

## Morphological and Physiological Responses of a Halophyte (Atriplex Halimus) to the Effect of Heavy Metal Case of Cadmium

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## Morphological and Physiological Responses of a Halophyte (*Atriplex Halimus*) to the Effect of Heavy Metal Case of Cadmium

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## MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF A HALOPHYTE (*ATRIPLEX HALIMUS*) TO THE EFFECT OF HEAVY METAL CASE OF CADMIUM

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### ABSTRACT

Today, cadmium (Cd) contamination challenges the environmental quality and food security. This experiment was realized to study the morphological and physiological response of the halophyte species *Atriplex halimus* L to cadmium toxicity by applying different concentrations of Cd (0, 500, 1000, 1500, 2000 ppm) on the plant *A. halimus* for two weeks after 60 days of seeding. The morphological parameters and physiological were evaluated the stem length (SL), root length (RL), leaf area (LA), chlorophylls (a, b, t and carotenoids), and the relative water content (RWC). The results show a decrease in stem elongation, ( $11.333 \pm 3.512$  cm in Cd treatments of 2000 ppm), roots ( $9,500 \pm 3,775$  cm in Cd treatments of 1000 ppm), and leaf area ( $1,675 \pm 0,816$  cm<sup>2</sup> in Cd treatments of 2000 ppm) compared to the control. As well as a decrease in the quantity of chlorophyll which ranged from 1088,308 to 972,383  $\mu\text{g/g}$  FM. Finally, it is observed that the accumulation of heavy metals affects the physiological and morphological status of plants. The findings suggest that *Atriplex halimus* L is tolerant to heavy metals, and could be used for phytoremediation of soils contaminated by trace elements.

**Keywords:** *Atriplex halimus* L. cadmium, morphological parameters, physiological parameters, phytoremediation.

### INTRODUCTION

Environmental harm caused by Heavy metals can bio-accumulate across biological chains, are persistent, non-biodegradable, and have a lengthy biological half-life. If consumed in excess, they can have adverse health effects on humans. (Haware and Pramod, 2011). The actual cause of rapid HMs pollution of arable soils and the environment is various anthropogenic activities and industrial processes, as well as chemical discharges into freshwaters (Souahi et al., 2021a). Because of its high water solubility, relative mobility, and long biological half-life, one of the most dangerous metals for

plants is Cadmium (Wang et al., 2014). When Cadmium is accumulated in the plant tissue, it causes symptoms such as cell death, chlorosis, wilting, and diminished growth (Benavides and Gallego, 2005; Finger-Teixeira et al., 2010). The process of photosynthesis is sensitive to this metal because it harms photosystems I and II (Li et al., 2015; Mesnoua et al., 2016). Recent studies show that *A. halimus* is a hyper tolerant species at high Cd concentrations up to 400 mM (Lefevre et al., 2010; Nedjimi and Daoud, 2009) and Perez-Esteban et al., 2013 indicated that it was a suitable species for phytostabilizing metals in mine soils.

*Atriplex halimus* L. (Chenopodiaceae) est une xérohalophyte pérenne et native des régions méditerranéennes arides et semi-arides (Lotmani and Mesnoui, 2011). This species has adaptations in its morphology, anatomy, biochemistry, and physiology to extreme conditions (Walker and Lutts, 2014). These plants have adapted to the harsh conditions of semi-arid environments (Souahi et al., 2022). The species of perennial plants exhibit strong resistance to a variety of abiotic stressors, such as salinity (Bajji et al., 2002), drought (Martinez et al., 2004), cold (Walker et al., 2008), and TMEs (Mateos-Naranjo et al., 2013; Bankaji et al., 2014). Their tolerance to heavy metals may, at least partly, rely on common physiological mechanisms (Souahi et al., 2021c). This study aims to examine the tolerance mechanisms of this plant to cadmium toxicity at increasing concentrations (0, 500, 1000, 1500, 2000 ppm) on morpho-physiological parameters of *Atriplex halimus* L. and the

phytoremediation potential in contaminated soils.

## MATERIALS AND METHODS

### *Plant Material*

Seeds of *A. halimus* were collected in December 2021 from a wild population growing in the semi-arid Aïn Zitoun area in the province of Oum El Bouaghi, North-East Algeria.

The seeds were placed in Alveolus for one month. Afterward, the seedlings were individually moved to fill pots with soil and placed in a glasshouse. Three times every week, the pots were irrigated (twice with distilled water and once with Hoagland nutrient solution (Hoagland and Arnon, 1950). Five Cd treatments were applied to the plants after 60 days of seedling culture: 0, 500, 1000, 1500, and 2000 ppm Cd (supplied as  $\text{CdSO}_4$ ), for 15 days.

