Environmental Health Engineering and Management Journal 2023, 10(1), 17-22
http://ehemj.com
doi 10.34172/EHEM.2023.02
Environmental Health Engineering and Management Journal

Original Article

Phytoremediation by using *Atriplex halimus* subsp. *schweinfurthii* as a bio-absorbent of Cadmium in the soil

Abdelghafour Doghbage[1*], Hassen Boukerker[2], Fathi Abdellatif Belhouadjeb[1], Bouzid Nedjimi[3]

[1]Centre de Recherche en Agropastoralisme (CRAPAST), Djelfa,17000, Algeria
[2]Centre for Scientific and Technical Research on Arid Regions (CRSTRA) Biskra, Algeria
[3]Faculty of Natural and Life Sciences, Ziane Achour University of Djelfa, Algeria

Abstract

Background: The problem of soil contamination is a major concern of emerging countries. Heavy metals such as lead, cadmium, copper, zinc, and mercury cannot be biodegraded, and therefore, persist in the environment for long periods. It has become essential to develop efficient techniques for decontamination of polluted soils. One of these techniques, phytoremediation, is economically viable and compatible with environmental preservation policies. The application of phytoremediation in salty areas requires the use of plants that are able to tolerate heavy metals and high soil salinity. *Atriplex halimus* is one of the species with a great purification potential, which is a spontaneous halophyte species, and endowed with a fairly important aerial and root biomass. This study aimed to determine the effect of cadmium sulfate ($CdSO_4$) on the germination and growth of *A. halimus*, and to assess its ability to accumulate cadmium and proline.

Methods: This is an experimental study of the tolerance of *A. halimus* to increasing concentrations of $CdSO_4$ by an in vivo test. This study aimed to assess the degree of tolerance of this perennial species towards different concentrations of $CdSO_4$.

Results: The results show a non-significant effect of $CdSO_4$ on the seed germination rate and growth of the aerial and root parts. However, a significant accumulation of proline was observed especially in the aerial part, whereas the highest cadmium content was recorded at the roots.

Conclusion: According to the results, this species can be used in phytoremediation to decontaminate soils contaminated by heavy metals.

Keywords: Heavy metals, Phytoremediation, *Atriplex*, Germination, Cadmium, Proline

Citation: Doghbage A, Boukerker H, Belhouadjeb FA, Nedjimi B. Phytoremediation by using *Atriplex halimus* subsp. *schweinfurthii* as a bio-absorbent of Cadmium in the soil. Environmental Health Engineering and Management Journal 2023; 10(1): 17-22. doi: 10.34172/EHEM.2023.02.

Article History:
Received: 2 February 2022
Accepted: 2 August 2022
ePublished: 12 February 2023

***Correspondence to:**
Abdelghafour Doghbage,
Email: doghbage_abdelghafour@yahoo.com

Introduction

Many parts of the world, particularly the vicinity of urban and industrial areas, are heavily polluted by heavy metals, usually produced by human activities. Among the metals present in nature, Cd, Cu, Hg, Ni, Pb, and Zn are considered as the most dangerous metals that make a major problem for the environment and human health. They persist in the environment and inevitably accumulate. They can migrate to surface water or groundwater or enter the food chain via plants to be found in animals, and eventually, in humans. Moreover, soil decontamination technology is often heavy and costly, which do not allow their application in large areas, another alternative may reside in the use of phytoremediation (1,2). This technique, which has only grown in importance for last years, is based on the use of hyperaccumulator plants able to absorb the metals from the soil and concentrating them in their parts, which will be harvested and subjected to treatment to recover the metals. *Atriplex halimus* subsp. *schweinfurthii* is one of these species with a great purification potential, which is a halophyte species that tolerates the conditions of aridity (drought and salinity) well, spontaneously in the arid and semi-arid Mediterranean region. It belongs to the Chenopodiaceae family, and has a fairly important aerial and root biomass.

In order to improve this technique, the laboratory study focuses on the research of new accumulating species with high biomass production, and also, the understanding of the mechanisms involved in the tolerance and accumulation of Cd.

