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# Soil salinity improves the capacity of *Atriplex nummularia* Lindl. to phytoextract cadmium

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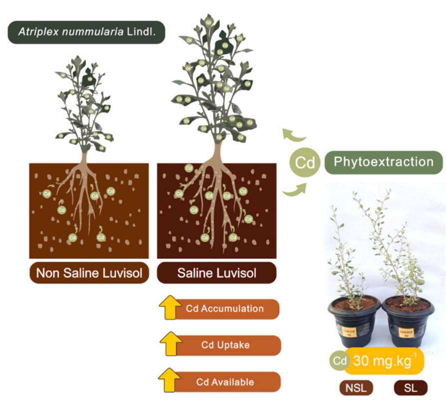
## HIGHLIGHTS

- Salinity enhances the capacity of *Atriplex nummularia* to accumulate cadmium.
- Salinity stimulates the production of dry biomass in *A. nummularia* grown in contaminated soil
- The *A. nummularia* has potential to phytoextract cadmium

## GRAPHICAL ABSTRACT

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## ABSTRACT

The halophyte *Atriplex nummularia* Lindl. is commonly used for remediating salt-affected soils and can accumulate heavy metals, yet studies on metal phytoextraction in saline soils are limited. This study makes a significant contribution to advancing the existing knowledge by exploring how salinity influences the cadmium (Cd) phytoextraction mechanisms in Atriplex, providing new insights into the effects of salinity on the absorption, translocation, and accumulation of heavy metals in halophytic plants. This research evaluated salinity's effect on Cd phytoextraction by Atriplex. The experiment used saline (SL, EC = 14.23 dS $m^{-1}$) and non-saline (NSL, EC = 1.89 dS $m^{-1}$) Luvisols with six Cd levels (0–50 mg $kg^{-1}$). Parameters assessed included dry biomass of aerial parts (DBAp) and roots, Cd in aerial parts (CdAp) and roots, translocation factor (TF), water relations, ion concentrations, and gas exchange. Cd did not affect DBAp in NSL, showing tolerance, while in SL, DBAp exceeded NSL up to 34 mg $kg^{-1}$ . Gas exchange decreased with Cd, but water relations were unaffected. CdAp reached 49 and

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25 mg kg$^{-1}$ in SL and NSL, respectively. Salinity provided a favorable TF up to the dose of 30 mg kg$^{-1}$ Cd. These results highlight the species' potential for Cd phytoextraction, especially in saline conditions.

## 1. Introduction

Cadmium (Cd) is one of the most commonly occurring heavy metals in contaminated soils, and its entry into the food chain has been exacerbated by human activities such as mining, metallurgy, the use of contaminated agricultural inputs, and irrigation with wastewater. Cadmium is regarded as one of the most bioavailable metals for plants, largely due to its high mobility in soil [49] and, even at relatively low concentrations in soil, Cd is toxic to plants and poses a significant risk to food safety [19]. Consequently, the remediation of soils contaminated by this element is of paramount importance.

A variety of strategies have been developed for the remediation of contaminated soils [34]. The conventional approach of removing polluted soils and replacing them with uncontaminated soils is financially impractical, unfeasible on a large scale, and has low public acceptance. This prompts the search for alternative methods, such as phytoremediation, which is distinguished by its low cost and sustainable characteristics [21,34,44]. This group of eco-friendly technologies has been identified as a promising approach for remediating metal-polluted soils. Among the techniques within the field of phytoremediation, phytoextraction employs the use of hyperaccumulator plants to remove Cd from contaminated soils. However, induced phytoextraction, which involves the application of chemical agents to enhance metal uptake by plants, can also be employed [23].

On a global scale, cadmium (Cd) emissions have reached 743.77 tons, with most of it accumulating in the soil [53]. In arable soils, the situation is particularly concerning due to the low affinity of Cd for soil colloids, making it readily available for crop uptake and transfer into the food chain. In Brazil, although data on soil contamination by heavy metals is scarce, several studies indicate that agricultural and urban areas have already been seriously affected by heavy metals [13,25,34]. For example, soil samples from 60 sugarcane fields exhibited Cd concentrations above natural background levels, clearly linked to anthropogenic sources, with phosphate fertilization being the most likely contributor [43]. Similarly, soils affected by metallurgical activities showed that children's daily exposure to Cd exceeded the acceptable daily intake [42].

Heavy metal contamination can occur alongside soil salinization, particularly in arid, semi-arid, and coastal regions impacted by urban activities [23]. These regions are therefore simultaneously affected by excess salts and heavy metals [27]. According to the Ministry of Environmental Protection of China, 16 % of all soils in the country and 19 % of its agricultural lands are classified as polluted [7], with different types of saline soils already impacted by heavy metal contamination. This trend of simultaneous pollution (salinization and heavy metals) has been intensifying over the years [50] and requires remediation practices adapted to saline soils.

Halophytic species have recently received increased attention as natural, cost-effective, and efficacious tools for phytoextraction, particularly in saline soils [39]. These plants can tolerate elevated concentrations of ions, particularly $Na^+$ and $Cl^-$, in such soils [54]. Recently, halophytic species have been evaluated for their potential in the removal of heavy metals, including Cd, Pb, Cu, and Zn [28]. *Atriplex nummularia* Lindl. has been the subject of extensive research into its salinity tolerance, demonstrating optimal growth and biomass production at NaCl concentrations ranging from 150 to 200 mmol·L$^{-1}$ [33,9]. In light of its promising performance in salt phytoextraction and evidence of its heavy metal tolerance, *A. nummularia* Lindl demonstrates significant potential for environmental remediation [23,35]. Due to the existence of environments impacted by both contaminants, an investigation into the potential of halophytic species for heavy metal removal from saline soils becomes a pertinent and environmentally sound solution for remediating these areas.

Physiological mechanisms, such as osmotic adjustment, water and osmotic potentials, and foliar gas exchanges, play essential roles in plant adaptation to saline environmental stresses [54]. Osmotic adjustment, for instance, enables the maintenance of cellular turgor and water absorption under water and saline stress conditions [26], creating favorable conditions for the uptake of metallic ions, such as Cd. Meanwhile, water and osmotic potentials regulate solute transport and the internal redistribution of water [38], potentially optimizing the efficiency of metal transport within plants [40,45]. Foliar gas exchanges, in turn, affect photosynthesis and energy metabolism, which are fundamental processes to sustain growth and biomass accumulation even under stress conditions [12,36]. Thus, understanding how these mechanisms interact and influence the absorption, translocation, and accumulation of Cd is essential to evaluating the potential of *A. nummularia* Lindl. in phytoremediation strategies for saline contaminated soils.

In this context, the objective of this study was to evaluate the influence of soil salinity on phytoextraction of Cd by *A. nummularia* Lindl. This was done by focusing on physiological mechanisms such as osmotic adjustment, water and osmotic potentials in leaves, leaf gas exchange, and Cd concentrations in aerial parts and roots. The hypothesis proposed is that the plant's physiological adaptations to saline soil not only modify its biomass but also enhance its efficiency in the removal of Cd from the soil. An understanding of these interactions is essential to enable the proposal of using *A. nummularia* Lindl. in phytoremediation strategies, especially in arid and semi-arid regions where salinity and heavy metal contamination often coexist.

## 2. Materials and methods

### 2.1. Location

The experiment was conducted in a protected environment at the Department of Agronomy, on the main campus of the Federal Rural University of Pernambuco (UFRPE), municipality of Recife, Pernambuco State, Brazil (8°01'01" S latitude, 34°56'41" W longitude, and 4 m altitude). The soil samples utilized in the present study were collected from the municipality of Belém do São Francisco, situated within the state of Pernambuco in the northeastern region of Brazil. The collection points were situated approximately 200 m apart (Fig. 1).

The soil samples utilized in the study were collected from the diagnostic horizons of two Luvisol orders (representative of the semi-arid region of Brazil), differentiated by the presence or absence of salinity. The soils were classified as Saline Luvisol (SL, with an electric conductivity (EC) of 14.23 dS m$^{-1}$ ) and Non-Saline Luvisol (NSL, with an EC of 1.89 dS m$^{-1}$) based on their physical and chemical characterization (Table 1).

### 2.2. Propagation of *A. nummularia* Lindl. seedlings

The seedlings of *A. nummularia* Lindl. were propagated by stem cuttings from a single mother plant to reduce genetic variability. They were cultivated in polyethylene bags containing washed sand and a nutrient solution of Hoagland and Arnon [22] at half ionic strength. After 60 days of planting, the seedlings were transferred to pots containing the contaminated soils.

