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Effect of salt and lead stress on seed germination and root anatomical structure of *Retama monosperma* (L.) Boiss. in Northwestern Algeria

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Abstract
Metal pollution and salinity are the most common problems currently encountered in soils. In this context, this study aimed to evaluate the influence of salt and metal stress on the germination, development and root structure of *Retama monosperma* (medicinal plant, *Fabaceae*) from the Oran (Cap-Falcon region, semi-arid zone).
The seeds were germinated under conditions of increasing salt (NaCl: 0-12 g/L) and lead stress (Pb3 (C6H5O7) 2: 25-500 ppm). The results revealed that the germination percentage decreased with increasing salinity, comparing with that of the control (0 g/L of NaCL). For lead citrate, the seeds present low resistance (reduced germination rate: 12.5%).
The roots reduced in length as the concentrations of the two stresses increased. A microscopic study showed that the structure of this organ was also affected in order to resist the stresses imposed. Thickness of the cortex and the number of xylem cells decreased significantly with salinity, while the cuticle increased in thickness. Bursting of certain parenchymal cells and conductive bundles was observed in the heavy metal treatment.

Key words: *Retama monosperma*, semi-arid, salt, lead stress, germination, structure.

Introduction

Arid and semi-arid zones are characterized by soil salinization which is among the most important factors limiting or inhibiting plant germination and growth. Salinity is due to evaporation of water from the soil and irregular, insufficient rainfall (Mezni et al. 2002) and poorly controlled cultural practices, such as the use of large quantities of water, often brackish (Mouhouche and Boulassal 1999). Added to this ecological constraint is the phenomenon of soil pollution by heavy metals such as lead (one of the most common heavy metals), which is due to industrial discharges. In humans, lead causes poisoning, a disease with serious consequences, even at low doses, especially on the nervous system (Brunet 2008). In general, heavy metals have adverse effects on the environment and human health; they can be the cause of the disappearance of certain animal and plant species (reduction of biodiversity, Gold 2002). In terms of plant physiology, firstly, germination is strongly or completely inhibited by lead (depending on its doses). This metal also strongly reduces the development of the seedling, primary roots and rootlets, enzymatic activities, limitation of root absorption of the soil solution (Bertrand 2008) thus the content of chlorophyll, total phenols, flavonoids, carbohydrates, proteins and proline (Gopal and Reizvi 2008; Banadka and Nagella 2023).

The Fabaceae constitute the basis of our alimentation and the nourishment of herbivores (grazing). In addition, they play an important role in agriculture, by improving soil nitrogen fertility, following their symbiotic association (Selami 2015), thereby limiting the use of chemical fertilizers which cause the environment pollution (Benidire et al. 2015). This is why our work aims to study a plant among the *Fabaceae*; *Retama monosperma* (L.) Boiss, endemic to the Mediterranean basin, which grows in coastal dunes, open forests, rocky pastures on hills and low mountains, semi-arid and arid (Maire 1952-1987). This species is involved in stabilising and revegetating dunes and contributing to the biofertilisation of semi-arid and arid ecosystems (Selami et al. 2014). It is a medicinal plant; used to treat various diseases such as skin diseases, diabetes, rheumatism and hypertension, of which their extracts showing four main pharmacological activities; anticancer, antioxidant, anti-inflammatory and antifungal effects (Benkhouili et al. 2022).

The Oran coast region is characterized by a semi-arid climate and so salinity causes a water deficit with physiological disturbances in seeds and plants, it is for this reason that we were interested in evaluating, the germination capacity of *R. monosperma* seeds, subjected to saline stress and on the other hand to metal stress. Lead was the subject of this present study, chosen for its toxicity. We also determined the impact of these stresses on the structure and growth of the roots of this plant's seeds given that the majority of heavy metals and salts are absorbed by these organs (Pourrut 2008). Aspects of responses of *R. monosperma* to the presence of different concentrations of salt and lead citrate were assessed using parameters of physiological and anatomical approaches.

Materials and methods

Plant material

Retama monosperma seeds used in this study were collected from the Cap-Falcon station (N. 35°45'08.9"N 0°. 47'40.4"W, Oran, Algeria). This site belongs to the semi-arid bioclimatic zone, characterized by hot, dry summers and harsh winters. Relative humidity is 70%. Average temperatures range from 18.5 to 32°C in summer and from 5 to 20°C in winter. Rainfall averages 322 mm. These climatic conditions are compounded by the influence of strong winds, which exacerbate the water deficit.