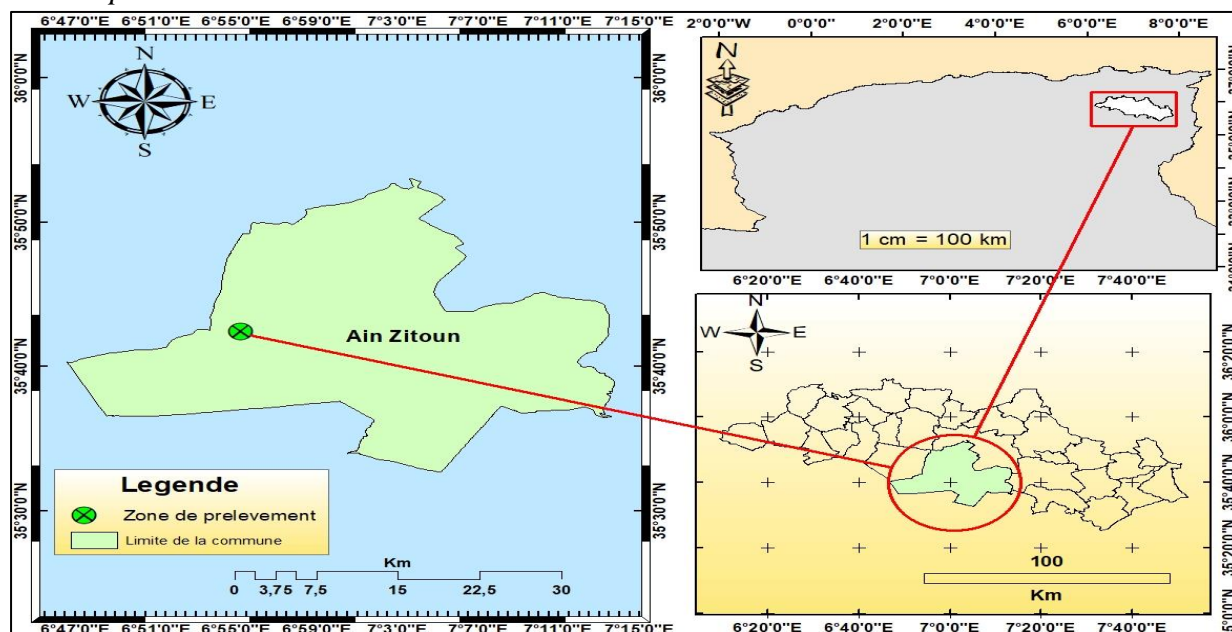


Figure 1: Geographical location of *Atriplex halimus* L. seed collection areas

### *Morphological Parameters*

#### *i. Growth in Length of the Stems and Roots of the Plant*

The growth in length of the aerial and root part of the plant was measured after the application of metal stress. Stem and root length were measured in centimeters (cm).

## ii. Leaf Area

Leaf area (LA) was determined according to the formula of Bezzala, (2005).

$$LA = (\pi \times a \times b) / 4$$

LA: Leaf area in (cm<sup>2</sup>).

a: The length of the limbal in (cm).

b: The width of the limbal in (cm).

## Physiological Parameters

### Relative Water Content (RWC %)

According to the method of Barrs and Weatherley (1962) and Scippa et al. (2004), the relative water content is determined. The following formula is used to calculate the relative water content RWC %:

$$RWC (\%) = [(Fw - dw) / (ftw - dw)] \times 100$$

Fw: fresh weight (g)

dw: dry weight (g)

ftw: full turgor weight (g)

### Determination of the Chlorophyll Content

The extraction of photosynthetic pigments was performed based on the technique of Arnon (1949) in the presence of 80% acetone. In fact 5 ml of 80 % acetone, 100 mg of fresh material was sliced into thin strips. The mixture is kept for 72 hours in total darkness and at 4°C. Chlorophylls "a" and "b" and carotenoids are determined using the extract that was obtained. Using a Jenway spectrophotometer (7035), the level of Chlorophyll (Chl) was determined by measuring the absorbance at wavelengths of 470 nm, 647 nm, and 663 nm. The pigment contents of the leaves were then determined according to the following method Lichtenthaler, (1987), the pigment contents of the leaves were then determined.

## Statistical Analysis

Data processing was performed using R Version 4.1.2. Shapiro-Wilk's and Levene's test were applied to test for normality and variance homogeneity across treatments, respectively.

