Chapter 14
Comparative Effect of Cadmium on Germination and Early Growth of Two Halophytes: *Atriplex halimus* L. and *A. nummularia* Lindl. for Phytoremediation Applications

Bouzid Nedjimi

Abstract Cadmium (Cd) is a heavy metal (HM), which is highly toxic and hazardous to all living organisms, even in low concentrations. The choice of the better species for seeding and sowing the Cd-ontaminated soils is of fundamental importance, especially in arid areas. *Atriplex* spp. are characterized by high tolerance to salinity, extreme temperatures, and HMs. In this work, the toxicity impacts of Cd on germinability characteristics and subsequent seedlings growth of two halophytic species (*Atriplex halimus* and *A. nummularia*) have been investigated. Seeds were treated with $CdCl_2$ at various concentrations (0, 100, 200, and 300 µM) for 15 days under controlled conditions (25 ± 1 °C with 16/8-h photoperiods). Results indicate that Cd significantly affected the final germination, germination rate, and both hypocotyl and radicle lengths of the studied halophytes. Both *Atriplex* seeds were usually tolerant to Cd at low concentrations, but high Cd concentrations significantly reduced all cited parameters. Based on the results of the tolerance index and degree of phytotoxicity, *A. halimus* seemed to be more resistant to Cd toxicity than *A. nummularia*.

Keywords Cadmium tolerance index · Germination bioassays · Phytoremediation · Phytotoxicity · Saltbushes · *Timson's* index

B. Nedjimi (✉)
Laboratory of Exploration and Valorization of Steppe Ecosystem, Faculty of Science of Nature and Life, Ziane Achour University of Djelfa, Djelfa, Algeria

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14.1 Introduction

Cadmium (Cd) pollution is among the most serious environmental threats to human health. Mine tailings, combustion emissions, phosphate fertilizers, pesticides, wastewater, atmospheric deposition, and sewage sludge are the principal sources of Cd pollution (Haider et al. 2021). Physiochemical procedures for Cd-decontamination of soils such as chemical leaching, excavation, precipitation, and heat treatment are quite very costly and may lead to soil modifications (Raza et al. 2020).

Germination inhibition, stunted growth, chlorosis, root browning, nutriments and water disturbance, and hormonal status failure were the major symptoms of Cd-toxicity in plant species (Moreira et al. 2020).

Phytoremediation using trees and shrubs is a promising, inexpensive, and environmentally friendly technique to eliminate pollutants and toxins from water and polluted soils (Nedjimi 2021). The success of this approach is related to the identification of promising shrub species to absorb, tolerate, and store a large amount of pollutants (HMs and toxins). In this context, the use of halophytic species with deep root systems and considerable green biomass constitutes an interesting tool for rehabilitating contaminated soils, especially in arid areas (Mujeeb et al. 2020; Joshi et al. 2020).

Seed halophyte germination and the subsequent seedlings' development are the initial delicate phases to environmental changes (Gul et al. 2013). The first critical phases of phytoremediation are the germination and seedling establishment in the HMs-polluted soils (Nedjimi 2020).

Atriplex spp. are group of the Amaranthaceae family (halophytes) growing indigenously in arid areas of the world, some of them are extremely resistant to harsh conditions such as salt and drought stresses, soil pollution, and extreme temperatures (Le Houérou 1992). They are persistent saltbushes that keep their leaves throughout the year and are usually used as forage by livestock in arid and semi-arid rangelands (El Shaer 2010; Nedjimi 2018). Though some works have examined the seed tolerance capacity of *Atriplex* species to salinity (Bhatt and Santo 2016; Shaygan et al. 2017; Bueno et al. 2017), little information is known about the effect of HMs on the germination of these halophytic species. Thus, the present investigation aims to evaluate the impact of Cd stress on the seed germinability and initial establishment of two *Atriplex* species widely cultivated in Algerian arid areas. This work is supposed to be useful in assessing the Cd tolerance and phytoremediation potential of *A. halimus* and *A. nummularia* to clean up polluted soils.

14.2 Materials and Methods

14.2.1 Species Description and Seed Source

Atriplex halimus L. (common name: Mediterranean saltbush) (Fig. 14.1a) is a perennial shrub reaching up to 1–3 m high, native to North African countries (Algeria, Tunisia, Morocco, and Libya). It is extremely resistant to water and salt

Fig. 14.1 (**a**) *Atriplex halimus* L. (Mediterranean saltbush) and (**b**) *A. nummularia* Lindl. (Old man saltbush)

stresses and can survive for several months without rainfall (Le Houérou 1992). *Atriplex nummularia* Lindl. (common name: Old man saltbush) introduced from Australia is erect evergreen saltbush that reaches 2.5–3 m high and grows in inland saline soils (Fig. 14.1b). It is very resistant to drought and grazing and produces a high leaf and wood biomass (Falasca et al. 2014).