### 2.3. Soil incubation with Cd

To determine the Cd doses corresponding to 0, 10, 20, 30, 40, and

50 mg kg$^{-1}$ , the soil was incubated with a $CdCl_2$ solution, distributed uniformly throughout the soil volume, for 20 days at 80 % of the maximum water retention capacity. The incubation was conducted in pots containing 5 kg of soil. Following a 20-day incubation period, the *Atriplex* seedlings were transferred to the pots and cultivated for a further 30 days.

### 2.3.1. Experimental design

The experiment was conducted according to a randomized block design in a 2 × 6 factorial arrangement. This refers to the two soil types, Saline Luvisol (SL) and Non-Saline Luvisol (NSL), and six concentrations of Cd (0, 10, 20, 30, 40, and 50 mg kg$^{-1}$), with four replications, resulting in a total of 48 experimental units. The data were subjected to analysis of variance (ANOVA), Tukey's mean comparison test at a 5 % probability level, and regression analysis.

## 2.4. Analyzed variables

### 2.4.1. Dry biomass

Thirty days following the transplantation procedure, the plants were harvested, with the aerial portion (comprising the leaves and stem) and roots separated. The dry mass was obtained by drying the plants in a drying oven at 65 ºC until a constant weight was reached. For data analysis, the relative biomass (RB) variable was calculated using the ratio between the production of the control dose (without Cd addition) and the dose of interest, multiplied by 100, according to equation 01:

$$RB(\%)\frac{Biomass\ of\ the\ dose\ of\ interest}{Biomass\ of\ the\ control}*100 \quad (1)$$

**Table 1**
Physical and chemical characterization of the soil samples utilized in the experiments.

| ATTRIBUTES | NSL | SL |
|---|---|---|
| | *Mean - Standard Deviation* | *Mean - Standard Deviation* |
| Bd (g.cm$^{-3}$) | 1.53 ± 0.01 | 1.68 ± 0.01 |
| Pd (g.cm$^{-3}$) | 2.70 ± 0.03 | 2.74 ± 0.03 |
| Tp (%) | 43.48 ± 0.01 | 38.94 ± 0.01 |
| TS (g.kg$^{-1}$) | 70.90 ± 0.60 | 63.00 ± 0.80 |
| Fs (g. kg$^{-1}$) | 54.70 ± 0.70 | 48.90 ± 1.00 |
| Cs (g.kg$^{-1}$) | 16.20 ± 0.50 | 14.10 ± 0.50 |
| Clay (g. kg$^{-1}$) | 506.70 ± 1.20 | 462.90 ± 0.80 |
| Silt (g.kg$^{-1}$) | 422.40 ± 0.01 | 474.10 ± 0 |
| CDW (%) | 124.93 ± 0.00 | 175.29 ± 0 |
| EC (dS.m$^{-1}$) | 1.89 ± 0.12 | 14.23 ± 0.61 |
| P (mg/dm$^3$) | 5 ± 0.01 | 2 ± 0.01 |
| Ca (cmolc/dm$^3$) | 6.50 ± 0.01 | 15.9 ± 00.01 |
| Mg (cmolc/dm$^3$) | 1.90 ± 0.01 | 4.30 ± 0.01 |
| Na (cmolc/dm$^3$) | 0.28 ± 0.01 | 1.11 ± 0.01 |
| K (cmolc/dm$^3$) | 0.40 ± 0.01 | 0.13 ± 0.01 |
| Al (cmolc/dm$^3$) | 0.00 ± 0.01 | 0.00 ± 0.01 |
| H (cmolc/dm$^3$) | 0.49 ± 0.01 | 1.07 ± 0.01 |
| S (cmolc/dm$^3$) | 9.1 ± 0.01 | 21.4 ± 0.01 |
| CEC (cmolc/dm$^3$) | 9.6 ± 0.01 | 22.5 ± 0.01 |
| V (%) | 95 ± 0.01 | 95 ± 0.01 |
| m (%) | 0 ± 0.01 | 0 ± 0.01 |
| pH ($H_2O$) 1:2.5 | 7.20 ± 0.04 | 6.21 ± 0.04 |
| FD (%) | 70.42 ± 0.00 | 63.03 ± 0 |
| Textural class | Silty clay | Silty clay |

**Bd**: Soil bulk density, determined using the graduated cylinder method; **Dp**: Particle density, determined using the volumetric flask method; **TP**: Total porosity; **TS**: Total sand; **CS**: Coarse sand; **FS**: Fine sand; **CDW**: Clay dispersed in water; **EC**: Electrical conductivity; **pH**: Hydrogen potential; **FD**: Flocculation degree; **CEC**: Cation exchange capacity; **V (%)**: Base saturation; **m(%)**: Aluminum saturation. Teixeira et al., [47].

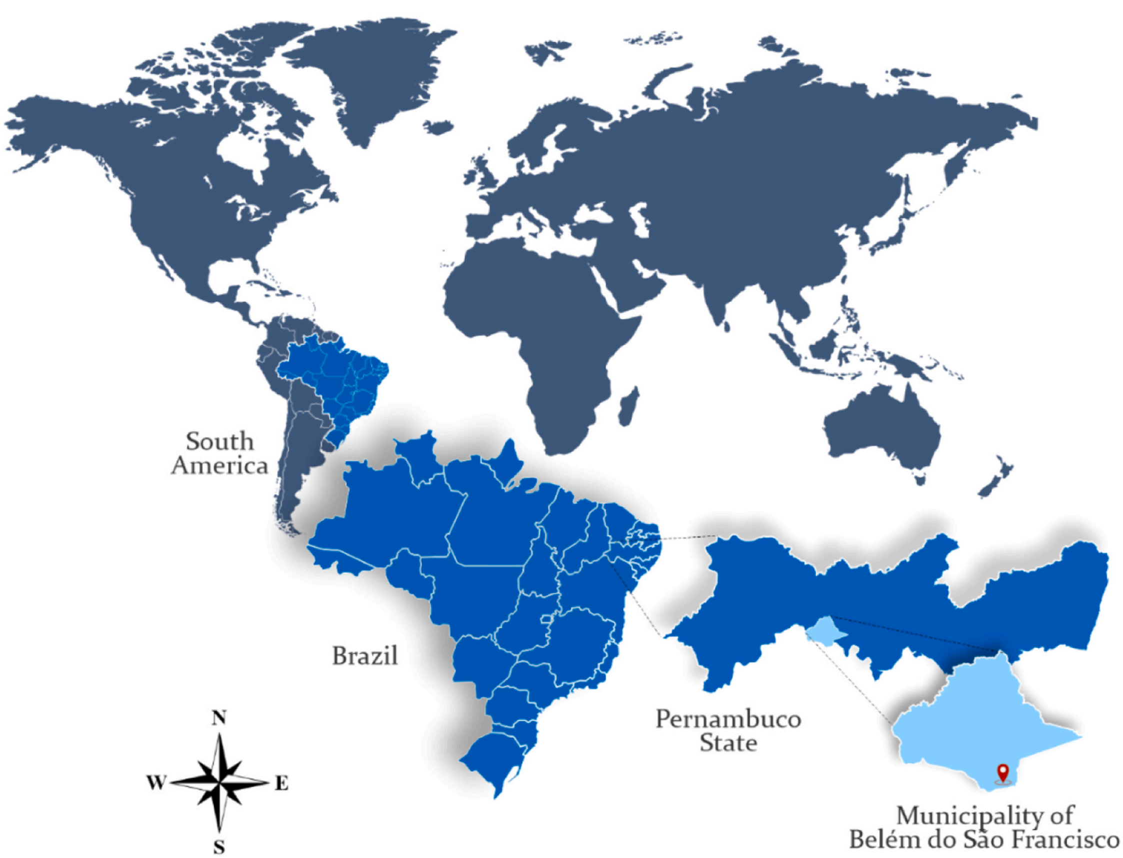


**Fig. 1.** Location of the municipality of Belém do São Francisco, where the Saline Luvisol (SL, with EC of 1.89 dS m$^{-1}$) and the Non-Saline Luvisol (NSL, with EC of 14.23 dS m$^{-1}$) were collected.