## RESULTS

### Stems and Roots Length

The results of the length (Stem, Root), Leaf area for the different concentrations of Cd applied to the demonstrated *Atriplex halimus* is shown in Table 1. In general, application of Cd decreases vegetative traits. Cd application decreased stem, root length and leaf area under 500 ppm to 2000 ppm treatments compared with the control.

#### i. Stems Length

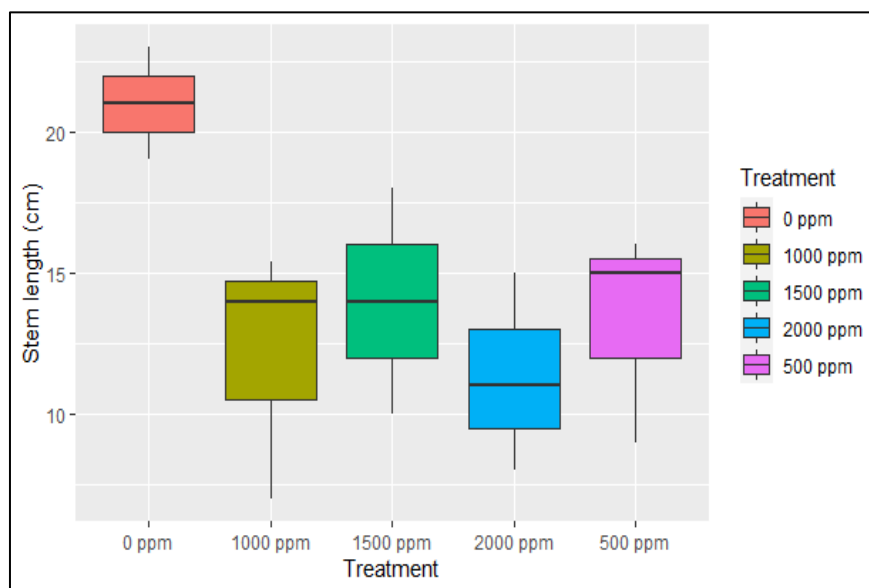
The Boxplots in Fig. 2 shows that treatment with high Cd reduced stem length in the plant *A. halimus*. The maximum stem length in *A. halimus* was shown in control (21.000 ± 2.000 cm). Stem length growth gradually decreased with increasing Cd concentration. Stem length was 13.33 ± 3.786, 12.133 ± 4.500, 14.000 ± 4000, and 11.333 ± 3.512 cm in Cd treatments of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, respectively. Our findings are consistent with those from previous results (Farooq et al., 2022; Abdal et al., 2021; Abdel Latef et al., 2021; Faiz et al., 2021; Haque et al., 2021; Luyckx et al., 2021; Mitra et al., 2018).

#### ii. Roots Length

Shapiro Wilk test showed that Cd concentration affected the root length, where it varied from 14.000 to 9.500 cm. The highest root length values were observed in control, Where averaged 14.000 ± 2.646 cm.

**Table 1: stem and root length, leaf area in *Atriplex halimus* plants after 15-d growth on a soil with different Cd concentrations**

Variable	0 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm
Stem length	21.000 ±	13.333 ±	12.133 ±	14.000 ±	11.333 ±
	2.000	3.786	4.500	4.000	3.512
Root length	14.000 ±	12.500 ±	9.500 ±	10.900 ±	10.600 ±
	2.646	1.500	3.775	3.651	4.084
Leaf area	3.794 ±	1.845 ±	3.164 ±	2.549 ±	1.675 ±
	1.673	0.981	1.775	0.640	0.816



**Figure 2: Boxplots displaying the effect of application high treatments of Cd (500 ppm, 1000 ppm,1500 ppm, 2000 ppm) on stems length in the plant *A. halimus* according to Shapiro Wilk test**

The root length of treatment 500 ppm was the least affected with a mean of  $12.200 \pm 1.500$  cm. the highest inhibition rate of the root length was observed at 1000 ppm. Compared with the control group with a mean of  $9.500 \pm 3.775$  cm. (Figure 3). Previous studies have shown that Cd exposure reduces root elongation. Cadmium limited root growth, according to prior studies (Ali et al., 2022; Abdel Latef et al., 2021; Han et al., 2021; Luyckx et al., 2021; Gao and Song, 2019; Ronzan et al., 2018; Rui et al., 2016).