Both *Atriplex* seeds were procured from the HCDS nursery of *Taâdmit*, located about 50 km from Djelfa province, Algeria (2° 59′ E long., 34° 17′ N lat., and 1049 m alt.). The annual precipitation in this region is about 250 mm. Minimum and maximum monthly temperatures occur respectively in January (3 °C) and July (34 °C). The seeds were kept in paper bags at 4 °C until the start of the germination test.

14.2.2 Germination Experiment and Seedling Measurements

Before germination, *Atriplex* seeds were disinfected for 10 min with 70% ethylic alcohol, treated with 8% H_2O_2 for 5 min, and finally rinsed with deionized water. Seeds of each species were deposed in petri dishes (90 mm diameter) lined with two disks of sterilized Whatman filter paper. The petri dishes were kept moistened with 5 mL of corresponding Cd-treatments (0, 100, 200, 300 μM $CdCl_2$). These concentrations were selected according to the Cd levels reported in Algerian arid soils

(Nedjimi and Daoud 2009). The petri dishes were arranged in a completely randomized design (CRD), with four replications, and each replicate contains 25 sterilized seeds (100 seeds/treatment).

Petri dishes were placed in an incubator in an alternating photoperiod of 16 h light/8 h obscurity at 25 °C temperature with photon flux intensity of 200 μmol m^{-2} s^{-1}. The germinated seeds were counted for 15 consecutive days till no further germination was detected. The protrusion of visible radicle by 2 mm through the seed coat was considered a germination criterion.

At the end of each experiment, the rate of germination (RG), using *Timson's* index, was determined by the following formula:

RG = Σ pg/t, where (pg) is the % of germination after 48 h interval, and (t) is the total time of the experiment (Nedjimi et al. 2020). Hypocotyl and radicle sizes were measured using a graduate scale.

The tolerance index (TI %) was measured by the equation given by Wilkins (1978):

TI % = [radicle size in Cd treatment/radicle size control] × 100.

The phytotoxicity index (PI %) was assessed using the method given by Hsu and Chou (1992):

PI % = [radicle size control − radicle size in Cd treatment/radicle size control] × 100.

14.2.3 Statistical Analysis

The statistical difference between the Cd-treatments was calculated using analysis of two-way ANOVA. Results are reported as mean ± standard error of four replicates for each treatment. Significance level was performed by post-hoc *Duncan*'s test ($P < 0.05$) using the software package STATISTICA 8.0.

14.3 Results and Discussion

14.3.1 Cadmium Effects on Germination Percentage

Results exhibited that the germination percentage was correlated to the Cd concentrations and the studied species (Fig. 14.2). The analysis of variance (ANOVA) indicates a significant effect of the *Atriplex* species ($F = 71.68$, $P < 0.001$), Cd-treatments ($F = 48.29$, $P < 0.001$) and their combination ($F = 802$, $P < 0.001$) on the seed germination percentage (Table 14.1). Seeds of both species are capable to germinating at all Cd concentrations (Fig. 14.2).The highest values of germination were detected in the control and low Cd treatments, but a gradual increase in Cd concentrations significantly reduced seed germination (Figs. 14.2 and 14.3). The prevention effect of this HM was more pronounced for *A. nummularia*, particularly when seeds were exposed to the highest concentrations (200 and 300 μM $CdCl_2$) (Fig. 14.2).