#### 2.4.2. Leaf water potential (Ψw), osmotic potential (Ψo), and turgor potential (Ψt)

The determination of leaf water potential (Ψw) was conducted prior to sunrise, 30 following transplantation, utilizing branches from the middle third and a Scholander pressure chamber [41]. To determine the osmotic potential (Ψo), leaves from the same branch selected for Ψw analysis were utilized. The sample was macerated, filtered, and subjected to centrifugation at 10,000 g for 15 min at 4 ºC [38]. From the supernatant, a 10 μL aliquot was taken for the determination of the tissue osmolality using a vapor pressure osmometer. The values obtained in mmol $kg^{-1}$ were converted into osmotic potential using the Van't Hoff equation:

$$\Psi o = -RTC \tag{2}$$

Where:

R = Universal gas constant (0.00831 kg MPa $mol^{-1}$ $K^{-1}$)
T = Temperature in Kelvin (K)
C = Solute concentration (mol $kg^{-1}$)

The turgor potential was estimated by subtracting the water potential value from the osmotic potential value [38]:

$$\Psi t = \Psi w - \Psi o \tag{3}$$

#### 2.4.3. Osmotic adjustment (OA)

Osmotic adjustment (OA) was calculated as the difference in osmotic potential between control and stressed plants after leaf saturation [6].

$$OA_{Total} = \Psi o^{100}{}_{Control} - \Psi o^{100}{}_{stress} \tag{4}$$

Where: $AO_{total}$ represents the total osmotic adjustment; $\Psi o^{100}_{control}$ denotes the osmotic potential of the plants that were not subjected to stress at full turgor; and $\Psi o^{100}$ signifies the osmotic potential of plants that were subjected to stress at full turgor.

#### 2.4.4. Relative water content (RWC)

The relative water content (RWC) was determined according to the methodology described by Barrs and Weatherley [4]. Ten leaf discs of approximately 8 mm in diameter were used. The fresh weight (FW), turgid weight (TW), and dry weight (DW) were obtained through weighing and calculated using equation 02.

$$RWC(\%) = \frac{FW - TW}{TW - DW} x100 \tag{5}$$

#### 2.4.5. Leaf succulence (FS)

The values of leaf succulence (FS) were obtained from the data collected for relative water content (RWC), using the relationship between the water mass of the samples and the area of the leaf discs (DA), represented by the equation below (Equation 03), as proposed by Delf [10].

$$FS = \frac{FW - DW}{DA} \tag{6}$$

#### 2.4.6. Leaf gas exchange

Leaf gas exchange assessments were conducted 30 days after transplantation using a portable infrared gas analyzer (IRGA), model LICOR LI-6400XT, between 8 and 11 a.m. The following variables were determined: net photosynthesis (A), stomatal conductance (gs), and transpiration (Tr). The subsequent calculations yielded the instantaneous water use efficiency (WUE - A/Tr) and intrinsic water use efficiency (WUE - A/gs).

#### 2.4.7. Determination of chloride (Cl-), sodium (Na+), potassium (K+), and cadmium (Cd) in plants

The samples were subjected to analysis for $Na^+$ and $K^+$ in the aerial parts (leaves + stems) employing the water extraction method proposed by Malavolta et al. [30]. The levels of $Na^+$ and $K^+$ were determined using a flame photometer. Chloride was determined by water extraction and titration with silver nitrate [30].

For the determination of Cd, the aerial parts (leaves + stems) and roots were digested using 0.5 g of plant material, 2 mL of $H_2O_2$, and 8 mL of $HNO_3$ in Teflon tubes [48]. The mixture was subjected to a temperature of 180 °C for 10 minutes in a microwave oven. Subsequently, the material was filtered, and the volume was adjusted to 25 mL. This solution was stored for determination of Cd concentration by inductively coupled plasma optical emission spectrometry (ICP-OES/Optima 7000 Perkin Elmer).

#### 2.4.8. Translocation factor (TF)

The equation proposed by Marchiol et al. [31] was employed to calculate the translocation factor (TF):

$$TF = \frac{Cd\ content\ in\ the\ aerial\ part}{Cd\ content\ in\ the\ root} \tag{8}$$

The translocation of metals to the aerial part is of paramount importance for the success of phytoextraction, as this is the fraction of the plant that is harvested and intended for removal, and it should have a high concentration of the target metal. Translocation factors (TF) greater than 1.0, in conjunction with significant biomass production, are indispensable for achieving efficient phytoextraction.

## 3. Results

### 3.1. Dry biomass

The variables dry biomass of the aerial part ($DB_{AP}$) and dry biomass of the root ($DB_R$) demonstrated a statistically significant effect ($p < 0.01$) with regard to the interaction between the factors Cd doses and soil salinity (Fig. 2).

In SL, a linear reduction of 0.22 g of $DB_{AP}$ per increase in Cd dose was observed, resulting in a level of the $DB_{AP}$ in SL and NSL that was equal at a Cd dose of 34.25 mg $kg^{-1}$. However, the $DB_{AP}$ in SL were observed to be approximately 55 %, 47 %, 34 %, and 14 % higher for the doses of 0, 10, 20, and 30 mg $kg^{-1}$, respectively (Fig. 2A).

The plant exhibited elevated biomass when cultivated in SL, particularly at low Cd doses, with relative yields (RB%) of 83.9 %, 67.8 %, 51.8 %, 35.7 %, and 19.7 % for 10, 20, 30, 40, and 50 mg $kg^{-1}$ of Cd, respectively (Fig. 2B). No regression adjustment was made for NSL, with an average of 6.02 g of $DB_{AP}$.

The roots of plants cultivated in SL exhibited a linear reduction in dry biomass of the root ($DB_R$) of 0.06 g per increase in Cd dose, with higher levels observed for the plants cultivated in NSL up to a dose of 20 mg $kg^{-1}$ of Cd. The plants cultivated in SL exhibited $DB_R$ values of approximately 38 % and 25 %, higher than those grown in NSL at the doses of 0 and 10 mg $kg^{-1}$ of Cd, respectively. The plants in NSL maintained a constant root biomass, with an average of 2.14 g of $DB_R$ (Fig. 2C). In contrast, the plants in SL exhibited a reduction in root biomass with increasing Cd doses, with RB% values of 81.7 %, 63.3 %, 45 %, 26.7 %, and 8.4 % for 10, 20, 30, 40, and 50 mg $kg^{-1}$ of Cd, respectively (Fig. 2D).

### 3.2. Water relations

The data indicated a statistically effect of the salinity factor on the water potential (Ψw), osmotic adjustment (OA), and relative water content (RWC) ($p < 0.05$). Regarding the Cd doses factor, a significant effect ($p < 0.05$) was observed for the variables Ψo and RWC. No significant difference was noted for the turgor potential (Ψt) between the