Seed preparation

The seeds used were first disinfected with 1% sodium hypochlorite for five min, rinsed with distilled water and were then chemically scarified with pure sulfuric acid for 4h to eliminate their tegumentary inhibition and promote germination (the tough envelopes make germination difficult). Afterward, these seeds were washed with distilled water in order to eliminate all traces of this acid (Bouredja et al. 2011).

Impact of salinity and lead on germination

The seeds were germinated in Petri dishes containing filter paper, in the dark and at ambient laboratory temperature (20°C), irrigating them daily with 5mL of different solution, for each batch studied. The germination was identified by the exit of the radicle.

For the salt stress, germination tests were realized using separate solutions of NaCl at concentrations of 0 (control), 2, 4, 6, 8, 10 and 12 g/L, prepared with sterile distilled water.

For the metal stress, the seeds were treated with different doses of lead citrate ($Pb_3(C_6H_5O_7)_2$: 0 (control), 25, 50, 75, 100, 300, and 500 ppm, prepared in sterile distilled water).

The parameters studied are as follows:

- **Germination rate (TG),** expressed as a percentage (%), by the number of seeds germinated (NSG) over the total number of seeds (TNS): TG%= NSG*100/TNS

- **Germination kinetics**, presented by the percentages of seeds germinated daily (Hajlaoui et al. 2007).
- **Germination speed**: a median duration parameter for the time required for 50% germination (T_{50}), calculated according to the following formula (Côme 1970): $T_{50} = t1 + (0.5 - sg1/sg2 - sg1) \times (t2 – t1)$.

  sg1: Percentage of seeds germinated at time t1 whose value is closest to 50% by lower value.

  sg2: Percentage of seeds germinated at time t2 whose value is closest to 50% by higher value

All experiments performed were repeated three times.

Impact of salinity and lead on root development and structure

Root length was measured using a graduated ruler. The histological structure of this organ was observed under a Zeiss optical microscope, using the double staining protocol of Langeron (1934): Transverse sections were made freehand and placed in sodium hypochlorite 6° for 5 min (in order to eliminate the cellular contents). After rinsing with distilled water, these cuts were mordanted in acetic acid during 2 min (for better fixation of stain), then stained with methyl green 1% for 05 min (for coloring lignified walls green). After washing with distilled water, they were stained with Congo red 1% pending 15 min (coloration of cellulosic walls pink), then rinsed with distilled water and mounted between slide and coverslip in a drop of distilled water.

Statistic study

The statistical analysis was performed using the SPSS software program (SPSS 26.0). The data were analyzed by one-way analyses of variance (ANOVA). To detect the statistical significance of differences ($p < 0.05$) between means, the Tukey test was used.

Results

Effect of salinity on germination kinetics, speed and rate of *R. monosperma*

The salinity affected the germination rate of *R. monosperma* seeds (Fig. 1); a decrease in germination rate was observed as the dose of NaCl increased. It should be noted that the seeds of this species have a short latency phase at 0 g/L (control, 4d), 2 g/L NaCl (4 d) and 4 g/L (5 d, comparable results with those of the seeds control). At 6 and 8 g/L, the seeds showed low tolerance with a longer latency period (8 d). No signs of germination were recorded for the doses of 10 and 12 g/L. Concerning the 0, 2, 4, 6 and 8 g/L concentrations, the final germination percentages were 83, 83, 73, 20 and 10%, respectively.

The T50 was estimated at 8, 9 d, respectively for control seeds and those irrigated with the stressed solutions of 2 and 4 g/L. For 6 and 8 g/L, the germination rates did not reach 50%.

