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ORIGINAL PAPER

Germination characteristics of *Peganum harmala* L. (Nitrariaceae) subjected to heavy metals: implications for the use in polluted dryland restoration

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Abstract

Phytoremediation is an effective and low-cost technique for the rehabilitation and cleanup of lands polluted with heavy metals. Selection of native plant species could avoid the ecological risks that are associated with the usage of non-native species. However, utilizing native species in phytoremediation and restoration of lands polluted by heavy metals requires information about their seed germinability and propagation requirements. The aim of this work was to assess the effects of four heavy metals (cadmium, chromium, lead and zinc) on the germination and early seedling growth of *Peganum harmala* L., a native Mediterranean species that has the potential to restore arid degraded lands. The results display that the germination characteristics (percent seed germination and *Timson's index*) and growth parameters (hypocotyl and radicle lengths) worsened as the concentrations of all the heavy metals increased. Cadmium was found to be the most toxic element regarding these parameters, with toxicity decreasing in the following pattern: Cd > Pb > Cr > Zn. Radicle growth was more affected by the heavy metals compared to hypocotyl growth and the seedlings appeared to be more resistant to Zn. The germination ability of *P. harmala* over a wide range of heavy metals suggests that this species can grow easily in polluted soils.

Keywords Arid land restoration · Native species · Metal tolerance index · *Timson*'s *index* · Phytoremediation

Introduction

Arid and semiarid lands around the world can support many woody and herbaceous species highly tolerant to harsh environmental conditions, e.g., drought, salinity and heavy metal contamination, which can be used for land rehabilitation and restoration (Barakat et al. 2013; Nedjimi 2016; Bhatt and Santo 2017).

Heavy metal pollution caused by anthropogenic practices like mine tailings, chemical applications (insecticides and fungicides), sludge and industrial waste production is one of the major environmental threats and leads to agro-ecosystem pollution and land degradation, particularly in arid areas (Liang et al. 2017; Dotaniya et al. 2018; Ghori et al. 2019).

Certain heavy metals (HMs) such as cadmium (Cd) and lead (Pb) are non-essential for plant growth and are highly toxic when their levels exceed critical threshold values (Sarwar et al. 2016). Other HMs such as zinc (Zn) and copper (Cu) are indispensible micro-nutrients for plants at low concentrations but at higher levels, they can lead to toxicity and induce metabolic perturbations and growth suppression for most plant species (Kabata-Pendias 2011).

Phytoremediation is a sustainable strategy that uses some hyper-accumulator plants and their rhizospheric microbes to stabilize, transform or degrade pollutants in air, soil, water and the environment (da Silva et al. 2018; Hesami et al. 2018; Khanoranga 2019). Removing HMs from soil, water or even from air using plants is considered an environmentally cost-effective approach (Morikawa and Erkin 2003; Branquinho et al. 2007). However, physical and chemical methods have several limitations or disadvantages due to higher cost and labor intensiveness (Hesami et al. 2018).

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Utilizing native species, which are well-adapted in terms of growth, survival and reproduction under such environmental stress, could be a better choice, and hence, identifying the native species which have tolerance ability to HMs is required. Until now, there have been few studies evaluating phytoremediation capacities of native desert plants (Badr et al. 2012; Ibrahim et al. 2013; Padmavathiamma et al. 2014).

Generally, seeds are well-protected against various stresses until the imbibition and subsequent seedling growth (Li et al. 2005). However, germinating seeds and initial seedling stage are much more vulnerable to HMs than mature plants, since their defense mechanisms are not yet fully developed (Li et al. 2005). Therefore, comprehending the plant sensitivity/tolerance to HMs stresses during germination and initial growth stage can determine its success or failure of propagation and survival in metal-contaminated lands.

Seed germination is the plant growth period that is most highly sensible to abiotic stresses including HMs pollution (Kranner and Colville 2011). Numerous reports have shown that HMs stresses dramatically affect the seed germinability and initial seedling development of many plant species such as *Miscanthus floridulus* (Hsu and Chou 1992), *Festuca rubra* (Hatamzadeh et al. 2012), *Linum usitatissimum* (Jain 2013) and *Pinus sylvestris* (Makhniova et al. 2019).