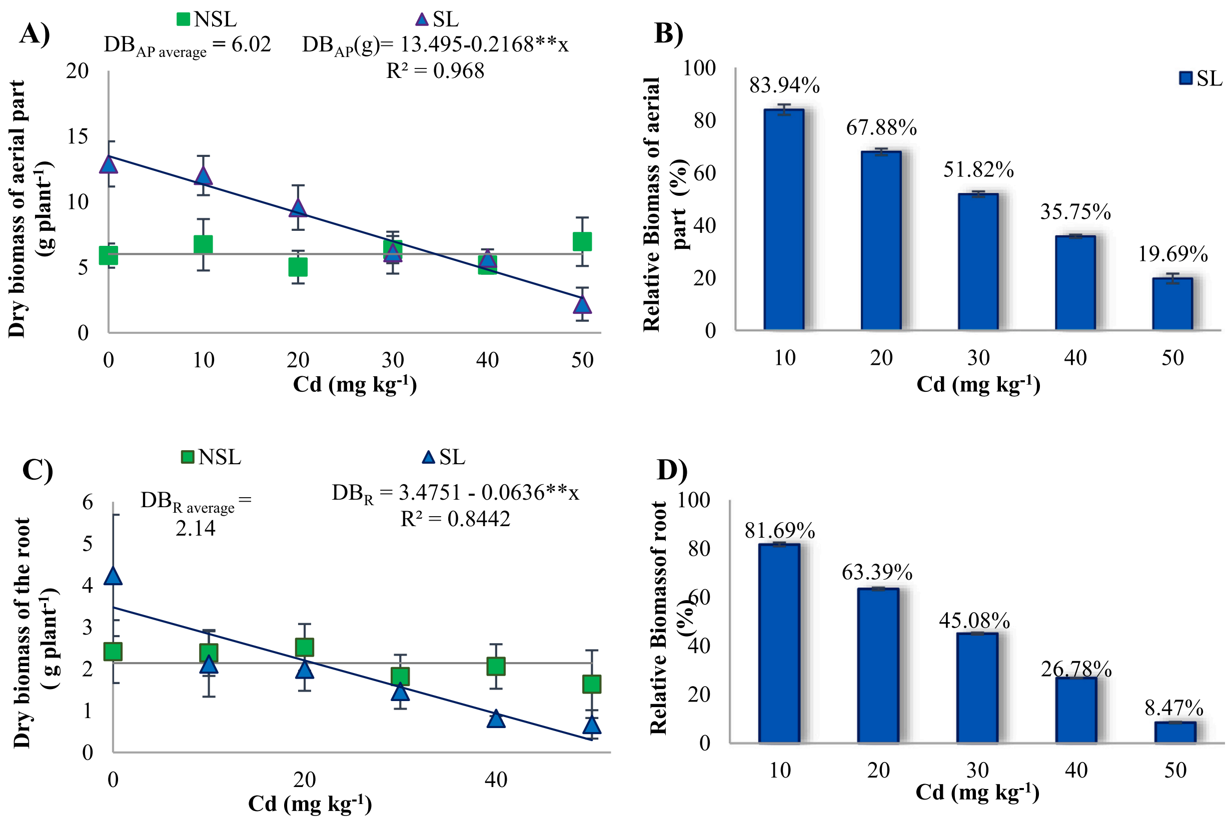


**Fig. 2.** Dry biomass of aerial part ($DB_{AP}$) (A); Relative Biomass (RB%) of aerial part (B); Dry biomass of the root ($DB_R$) (C); Relative Biomass (RB%) of root (D) of *Atriplex nummularia Lindl.* cultivated under increasing doses of cadmium. Significant regression coefficients with $p \leq 0.01$ (**) and $p \leq 0.05$ (*).

studied factors (Fig. 3). With respect to the interaction, a significant effect ($p < 0.05$) was observed for the osmotic potential (Ψo).

(OA) (C); Relative Water Content (RWC) (D) and (E); Leaf succulence (LS) (F) of *Atriplex nummularia* Lindl. cultivated under increasing doses of cadmium in the saline and non-saline soils. Means followed by the same letter, capital letters for soils, and lowercase letters for Cd rates, do not differ by Tukey's test at the 5 % significance level. The error bars correspond to the standard deviation.

The Ψw was −26.25 and −25.43 bar for NSL and SL, respectively (Fig. 3A). The *A. nummularia* Lindl. plants grown in SL exhibited significantly lower Ψo values compared to those grown in NSL (Fig. 3B), with a minimum value of −45.1 bar for SL at doses of 26.5 mg $kg^{-1}$ and an average of −34.80 bar for NSL.

The osmotic adjustment (OA) was found to be 29.79 % higher for SL compared to NSL (Fig. 3C). Nevertheless, no notable discrepancies in OA were observed between soil types at increasing Cd doses.

With regard to the RWC values, it was observed that, in response to the Cd doses, the RWC exhibited an average of 77.91 % (Fig. 3D). Regarding the soil factor, the RWC was 79.77 % for SL and 76.05 % for NSL, resulting in a 4.88 % difference (Fig. 3E). Leaf succulence (FS) in plants grown in SL was approximately 11.47 % higher compared to those grown in NSL (Fig. 3F).

### 3.3. Leaf gas exchange

The results of the assessments conducted regarding photosynthetic performance revealed a significant effect ($p < 0.01$) of the soil salinity factor on the variables stomatal conductance (gs) and transpiration (Tr). With regard to the factor of Cd doses, a significant effect ($p < 0.01$) was observed for gs ($p < 0.05$) and for water use efficiency (WUE). As for the interaction, a significant effect ($p < 0.05$) was found for liquid photosynthesis (A) (Fig. 4).

In plants grown in NSL, A decreased linearly by 0.08 μmol $CO_2$ $m^{-2}s^{-1}$ for each increase in the Cd dose, resulting in a reduction of over 65 % between the control dose and the maximum dose of 50 mg $kg^{-1}$ (Fig. 4A). In contrast, plants in SL exhibited a quadratic response, with liquid photosynthesis reaching a minimum of 2.77 μmol $CO_2$ $m^{-2}s^{-1}$ at the dose of 29.60 mg $kg^{-1}$ of Cd.

The WUE in NSL exhibited a liner decline of 0.0094 with each increase in the Cd dose, resulting in a reduction of over 46 % between the control dose and the highest dose of 50 mg $kg^{-1}$ (Fig. 4B). In SL, no regression adjustment was observed, with an average of 0.82. Regarding gs, there was a linear reduction of 0.0016 mol $m^{-2}$ $s^{-1}$ for each increase in the Cd dose, resulting in a 48 % decrease between the control dose and the highest Cd dose (Fig. 4C). Plants grown in NSL exhibited a 21 % higher stomatal opening than those in SL (Fig. 4D).

The transpiration (Tr) in plants in NSL was 18 % higher than in SL, with a linear reduction of 0.032 mmol $H_2O$ $m^{-2}$ $s^{-1}$ for each increase in the Cd dose, resulting in a 27 % decrease between the control dose and the maximum Cd dose (Fig. 4E and 4F).

### 3.4. Contents of sodium, potassium, and chlorine in the aerial part

A significant effect ($p < 0.01$) was observed for the soil salinity factor on the variables sodium (Na) and chlorine (Cl) content in the aerial part. Furthermore, a significant effect ($p < 0.01$) was found for potassium (K) content considering the interaction (Fig. 5).

The sodium ($Na^+$) content in plants cultivated in SL was approximately 48 % higher than in those grown in NSL (Fig. 5A). a similar trend was observed for chlorine (Cl) accumulation, with plants from SL exhibiting a 27 % higher accumulation compared to plants grown in NSL (Fig. 5B).

The potassium (K) content in the aerial part of plants in SL exhibited a significant linear reduction of approximately 57 % between the control dose and the 50 mg $kg^{-1}$ Cd dose. From the 30 mg $kg^{-1}$ dose onwards, the K content exhibited a decline of 16 % and 34 % at the 40 and