### iii. Leaf Area

The application of Cd (500, 1000, 1500, 2000 ppm) decreased leaf area, The order of Cd treatments 1000 ppm, 1500 ppm, 500 ppm, 2000 ppm with a mean of 3.164

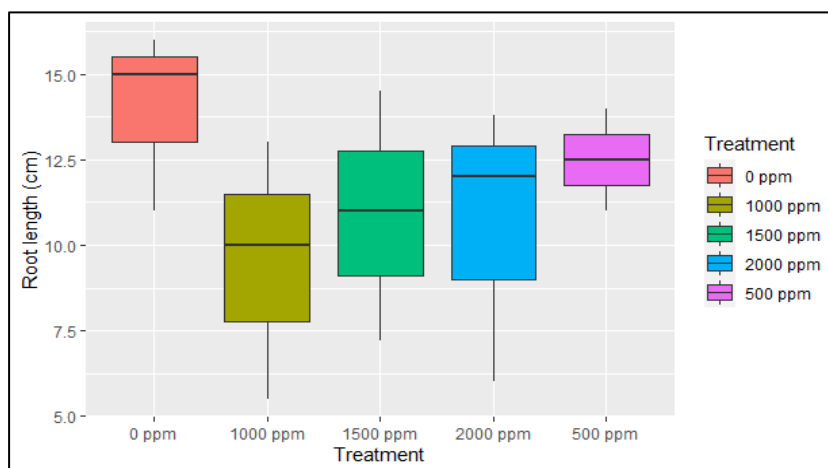
$\pm 1.755$ ,  $2.549 \pm 0.640$ ,  $1.845 \pm 0.981$ ,  $1.675 \pm 0.816$  respectively, compared to the control (Figure 4). Physiology has reportedly been negatively influenced by cd. Other research has also shown that Cadmium can significantly reduce total leaf area, which confirms our results (Zea et al., 2022; Azizi et al., 2021; Shah et al., 2021; Dai et al., 2020; Shiyu et al., 2020; Ronzan et al., 2018; Jinadasa et al., 2016).

### i. Chlorophyll Content

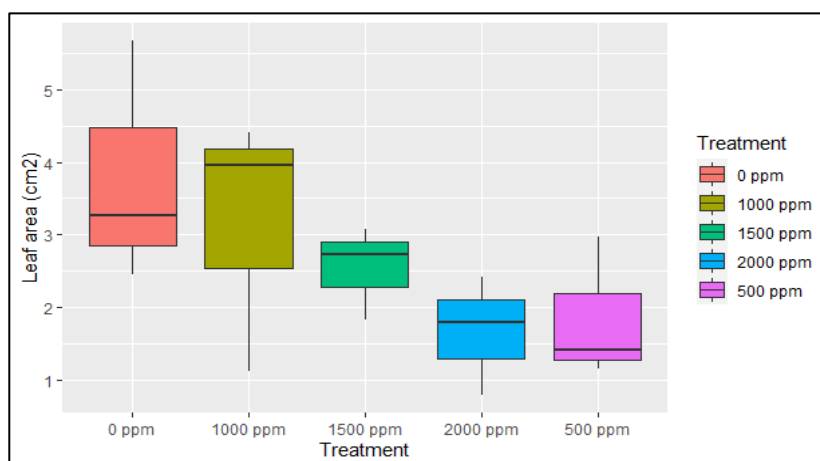
An analysis of Chlorophyll content in *A. halimus* was performed using software R, employing the data that are shown in Table 2. With the Shapiro-Wilk test, Chl content and RWC index decreased in *A. halimus* leaves when exposed to Cd. This decrease differed

according to the concentration of Cd. Disruption of photosynthesis under high levels of Cd mainly was attributed to a reduction of growth and biomass. Cd

interferes with photosynthesis by damaging chlorophyll biosynthesis (Malec et al., 2010).



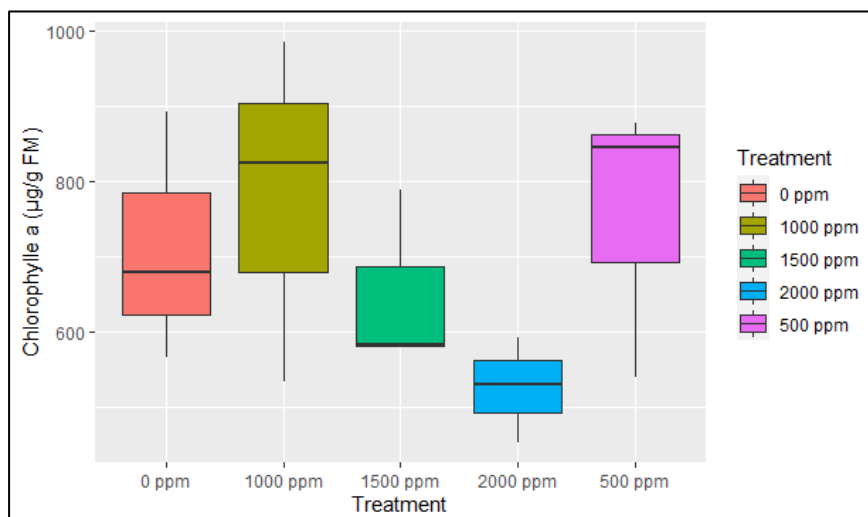
**Figure 3: Boxplots displaying the effect of high application treatments of Cd (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm) on roots length in the plant *A.halimus* according to Shapiro Wilk test**