HMs can affect growth and plant productivity (Hasanuzzaman and Fujita 2012). However, some plants known as hyper-accumulator species survive spontaneously in a wide range of polluted lands (Maestri et al. 2010; Hesami et al. 2018). These species, which comprise annuals and perennial plants, exhibit different levels of tolerance against HMs (Peer et al. 2003; Nedjimi 2018). For landscaping programs, it is very useful to select plants that have spontaneously colonized polluted soils (Conesa et al. 2007; Nedjimi 2016).

Peganum harmala L. (family of Nitrariaceae ex. Zygophyllaceae) is a native species that occurs naturally in degraded and metalliferous lands in arid and semiarid regions around the Mediterranean basin (Suleiman et al. 2011; Nedjimi et al. 2012). Due its high contents of alkaloids, including harmine, harmol, harmaline and peganol (Moloudizargari et al. 2013), seeds of this species are used in traditional medicine from ancient times to treat large human diseases. These include hypoglycemic, antispasmodic, antidepression (epilepsy and Parkinson' disease), antitumoral and antileishmaniasis effects (Zaker et al. 2007; Singh et al. 2008; Astulla et al. 2008; Rahimi-Moghaddam et al. 2011). *Peganum harmala* is found in polluted soils, which presumably means it is more able to cope with HMs than other plants species. However, information about its germinability and initial growth characteristics are scarce. Thus, conducting the seed germination of *P. harmala* under different metal stresses could be helpful to evaluate their potential for utilizing them in metal-contaminated areas of arid regions.

Therefore, the objective of the present investigation was to assess the phytotoxicity of Cd, Pb, Cr and Zn on germination and initial seedling growth of *P. harmala*, thereby testing its tolerance to HMs for the possible use in rehabilitation of Algerian arid lands where the occurrence of these HMs is frequent. This information could be useful to establish restoration programs by selecting the most suitable plant species to revegetate the contaminated lands. This study was conducted at the Faculty of Science of Nature and Life, University Ziane Achour of Djelfa (Algeria) from 2015 to 2016.

Materials and methods

Study species and seed collection

Peganum harmala is a native herbaceous plant (Fig. 1) that can grow up to 0.30–0.80 m tall. The dark-green leaves are arranged alternately on stiff twigs. In xeric soils, the root system can reach a depth of 5–6 m. *Peganum harmala* flowers in late spring, from April to June. After maturation, the fruit is a dry dehiscent capsule constituted by three carpels containing about 50 dark-brown seeds (3 mm in length) (Quézel and Santa 1963). A mature plant produces 1000–2500 seeds per year. For this work, the average seed mass was determined by weighing three replicates: the mean dry weight of 100 seeds was 5 ± 0.03 g.

Fig. 1 *Peganum harmala* L. (Nitrariaceae ex. Zygophyllaceae) "Common names: Harmal, Syrian Rue". Whole plant (**a**), flower (**b**), fruit capsule (**c**) and seeds (**d**)

This species can tolerate very harsh ecological conditions such as salinity and drought; native populations of *P. harmala* can be found in very alkaline and salty soils, with low annual rainfall (100–250 mm) (Ahmed and Khan 2010).

Mature seeds of *P. harmala* were hand-collected in September 2015 from the *Dar Chioukh* region (Djelfa province) located in the central drylands of Algeria (3° 29′ E longitude, 34° 53′ N latitude and 1103 m a.s.l., Northern Algeria). Seeds were randomly harvested from 50 individual plants in order to reduce the influence of genetic variation. After cleaning the fruit capsules, the seeds were kept in a cold room at 4 °C until the germination test.

Germination bioassays and seedling measurements

Before use, the seeds were sterilized externally by soaking in 5% sodium hypochlorite (NaClO) for 10 min and were then washed abundantly with deionized H_2O. The germination assay was performed in plastic Petri dishes (9 cm Ø) included two sterile Whatman filter papers wetted with 5 ml of the different treatment solutions (0, 100, 200, 300 µM) of the HMs (Zn, Cr, Pb and Cd), added as zinc sulfate ($ZnSO_4$), potassium dichromate ($K_2Cr_2O_7$) and lead and cadmium nitrate, $Pb(NO_3)_2$ and $Cd(NO_3)_2$, respectively. The dishes were sealed with adhesive tape (Parafilm™) to avoid evaporation loss and incubated for 15 days.