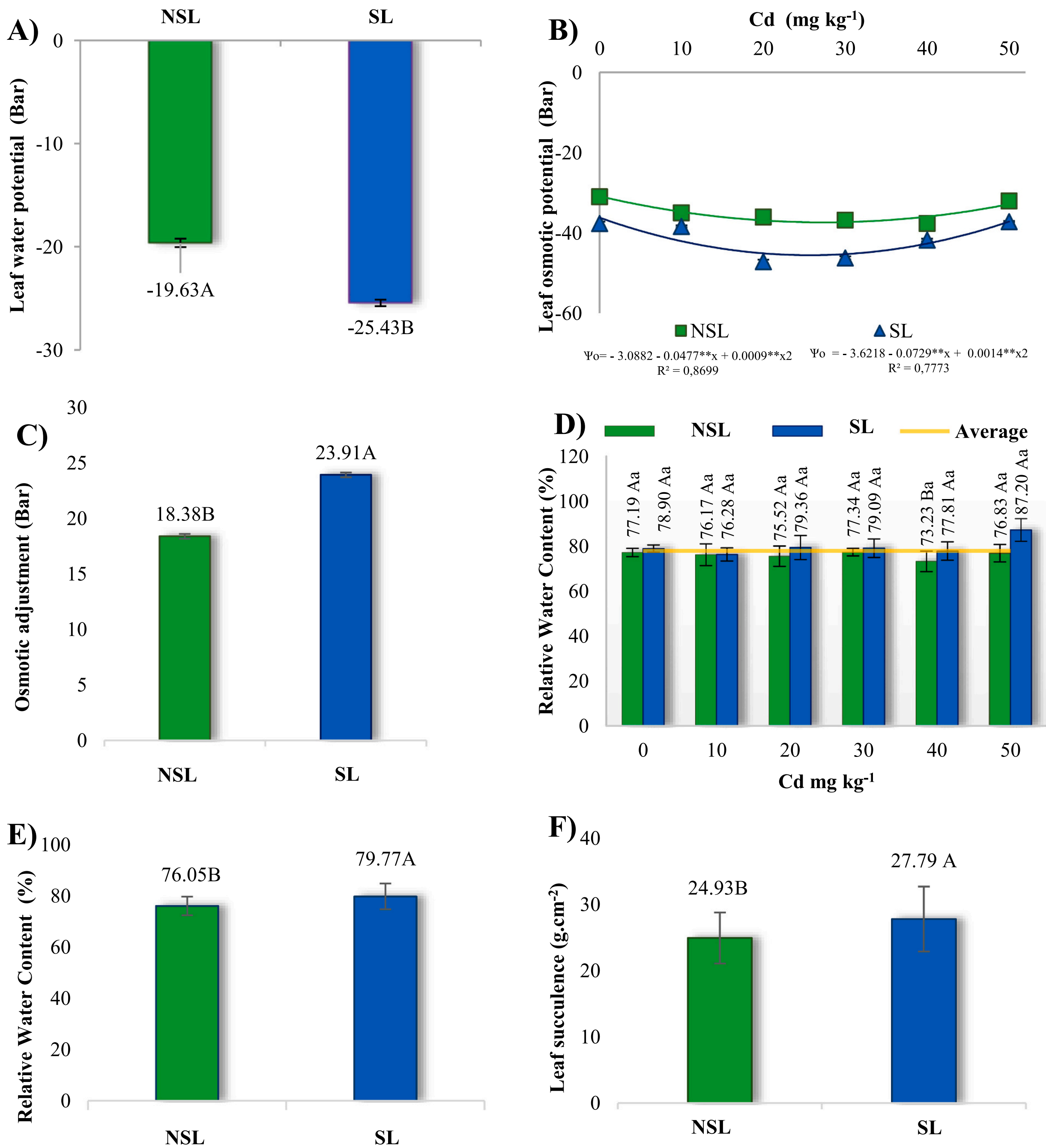


**Fig. 3.** Leaf water potential (Ψw) (A); Leaf osmotic potential (Ψo) (B); Osmotic adjustment.

50 mg kg$^{-1}$ doses, respectively, in comparison to the NSL control. In contrast, in NSL, the K content demonstrated a consistent trend with increasing Cd doses, with an average of 18.76 mg kg$^{-1}$ (Fig. 5C).

### 3.5. *Cadmium content in the aerial part and roots*

A significant effect ($p < 0.01$) of the soil salinity factor was observed for the cadmium content in the aerial part ($Cd_{PA}$). With respect to the Cd doses factor, a significant effect ($p < 0.01$) was observed for the cadmium content in the aerial part ($Cd_{PA}$), and a notable interaction was identified for the Cd content in the roots($Cd_R$) (Fig. 6).

There was an increase in the Cd content in the aerial part in response to elevated Cd doses (Fig. 6A). The accumulation of Cd exhibited a quadratic behavior, with the highest accumulation occurring at the dose of 39.58 mg kg$^{-1}$ . In the areal part, the Cd content reached a value of 52.80 mg kg$^{-1}$ . The Cd content was higher for the SL plants, with those cultivated under these conditions accumulating approximately 48 % more Cd than those grown in NSL (Fig. 6B).

The Cd content in the roots exhibited a response to increasing Cd doses in the soil, with a pronounced impact observed for SL. The NSL demonstrated a linear response, with Cd accumulation increasing in proportion to the dose, exhibiting a content 98 % higher at the Cd dose of 50 mg kg$^{-1}$ in comparison to the control. SL demonstrated a quadratic response, exhibiting the highest Cd accumulation (69.51 mg kg$^{-1}$) at the Cd dose of 40.25 mg kg$^{-1}$ . The accumulation in both soil conditions reached equilibrium equal at the Cd dose of 46.25 mg kg$^{-1}$ , with 67.89 mg kg$^{-1}$ (Fig. 6C).

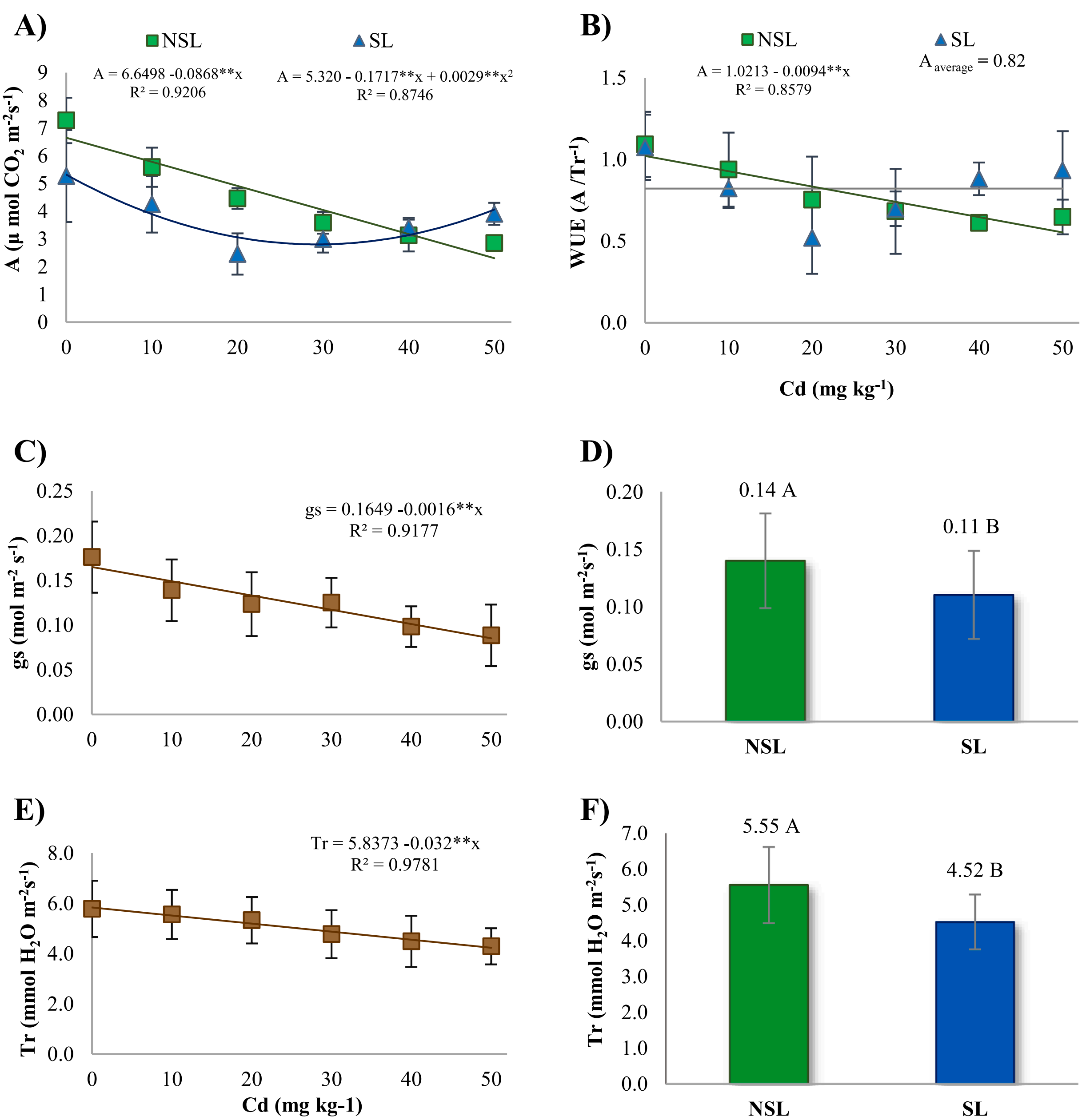


**Fig. 4.** Net photosynthesis (A); Water Use Efficiency (WUE) (B); Stomatal conductance (gs) (C), (D); Transpiration (Tr) (E) (F) of *Atriplex nummularia* Lindl. cultivated under increasing doses of cadmium in the presence and absence of salinity. The means followed by the same letter do not differ by Tukey's test at the 5 % significance level. The bars correspond to the standard deviation.

### *3.6. Translocation factor (TF)*

A significant effect ($p < 0.01$) of soil salinity on the translocation factor (TF) was observed. The TF was 36 % higher in plants grown in SL a compared to those grown in NSL, indicating greater efficiency in phytoextraction. The highest TF values were observed at doses of 10, 20, and 30 mg kg$^{-1}$ , with values of 2.37, 1.14, and 1, respectively, for SL (Fig. 7).

## 4. Discussion

### *4.1. Dry biomass*

The results indicate that, in the absence of Cd (dose 0), plants of *A. nummularia* Lindl. exhibited higher dry biomass when grown in SL compared to those grown in NSL. This positive effect of salinity is consistent with the expectation that halophytic species will respond positively to salinity levels that do not yet reach the levels associated with osmotic stress [18]. *A. nummularia* Lindl. is widely recognized for its high tolerance to salinity, with optimal growth and biomass production occurring in NaCl concentrations between 150 and 200 mmol L$^{-1}$ (~20 dS m$^{-1}$) [33,38,9]. These findings underscore the beneficial role of salinity in promoting the growth and development of *A. nummularia* Lindl, even in the presence of contaminants.

