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EFFECTS OF HEAVY METALS ON THE GERMINATION AND RADICLE GROWTH OF HALOPHYTES SPECIES (*ATRIPLEX HALIMUS* L.)

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Abstract: The present work deals with the effect of three heavy metals on germination of *Atriplex halimus* L. seeds. The experiments were conducted during 10 days, under strictly controlled laboratory conditions. Precocity of germination (PG), Germination Percentage (GP), Kinetics of germination, Germination index (GI) and Radicle length (RL) were estimated. The results showed that Kinetics of germination, GI and RL were significantly affected by heavy metal stress. In contrast, the increase of applied heavy metal dose resulted in prolongation of GI, and therefore, in significant decrease in RL. Root growth is more sensitive to metals than germination; the inhibitory effect of cadmium (Cd) on growth is earlier than those of zinc (Zn) and lead (Pb). It should be noted that *A. halimus* L. seeds were able to germinate even at 8000 ppm, which is a concentration higher than critical limits for agricultural soils and irrigation water. This suggests that it could be considered as a moderately tolerant species, at least during the germination phase, to metal stress.

Keywords: abiotic stress, *Atriplex halimus* L, germination, heavy metal, seeds.

INTRODUCTION

Plants are frequently subjected of many stressors such as drought, freezing, heat shock, toxic metals/metalloids, ultraviolet, radiation, air pollutants, nutrient deficiency, pathogen attack (Skoneczny *et al.*, 2019; Hasanuzzaman *et al.,* 2020). Heavy metals (HMs), as one of the major contaminants for the environment (Krishna and Mohan, 2016), they can be transported to different locations over waters and soils by erosion or acid rain (Sharma *et al.*, 2017). However, since most HMs are not degraded by chemical and microbial processes (Kirpichtchikova *et al.,* 2006), their high concentrations remain in the soil for a long time and reach animals and humans via the food chain (Nagajyoti et *al.,* 2010).

Halophytes are plants that naturally grow in high salinity regions, can withstand harsh environmental conditions such as salt stress and drought, and can tolerate and/or accumulate toxic ions, including those of heavy metals (Adhikari *et al.*, 2010; Suelee *et al.*, 2017). Many species belonging to the genus *Atriplex* are well adapted to extreme environmental conditions (Martinez et *al.,* 2003). *Atriplex halimus* L. is one of the most abundant perennial halophytes present in Algerian saline steppes in association with *Salsola vermiculata* and *Suaeda fruticoza* (Ortiz-Dorda et *al.*, 2005). The ability of this species to physically stabilize soils and its high tolerance to trace metals should be exploited for phytoremediation of metal-contaminated sites in arid and semi-arid areas (Walker and Lutts, 2014). The study of physiological mechanisms ensuring the survival of halophytes under conditions of heavy metal excess has become an urgent task (Jordan et *al.,* 2002; Shevyakova et *al.,* 2003). Germination is a critical stage in the life cycle and development of many desert plants. It ensures reproduction and therefore controls population dynamics (Radosovich et *al.,* 1997). It can be affected by metals in two ways: direct toxicity and/or water uptake inhibition (Kranner and Colville, 2011). However, no data are available for us to learn about seed germination behavior under multiple stress conditions.

Accordingly, the aim of the present study was to investigate the effect of heavy metals on halophytes species (*Atriplex halimus* L.) seed germination and radicle growth under high concentrations and to unveil the possible mechanisms underlying it. In this experiment, one essential (Zn) and two nonessential elements (Pb and Cd) were used.

MATERIALS AND METHODS

The plant material having been the object of the present study concerns the seeds of *Atriplex halimus* L. which come from High Commissariat for the Development of Steppe (HCDS) of Tebessa (northeastern Algeria). Seeds were soaked for 10 minutes in 10% (v/v) solution of sodium hypochlorite (NaOCl), after rinsing three times in distilled water. Next, 20 seeds were placed in petri dishes (90-mm diameter) on filter paper and were treated separately with solutions containing Zn (0 ppm, 2000 ppm, 4000 ppm, 6000 ppm and 8000 ppm), Cd (0 ppm, 2000 ppm, 4000 ppm, 6000 ppm and 8000 ppm) and Pb (0 ppm, 2000 ppm, 4000 ppm, 6000 ppm and 8000 ppm). Control treatments were supplied with distilled water. Germination was continued for 8 days and germinated seeds were counted on a daily. Seeds were considered germinated when their radical length was 2 mm (ISTA,

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2003). After 10 days of treatment, following parameters were measured.