For each treatment, four replications of 25 seeds were used. The experiment protocol was conducted on complete randomized design. The germination process (protrusion of the radicle) was recorded when the radicle length reached 2–3 mm.

The seeds were germinated in a phytotron with controlled photoperiods of 12-h dark and 12-h light, and a temperature regime of 15 °C and 25 °C (night/day); these conditions were found to be appropriate to enhance the germination potential of this Mediterranean species (Nedjimi 2013).

The germination rate (*Timson's index*) was assessed using the formula described by Nedjimi (2019). *Timson's index* $= \Sigma pg/t$, where (pg) is the percent of germination after 2-d interval and (t) is the total germination period.

To study the influence of the different metals on initial seedling growth, the lengths of the radicles and roots were measured.

The metal tolerance index (MTI %) was calculated using the method of Wilkins (1978): MTI % = (radicle length in metal solution/radicle length control) × 100.

Statistical analysis

The results were subjected to two-way ANOVA to determine the effects of the HMs, concentrations and their interaction (HMs × C) on germination parameters (percent seed germination and *Timson's index*) and seedlings measurements (hypocotyl and radicle lengths). *Duncan*'s multiple-range test was applied to evaluate significant variations between the treatments at the $P < 0.001$ level. The data were arcsine converted before the statistical analysis to ensure the uniformity of variance. Linear regressions were used to determine the relationships between HM concentrations and germination. Statistical evaluation was performed using SPSS software, version 17.0 (SPSS Inc., Chicago, USA).

Results and discussion

Heavy metal effects on percent seed germination

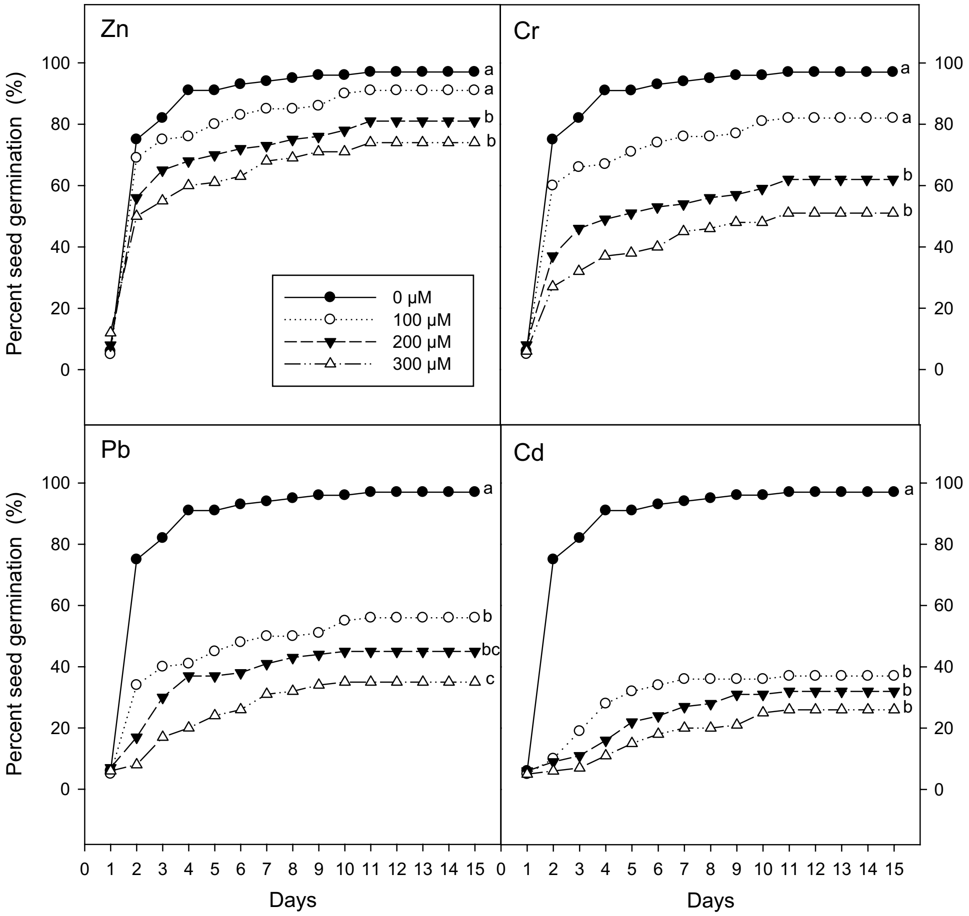
HMs pollution has been known as a major environmental threat due to their pervasiveness and persistence. Accumulation of HMs in soil can create serious threat to plants due to their toxicity (Benavides et al. 2005). Therefore, in this work, we assessed the phytotoxicity of selected HMs (Cd, Cr, Pb and Zn) with regard to the seed germinability of *P. harmala*. A two-way ANOVA indicates a significant impact of the