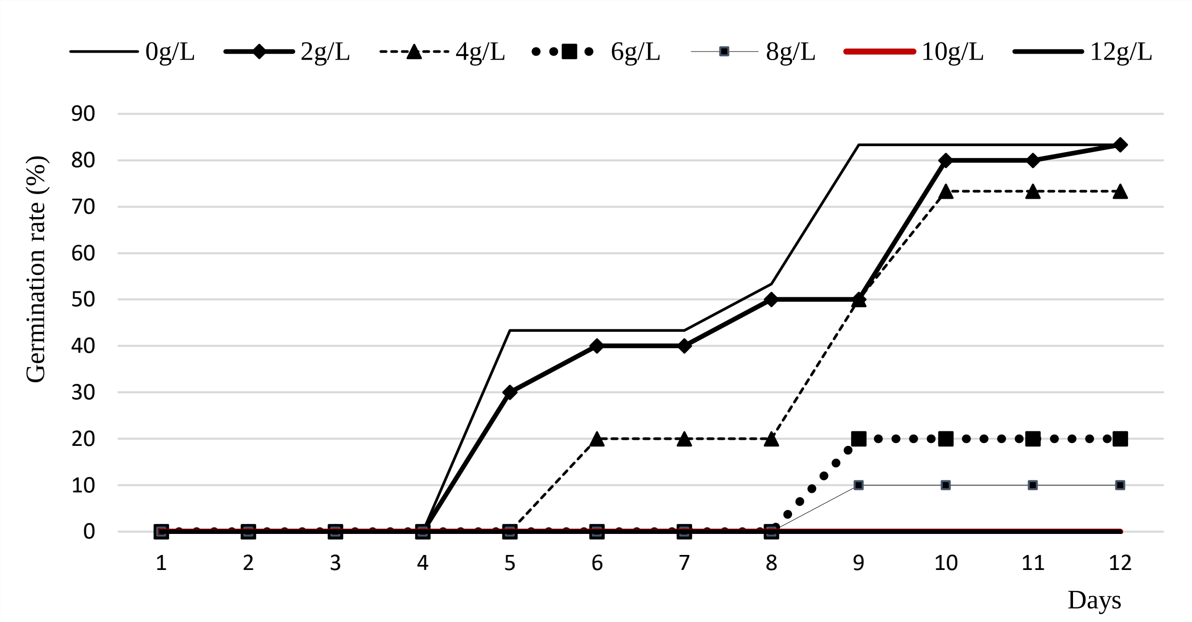

Fig.1 Germination kinetics of *R. monosperma* seeds under the effect of salinity

Effect of salinity on root development and anatomical structure

Analysis of root length measurements (7 days after germination) of *R. monosperma* seedlings, stressed with saline solutions at different concentrations, indicated that the length of this organ is increasingly sensitive to NaCl. The highest value was recorded in the control (5.5±0.5 cm) and the lowest in the case of 8 g/L (1.23 ±0.68 cm, Fig. 2). It appears that salt had a negative impact on root development.

In terms of root histology, distinct structural alterations are visible. In order to resist this stress, particularly at 8 g/L NaCl, we observed a reduction in the thickness of the cortical parenchyma zone, a decrease in the number of xylem cells, and an increase in the thickness of the cuticle (lipid layers, Fig. 3).

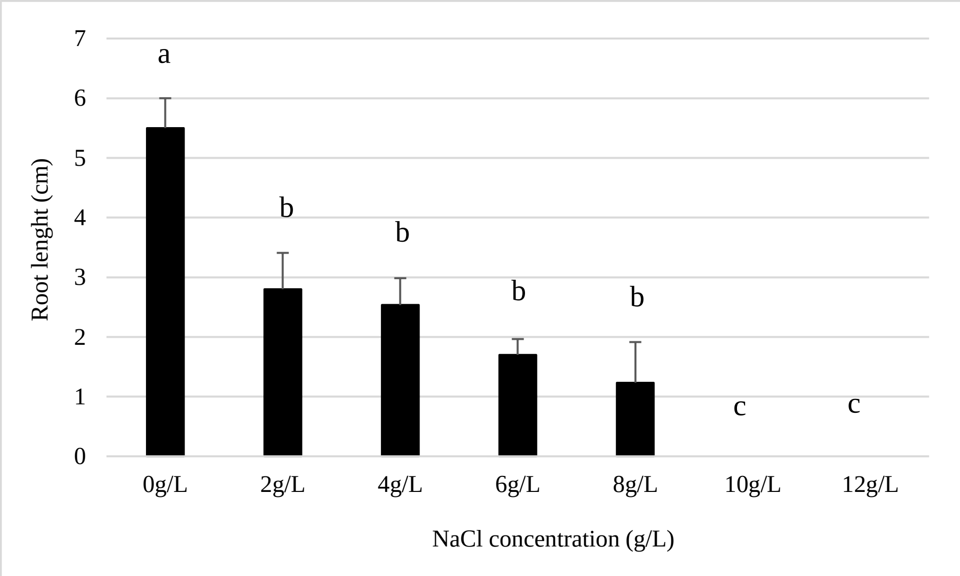

Fig.2: Length of 7-days-old *R. monosperma* roots as a function of different NaCl concentrations.