**Figure 4: Boxplots displaying the effect of high application treatments of Cd (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm) on leaf area in the plant *A.halimus* according to Shapiro Wilk test.**

**Table 2: Total chlorophyll (Chl) and relative water content in *Atriplex halimus* plants after 15-d growth on a soil with different Cd concentrations**

Variable	0 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm
Chlorophyll a	712.050±	753.701 ±	780.761 ±	650.501 ±	524.825 ±
Chlorophyll b	165.507	186.675	228.246	119.349	69.555
Chlorophyll t	361.640 ±	334.607 ±	354.212 ±	321.881 ±	236.741 ±
Carotenoids	88.597	39.932	109.340	62.313	63.462
RWC	1073.690 ±	1088.308 ±	1134.973 ±	972.383 ±	972.383 ±
	239.417	226.581	337.020	170.003	170.003
	28.053 ±	53.455 ±	52.093 ±	50.413 ±	31.130 ±
	7.451	11.036	20.257	17.296	20.694
	55.423 ±	53.570 ±	64.348 ±	51.230 ±	52.194 ±
	6.549	19.424	8.141	8.156	4.189



**Figure 5: Boxplots graph with Shapiro Wilk test displaying the variation of Chlorophyll a in different treatments of Cd on the plant *A. halimus***

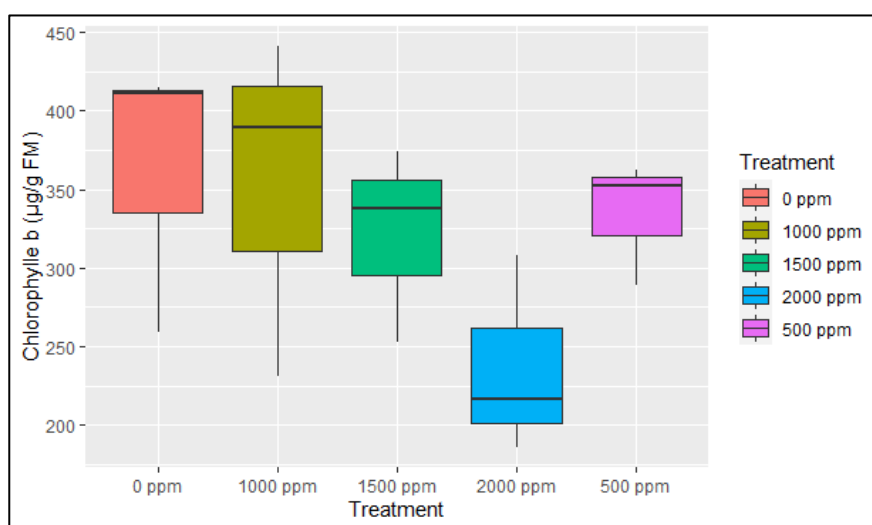
**Chlorophyll A**

Values of Chl a ranged between 524.825 and 780.761 µg/g FM (Fig. 5). The Shapiro Wilk test showed the variation of Chl a between the different concentrations of Cd on the plant *A. halimus* where Chl a in treatments 500 ppm and 1000 ppm was higher compared to the control  $753.701 \pm 186.675$  µg/g FM and  $780.761 \pm 228.246$  µg/g FM respectively. At the same time, treatments 2000 ppm of Cd indicated Chl a values  $524.825 \pm 69.555$  µg/g FM lower than the control

(table2).

**Chlorophyll B**

Chl b averaged  $354.212 \pm 109.340$  in the treatments 1500 ppm, where it is ranged from 361.640 to 236.741 µg/g FM (Fig. 6). The highest Chl b values observed in control, where the Chl b averaged  $361.640 \pm 88.597$  µg/g FM. While Chl b values in the treatments 2000 ppm were the lowest  $236.741 \pm 63.462$  µg/g FM.