This study aimed to enhance an indigenous halophyte species through a germination test and in vivo culture applying stress based on cadmium sulfate cadmium sulfate ($CdSO_4$), to use it in phytoremediation purpose.

Materials and Methods

Materials

Plant material

The seeds of *Atriplex halimus* come from El Mesrane region in Djelfa (Algeria), specifically in the Chott Zahrez area. The seeds were harvested on a tuft of *A. halimus* showing a good vegetative state. After manual dehulling of their fruiting valves (bracteoles), the seeds were disinfected by a 10-minute stay in 70% ethanol, followed by 10 minutes in 8% oxygenated water, and then, they were rinsed five times with distilled water to remove any trace of disinfectant.

Cadmium salt

The variation factor considered is the concentration of Cd SO_4, this salt was chosen because of its predominance in the soil solution in its natural state (3). To determine the effect of $CdSO_4$ on the germination and growth of *A. halimus* and to define the critical sensitivity level of this halophyte species, different concentrations of $CdSO_4$ were used, thus, four concentrations of cadmium salt (0, 0.01, 0.1, and 1 mmol $CdSO_4$) were retained in this study.

Methods

Germinative test

The seeds were germinated in lots of 100 seeds for each treatment in the Petri dishes, with to 25 seeds/Petri dish, and four repetitions per treatment (0, 0.01, 0.1, and 1 mmol $CdSO_4$). The filter paper was moistened initially, and then, with 5 ml of distilled water (witness) or different solutions of $CdSO_4$ every 24 hours. The Petri dishes were placed in an incubator (Figure 1), whose temperature was set at 20 ± 1°C. The germinated seeds were counted every 24 hours, as a germination criterion, until the appearance of a 1-mm root.

In vivo cultivation

The seeds were sown directly in plastic pots (10 cm of diameter) filled with a peat-sand mixture (Figure 1), at a rate of 10 seedlings per pot. These seedlings are irrigated by distilled water for 30 days after sowing, and then, the stress was applied for 7 days by different solutions of $CdSO_4$. The laboratory experiments took place in controlled environments with an ambient temperature and a photoperiod of 12 hours of lighting ensured by a luminous device at the Institute of Agro Pastoralism (Djelfa).

The measurements were performed after 37 days of cultivation, and on 10 seedlings for each treatment. The growth indicators considered include:

- The fresh matter of the shoot and root part
- The dry matter of the shoot and root part
- The water content of the shoot and root part

The method used for the determination of proline is that mentioned by Troll and Lindsley (4) simplified by Dreier and Goring (5), and the content of cadmium in the plant was determined using the method of Chardonnens et al (6).

The experiment corresponds to 4 treatments, and each concentration was tested on 10 plants with 10 repetitions. The experimental plan used was the randomized complete block design (RCBD), where the treatments correspond to the different concentrations of $CdSO_4$ (Table 1).

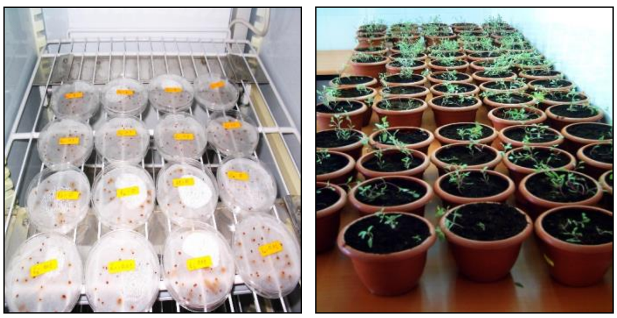

Figure 1. Germination test and in vivo cultivation of *Atriplex halimus* seeds

Results

The germination test

The results show that the germination percentage of *Atriplex halimus* seeds obtained after 7 days varied with different concentrations of $CdSO_4$ (Figure 2). In the absence of $CdSO_4$, the germination rate was equal to 97%. Increasing the concentration of cadmium sulphate slightly decreased the rate of germination compared to the witness. For treatments of 0.01 and 0.1 mmol, the germination percentages were 88% and 91%, respectively.