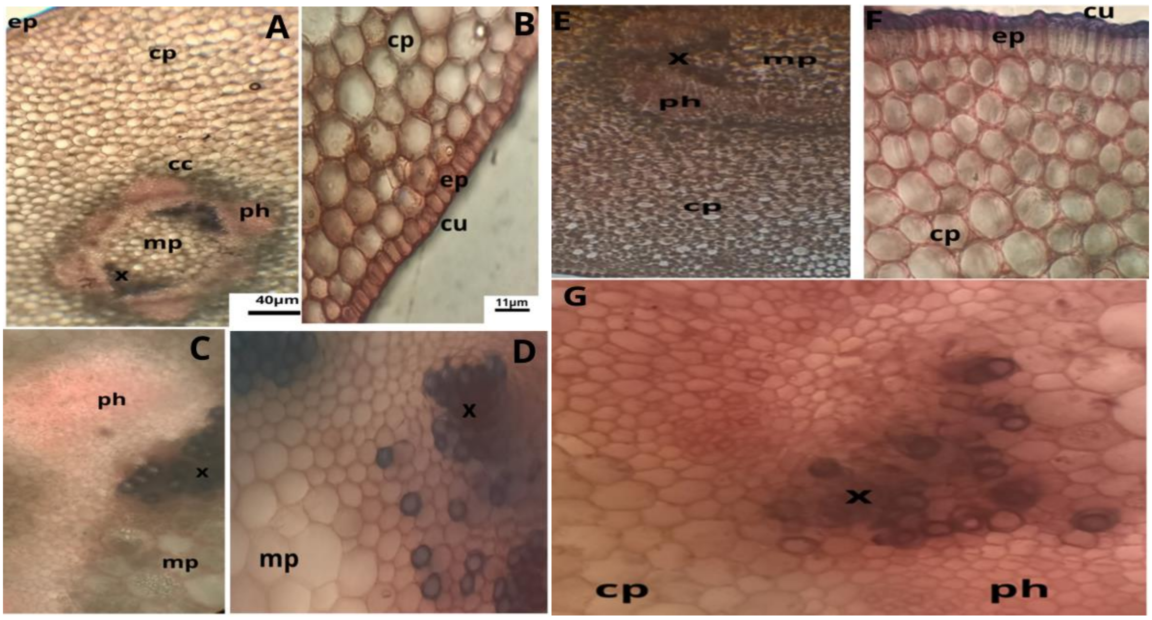

Fig.3: Anatomical structure of *R. monosperma* roots aged seven days as a function of salinity. cc, central cylinder; cp, cortical parenchyma; cu, cuticle; ep, epidermis; mp, medullary parenchyma; ph, phloem; x, xylem. A, photo overview of control root (x100); B, detail of control root cuticle (x400); C, detail of control root phloem (x400); D, detail of control root xylem (x400); E, photo overview of root (reduction in cortical parenchyma) in the presence of 8g/L NaCl (x100); F, detail of root cuticle (increase in thickness) in the presence of 8g/L NaCl

(x400); G, detail of root xylem (reduction in xylem cell numbers) in the presence of 8g/L NaCl (x400).

Effect of lead on germination kinetics and rate of *R. monosperma*

The germination curves for *R. monosperma* seeds stressed by lead citrate at different concentrations (25, 50, 75, 100, 300 and 500 ppm, Fig. 4) appeared very low germination percentages (12.5%). The lag phase was also significantly increased by this metal stress, of which the longest duration observed in the 50, 75, 100, 300 and 500 ppm concentrations (22 d), compared with the controls (4d) and 25 ppm dose (10 d). Our results therefore revealed the negative effect of this heavy metal on germination kinetics. All the seeds germinated in the presence of $Pb_3(C6H5O7)_2$ at different concentrations did not reach 50%. The germination rate therefore decreased.