Precocity of germination (%)

The precocity of seeding which corresponds to the rate of seeds germinated from the 1st day. In this case, the precocity of germination is expressed by the rate of the first germinated seeds.

Germination Percentage (%)

Seed germination of cereals was recorded every 24 h according to the seedling evaluation procedure up to ten days. The germination percentage (GP) was calculated using the formula below (ISTA, 2003) for each replication of the treatment:

$$GP = \frac{Number\ of\ germinated\ seeds}{total\ number\ of\ seeds} x\ 100$$

Germination index (germination speed)

Was calculated as the sum of the percentage of seeds germinated on each day divided by the number of days since the germination test started (Bradbeer, 1988; Wardle et *al.,* 1991).

$$S = (N1 \times 1) + (N2 - N1) \times 1/2 + (N3 - N2) \times 1/3 + \dots (Nn - Nn\text{-}1) \times 1/n$$

N1, N2, N3, Nn-1, Nn: Proportion of germinated seeds observed at first, second, third … (n – 1), (n) days or hours.

Kinetics of germination

The number of sprouted grains was counted daily until the 7th day of the experiment, to better understand the physiological significance of the germ behaviour of the studied varieties (Hajlaoui et *al.,* 2007).

Radicle length

Radicle parts of the seeds in petri dishes were separated after germination, and measured in centimeters from the point where the radicle and plumule joins together at the end of the radicle and to the top of the plumule.

Statistical Analysis

Data processing was performed using Microsoft Excel 2010, ExcelStat 2014 and R Version 3.6.2. Shapiro-Wilk’s and Levene’s test were applied to test for normality and variance homogeneity across treatments, respectively.

Nonparametric tests and specifically, the Kruskal-Wallis test were used for non-normal data. The level of significance was considered less than 0.05. When Kruskal-Wallis tests indicated significant differences between the analysed groups, the analysis was further developed using Conover-Iman test for multiple comparisons. Pearson’s correlation coefficient was also calculated to find out the effect of different dependent variables on each other and was represented using scatter plots.

RESULTS

Kruskal-Wallis tests in Table 1 showed that heavy metal concentration had significant effect on all parameters studied. This test indicated the presence of a very highly significant treatment effect on the precocity of germination and the length of the roots ($P < 0.001$), a significant effect on the germination percentage ($P < 0.05$) and a highly significant effect on the germination index ($P < 0.01$).

Table 1.

Sample Data for Kruskal-Wallis mean rank test

Parameters	chi-squared	p-value
Precocity of germination	27.64	0.0006***
Germination Percentage	21.58	0.042*
Germination index	28.86	0.004**
Radicle length	33.77	0.0007***

Note: *** Significant at the 0.001 Confidence Level.****** Significant at the 0.01 Confidence Level. * Significant at the 0.05 Confidence Level.

The precocity of germination and the germination percentage of *A. halimus* L. were minimally affected by the presence of metals in the germination media, with some differences depending on the metal (Figure 1 and Figure 2).