**Figure 6: Boxplots graph with Shapiro Wilk test displaying the variation of Chlorophyll b in different treatments of Cd on the plant *A. halimus***



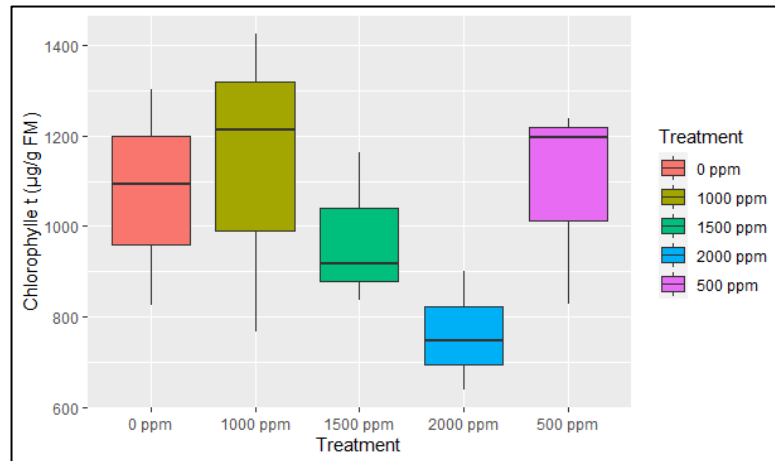


Figure 7: Boxplots graph with Shapiro Wilk test displaying the variation of Chlorophyll t in different treatments of Cd on the plant *A. halimus*

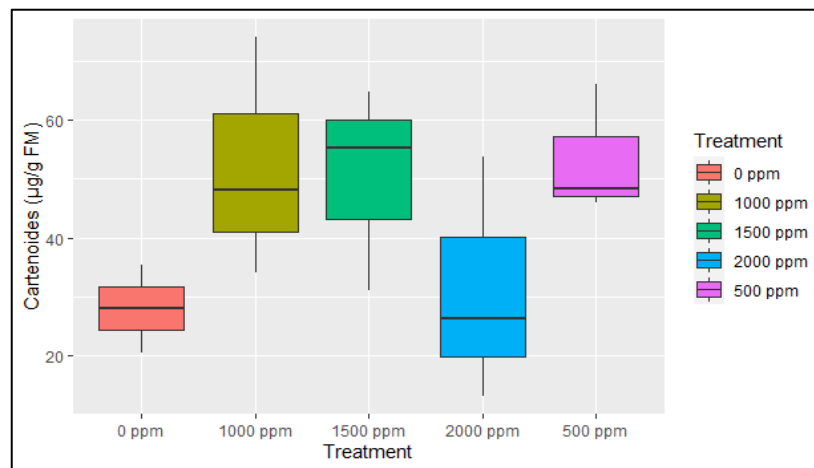


Figure 8: Boxplots graph with Shapiro Wilk test displaying the variation of Carotenoids in different treatments of Cd on the plant *A. halimus*

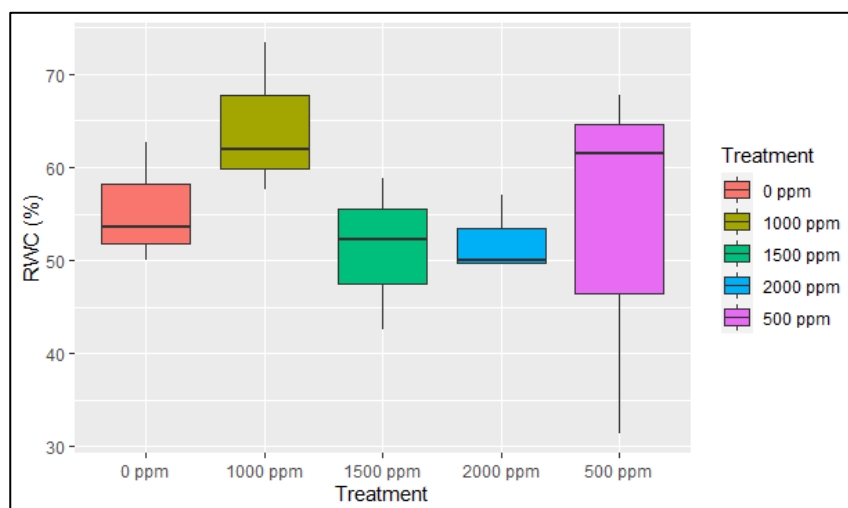


Figure 9: Boxplots graph with Shapiro Wilk test displaying the variation of RWC in different treatments of Cd on the plant *A. halimus*

### ***Chlorophyll T***

The maximum level of chl t was recorded in the treatments 1000 ppm  $1134.973 \pm 337.020 \mu\text{g/g FM}$ . the control followed with a mean of  $1073.690 \pm 239.417 \mu\text{g/g FM}$ , the treatment of 500 ppm ( $1088.308 \pm 226.581$ ) was classified in the third position. In contrast, the lowest was measured in treatments 1000 ppm with  $761.566 \pm 130.976 \mu\text{g/g FM}$  (Figure 7).