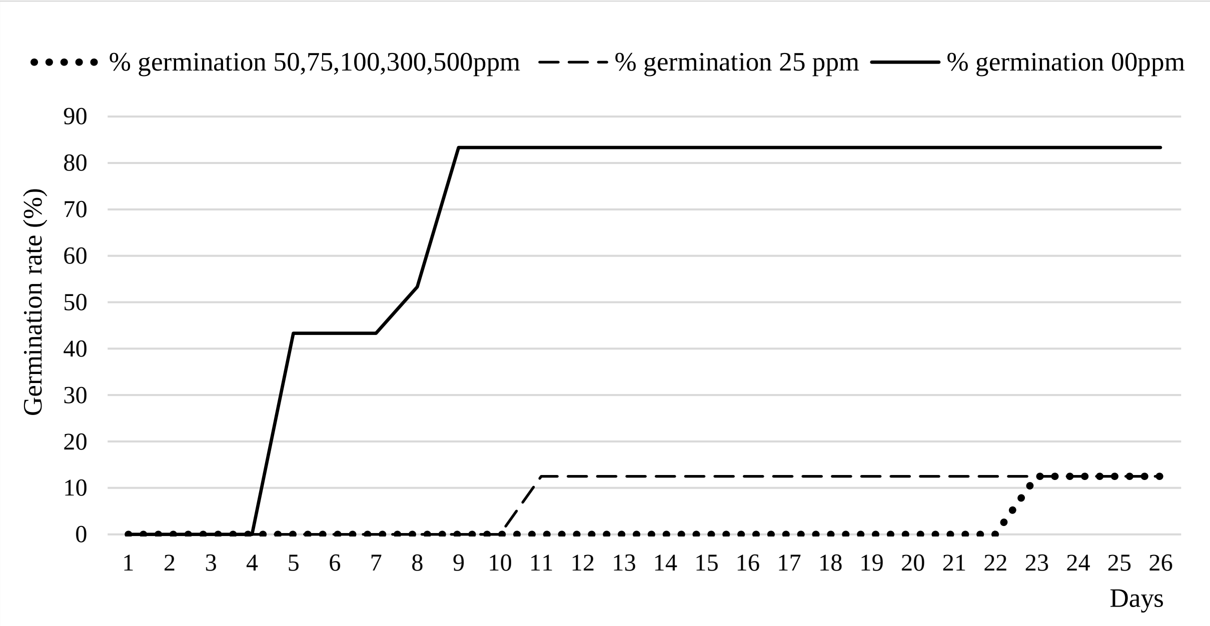

Fig.4: Germination kinetics of *R. monosperma* under the effect of lead

Effect of lead on root development and histological structure

Compared with control, A highly significant decrease in root length (aged 7 days after germination) was noted as a function of the increase in the concentration of $Pb_3(C6H5O7)_2$. For the lot irrigated with 500 ppm, we have mentioned 1.5±0.5 cm of length (versus 5 cm at 0 ppm, Fig. 5).

Microscopic observation of the roots of *R. monosperma* aged 7 days (Fig. 6) has highlighted several structural modifications in this organ as the lead dose increases, such as rupture of certain cells of cortical, medullary parenchyma, and phloem, thus change in xylem structure and arrangement.

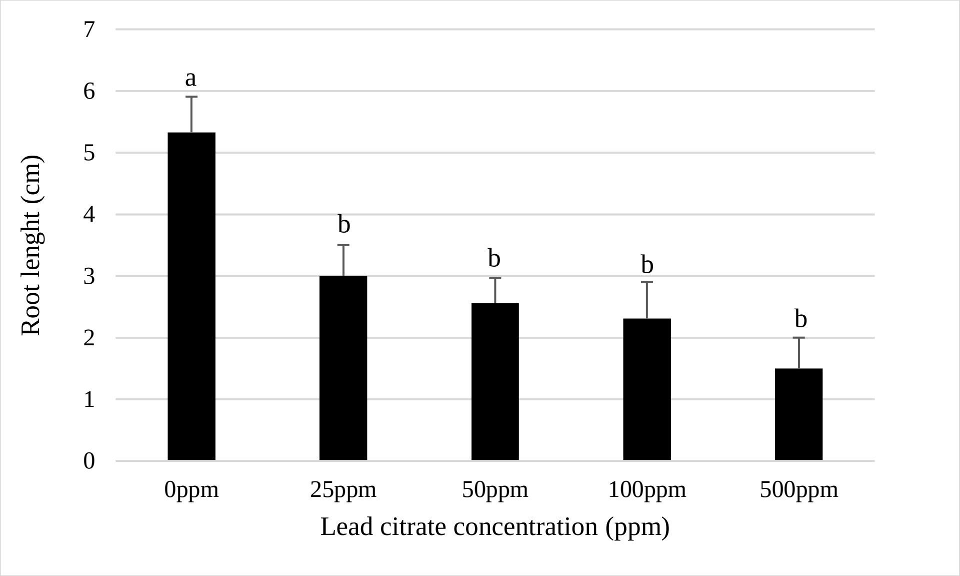

Fig.5: Length of 7-day-old *R. monosperma* roots as a function of different lead concentrations.