In SL, an interaction between salinity and Cd concentration was observed with the increase in Cd doses, resulting in a reduction in plant dry biomass. This effect was not observed in NSL, indicating that salinity may intensify the negative effects of Cd on plants. It was observed that, up to a Cd dose of 30 mg kg$^{-1}$ , the dry biomass in SL was greater than in NSL and remained similar up to this threshold. However, beyond this dose, the Cd doses in SL appeared to potentiate the reduction in biomass, suggesting that, while salinity may favor growth under Cd-free

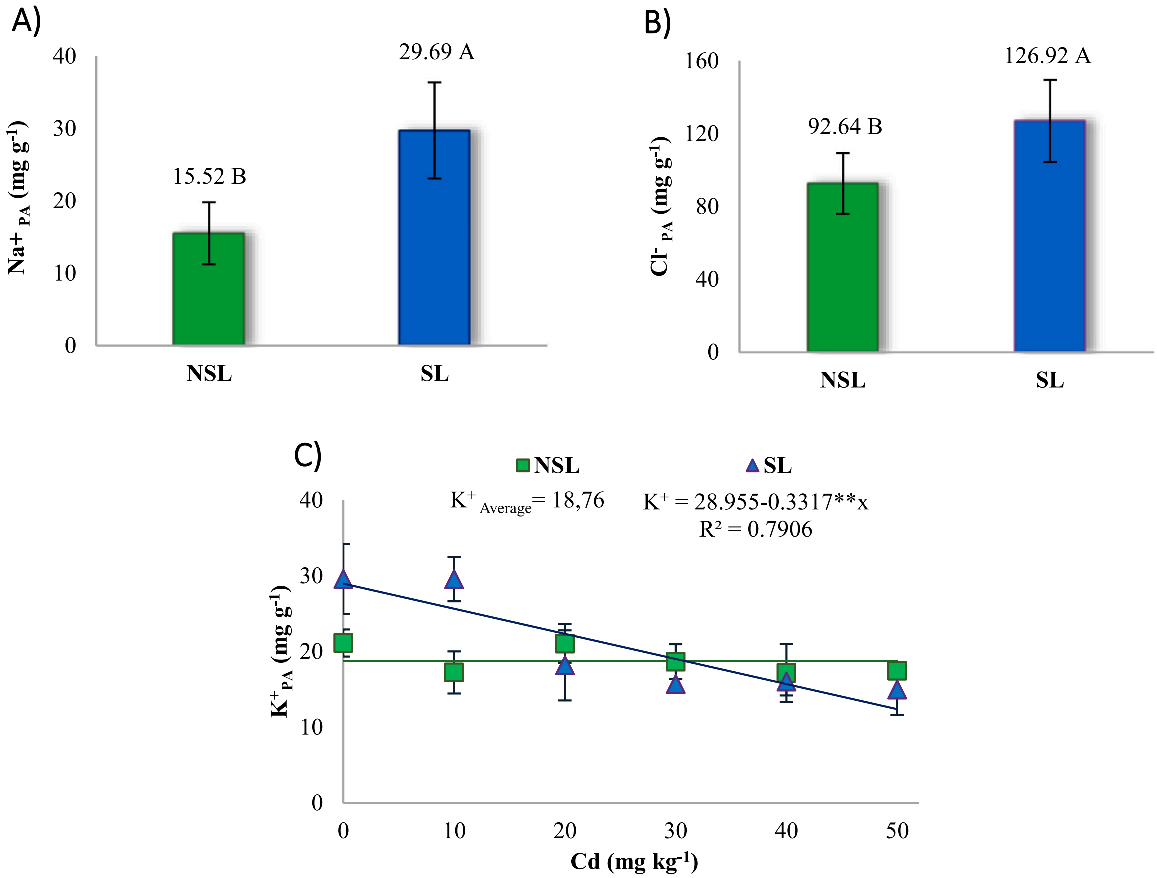


**Fig. 5.** Sodium ($Na^{+}$) (A), chlorine ($Cl^{-}$) (B), and Potassium ($K^{+}$) (C) contents of the aerial part of *Atriplex nummularia* Lindl. cultivated under increasing doses of cadmium in saline and non-saline soils. Significant regression coefficients with $p \leq 0.01$ (**) and $p \leq 0.05$ (*) error bars correspond to the standard deviation.

conditions, it amplifies negative effects when Cd is present.

The relationship between salinity and Cd accumulation in *A. nummularia* Lindl. and other halophytes is complex, involving multiple physiological, biochemical, and molecular mechanisms, including enhanced Cd bioavailability in the rhizosphere and osmotic adjustment and co-transport of Cd. For example, chloride ions can interact with Cd to form soluble Cd-Cl complexes, which favor the mobilization of Cd into the soil solution. It has been observed that higher ionic strength in the soil enhances Cd release more effectively than other metals [24,51]. Therefore, the formation of highly soluble Cd-chloro species ($CdCln^{2-n}$) significantly increases the availability of Cd in the soil, thereby promoting its uptake by plants [17,29]. Several studies have shown that salinity enhances metal mobility by promoting anion-metal complexation and competition between salt cations and metals for sorption sites [2,20]. This effect is well-documented for Cd, which tends to accumulate in Atriplex species under saline conditions, even at low soil Cd levels (50 μM) [23].

*A. nummularia* Lindl. exhibits specialized ion transport systems, characterized by increased activity of membrane transporters and efficient ion sequestration within vacuoles, which favor not only $Na^{+}$ uptake [37] but potentially also $Cd^{2+}$ absorption. Additionally, enhanced osmotic adjustment under saline conditions contributes to the species' capacity to tolerate salinity while simultaneously accumulating Cd. These findings indicate that the combined effect of soil chemical processes and the intrinsic physiological mechanisms of *A. nummularia* Lindl. confer a significant capacity for Cd phytoextraction.

### 4.2. Water relations

The results of the water relation variables indicated that there was no significant variation in water potential (Ψw) as a function of Cd doses, suggesting that *A. nummularia* Lindl. may exhibit tolerance to Cd. Given that *A. nummularia* Lindl is a halophytic species, the notable discrepancy in Ψw between SL and NSL can be attributed to its adaptation to saline environments [33]. Soil salinity typically diminishes osmotic potential (Ψo), which, in turn, facilitates osmotic adjustment in plants, allowing them to sustain water absorption even under saline conditions [26].

This osmotic adjustment is essential for survival in saline environments. It involves the compartmentalization of salts in vacuoles and a reduction in their concentration in the cytoplasm, which preserves cellular functionality [38]. The maintenance of cellular hydration in foliar tissues in the presence of Cd may be attributed to the significant reduction in transpiration rate, which is mediated by stomatal closure [40]. This author proposes that Cd affects water flow efficiency by decreasing the transpiration rate and/or by modifying stomatal closure in leaves.

Additionally, it was observed that the leaf succulence was approximately 11.47 % higher in SL compared to NSL. This development of succulence in response to soil salinity may facilitate the regulation of salt concentrations, thereby contributing to the maintenance of cellular turgor [5]. In halophytic species, both succulence and osmoregulation are pivotal for growth under abiotic stress. Succulence, an anatomical adaptation, enhances vacuolar capacity, facilitating the accumulation of substantial quantities of water and dissolved ions in plant tissues [46].