Table 1 A two-way ANOVA of the effects of heavy metals (HMs), concentrations (C) and their interaction (HMs × C) on germination, growth parameters and tolerance index of *P. harmala*

Independent variables	Heavy metals (HMs)		Concentrations (C)		Interaction (HMs × C)	
	df	*F* values	*df*	*F* values	*df*	*F* values
Percent germination	3	190.18***	3	336.50***	9	23.19***
Rate of germination	3	84.04***	3	168.37***	9	10.25***
Hypocotyl length	3	20.91***	3	37.20***	9	3.27**
Radicle length	3	1.40**	3	61.15***	9	0.37 ^{ns}
Tolerance index	3	26.34***	3	1231.41***	9	8.10***

Data represent degree of freedom (*df*) and *F* values significant at $^{**}P < 0.01$; $^{***}P < 0.001$, *ns* not significant

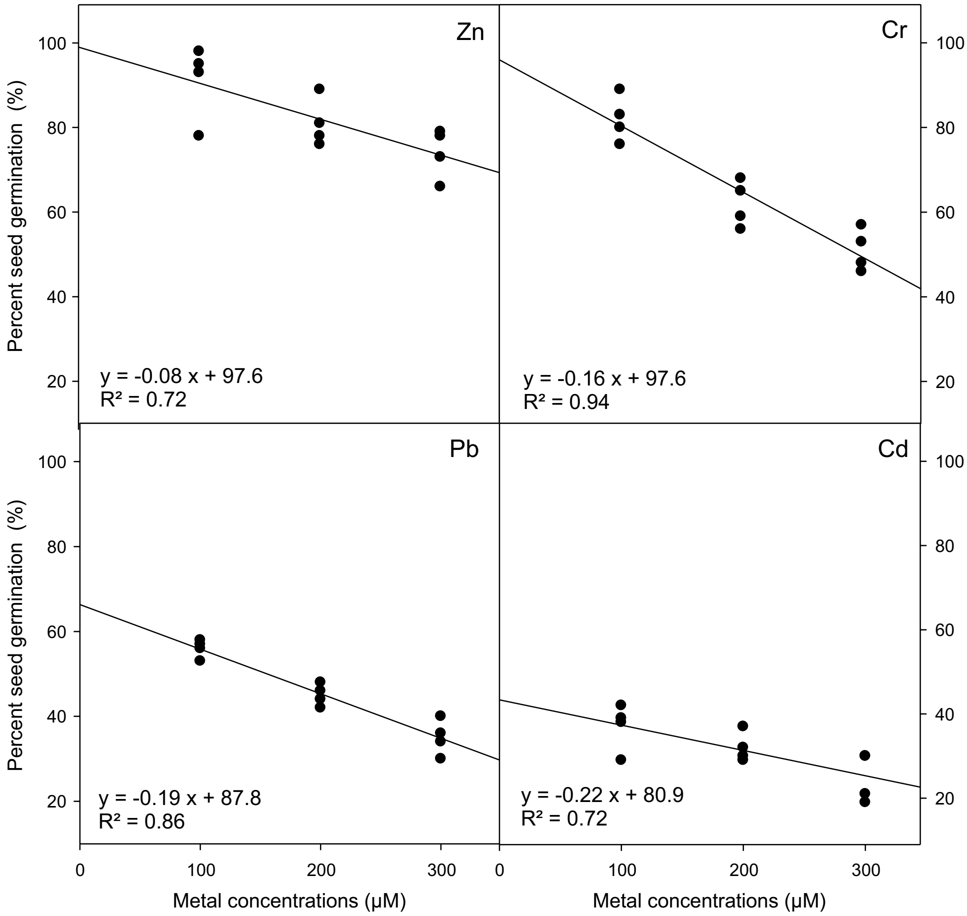
Fig. 2 Cumulative percent germination as a function of time of *P. harmala* seeds treated with Zn, Cr, Pb or Cd. Different letters indicate significant difference between treatments ($P < 0.001$, *Duncan*'s multiple-range test)