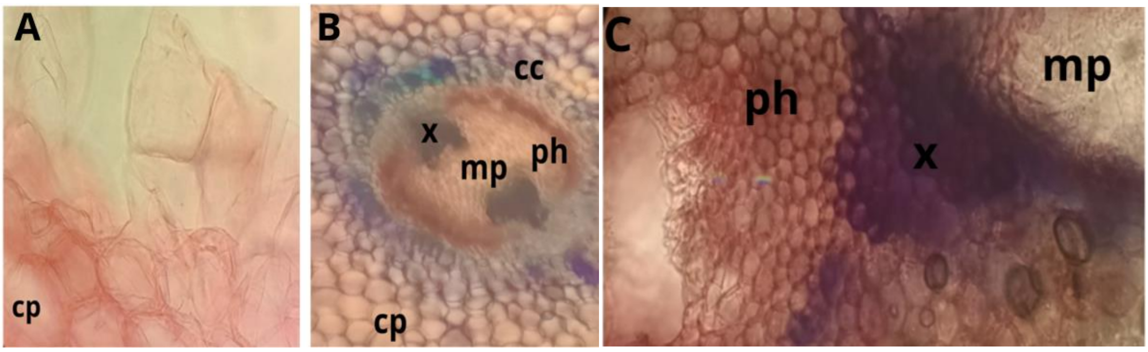

Fig.6: Histological structure of of *R. monosperma* roots aged seven days, in the presence of lead citrate at 500ppm. cc, central cylinder; cp, cortical parenchyma; mp, medullary parenchyma; ph, phloem; x, xylem. A, detail of root cortical parenchyma (rupture of certain cells) (x400); B, overview of root central cylinder (changing the structure and layout of the conductor bundles) (x100); C, detail of root xylem (modification of cell arrangement), phloem and medullary parenchyma (rupture of certain cells) (x400).

Discussion

R. monosperma could be a good indicator of the presence of salt and heavy metals in soils. These two stresses had a negative influence on the seeds germination and root development of this plant, like most other plant species. The reduction in germination capacity could be explained by an alteration of the enzymes present in the seed by the doses of NaCl and Pb3 (C6H5O7)2 used (Bertrand 2008).

According to Muñoz Vallés et al. (2013), *R. monosperma* is able to exhibit some tolerance to soil salinity, having leaves falling rapidly after emergence and carrying out gas exchange in photosynthetic stems (cladodes), thereby reducing salt-induced stress. However, the morphology of the shoots with branching of the stems and the small size of the leaves (numerous and pubescent), minimize the quantity of salt deposited on this plant species. The salinity of dune soil is generally low due to the very rapid leaching of salt from the sand by rain (Muñoz Vallés et al. 2011).

By comparing the species studied with other species of the *Fabaceae* family, we find that our results of salt stress agree with those obtained by several researchers, such as the case of *Retama raetam* (whose the tolerance threshold was recorded inhibited at 272 mM, Mehdadi et al. 2017), soybean (*Glycine max,* tolerant to 15g/L with a rate of 40%) and cowpea (*Vigna unguiculata*, tolerant to 15g/L with a rate of 13%, Brahima et al. 2018), beans (*Phaseolus vulgaris,* the absence of germination was recorded at concentrations >5000 mg/L, Tsamo et al. 2022). In faba beans (*Vicia faba*), the effect of salinity is characterized by an increase in latency time, a decrease in germination speed and rate, especially at the concentrations of 150 and 200 mM (Benidire et al. 2015). According to these authors, there are two effects of this stress; osmotic (reversible) and toxic (irreversible). For *Lotus corniculatus* (forage species), is moderately salt tolerant, of which the germination rate decreased, shoot length decreased and higher tannin content remarked under NaCl stress in vitro (Beyaz and Kazankaya 2024). Moreover, Fernandes et al. (2004) have reported that in lupine (*Lupinus albus*), the glucose content decreased with salt increase (glucose demand by respiration cells in order to ensure a supply of ATP and NAD (P) H in repairing the damage caused by salt), but the sucrose content increased (a physiological adaptation mechanism) in plants treated with 150 mM NaCl (rise of sucrose synthase activity).

