits a higher tolerance to metals than other halophylous *Chenopodiaceae* species studied, such as *Atriplex halimus* and *Salicornia ramosissima*, both analyzed at the same location [21]; and higher than *Salsola passerine*, which is an accumulator for Ni, Cu, Cd, Cr, and Co [9].

Author Contributions: Conceptualization, I.S., F.N.-R. and A.F.M.-R.; methodology, I.S., F.N.-R., M.D.I.-I., G.M.-S., J.A.D., A.P., S.R.-A., E.S.-G. and F.J.J.-N.; software, validation, and formal analysis, M.D.I.-I. and A.P; investigation, I.S. and A.F.M.-R.; resources, F.N.-R., S.R.-A. and A.F.M.-R.; Data-curation, F.N.-R. and S.R.-A.; Writing—original draft preparation: I.S.; Writing—review and editing, F.N.-R.; visualization, F.N.-R.; supervision, F.N.-R. and A.F.M.-R.; project administration and funding acquisition, F.N.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Spain's Ministry of Education, Science and Sport, FPU Grant (Ref. FPU14/06556) and by Spain's Ministry of Economy and Competitiveness, predoctoral grant (Ref. BES-2012-059366).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank the institution and staff of the Odiel Marshes Natural Park. Infante-Izquierdo wishes to thank Spain's Ministry of Education, Science and Sport for awarding the FPU Grant (Ref. FPU14/06556). Martínez-Sagarra wishes to thank Spain's Ministry of Economy and Competitiveness for the award of a predoctoral grant (Ref. BES-2012-059366).

Conflicts of Interest: The authors declare that they have no conflict of interest.

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