HMs ($F = 190.18$, $P < 0.001$), concentrations ($F = 336.50$, $P < 0.001$) and their interaction (HMS × C) ($F = 23.19$, $P < 0.001$) on the percent seed germination (Table 1). Germination decreased with the increase in the Zn, Cr, Pb and Cd concentrations (Fig. 2). For Zn and Cr, this parameter did not change significantly at 100 μM ($P > 0.05$) in comparison with the control. The most pronounced suppressive effect of the HMs was recorded for Pb and Cd (Fig. 2). Strong statistical correlations were found between percent seed germination and the HMs concentrations, with R^2 ranging from 0.72 to 0.94 (Fig. 3). The phytotoxicity of the HMs can be ranked in the order of the suppression effects as follows: Cd > Pb > Cr > Zn.

Germination and embryo growth bioassays are the two first stages widely used as basic experimental tests of the phytotoxicological effect of HMs on different crops and plant species (Kranner and Colville 2011). All the tested HMs significantly affected seed germination of *P. harmala*. However, the precise effect depends on the particular HM and its concentration. At lower concentration, Zn and Cr did not reduce the germination which indicates that probably lower concentrations of Zn and Cr did not interfere with the respiratory activity and mobilization of seed reserves such as starch, proteins and phytate (Bishnoi et al. 1993). In general, the percent seed germination decreased as the concentrations of HMs increased. These results are in conformity with those reported in other investigations. For example, Pandey et al. (2007) observed a considerable reduction in percent seed germination of *Catharanthus roseus* treated with 50–500 μM of $CdCl_2$ or $PbCl_2$. Abraham et al. (2013) stated that exposure of *Arachis hypogaea* seeds to increasing concentrations of Cd, Pb or Cu (0, 75 and 100 mg L^{-1}) reduced significantly the germination percentage. Also, Li et al. (2005) reported that the seed germinability of *Arabidopsis thaliana* ecotype Columbia was negatively affected by Cd, Pb and Zn provided as chloride salts. Shaukat et al. (1999) found comparable response when examining the seed germinability of *Parkinsonia aculeata* and *Pennisetum americanum* exposed to Cd, Pb and Cr treatments. Mbadra et al. (2019) indicated that 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.

Fig. 3 Regression plots of mean percent germination of *P. harmala* seeds treated with Zn, Cr, Pb or Cd



Both the osmotic and toxic effects of HMs have been implicated in the inhibition of the germination in polluted media. Street et al. (2007) indicated that high levels of Cu, Zn, Cd, Pb and Hg reduced the seed germination in various species such as *Bowiea volubilis*, *Eucomis autumnalis* and *Merwilla natalensis* due to abnormalities in the embryo growth process.

HMs inhibit or cease germination by various mechanisms such as (1) by embryonic damage and loss of coleoptile vitality (Wierzbicka and Obidzinska 1998) and (2) by decreasing α-amylase activity, responsible for starch hydrolysis, which interrupted the sugar supply to the embryo (Mihoub et al. 2005). Li et al. (2005) demonstrated that *Arabidopsis* seeds that had not germinated in Cu treatments were able to recover their germinability after transferring them to distilled H_2O, confirming the osmotic effects of Cu.

Heavy metal effects on the rate of germination (*Timson's index*)

A two-way ANOVA shows that *Timson's index* (germination rate) for the *P. harmala* seeds was significantly affected by the HMs ($F=84.04$, $P<0.001$), concentrations ($F=168.37$, $P<0.001$) and the interaction of these two factors ($F=10.25$, $P<0.001$) (Table 1). An increase in the HMs concentrations significantly decreased the *Timson's index*. This suppression was apparent at the highest concentration (300 μM), for which this index was reduced by about 68% and 78.83% as compared to the control, respectively, for Pb and Cd (Fig. 4).