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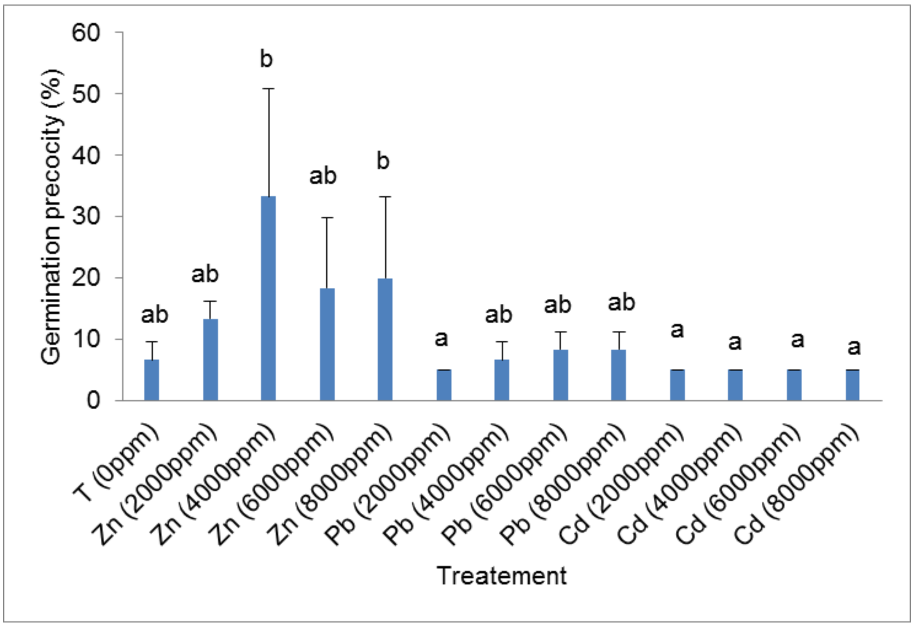

Fig. 1. Effect of different concentrations of Zn, Cd and Pb on germination precocity of *A. halimus* seeds. Results are means ± sd (n = 3). Different letters denote significant differences between treatments (P < 0.05).

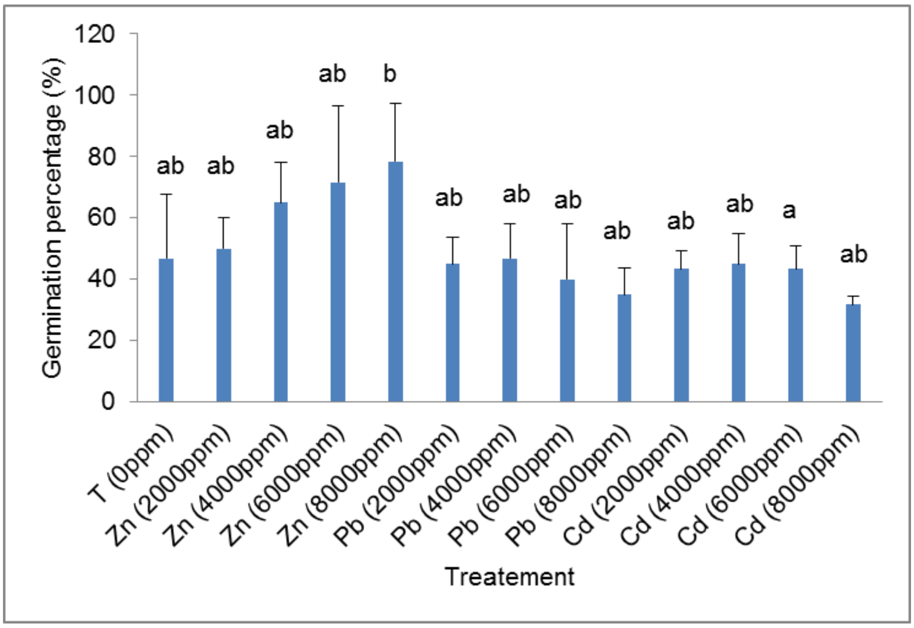

Fig. 2. Effect of different concentrations of Zn, Cd and Pb on germination percentage of *A. halimus* seeds. Results are means ± sd (n = 3). Different letters denote significant differences between treatments (P < 0.05).

The germination index (GI) of the seedlings was affected by the presence of metals in different ways (Table 1, Figure 3). Zinc did not affect the germination index at any of the concentrations tested, but cadmium and lead significantly reduced the germination index compared to the control when present at the highest concentration. The presence of lead significantly reduced GI at 4000 ppm to 53.57 % of the control, and decreased from 6000 ppm to around 48.77 % of the control; however, cadmium had a negative effect at 2000 ppm, 6000 ppm and 8000 ppm with a significant reduction in GI to 47.07 %, 51.62 % and 47.95 % respectively of the control.