Table 1. The experimental plan of the different concentrations of $CdSO_4$ treatments

T_2	T_0	T_0	T_2	T_1	T_0	T_0	T_3	T_3	T_0
r_9	r_9	r_3	r_7	r_{10}	r_{10}	r_4	r_3	r_7	r_2
T_3	T_1	T_3	T_1	T_0	T_1	T_2	T_1	T_3	T_2
r_9	r_7	r_{10}	r_1	r_5	r_2	r_3	r_5	r_4	r_8
T_0	T_0	T_1	T_0	T_3	T_1	T_3	T_1	T_2	T_2
r_8	r_1	r_6	r_7	r_1	r_8	r_2	r_4	r_5	r_{10}
T_0	T_1	T_3	T_2	T_2	T_2	T_3	T_3	T_1	T_2
r_6	r_3	r_8	r_2	r_4	r_6	r_5	r_6	r_9	r_1

T_0: Distilled water (witness). T_1: 0.01 mmol $CdSO_4$. T_2: 0.1 mmol $CdSO_4$. T_3: 1 mmol $CdSO_4$.
r: Repetition.

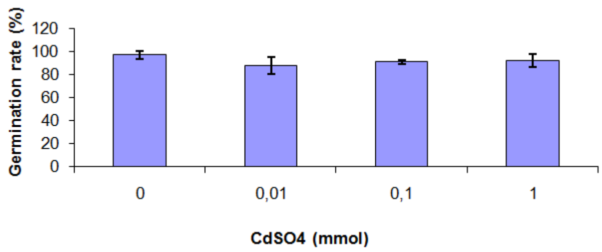

Figure 2. Effect of increasing doses of $CdSO_4$ on germination percentage of *Atriplex halimus* subsp. *schweinfurthii* seeds

With a quantity of $CdSO_4$ equal to 1 mmol, the germination rate remained fairly high (92%). The analysis of variance shows a non-significant difference between the different treatments used of $CaSO_4$ ($P > 0.05$, $F = 2.15$).

In vivo growth and development of plants

Fresh matter (shoot and root part)

In the absence of Cd SO_4, the average weight of the fresh matter (aerial part) was equal to 111.39 mg/plant, the addition of $CdSO_4$ is manifested by a very slight decrease in seedling biomass compared to the witness particularly at 0.01 mmol and 0.1 mmol of $CdSO_4$ (Figure 3). With a concentration of 1 mmol ($CdSO_4$), there was a decrease in fresh matter up to 86.1 mg/plant. According to the result of the analysis of variance, there was no significant difference between treatments of $CdSO_4$ used ($P > 0.05$, $F = 0.65$).

The effect of the addition of $CdSO_4$ is noticed by a slight increase in the production of fresh matter in the root part compared to the witness, it was up to 2.94 mg/plant for a concentration of 1 mmol of $CdSO_4$ (Figure 3). The ANOVA table shows that the factor studied did not have a significant influence on the production of root fresh matter ($P > 0.05$, $F = 1.59$).

Dry matter (aerial and root part)

Based on the analysis of variance, the different concentrations of $CdSO_4$ did not have a significant effect on the dry matter aerial part ($P > 0.05$, $F = 0.19$). Increasing concentrations of $CdSO_4$ particularly at 1 mmol led to a slight decrease in aerial dry matter (Figure 4). The results obtained show that the concentrations of 0.01 and 0.1 mmol $CdSO_4$ increased the production of the dry matter in the roots compared to the witness, with respective averages equal to 0.47 and 0.49 mg/plant. Also, when the concentration increased to 1 mmol of $CdSO_4$, the production of dry matter (root part) decreased to 0.38 mg/plant (Figure 4).