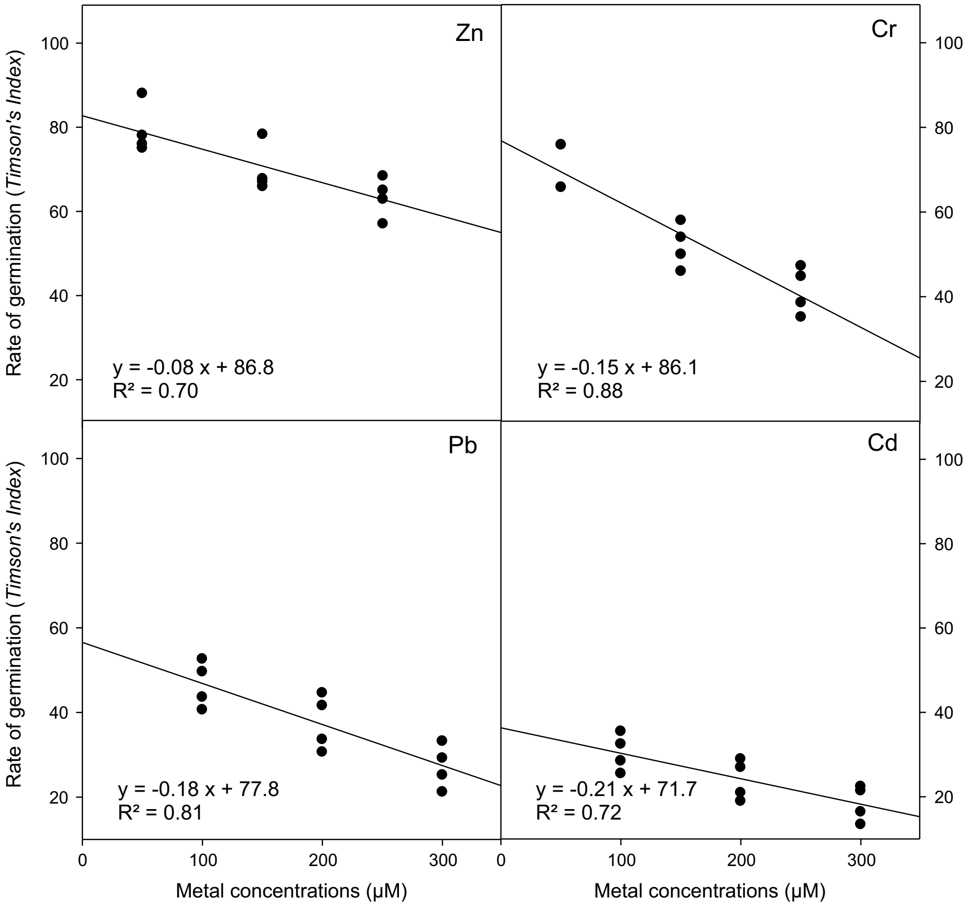
The phytotoxicity of HMs is influenced by many factors such as (1) the type of HMs, (2) plant species (3) development stage and (4) duration of exposure to the HMs (s) (Kranner and Colville 2011). In the present study, the application of Cd, Pb, Cr or Zn adversely affected the *Timson's index* of *P. harmala* seeds.

These results are consistent with the previous study, who reported that Cd, Cu, Pb and Zn, added as chlorides, decreased the germination rate of *Cucumis sativus* (Munzuroglu and Geckil 2002). Similarly, a study conducted by Ćurguz et al. (2012) showed that the *Timson's index* of *Picea abies* was affected by Cd, Pb and Zn application, although they used lower concentrations of these metals.

Heavy metal effects on early seedling growth

Figure 5 shows that a clear inhibitory effect on hypocotyl elongation begins at 100, 200 and 300 μM, respectively, for

Fig. 4 Regression plots of the rate of germination (*Timson's index*) of *P. harmala* seeds treated with Zn, Cr, Pb or Cd