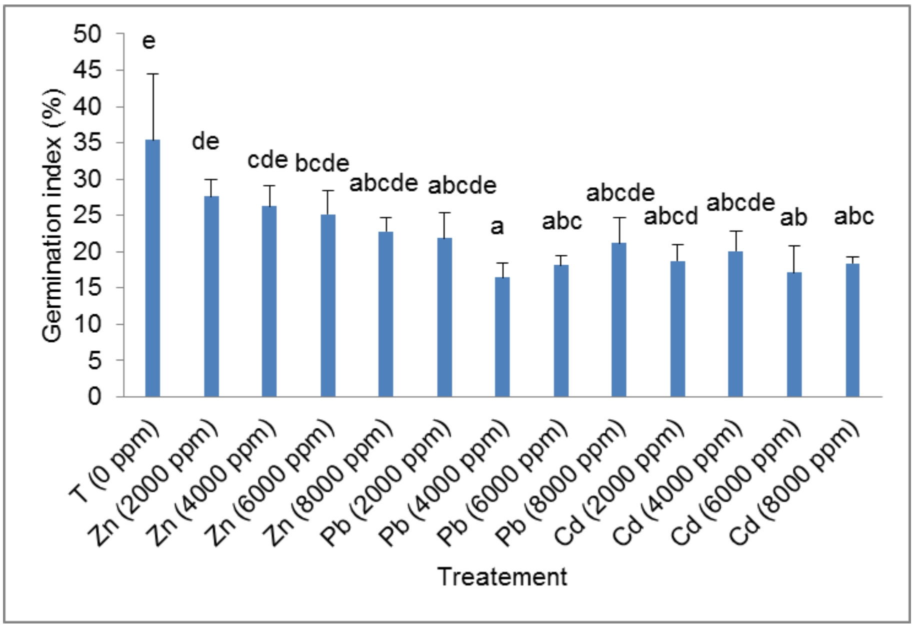

Fig. 3. Effect of different concentrations of Zn, Cd and Pb on germination index of *A. halimus* seeds. Results are means ± sd (n = 3). Different letters denote significant differences between treatments (P < 0.05).

As shown in Figure 4, zinc, at the concentrations tested, did not statistically affect the initial development of *A. halimus* L.seedlings, but the length of the radicle was significantly affected by lead, with a reduction of 60.78 % at 4000 ppm and 75.10 % at 8000 ppm. Cadmium, at all concentrations tested, significantly reduced the growth of the radicle, and the length decreased from 2000 ppm to around 71.96 % of the control; it was 75.88 % of the control at 4000 ppm, 89.61 % at 6000 ppm and 82.35 % at 8000 ppm.

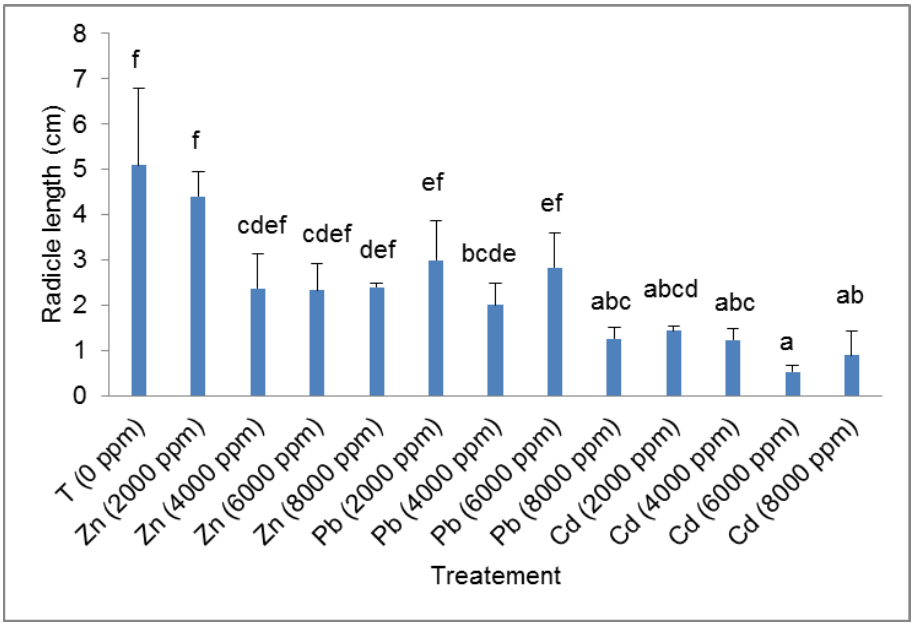

Fig. 4. Effect of different concentrations of Zn, Cd and Pb on radicle length of *A. halimus* seeds. Results are means ± sd (n = 3). Different letters denote significant differences between treatments (P < 0.05).