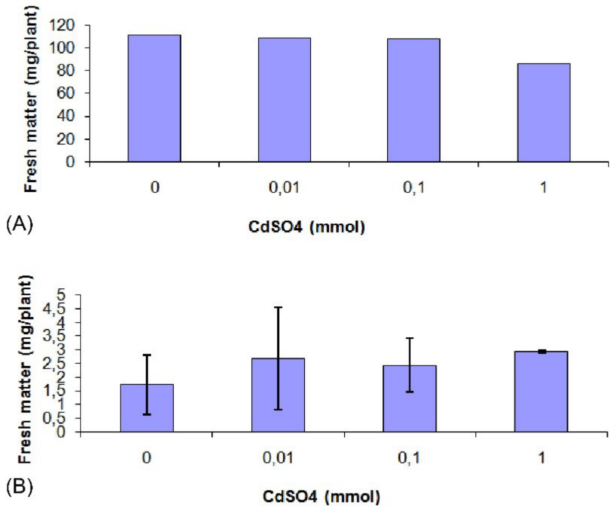

Figure 3. The weight of the fresh matter aerial part (A) and root (B) (mg/plant) of seedlings of *Atriplex halimus* subsp. *schweinfurthii* grown in vivo with different concentrations of $CdSO_4$

Accumulation of proline

The aerial part

The accumulation of proline in the aerial part progressed according to the $CdSO_4$ concentration, it started at 0.01 mmol where its content was doubled compared to the witness (0.06 and 0.11 µg), this increase continued up to 0.22 µg for the highest concentration (1 mmol $CdSO_4$) (Figure 5). The results of the analysis of variance show that the increased concentration of $CdSO_4$ had a highly significant effect on the accumulation of proline in the tissues of the aerial part of *A. halimus* subsp. *schweinfurthii* ($P < 0.001$, $F = 163.68$).

The root part

Concerning the root part, the results show that the proline content in the roots recorded an increase as soon as the plant received 0.01 mmol of $CdSO_4$, until it reached 0.12 µg at a concentration of 1 mmol of $CdSO_4$ (Figure 5). The results of the analysis of variance show that there is a highly significant difference between the different

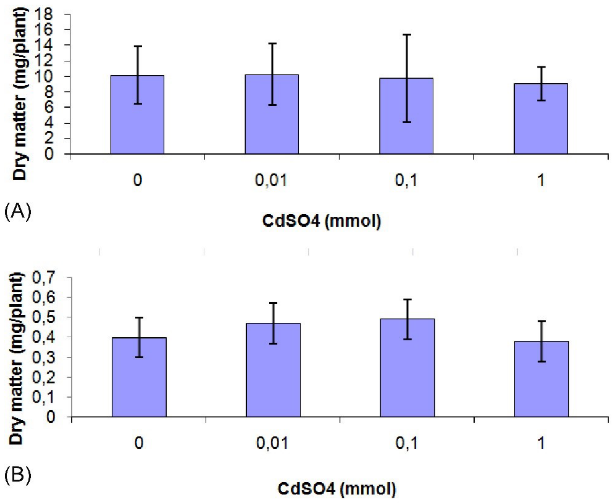

Figure 4. The weight of the dry matter aerial part (A) and root (B) (mg/plant) of seedlings of *Atriplex halimus* subsp. *schweinfurthii* grown in vivo with different concentrations of $CdSO_4$

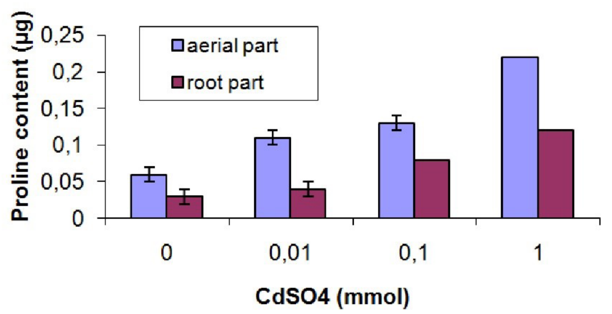

Figure 5. Proline content (µg) in the aerial and root part of *Atriplex halimus* subsp. *schweinfurthii* grown in vivo with different concentrations of $CdSO_4$

treatments tested ($P < 0.001$, $F = 77.08$).

Accumulation of cadmium

The aerial part

The cadmium contents measured in the tissues of the aerial part of *A. halimus* seedlings show a very significant increase in the Cd content at 1 mmol $CdSO_4$ with an average of around 8.97 mg/L compared to the rest of the treatments (Figure 6). The analysis of variance data (Table 2) reveals a very highly significant difference between the different $CdSO_4$ treatments ($P < 0.001$, $F = 554353.31$).