In seeds from other botanical families, the same findings have been reported, such as Aleppo pine (*Pinus halepensis*, *Pinaceae*, Zouidi et al. 2019) and *Lygeum spartum* (*Poaceae*, Zouidi et al. 2023). By Ayarpadikannan et al. (2012), *Suaeda asparagoides* (*Chenopodiaceae*) genes (*SaRBP1* and *SaDhn*) exert abiotic salt stress tolerance effects in yeast that could contribute to accumulating genetic resources to improve osmotic tolerance in plants.

The conclusions obtained concerning of NaCl effect on root development are in accordance with those of previous works such as those of Benidire et al. (2015), Dolatabadian et al. (2011), Tsamo et al. (2022) and Beyaz and Kazankaya (2024). The observations of microscopic structure are consistent with those of Benidire et al. (2015). According to these authors, the length and density of absorbent root hairs of faba bean are also strongly affected by salinity. Dolatabadian et al. (2011) mentioned that this stress significantly increased the thickness of the cuticle and decreased the cortex in soybean stem. Furthermore, the formation and arrangement of xylem were modified with increased deposition of lignins. These authors suggested that the cuticle plays an essential role in plant tolerance through its ability to delay the onset of cellular dehydration disorders during stress. In study of Cachorro et al. (1993), the salt stress was also associated with a greater deposition of lignin in the development of the xylem of the bean root, which was suggested to be a factor inhibiting the growth of this organ and therefore, representing an adaptation mechanism.

The decrease in germination rate as a function of increasing lead may be due to the inhibitory effect of this heavy metal on the germination of *R. monosperma*. Similar results were noted on the seeds of several plant species, by increasing the doses of lead, such as *Nigella sativa*, *Nigella damascena* (*Ranunculaceae*, Gedik et al. 2023) and beans (level zero was at a dose of 200 µmol/L, Tsamo et al. 2022). Souahi et al. (2021) cited that the germination kinetics of *Atriplex halimus (Amaranthaceae)* were significantly affected by heavy metals. In addition, several studies have shown that the addition of Ca2+, Mg2+ or K+ ions to the external environment significantly inhibits the penetration of lead into plant roots (Bertrand 2008). The findings of roots development in presence of this metal joined those of Gedik et al. (2023), Souahi et al. (2021). The same declarations were reported on the growth of bean shoots (Tsamo et al. 2022). By Bertrand (2008), lead has a strong affinity for soil constituents. It mainly enters the plant via the roots. It can bind to the carboxyl groups of the uronic acids composing the root mucilage or directly to the polysaccharides present on the surface of the rhizodermal cells. This heavy metal, after attaching to this tissue, penetrated the root system passively and followed the water conduction system. This absorption is not uniform along the root, since there is a lead concentration gradient in the tissues from the apex, which is the most concentrated zone. Young tissues, and in particular the apical zone where the cells still have thin walls, are the areas absorbing the most lead.

Conclusion

This research has revealed that salinity and lead reduced the germination thus root development of *Retama monosperma*. The germination kinetics in the presence of NaCl solutions at 2 and 4 g/L followed those of the control, whose seeds had a significant germination capacity (about 70-80%). At concentrations of 6 and 8 g/L, a weakly tolerance to this salt has been noted (10-20% of germination). Under lead stress, these seeds presented a reduction of germination starting from 25 ppm dose. The histological structure of root was also affected by these two stresses such as decrease in conductive bundles number. To better understand the development of this plant, we propose to study other morphological, physiological and biochemical parameters such as the effect of saline and metal stress on the number and surface of leaf, the height and structure of the seedlings, the content of chlorophyll, total phenols, flavonoids, proline and soluble sugars (stress metabolites), highlighting the necessity of further investigation into its mechanisms of adaptation and its use in phytoremediation techniques.

Statements and Declarations

Ethical Statement

This research does not involve the use of any type of animal, human, blood or cell line.

Conflict of interest

The authors declare no conflicts of interest.

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