### 4.3. Leaf gas exchange

The application of Cd to plants resulted in a reduction in gas exchange and $CO_2$ assimilation, which in turn affected photosynthesis (Fig. 3A). These findings indicate that Cd toxicity impairs essential

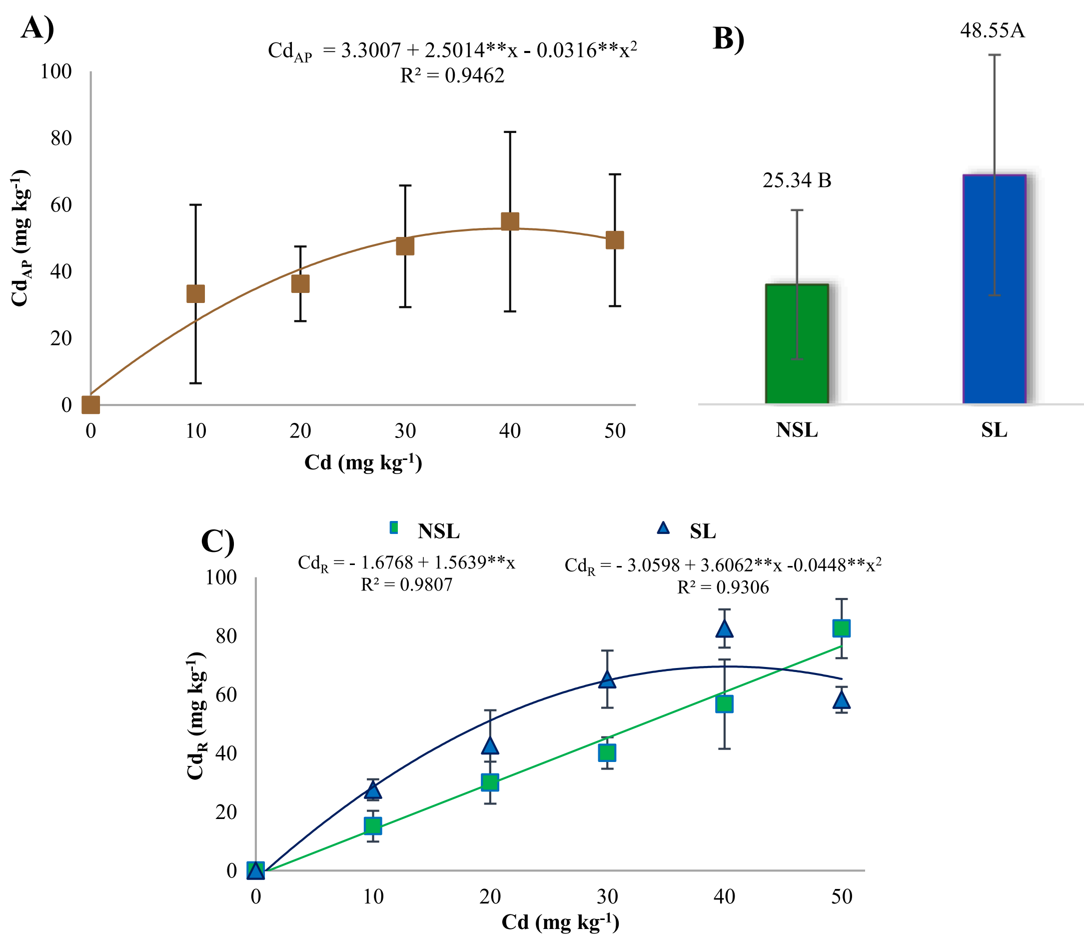


**Fig. 6.** Cadmium (Cd) content of the aerial part ($Cd_{AP}$) of *A. nummularia* Lindl. cultivated under increasing doses of cadmium (A) in the presence and absence of salinity (B) and Root Cadmium Content ($Cd_R$) of *A. nummularia* Lindl. cultivated in a saline and non-saline Luvisol contaminated with increasing doses of cadmium. Significance of regression coefficients: ** ($p \leq 0.01$) and * ($p \leq 0.05$). The means followed by the same letter do not differ by Tukey's test at the 5 % significance level. The error bars correspond to the standard deviation.

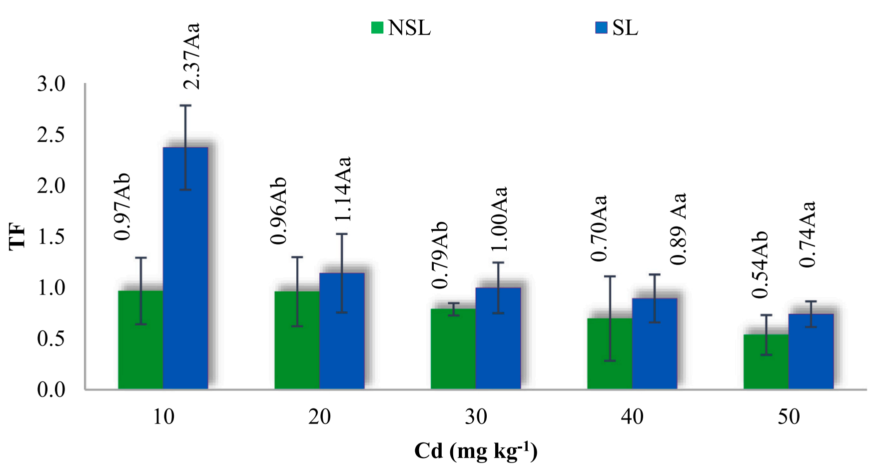


**Fig. 7.** Cd translocation factor (TF) of *Atriplex nummularia* Lindl. grown in saline (SL) and non-saline (NSL) soils under increasing Cd rates. Means followed by the same letter, capital letters for soils, and lowercase letters for Cd rates, do not differ by Tukey's test at the 5 % significance level. Error bars correspond to the standard deviation.

physiological processes that are crucial for plant growth [19]. However, in SL, a quadratic photosynthetic response was observed in the plants, suggesting that they have developed a degree of adaptation or tolerance to Cd at intermediate doses, before photosynthesis is severely affected (2.77 μmol $CO_2$ $m^{-2}s^{-1}$ at the dose of 29.60 mg $kg^{-1}$).

The water use efficiency (WUE) remained stable in SL, indicating that the plants were able to maintain a balance between $CO_2$ absorption and water loss. In contrast, the data from the NSL revealed a vulnerability to increasing Cd, with a linear reduction in WUE. This reduction reflects the decrease in physiological efficiency due to Cd toxicity. These findings suggest that saline stress may modulate the response to Cd, influencing photosynthetic efficiency and osmotic management [36].

Cadmium (Cd) is recognized as one of the most toxic heavy metals to the photosynthetic apparatus, causing a series of physiological dysfunctions in the plant [19]. The stress caused by Cd affects the osmotic balance, thereby interfering with the rate of transpiration and the hydration of the plant. These changes can result in a reduction in the leaf surface area, deterioration of the main transpiring tissues, and impairment of stomatal conductance. Consequently, Cd induces dysfunction in transpiration in various species, including *Atriplex nummularia* [35], *Suaeda glauca*, and *Limonium aureum* [52].

Plants belonging to the Atriplex genus, which display a diminished transpiration rate and elevated water use efficiency, are distinguished by C4 photosynthetic metabolism, categorizing them as halophytic plants [33,38,8].

In the reduction of transpiration, for example, when stomata close, water movement is driven by active solute pumping in the roots via symplastic pathways, transferred intracellularly and cell-to-cell through plasmodesmata. Metal ATPases are capable of transporting Cd across membranes and play a significant role in Cd translocation from the roots to the leaves [19,40].

### 4.4. Sodium, potassium, and chlorine content in the aerial part

The findings of our study indicate that in saline soil (SL), there was an interaction between salinity and Cd concentration, resulting in a reduction in $K^+$ levels. This effect was not observed in non-saline soil (NSL), suggesting that salinity amplifies the negative effects of Cd on $K^+$ absorption.

The decline in $K^+$ levels may be linked to the observed reduction in dry biomass of the aerial plant parts in SL. The reduction in dry biomass is likely associated with the depletion of essential cations, such as $K^+$, caused by the accumulation of Cd in the aerial part of the plant [35]. In SL, the elevated concentration of soluble salts facilitates the mobility of Cd, thereby promoting the formation of complexes between Cd and chloride. These complexes may penetrate the roots or dissociate, leading to competition between Cd and sodium at adsorption sites. This process increases the activity and bioavailability of Cd, facilitating its absorption by the roots and resulting in the reduction of $K^+$ as Cd doses increase [17,29].

The concentrations of $Na^+$ and $Cl^-$ were elevated in plants cultivated in SL, which resulted in more negative osmotic (Ψo) and water (Ψw) potentials, thereby facilitating osmotic adjustment (OA) in SL. Therefore, the data obtained demonstrated that *A. nummularia* Lindl. retained its capacity for $Na^+$ and $Cl^-$ phytoextraction in SL, regardless of the increase in Cd doses. Overall, the literature indicates that $Cl^-$ and $Na^+$ are the two primary elements extracted in saline soils, with an increase in these ions observed in the leaf content of *A. nummularia* Lindl. cultivated under saline conditions [18,33].