The root part

The results obtained from the root part are similar to those recorded for the aerial part, they show that the presence of 1 mmol $CdSO_4$ led to a significant increase in the cadmium content in the root part (13.66 mg/L) compared to the witness (0.36 mg/L) (Figure 6). According to the results of the analysis of variance, the different doses of $CdSO_4$ had a highly significant effect on the Cd content accumulated in the roots ($P < 0.001$, $F = 623279.31$).

Discussion

The effect of different concentrations of $CdSO_4$ (0.01, 0.1, and 1 mmol) on the germination of *A. halimus* subsp. *schweinfurthii* was evaluated during one week of treatment. The results show that seeds of this species were characterized by their rapid germination and low sensitivity to high concentrations of $CdSO_4$. The germination rate remained above 80% for all the treatments applied. The results are consistent with those reported by Yamaguchi et al (7) who worked on another halophyte *Arabidopsis thaliana* cultivated in the presence of cadmium. On the other hand, the results of Huybrechts et al (8) showed the impossibility of some species to germinate in soils polluted by cadmium.

The study of the growth of *A. halimus* subsp. *schweinfurthii* seedlings during the first month cultivated in vivo in the presence of the different concentrations of $CdSO_4$ showed that the recorded change in the fresh and dry biomass of the seedlings is not very important compared to the witness, this fact demonstrates a certain tolerance of this species towards cadmium. It should be noted that the tolerance of the root part remains higher than the aerial part, as the most important values of the fresh and dry matter were recorded by the concentrations of 1 mmol and 0.1 mmol $CdSO_4$, respectively. These results are consistent with those reported by Shevyakova et al (9) who investigated the adaptation of a halophyte species (*Mesembryanthemum crystallinum*) in the presence of cadmium chloride. They found that there was only a very low correlation between the decrease in aerial biomass, and also, in the root system in a concentration of 1 mmol of $CdCl_2$, and the accumulation of heavy metals in these organs. Moreover, during the experiment of the present study, the applied stress had no effect on the survival of the seedlings of *A. halimus* subsp. *schweinfurthii*, and all seedlings were kept alive until the end of treatment.

From an ionic point of view, it has been found that the more stress intensity of $CdSO_4$ is growing the more cadmium accumulation increases in the different organs. Indeed, the highest cadmium contents were recorded for the dose of 1 mmol $CdSO_4$ (13.66 mg/L at root part

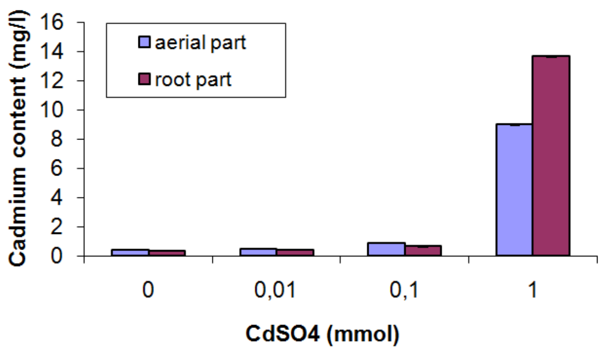

Figure 6. Cadmium content (mg/L) in the aerial and root part of *Atriplex halimus* subsp. *schweinfurthii* grown in vivo with different concentrations of $CdSO_4$

Table 2. Analysis of the variance of the parameters measured for different concentrations of $CdSO_4$

$CdSO_4$ (mmol)	Concentrations			
	0	0.01	0.1	1
Germination rate (%)	97±3.8[a]	88±7.3[a]	91±2[a]	92±5.6[a]
Fresh matter aerial part (mg/plant)	111.39±0.03[a]	108.65±0.04[a]	108.34±0.06[a]	86.1±0.02[a]
Fresh matter root part (mg/plant)	1.73±1.09[a]	2.68±1.88[a]	2.44±0.99[a]	2.94±0.05[a]
Dry matter aerial part (mg/plant)	10.15±3.7[a]	10.23±3.9[a]	9.76±5.65[a]	9.01±2.14[a]
Dry matter root part (mg/plant)	0.4±0.21[a]	0.47±0.14[a]	0.49±0.15[a]	0.38±0.13[a]
Proline content (µg) (aerial part)	0.06±0.01[d]	0.11±0.01[c]	0.13±0.01[b]	0.22±0.001[a]
Proline content (root part) (µg)	0.03±0.01[c]	0.04±0.01[c]	0.08±0.001[b]	0.12±0.001[a]
Cadmium content (aerial part) (mg/L)	0.43±0.001[c]	0.44±0.008[c]	0.88±0.001[b]	8.97±0.017[a]
Cadmium content (root part) (mg/L)	0.36±0.012[c]	0.37±0.007[c]	0.65±0.01[b]	13.66±0.02[a]