### ***Carotenoids***

All The treatments of Cd revealed significantly higher carotenoids than the control, except the treatment 2000 ppm (Table 2). The highest value was detected in the treatment 500 ppm  $53.455 \pm 11.036$ , the treatment 1000 ppm  $52.039 \pm 20.257$ , and the treatment 1500 ppm  $50.413 \pm 17.296 \mu\text{g/g FM}$ . Whereas the Carotenoids ratio was  $28.0537.451 \mu\text{g/g FM}$  in control. Values of Carotenoids  $31.130 \pm 20.694 \mu\text{g/g FM}$  in the treatments 2000 ppm were not different compared to the control (Figure 8)

### ***Relative Water Content***

Boxplots graph for RWC showed high concentrations Cd (500, 1000, 1500, 2000 ppm) decreased RWC by about 53.570 %, 64.348 %, 51.230 %, 52.194 %, respectively compared to the control (Figure 9).

## **DISCUSSION**

Reduction of physiological and biochemical processes of plant growth, usually caused by high concentrations of toxic elements (Souahi et al., 2014; Souahi et al., 2016).

The heavy metal cadmium (Cd) affects the physiological and morphological characteristics and pollutes the environment (Azizi et al., 2021). Seed germination, early seedling development, and plant biomass are all reduced by

Cadmium. It affects stomatal conductance, root and shoot length, fresh and dry mass, chlorophyll levels, and gas exchange properties, as well as altering photosynthesis and other physiological processes (Hussain et al., 2021; El Rasafi et al., 2021; Rizwan et al., 2016).

There were plant species that responded to stress conditions among the ones that were researched to varying degrees, as well as plant species that significantly responded to stress conditions. (Souahi, 2021). Halophytes are widely distributed worldwide. Due to their developed morphological and physiological characteristics, such as limiting the penetration of heavy metals, they can also manage the stress related to heavy metals (Caparrós et al., 2022). The high resistance of halophytes to heavy metals, such as *Atriplex* have very efficient Cd ion exclusion mechanisms (Wang et al., 2013). Recent research on *A. halimus* L has demonstrated that these plants can accumulate significant amounts of Cd. (Kahli et al., 2021; Pérez-esteban et al., 2013).

As a non-essential mineral, Cd hurts plants. Hence it is necessary to modify the concentration of Cd in cells to support plant growth and development (Qian et al., 2022; Haque et al., 2021) Cd treatment significantly reduced biomass in all tissues (roots, stems, and leaves) (Yu et al., 2022). As the Cd level increased, the inhibition degree of growth gradually increased (Qian et al., 2022; Dai et al., 2020). The root turns into necrotic, decomposing, and mucilaginous after a long time of Cd exposure, decreasing the elongation of roots and shoots of plant life and causing leaf rolling and chlorosis (Abbas et al., 2018). Decreases in root length, dry mass, and enlargement of root diameter are due to cadmium toxicity (Gratão et al., 2009). One of the precocious and apparent signs of Cd toxicity is the inhibition of root elongation (Lux et al., 2011). cadmium provokes the inhibition of root elongation; this

inhibition could be attributed to the depolymerization of the cellular cytoskeleton microtubules and the formation of chromosomal aberrations, which result in a decrease of mitotic activities of meristematic cells (Seth et al., 2008). Cd adversely impacts the homeostasis of auxins, inhibiting the cell division inside the apical meristem of roots, resulting in reduced plant increase (Krishnamurthy and Rathinasabapathi, 2013).