The impact of different concentrations of Zn, Cd and Pb on germination kinetics of *A. halimus* L. seeds was also examined (Figure 5). The kinetics of germination expresses three phases, a first phase of latency, which had with the imbibition of seeds; a second exponential phase where one attends an acceleration of germination and finally a third phase characterized by a stationary stage indicating a break of germination. Under Cd and Pb condition, the seeds expressed their sensitivity starting from 2000 ppm by expressing a reduced percentage of germination with a slow exponential phase and which lasts much longer. With the highest levels of Cd and Pb (4000 ppm, 6000 ppm and 8000 ppm), the seeds seem too affected by these conditions and show a dynamics of very slow germination and this throughout the experiment. On the other side, under condition of Zn treatment; evolution of germination with (2000 ppm) is close to the control and reached 46.67% at the 7th day. This progression tends to mount as the concentrations in Zn increase.

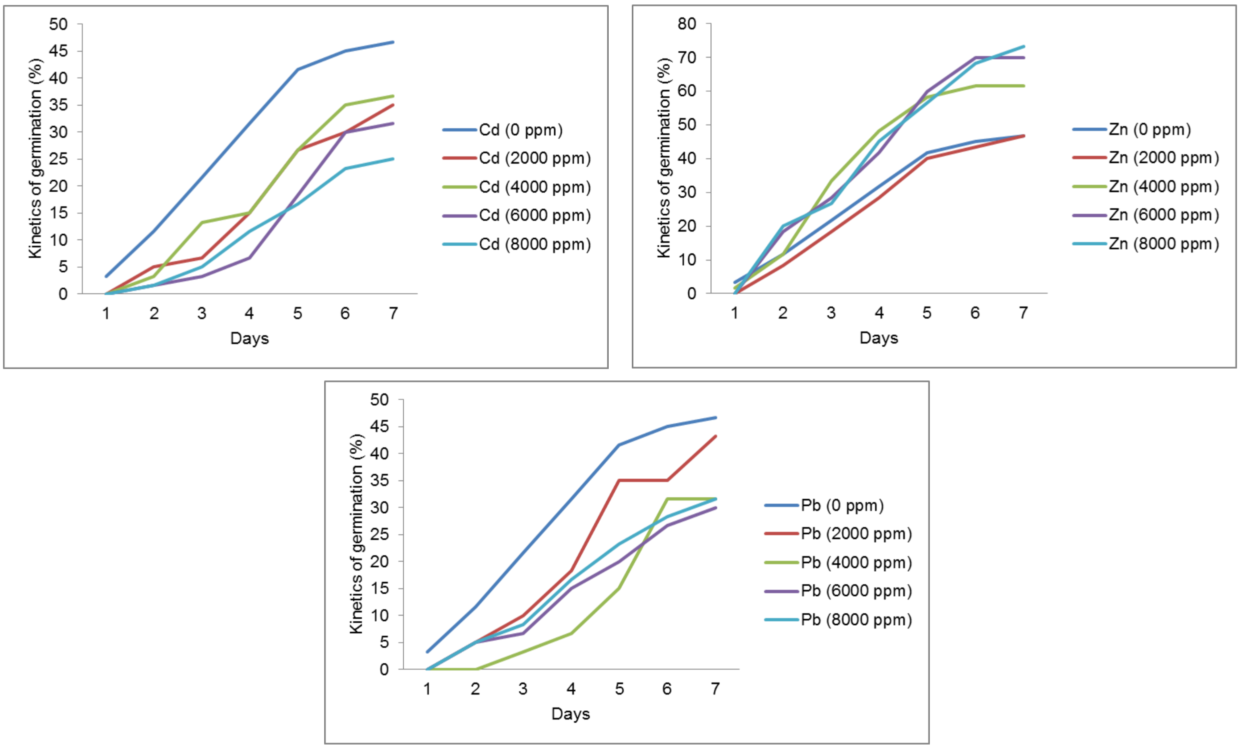

Fig. 5. Effect of different concentrations of Cd, Pb and Zn on germination Kinetics of *A. halimus* seeds.