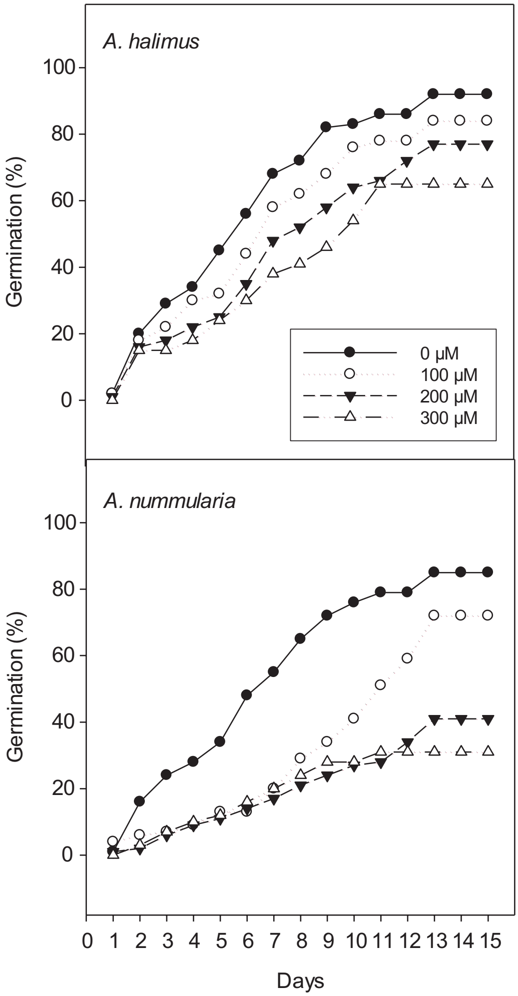

Fig. 14.2 Cumulative germination percentage as a function of time of *Atriplex halimus* and *A. nummularia* seeds treated with $CdCl_2$ concentrations (0, 100, 200, and 300 µM)

In this work, the impact of Cd application on the germination characteristics of two halophytes was examined. The findings showed that this HM affected the final germination in both halophytes. The depressive effect of Cd on germinability was more pronounced on *A. nummularia* seeds compared to *A. halimus.* These results were found in agreement with previously reported results such as *Arabidopsis thaliana* (Li et al. 2005), *Salicornia brachiata* (Sharma et al. 2011), and *Suaeda salsa* (Liu et al. 2012). Heavy metals may impair seed germination and seedling establishment by water imbibition failure, metal harmfulness, mineral nutrition imbalance, or the interaction of these factors (Kranner and Colville 2011). Cadmium possesses an effect on seed–water exchanges, causing a direct decrease in water uptake. If Cd crosses the seed coat, it affects the metabolic activities (mobilization of nutrients) that occur in the germination process (Nedjimi and Daoud 2009; Tran and Popova

Table 14.1 A two-way ANOVA of the effects of species (Sp), concentrations (C), and their combination (Sp × C) on germination, seedling growth, tolerance index, and phytotoxicity index of *Atriplex halimus* and *A. nummularia*

Independent variables	Species (Sp)	Concentrations (C)	Interaction (Sp × C)
Germination percentage	71.68^{***}	48.29^{***}	8.02^{***}
Rate of germination	133.39^{***}	62.97^{***}	6.28^{**}
Hypocotyl length	4.87^{*}	4.07^{*}	0.11^{ns}
Radicle length	48.23^{***}	43.03^{***}	8.01^{**}
Tolerance index	126.49^{***}	313.97^{***}	20.12^{***}
Phytotoxicity index	54.51^{***}	135.07^{***}	8.66^{**}

Note: Data represent *F*–values significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant

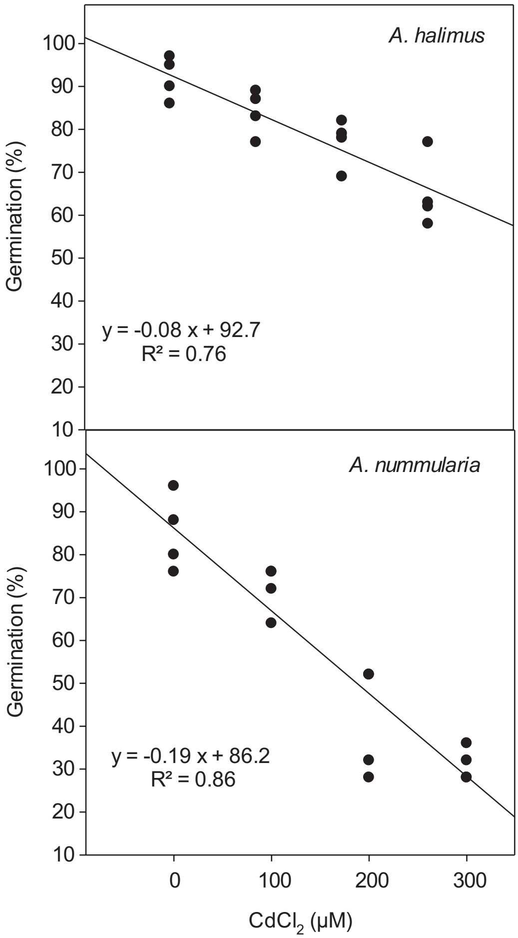

Fig. 14.3 Regression plots of mean germination percentage of *Atriplex halimus* and *A. nummularia* seeds treated with $CdCl_2$ concentrations (0, 100, 200, and 300 µM)