Previously, plants' decrease in chlorophyll content was related to metal stress (Souahi et al., 2017; Souahi, 2021b). Cd exposure caused a decrease in the content of the total chlorophylls, significantly compromising the growth (Singh et al., 2020; Carfagna et al., 2013). Cadmium is one of the toxic heavy metals that interacts with the photosynthetic components and damage photosystems. (Bashir et al., 2015). Changes in chloroplast ultrastructure with low chlorophyll content result from changes in leaf phenology caused by Cd toxicity, leading to chlorosis and reduced photosynthetic activity (Gallego et al., 2012; Miyadate et al., 2011). The tolerance of plants to different stresses can be evaluated by changes in pigment composition (Rong-hua et al., 2006; Ramirez et al., 2014; Xue et al., 2018; Souahi, 2021). The synthesis and/or accumulation of photosynthetic pigments is negatively affected by heavy metal stress (Parmar et al., 2013; Souahi et al., 2017). For example, in a previous study on peanuts under Cd-stressed conditions, a significant decrease in chlorophyll content was observed (Dong et al., 2019). In the current study, we observed a decrease in chlorophyll a, b chlorophyll contents in *Atriplex halimus* under Cd stress at 8000ppm (Souahi et al., 2022). Our results indicate that the increase of Cd concentration induces a reduction in the process of photosynthesis and it affects the content of photosynthetic pigments in *Atriplex halimus*. All these changes can be

considered as biomarkers of Cadmium toxicity on the plant.

The quantity of reactive oxygen species (ROS) increases in response to Cd stress, which damages structural proteins in chloroplasts (Azizi et al., 2021). Cadmium inhibits photosynthesis in most plants; the high level of Cd reduces Chl contents and inhibits electron transport in the leaves, resulting in reduced photosynthetic activity (Xue et al., 2018; Zhou and Qiu, 2005). Cadmium-responsive reduced Chlorophyll and carotenoid content may be because of the inhibition of enzymes answerable for pigment biosynthesis, and this inhibition triggered a type of senescence (Qian et al., 2009). Different concentrations of Cd significantly affect the total chlorophyll content (Małkowski et al., 2019; Sajeev et al., 2012). As a result of Cd-induced oxidative stress, Chlorophyll and carotenoid levels may fall (Cherif et al., 2012). Generally, the ratio of carotenoids to Chlorophyll is more critical in Cd-stressed plants therefore, carotenoids are less affected by Cd than chlorophylls (Dobrikova et al., 2017). At the same time, mature leaves lose their chlorophyll b fast due to the conversion into chlorophyll a. The reason is the interplay with the senescence signaling resulting in the interconversion of chlorophyll b into a before degradation (Ebbs and Uchil, 2008). The Cd affects photosynthesis in higher plants. As a result, in our earlier experiments, the Cd strain significantly decreased the chlorophyll contents (Chl a and b) in *Atriplex* (Nedjimi, 2018).

Cadmium reduces the leaf water content (Anwar et al., 2019; Mostofa et al., 2019). Due to stomatal closure in the presence of high Cd, low transpiration rate and high stomatal resistance are observed. A decrease in leaf osmotic capacity revealed an adaptive response of the plant to water stress (Rucinska-Sobkowiak, 2016). A water deficit in the leaves is a result of the reluctance of long-distance water shipment, which causes a depletion

of water in the leaves (Osakabe et al., 2014). A complicated collection of water-related changes are brought about by cadmium exposure across the entire plant. Due to Cd toxicity, water absorption is reduced, and water transport is inhibited in roots (Kudoyarova et al., 2015). Cd reasons for multifaceted changes in plant life at morphological and physiological stages (Kaur and Hussain, 2020; Bączek-Kwinta, 2019). Therefore, the cultivation of *A. halimus*, which is often recommended for the phytostabilisation of metal-polluted sites, could be started by seeding.

## CONCLUSION

Contamination by trace metals (TME) has become a significant environmental issue that limits plant production and poses health risks to humans. In this context that our study was conducted, which led us to analyze the effect of Cadmium on young plantlets of *Atriplex halimus*. In plants, the most directly visible effect of Cadmium is an inhibition of the growth of the aerial and root parts of *Atriplex halimus*.

The increase of Cd concentration induces a reduction of photosynthesis and inhibits chlorophyll synthesis, and all these observed changes could be taken as biomarkers of heavy metal toxicity on the plant. The Cd-induced metal stress effect is reflected in a decrease in the relative water content in *Atriplex halimus*.

Finally, *Atriplex halimus* should be exploited for soil remediation using phytoremediation approaches. These plants could constitute an attractive model for studying metal exclusion mechanisms but could also be used for cultivation on metal-contaminated soils. Our results support the hypothesis that *Atriplex halimus* L could be implicated in projects to rehabilitate Cd-contaminated soils or agro-ecosystems.

## AUTHORS CONTRIBUTION

Authors' contributions CH A designed the study, extracted the data and wrote the study manuscript. S H contributed to data analysis and interpretation the manuscript. K Z and G R read the manuscript and participated in the preparation of the final version of the manuscript. All authors read and approved the final manuscript.

## CONFLICT OF INTEREST

A. CH, H. S, Z. K and R. G declare that they have no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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