Results of Pearson's correlation analyses between different germination parameters are given in Figure 6. According to correlation matrices, precocity of germination was positively correlated with germination percentage (r = 0.66, P < 0.001) and with germination index (r = 0.36, P = 0.02). In addition, germination percentage was positively correlated with germination index (r = 0.34, P = 0.03). Finally, germination index was positively correlated with radicle length (r = 0.70, P < 0.001).

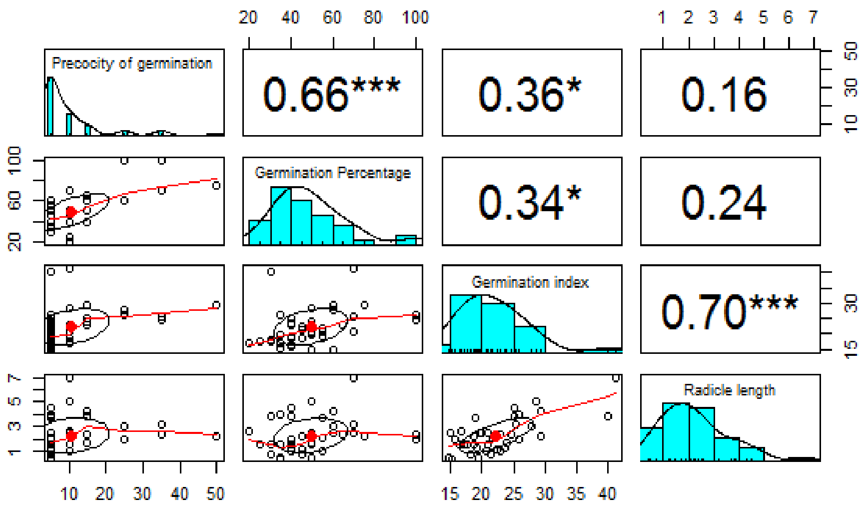

Fig. 6. Correlation matrix showing correlation among different germination characteristics from *Atriplex halimus* exposed to heavy metals stress.

DISCUSSION

The germination and embryonic growth bioassays are the first two steps largely used as basic experimental tests for the phytotoxicological effect of TMEs on different crops and plant species (Kranner and Colville, 2011; Souahi et *al.*, 2017). Seed germination and seedling growth can be diminished by high metal concentrations (Souahi et *al.*, 2021).

In our study, remarkable tolerance to heavy metals has been reported for the genus *Atriplex* (a member of the Chenopodioideae subfamily in the Amaranthaceae family). Germination percentage and precocity of seed germination of *A. halimus* did not change with increasing metal concentrations, though germination was significantly accelerated in the presence of Zn, Pb and Cd, but germination index and root growth kinetics change with increasing cadmium concentrations, *A. halimus* seedlings were shown to tolerate up to 2000 ppm Pb and high levels of Zn, remaining unaffected at concentrations as high as 8000 ppm. Similar to our results, high concentrations of Cu and Zn (up to 2000 μM) did not affect seed germination in the halophytic herb *Salicornia ramosissima* J. Woods and in *Atriplex halimus* L. (Márquez-García et *al.,* 2013). Soil metallic pollution with Pb, Zn, Cu and Cr affected the percentage of germination of *Solanum lycopersicum* and *Cicer arietinum*, whereas these metals did not affect *Cucumis sativus* germination (Mbadra et *al.,* 2019). Although seed coat may act as a barrier to metal uptake by other grasses and forbs (Munzuroglu and Geckil, 2002; Li et *al.,* 2005; Kranner and Colville, 2011). According to Moise *et al.* (2005), the integument provides very high protection against abiotic stresses in many plant species, and the strong interspecific variation in the morphologies of these integuments can affect their permeability to metals.