2013). This difference in the germination failure between species can be attributed to their different selective absorption across the seed teguments (anatomy and structure of coat) and embryo metal-tolerance (Siddiqui et al. 2014). The external coat of the seed acts like a barrier that avoids the penetration of a considerable amount of Cd inside the embryo from the polluted soil and protects the embryo from Cd toxicity (Amin et al. 2018).

14.3.2 Cadmium Effects on the Timson's *Index*

Analysis of variance (ANOVA) shows that *Timson's* index (germination rate) was significantly affected by the *Atriplex* species ($F = 133.39$, $P < 0.001$), Cd-treatments ($F = 62.97$, $P < 0.001$) and the combination of these two factors ($F = 6.28$, $P < 0.01$) (Table 14.1). The rate of germination of both halophytic species was affected significantly by Cd application as compared to control (Fig. 14.4). Rate of germination decreased with exposure to increasing Cd treatments, and this reduction was more apparent for the highest concentrations. At high Cd concentration (300 μM $CdCl_2$), the rate of germination was reduced by 35.46% and 63.58%, respectively, for *A. halimus* and *A. nummularia*. The depressive effect of Cd on seed germination rate was also reported in other halophytes such as *Spartina alterniflora* (Mrozek 1980); *Suaeda salsa* (Liu et al. 2012).

The influence of Cd on the seed germinability depends on their aptitude to penetrate embryonic tissues across the seed teguments (coats). The anatomy and thickness of the coat differ between plant species, and consequently, the same concentration of Cd had a different impact among species. The germination depends on the cotyledon reserve mobilization (starch and polysaccharides) for the embryo development; however, the presence of Cd can cause oxidative stress (ROS) and disturbs the hydrolyzing enzyme involved in the germination event (Kuriakose and Prasad 2008; Kranner and Colville 2011).

14.3.3 Cadmium Effects on Early Seedling Growth

Plant morphology is a better character for the description of plant growth changes in the presence of HMs (Amin et al. 2018). The impact of different treatments of Cd on hypocotyl length of *Atriplex* species is presented in Fig. 14.5. The analysis of variance (ANOVA) indicated that the *Atriplex* species ($F = 4.87$, $P < 0.05$) and Cd-treatments ($F = 4.07$, $P < 0.05$) had a significant effect on hypocotyl elongation, but their combination was not significant ($F = 0.11$, $P > 0.05$) (Table 14.1). The increase of Cd doses produced a significant reduction in the hypocotyl elongation of both species. The highest concentrations of Cd clearly affected the hypocotyl elongation of *A. nummularia*, while *A. halimus* was less affected (Fig. 14.5).

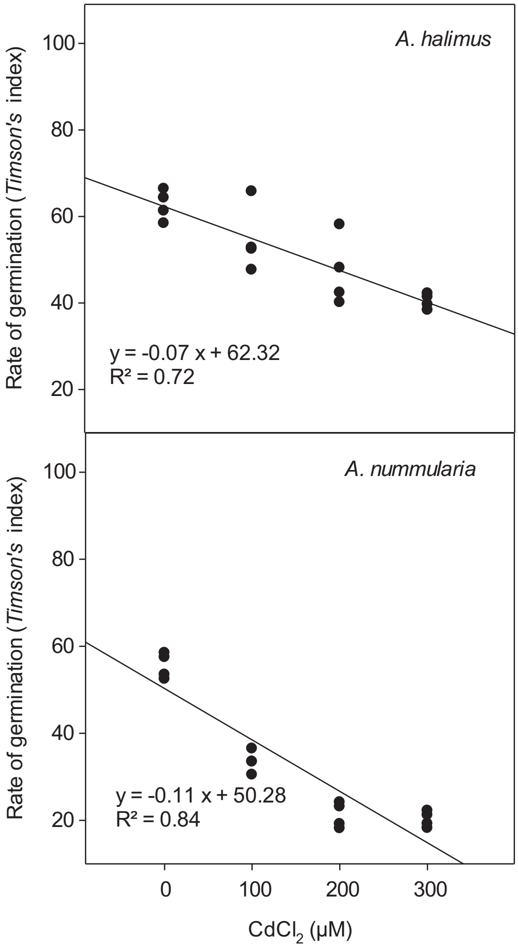

Fig. 14.4 Regression plots of the *Timson's* Index (rate of germination) of *Atriplex halimus* and *A. nummularia* seeds treated with $CdCl_2$ concentrations (0, 100, 200, and 300 μM)