[a,b,c,d] Mean separation within lines, by Tukey's test ($P < 0.05$). Values with the same letters are not significantly different. Significance at $P < 0.05$.

and 8.97 mg/L in the aerial part), it should be noted that the roots were characterized by a large capacity of accumulation of the Cd compared to the aerial part. All these facts clearly demonstrate a significant protective function (barrier) of the root system, which limits the influx of cadmium into the upper organs of the plant even in the presence of toxic concentrations. Similar results were reported in *Brassica juncea* by Liang Zhu et al (10) and in *Lupinus albus* by Costa and Spitz (11). The study of Das et al(12) shows that intracellular capture of cadmium is carried out by Phytochelatins, which are small peptides rich in sulfur, these peptides are strongly synthesized in the presence of cadmium by a cytoplasm enzyme. The Cd-PC complexes make it possible to reduce the toxicity of Cd, they are accumulated preferentially in the vacuoles of the roots, and this mechanism delays the translocation of Cd to the aerial parts of the plant.

In the presence of cadmium, in "resistant" plants, the activity of acid phosphatase and peroxidase is increased, and these materials accumulate preferentially the cadmium in their roots compared to non-tolerant plants. Similar responses have been described for maize (13), pea (14,15), poplar (16) and for barley (17) in the presence of cadmium. Similarly, the applied stress levels induced a significant increase in proline levels in the aerial and root parts of seedlings of *A. halimus* subsp. *schweinfurthii*. In fact, the accumulation of proline increases gradually as the concentration of $CdSO_4$ increases. It was found that the highest proline contents are recorded at the leaves. However, these contents are lower at the root part.

At the cellular level, plant stress tolerance can be expressed through the accumulation of organic osmolytes (18). Most of these osmolytes are nitrogenous components and free amino acids, in particular proline, which contributes to osmotic adjustment (19). Shevyakova et al observed the accumulation of proline in the roots of Mesembryanthemum crystallinum only in the presence of 0.01 mmol $CdCl_2$, compared to the leaves, where the most important quantities of proline are recorded at 1 mmol of concentration of $CdCl_2$ (9).

The accumulation of proline can be caused not only by a direct effect of cadmium ions, but also by the insufficient water absorption, this insufficiency is developed in leaves and roots under stressful conditions of cadmium, the latter causes water absorption damage by the roots (20). Proline plays an important role in the tolerance of plants towards cadmium; it is synthesized as a response to any stress. It can intervene by regulating the internal osmotic pressure by increasing its concentration (21). Additionally, the synthesis of proline can be included in the regulation of cytoplasmic pH. Therefore, it helps in the stabilization of membrane proteins and free proteins, indicating its osmoprotective role (22-24).

Conclusion

This is an experimental study of the tolerance of *A. halimus* subsp. *schweinfurthii* to increasing the concentrations of $CdSO_4$ by an in vivo test. This study aimed to assess the degree of tolerance of this steppe perennial species towards different concentrations of $CdSO_4$.

Regarding the germination of seeds of *A. halimus* subsp. *schweinfurthii*, the $CdSO_4$ concentrations cause a very slight decrease in the germination rate compared to the witness. This shows that the seeds of *A. halimus* subsp. *schweinfurthii*, exhibit a great ability to germinate under highly stressful conditions with a germination percentage greater than 90% at fairly high concentration levels.