In some species, germination acceleration could be attributed to an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in plants exposed to metals, causing a slightly enhanced level of oxidative stress that stimulates germination (Kranner and Colville, 2011).

There is a linear relation between seed germination and Cd concentration (Cheng and Zhou, 2002). Eghareba and Omoregie (2010) demonstrated that Cd decreased the germination percentage and plant height in *Vigna unguiculata*.

High levels of Cu, Zn, Cd, Pb and Hg reduced the seed germination in various species due to abnormalities in the embryo growth process (Street et *al.,* 2007). In addition, dormancy release and seed germination are also tightly associated with two important phytohormones: abscisic acid and gibberellins (White et *al.,* 2000). Interestingly, ROS can affect their biosynthesis and catabolism during seed germination (Liu et *al.,* 2010; Bahin et *al.,* 2011).

Germination rate of Zn-rich seeds was lower than the others; nevertheless, such seed lots had higher germination percentage and produced vigorous seedlings. Thus, it is recommended the application of zinc under Zn- and water-deficient soil conditions, to produce vigorous seed lots (Karami et *al.,* 2016).

Our results show that there is a very highly significant effect of metal treatments on root length. It was observed that radicle length decreased significantly with increasing Cd and Pb concentrations. Similar to our results, Kranner and Colville (2011) report that root growth is inhibited in more than 15 plant species in the presence of increasing concentrations of metals (Cr, Cd, Cu, Zn, Pb, Ni and Hg). Concentrations of Cu, Pb, Zn and Hg, which cause inhibition of *Arabidopsis thaliana* radicle growth, do not inhibit germination, unlike Cd, which inhibits germination and radicle growth at similar concentrations (Li *et al.,* 2005).

Some of the promising halophytes candidates e.g., *Tamarix smyrnensis* (Lefèvre *et al.,* 2009), *Limoniastrum monopetalum* (Manousaki and Kalogerakis 2009), and *Suaeda salsa* (Wu et *al.,* 2012) have been identified that may confer metal resistance by accumulating heavy metals from roots to shoots. Hypocotyl and radicle growth is more sensitive to the toxic action of metals and is often completely inhibited by low concentrations that have little effect on germination (Mahmood et *al.,* 2005; Aydinalp and Marinova, 2009; Kranner and Colville 2011). The stressful situation, associated with excess ROS generation, would prevent radicle emergence. Thus, ROS play a dual role alternating between having a signaling role and being deleterious during seed dormancy release (Bailly *et al.,* 2008).

Many of the physiological and molecular mechanisms that contribute to salt tolerance in halophytes, including ions compartmentalization, synthesis of organic solutes and a robust antioxidative system (Freeman *et al.,* 2004; Flowers and Colmer, 2008) are also found in heavy metal tolerant species (Souahi, 2021).

Halophyte adapts to heavy metal stress with two distinct types: harmful toxic ions excreted by salt bladders, trichomes and salt glands, or various tissues accumulated and diluted toxic ions by succulent tissues (Manousaki and Kalogerakis 2011).

Therefore, the cultivation of *A. halimus*, which is often recommended for the phytostabilisation of metal polluted sites, could be established by sowing. The imbibition kinetics, germination rate and root growth kinetics would be interesting markers for their tolerance to heavy metals during germination (Fatarna *et al.,* 2017).

CONCLUSION

This study demonstrates for the first time the effect of trace amount of heavy metal (Zn, Cd and Pb) on the seed germination of *A. halimus* plant under high concentrations. Cadmium and lead are not required for plant growth compared with the essential element (Zn). Seeds from *A. halimus* species were metal-tolerant, since their germination percentage was not affected, even at high metal concentrations.

Halophytes are of significant interest since these plants are naturally present in environments with an excess of toxic ions and tolerate other environmental stresses, especially heavy metals as their tolerance to salt and to heavy metals may, at least partly, rely on common physiological mechanisms.

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AUTHORS CONTRIBUTIONS

HS, and AC designed the study. NA performed field and laboratory work. HS carried out the statistical analyses and wrote the paper. HS and AC elaborate the discussion. All authors contributed to the revision of the paper and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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