Radicle elongation was also affected by Cd application (Fig. 14.5). The analysis of variance (ANOVA) demonstrated that the *Atriplex* species ($F = 48.23$, $P < 0.001$), Cd-treatments ($F = 43.03$, $P < 0.001$) and their combination ($F = 8.01$, $P < 0.01$) significantly affected radicle elongation (Table 14.1). An elevation in Cd concentrations significantly reduced the radicle elongation of both species. At 300 μM $CdCl_2$ the radicle length was reduced by 46.97% and 57.71%, respectively, for *A. halimus* and *A. nummularia.* This aspect has also been mentioned in other halophytic species belonging to the Amaranthaceae family such as *Salicornia brachiata* (Sharma et al. 2011) and *Halogeton glomeratus* (Yao et al. 2021). However, Santos et al. (2015) showed an opposite pattern in the halophyte *Juncus acutus*, who found that application of 0.05, 0.1, 0.5, and 1 μM $CdSO_4$ significantly increased seedling length compared to control. This impairment in radical growth caused by Cd application can be due to its effect on cell division and/or cell wall elasticity (Kranner and Colville

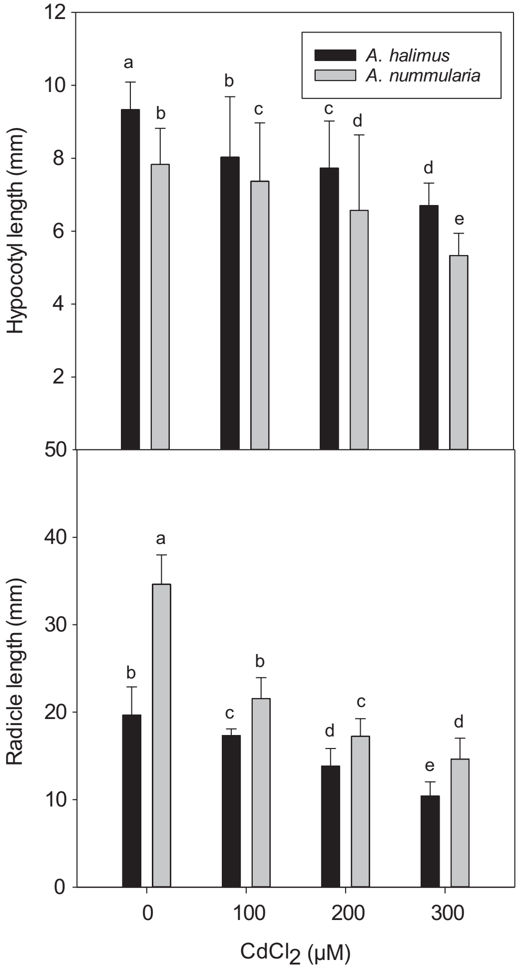

Fig. 14.5 Hypocotyl and radicle elongations of *Atriplex halimus* and *A. nummularia* seedlings treated with $CdCl_2$ concentrations (0, 100, 200, and 400 µM). Bars represent means ± S.E. ($n = 3$). Different letters indicate a significant difference between treatments ($P < 0.001$, *Duncan*'s multiple–range test)

2011). The root system is the first plant organ exposed to pollutants and is more sensitive to metal exposure than the aerial part (Zhang et al. 2020). Root growth reduction, underdeveloped root hair, and browning of roots were the main symptoms of Cd toxicity (Mahmood et al. 2007; Moreira et al. 2020). Cadmium stress blocks cell division, thus decreasing plant elongation, preventing mineral nutrition and water improvement, and leads to reducing aerial part development (Yao et al. 2021). Several reasons can be anticipated to explain the stunted growth of hypocotyl induced by Cd application: inhibition of mitosis, polysaccharide metabolism breakdown, changes in photosynthesis, and reduction in the water potential (Nagajyoti et al. 2010).