The effect of increasing doses of $CdSO_4$ on the growth of *A. halimus* subsp. *schweinfurthii* seedlings grown in vivo was not significant. It was found for the aerial part that the highest fresh and dry matter were recorded for the concentrations of 0.01 and 0.1 mmol $CdSO_4$, so that adding a severe dose equal to 1 mmol induces a slight reduction of the fresh and dry biomass compared to the witness. It was also noted that there is an increase in fresh and dry biomasses compared to the witness for the root part at plants subjected to high concentrations (0.01, 0.1, and 1 mmol $CdSO_4$).

Considering the influence of increasing doses of $CdSO_4$ on the accumulation of Cd, it has been observed that the more the intensity of stress, the more the content of Cd in organs of the plant, this accumulation is greater in the root (13.66 mg/L), which shows the capacity of this plant to accumulate cadmium in its organs, especially at roots. Similarly, the accumulation of proline is one of the most notable manifestations of salinity and water stress, the applied stress levels induced a significant increase in proline content in the leaves and roots of young plants of *A. halimus* subsp. *schweinfurthii*. The highest proline contents were recorded in the part of the leaves with values from 0.11 µg/g dry weight of plant (DW) to 0.22 µg/g DW.

The present study shows that *A. halimus* subsp. *schweinfurthii*, which is a native shrub forage, tolerates high concentrations of Cd, and it is capable of accumulating significant quantities of cadmium, especially at the roots. This study provides the opportunity to use this halophyte species in phytoremediation to decontaminate soils polluted by heavy metals.

Acknowledgments

The authors would like to thank the Directorate General for Scientific Research and Technological Development (DGRSDT), Algeria, for their encouragement to perform this study.

Special thanks addressed to Mr. Abdallah BOUMAKHLEB and Mr. Amar KHADOUMI for the collection of seeds and also to Mr. Safieddine HASSANI for his assistance in the experimental part.

Ethical issues

The authors hereby certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere.

Competing interests

The authors declare that there is no conflict of interests.

Authors' contributions

Conceptualization: Abdelghafour Doghbage, Bouzid Nedjimi.
Data curation: Abdelghafour Doghbage, Bouzid Nedjimi.
Formal Analysis: Abdelghafour Doghbage
Funding acquisition: Abdelghafour Doghbage.
Investigation: Abdelghafour Doghbage.
Methodology: Abdelghafour Doghbage, Bouzid Nedjimi.
Project administration: Abdelghafour Doghbage.
Resources: Abdelghafour Doghbage, Fathi Abdellatif Belhouadjeb.
Supervision: Bouzid Nedjimi.
Validation: Abdelghafour Doghbage, Fathi Abdellatif Belhouadjeb, Bouzid Nedjimi.
Visualization: Abdelghafour Doghbage.
Writing – original draft: Abdelghafour Doghbage, Hassen Boukerker, Fathi Abdellatif Belhouadjeb, Bouzid Nedjimi.
Writing – review & editing: Abdelghafour Doghbage, Hassen Boukerker, Fathi Abdellatif Belhouadjeb.