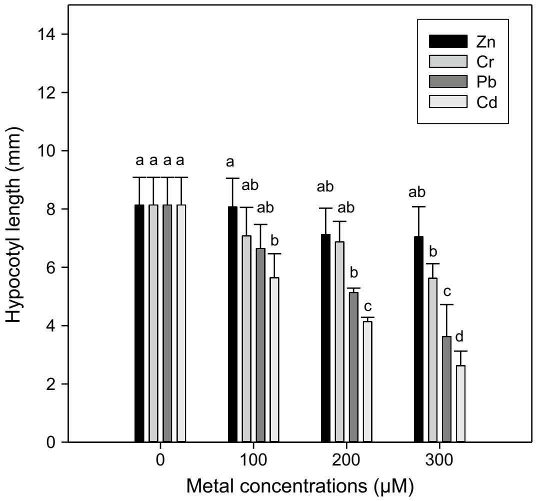


Fig. 5 Hypocotyl length of *P. harmala* seedlings treated with Zn, Cr, Pb or Cd. Bars represent mean ± S.E. (n = 3). Different letters indicate a significant difference between treatments ($P < 0.001$, *Duncan*'s multiple-range test)

Cd, Pb and Cr. However, the increasing Zn concentration did not produce a significant ($P < 0.05$) effect on hypocotyl length. For the same concentrations, Cd had a stronger adverse effect on hypocotyl length when compared with the other HMs. A two-way ANOVA displayed that the presence of HMs ($F = 20.91$, $P < 0.001$), concentrations ($F = 37.20$, $P < 0.001$) and their interaction (HMS × C) ($F = 3.27$, $P < 0.01$) significantly affected hypocotyl length (Table 1).

The inhibition of hypocotyl growth decreased in the order, Cd > Pb > Cr > Zn, and was probably the consequence of direct effects (toxicity of metals accumulated in tissues) and/or indirect effects (mineral nutrition deficiencies) of the HMs (Kranner and Colville 2011).

The two-way ANOVA shows that the HMs ($F = 1.40$, $P < 0.01$) and concentrations ($F = 61.15$, $P < 0.001$) had a significant effect on radicle length, but their interaction (HMS × C) was not significant ($F = 0.37$, $P > 0.05$) (Table 1). The exposure of *P. harmala* to HMs decreased radicle length, with the highest concentration (300 μM) causing a reduction of 84.1%, 87.34%, 85.20% and 93.20%, respectively, for Zn, Cr, Pb and Cd (Fig. 6). This suppression of

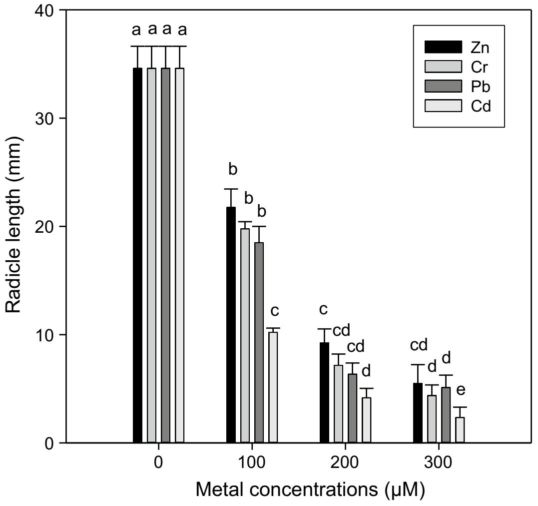


Fig. 6 Radicle length of *P. harmala* seedlings treated with Zn, Cr, Pb or Cd. Bars represent mean ± S.E. ($n = 3$). Different letters indicate a significant difference between treatments ($P < 0.001$, *Duncan*'s multiple-range test)

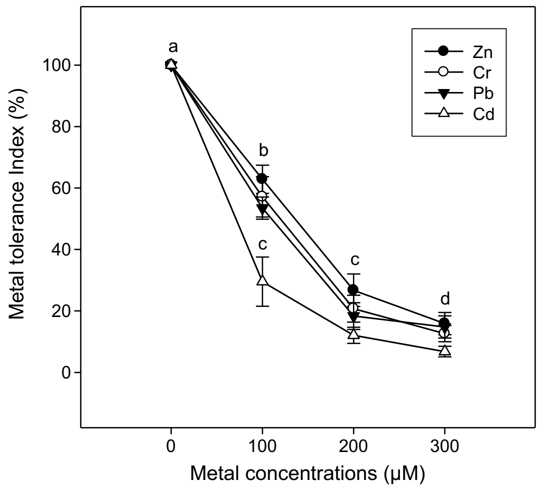


Fig. 7 Metal tolerance index of *P. harmala* seedlings treated with Zn, Cr, Pb or Cd. Values represent mean ± S.E. ($n = 3$). Different letters indicate a significant difference between treatments ($P < 0.001$, *Duncan*'s multiple-range test)

radicle growth was most apparent for Cd $(NO_3)_2$ but was less so in the case of Zn.