14.3.4 Cadmium Tolerance Index (TI %)

Tolerance index (TI %) was significantly affected by *Atriplex* species ($F = 126.49$, $P < 0.001$), Cd-treatments ($F = 313.97$, $P < 0.001$), and their combination ($F = 20.12$, $P < 0.001$) (Table 14.1). In both halophytes, this parameter was significantly reduced in the presence of Cd treatments (Fig. 14.6). At 300 μM $CdCl_2$, *A. nummularia* and *A. halimus* had a tolerance index of 42% and 53%, respectively. *Atriplex halimus* seems to be more tolerant to Cd than *A. nummularia.* Zhang et al. (2020) mentioned that the tolerance index was a good parameter to classify a plant species as tolerant at germination stage and initial seedling growth. In our investigation, significant impairment in germination and early seedling growth induced by Cd-stress indicated that *A. nummularia* is less tolerant to Cd compared to *A. halimus*.

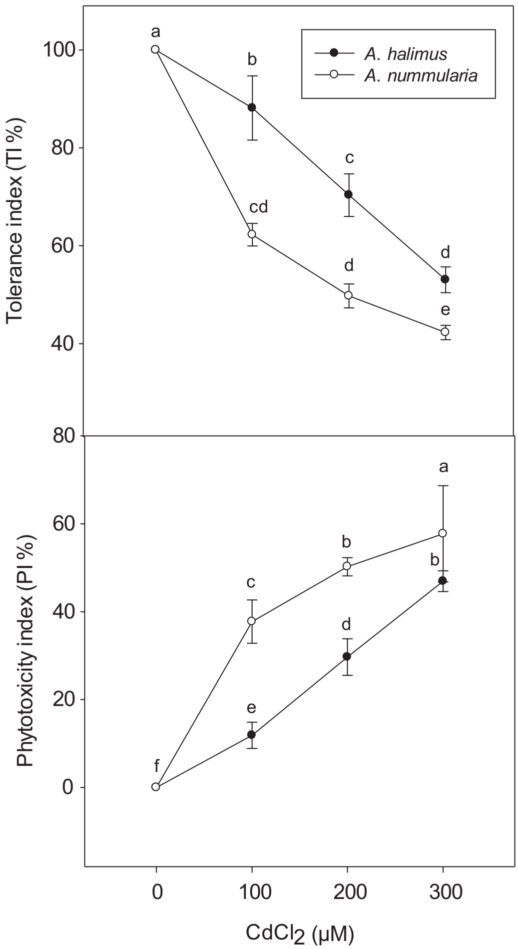

Fig. 14.6 Phytotoxicity and tolerance index of *Atriplex halimus* and *A. nummularia* seedlings treated with $CdCl_2$ concentrations (0, 100, 200, and 300 μM). Values represent means ± S.E. ($n = 3$). Different letters indicate a significant difference between treatments ($P < 0.001$, *Duncan*'s multiple-range test)

14.3.5 Phytotoxicity Index (PI %)

The analysis of variance (ANOVA) indicates a significant effect of the *Atriplex* species ($F = 54.51$, $P < 0.001$), Cd-treatments ($F = 135.07$, $P < 0.001$), and the combination of these two factors ($F = 8.66$, $P < 0.01$) on the PI % (Table 14.1). The phytotoxicity of Cd on seedling growth of *Atriplex* species is presented in Fig. 14.6. This parameter in both species increased significantly with the increasing concentration of $CdCl_2$.The lowest phytotoxicity was recorded for *A. halimus.* These findings suggest that *A. halimus* have a higher tolerance to Cd toxicity than *A. nummularia.* These facts were in conformity with the results found by Sharma et al. (2011) in *Salicornia brachiate* subjected to Cd stress. The phytotoxic impact of Cd on the germinability and early growth of plants is well recognized; however, the degree of Cd-phytotoxicity differs substantially depending on the plant species, varieties, and Cd concentration in the growth medium (Haider et al. 2021).When Cd enters across the cytoplasmic membrane, it affects the cellular metabolic processes in the cytosol by interacting with lipids and proteins, which affects the membrane conductivity, the enzymatic reactions, and induces oxidative stress (ROS) by free radical production (Raza et al. 2020).

14.4 Conclusion

This work indicated that Cd had an adverse impact on *Atriplex* seed germination and initial stage growth of two halophytes. The increase of this HM leads to decrease germination and initial growth parameters in both species. However, *A. halimus* seems to be more tolerant to Cd than *A. nummularia.* These saltbushes can be used for phyto-rehabilitation of soils affected by Cd. Further study is required to confirm these findings in field conditions.

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Conflict of Interest The author declares no conflict of interest.

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