References

1. Saadallah K, Drevon JJ, Hajji M, Abdelly C. Genotypic variability for tolerance to salinity of N2-fixing common bean (*Phaseolus vulgaris*). Agronomie. 2001;21(6-7):675-82. doi: 10.1051/agro:2001160.
2. Nedjimi B, Daoud Y, Touati M. Growth, water relations, proline and ion content of in vitro cultured *Atriplex halimus* subsp. *schweinfurthii* as affected by CaCl2. Commun Biometry Crop Sci. 2006;1(2):79-89.
3. Sposito G. The Chemistry of Soils. Oxford University Press; 1989. p. 277.
4. Troll W, Lindsley J. A photometric method for the determination of proline. J Biol Chem. 1955;215(2):655-60.
5. Dreier W, Göring H. Der Einfluß hoher Salzkonzentrationen auf verschiedene physiologische Parameter. Wissenschaftliche Zeitschrift der Humboldt-Universität Berlin. 1974;23(6):641-4. [In German].
6. Chardonnens AN, Ten Bookum WM, Kuijper LDJ, Verkleij JAC, Ernst WHO. Distribution of cadmium in leaves of cadmium tolerant and sensitive ecotypes of *Silene vulgaris*. Physiol Plant. 1998;104(1):75-80. doi: 10.1034/j.1399-3054.1998.1040110.x.
7. Yamaguchi S, Smith MW, Brown RG, Kamiya Y, Sun T. Phytochrome regulation and differential expression of gibberellin 3beta-hydroxylase genes in germinating *Arabidopsis* seeds. Plant Cell. 1998;10(12):2115-26. doi: 10.1105/tpc.10.12.2115.
8. Huybrechts M, Cuypers A, Deckers J, Iven V, Vandionant S, Jozefczak M, Hendrix S. Cadmium and plant development: An agony from seed to seed. International journal of molecular sciences. 2019; 20(16), 3971. doi: 10.3390/ijms20163971.
9. Shevyakova IY, Buravov LI, Kushch LA, Yagubskii EB, Khasanov SS, Zorina LV, et al. Radical cation salts of tetrathiotetracene and tetraselenotetracene with photochromic anion [Fe(NO)(CN)5]2–. Russ J Coord Chem. 2002;28(7):487-95. doi: 10.1023/a:1016253328624.
10. Liang Zhu Y, Pilon-Smits EA, Jouanin L, Terry N. Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol. 1999;119(1):73-80. doi: 10.1104/pp.119.1.73.
11. Costa G, Spitz E. Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. Plant Sci. 1997;128(2):131-40. doi: 10.1016/s0168-9452(97)00148-9.
12. Das P, Samantaray S, Rout GR. Studies on cadmium toxicity in plants: a review. Environ Pollut. 1997;98(1):29-36. doi: 10.1016/s0269-7491(97)00110-3.
13. Lozano-Rodriguez E, Hernàndez LE, Bonay P, Carpena-Ruiz RO. Distribution of cadmium in shoot and root tissues1. J Exp Bot. 1997;48(1):123-8. doi: 10.1093/jxb/48.1.123.
14. Dixit V, Pandey V, Shyam R. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J Exp Bot. 2001;52(358):1101-9. doi: 10.1093/jexbot/52.358.1101.
15. Sandalio LM, Dalurzo HC, Gómez M, Romero-Puertas MC, del Río LA. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot. 2001;52(364):2115-26. doi: 10.1093/jexbot/52.364.2115.
16. Schützendübel A, Nikolova P, Rudolf C, Polle A. Cadmium and H2O2-induced oxidative stress in *Populus × canescens* roots. Plant Physiol Biochem. 2002;40(6-8):577-84. doi: 10.1016/s0981-9428(02)01411-0.
17. Guo T, Zhang G, Zhou M, Wu F, Chen J. Effects of aluminum and cadmium toxicity on growth and antioxidant enzyme activities of two barley genotypes with different Al resistance. Plant Soil. 2004;258(1):241-8. doi: 10.1023/B:PLSO.0000016554.87519.d6.
18. Serraj R, Sinclair TR. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 2002;25(2):333-41. doi: 10.1046/j.1365-3040.2002.00754.x.
19. Ashraf M, Harris PJC. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 2004;166(1):3-16. doi: 10.1016/j.plantsci.2003.10.024.
20. Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J. 2002;32(4):539-48. doi: 10.1046/j.1365-313x.2002.01442.x.
21. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59(2):206-16. doi: 10.1016/j.envexpbot.2005.12.006.
22. Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, et al. Does proline accumulation play an active role in stress-induced growth reduction? Plant J. 2002;31(6):699-712. doi: 10.1046/j.1365-313x.2002.01389.x.
23. Baghaie AH, Daliri A. The effect of organic chelates and gibberellic acid on petroleum hydrocarbons degradation in the soil co-contaminated with Ni and crude oil under canola cultivation. Environ Health Eng Manag. 2020;7(1):15-22. doi: 10.34172/ehem.2020.03.
24. Fooladi M, Moogouei R, Jozi SA, Golbabaei F, Tajadod G. Phytoremediation of BTEX from indoor air by Hyrcanian plants. Environ Health Eng Manag. 2019;6(4):233-40. doi: 10.15171/ehem.2019.26.