The radicle is the primary plant organ most affected by metal uptake and its growth is commonly stunted compared to aboveground part. Therefore, the measurement of radicles is often used for evaluating the degree of HM toxicity (Wilkins 1978). This concept was confirmed here by the present results, the radicles being affected before the hypocotyls by all the HMs tested. This high sensitivity of the radicle to HMs can be explicated by the fact that the root system is the first organ of the plant that is in direct contact with toxins in the rhizospheric medium. The inhibition of root growth by HMs might be due to abnormal mitosis and blockage of cell division (Jiang et al. 2001). Similar results have been found for other plant species such as *Pimpinella anisum* (Jeliazkova and Craker 2003) and *Ambrosia artemisiifolia* (Bae et al. 2016).

In this study, germination suppression and seedling growth inhibition of *P. harmala* were more affected by the highest Cd and Zn treatments. Cadmium is known for its phytotoxicity by inducing failure in seed imbibition, nutrient uptake and growth restriction (Li et al. 2005). However, Pb has severe effects on many physiological processes such as prevention of water absorption, cell membrane dysfunction and interaction with many enzymes necessary for normal seedling growth (Nagajyoti et al. 2010).

Metal tolerance index

The two-way ANOVA indicates a significant effect of the HMs ($F = 26.34$, $P < 0.001$), concentrations ($F = 1231.41$, $P < 0.001$) and their interaction ($F = 8.10$, $P < 0.001$) on the MTI (Table 1). Figure 7 displays the values of the MTI (%) of the *P. harmala* seedlings. The increase in the HMs concentrations substantially reduced this index, but it declined more quickly for Cd compared to the other HMs. At the highest concentration (300 µM), the MTI (%) was 15.89%, 12.64%, 14.80% and 6.79%, respectively, for Zn, Cr, Pb and Cd, relative to the untreated seedlings (control) (Fig. 7).

The present report shows that Cd, as Cd $(NO_3)_2$, was the most toxic HM with respect to the seed germinability and initial growth of *P. harmala*, compared to Pb, Cr and Zn. A comparable conclusion was drawn by Shafiq and Iqbal (2006), who reported that Cd was more inhibitory than Pb regarding the germinability and initial plant growth of *Cassia siamea*. Cadmium is a non-essential trace element (Shahid et al. 2016). Its uptake alters the assimilation of mineral nutrients (like Fe and Ca), inhibits stomatal conductance and consequently suppresses the root hydraulic conductivity (Nedjimi and Daoud 2009; Nedjimi 2018).

Among the HMs tested here, Zn showed the lowest inhibitory effect on the germination and hypocotyl length of *P. harmala*. Similarly, an experiment carried out with *Salicornia ramosissima* did not show any impact of $ZnSO_4$, at concentrations from 10 to 2000 µM, on the final germination or on cotyledon and hypocotyl growth (Márquez-García et al. 2013). The highest tolerance of Zn was also reported by Ozdener and Kutbay (2009), who investigated the effects of Cu, Cd, Ni, Pb and Zn on seed germination of *Eruca sativa*. Zinc is an essential trace element implicated in protein and tryptophan synthesis (the precursor of auxin), which is indispensable for meristem cell division (Marschner 1995). It plays a role as a stimulator of several enzymes such as RNA polymerase and superoxide dismutase (SOD). Its deficiency causes growth reduction and leaf chlorosis (Kabata-Pendias 2011).

Conclusion

The results found in this study show that seeds of *P. harmala* harvested from the central drylands of Algeria were able to germinate in moderate concentrations of HMs and appear to be more tolerant/resistant to Zn than to the other HMs tested (Cd, Cr and Pb). The phytotoxicity of the HMs regarding germination and seedling growth, in descending order of damage, was Cd > Pb > Cr > Zn. This information can be considered a contributing step in finding of the tolerance limit of *P. harmala* at different concentrations of treated metals. However, we will conduct further study in near future to evaluate the biomass production and ability to uptake different HMs by this species.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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