### 4.5. Cadmium Content in the Aerial Part and Roots

A plant is classified as a hyperaccumulator of Cd when the concentrations in the aerial part exceed 100 mg $kg^{-1}$. This threshold was established by Baker et al. [3] for non-artificially contaminated soils. In the present study, the values observed in the aerial part of the Atriplex were higher than 50 mg $kg^{-1}$, particularly at higher Cd doses, indicating a significant potential for Cd accumulation. However, the observed concentrations and artificial contamination did not reach the threshold for classification as a hyperaccumulator. However, this result indicates that the Atriplex displays a remarkable capacity to tolerate and accumulate Cd, as evidenced by the absence of a reduction in dry biomass of aerial parts ($DB_{AP}$) in plants grown in non-saline soil (NSL) with increasing Cd doses. Furthermore, in saline soil (SL), up to the dose of 34.25 mg $kg^{-1}$ of Cd, plants exhibited higher $DB_{AP}$ compared to those grown in NSL. This suggests that this species may be a promising candidate for phytoremediation programs in areas contaminated with Cd and salinized soils.

Eissa [15] conducted a study to evaluate the halophyte *Atriplex lentiformis* grown in soil contaminated with 50 mg $kg^{-1}$ of Cd and treated with different doses of vinasse and EDTA. The authors observed a notable accumulation of 130 mg $kg^{-1}$ of Cd in the aerial parts and 230 mg $kg^{-1}$ in the roots of the control plants, which did not display any toxicity symptoms.

The significant accumulation of Cd, even in the absence of chelating agents, indicates that the halophyte *Atriplex lentiformis* possesses natural mechanisms for tolerance and accumulation of heavy metals, rendering it a promising candidate for phytoextraction of soil contaminants. It is important to note that the experiment was conducted over a 30-day period, and the plants were cultivated in pots with a capacity of 5 kg of soil. This suggests that, with prolonged cultivation, greater soil volume available for the root system and higher Cd concentrations, the phytoextraction capacity could be significantly enhanced.

Plants of the *Atriplex* genus possess several characteristics that make them suitable for the phytoremediation of soils contaminated by heavy metals. Among these advantages are their robust root system, high biomass production, and ability to thrive in soils with high salt concentrations, such as NaCl, which are essential factors for their adaptability to saline environments [33].

Additionally, their advanced physiological and biochemical mechanisms contribute to their tolerance to osmotic stress and metal toxicity. These mechanisms include osmotic adjustment through the accumulation of compatible solutes (proline, glycine, and sugars), ion compartmentalization in vacuoles, and salt excretion via leaf glands [26,8]. Leaf succulence and the rigidity of cell walls also help dilute toxic salts and maintain cellular integrity [38]. All these traits of the species assist in mitigating heavy metal stress.

In an experimental context with nutrient solution cultivation, Kahli et al. [23] observed Cd levels of 500 mg $kg^{-1}$ in the roots and 1900 mg $kg^{-1}$ in the leaves of *A. nummularia* Lindl., thereby further reinforcing the high capacity of this species to accumulate Cd. It is important to emphasize that the availability of Cd in a hydroponic system is significantly higher than in soil cultivation, leading to enhanced accumulation.

In another scenario, when comparing the performance of *Atriplex nummularia* and *Atriplex amnicola* with traditional forage crops (millet and cowpea) in sandy soils polluted and non-polluted with heavy metals, Ding et al. [11] observed that the roots of traditional forage crops were more sensitive to metal toxicity, experiencing a 15–17 % reduction in root biomass. In contrast, *Atriplex* species maintained root biomass 54 % higher than that of forage crops, even in contaminated soils.

In addition, *Atriplex* also promotes improvements in the quality of contaminated soils by increasing aggregation and porosity, reducing salinity, and decreasing the bioavailability of toxic elements. Specifically, *A. nummularia* cultivated in polluted soil demonstrated higher efficiency in the immobilization of heavy metals compared to uncultivated areas [16]. This effect can be attributed to different mechanisms, such as metal adsorption by roots, complexation with root exudates, metal precipitation in the rhizosphere, and changes in element valence [14].

In a study conducted by Acosta et al. [1], the potential of *Atriplex halimus* for phytoremediation of mining tailings contaminated with heavy metals in a saline environment was evaluated. Over a five-year experiment, the use of marble waste ($CaCO_3$) and pig slurry improved the soil, favoring plant growth and the stabilization of metals such as Cd, Zn, and Pb. *Atriplex halimus* accumulated significant concentrations of metals in its aerial parts. The study highlights the feasibility of using halophytes to restore saline and contaminated areas.

Similarly, *Atriplex nummularia* can be applied in comparable scenarios, leveraging its high tolerance to salinity and its capacity to translocate Cd to the aerial parts. Furthermore, combining the plant with management strategies that consider salinity as a positive factor for metal mobilization can enhance its effectiveness, especially under semiarid conditions. These scenarios underscore the role of *Atriplex nummularia* as a sustainable tool for restoring degraded environments.

### 4.6. Translocation factor (TF)

The findings of this study indicate that salinity exerts a significant influence on the translocation of Cd by *A. nummularia* Lindl. This suggests that these plants exhibit an adaptive response when cultivated in saline soil (SL). This positive effect was evidenced by an enhanced capacity to translocate Cd from the roots to the aerial parts, thereby improving the efficiency of phytoremediation.

As stated by Mcgrath and Zhao [32], plants exhibiting a translocation factor (TF) exceeding 1.0, high biomass production, and a metal content of less than 1 % in the aerial parts are considered metal accumulators and deemed suitable for phytoremediation. In our study, the TF values exceeded 1.0, indicating that *A. nummularia* Lindl. has the ability to accumulate and translocate Cd from the roots to the aerial parts. This renders it a promising candidate for Cd phytoremediation, although further validation in soils naturally contaminated with Cd is required.

Furthermore, the plants exhibited effective water status regulation, effectively mitigating osmotic stress while demonstrating robust dry biomass production without any discernible indications of Cd phytotoxicity. Similar outcomes were documented by Nedjimi et al. [35], wherein most of the absorbed Cd was translocated to the aerial parts (146.59 mg $kg^{-1}$ of Cd with 34 mg $kg^{-1}$ of $CdCl_2$), with TF values exceeding 1.0, substantiating the potential of Atriplex for Cd phytoremediation.

The findings of studies such as those conducted by Zhang et al. [52] serve to reinforce the influence of salinity on the translocation of Cd. In their investigation of the halophytes *Suaeda glauca* and *Limonium aureum*, the authors observed that an increase in salinity resulted in an enhanced absorption and translocation of Cd. In the case of *Limonium aureum*, an elevated level of Cd was excreted through salt glands in the leaves, thereby underscoring the role of salinity in regulating the transport of heavy metals.

Similarly, Kahli et al. [23] conducted an analysis of three Atriplex species (*canescens, halimus,* and *nummularia*) at varying salinity concentrations. Their findings indicated that the introduction of salt led to an increase in Cd accumulation in all three species, with the most pronounced impact observed at lower $CdCl_2$ concentrations (2 mg $kg^{-1}$). *Atriplex nummularia* Lindl. exhibited a Cd accumulation of approximately 1900 mg $kg^{-1}$ in the leaves, with the highest TF values (up to 4) observed in treatments with 5 mg $kg^{-1}$ of $CdCl_2$.

The findings indicate that, in saline conditions, *A. nummularia* Lindl. exhibits enhanced Cd translocation from roots to aerial parts, particularly up to 30 mg $kg^{-1}$ . This illustrates a physiological adaptation of the plants to the saline environment, emphasizing the potential of salinity to enhance the efficiency of Cd phytoremediation.

The results of this study highlight the potential of *A. nummularia* Lindl. for Cd phytoextraction in saline soils, emphasizing the relevance of this halophyte in environmental remediation strategies. To ensure data reliability and avoid confounding factors, a rigorous methodological approach was adopted, selecting soil samples of the same type with similar clay content and physicochemical characteristics. This precaution was essential to isolate the effects of Cd and salinity on plant performance. Therefore, future studies should explore a broader range of soil types and edaphic conditions, allowing for greater applicability of the results obtained in different environmental scenarios.

## 5. Conclusions

The findings of this study indicate that *Atriplex nummularia* Lindl. is an effective phytoremediation agent for Cd in saline soils, with particular emphasis on the maintenance of dry biomass and the capacity to accumulate the metal. Plants cultivated in saline soil exhibited elevated levels of Na and Cl in the aerial parts, suggesting that these ions play a pivotal role in osmotic adjustment and in the plant's adaptation to salinity.

Regarding the accumulation of Cd, the concentrations in the aerial parts exceeded 50 mg $kg^{-1}$ at a dose of 34.25 mg $kg^{-1}$ of Cd, and the translocation factor (TF) remained above 1.0 up to a dose of 30 mg $kg^{-1}$ when cultivated in saline soil. This suggests that the plant has an efficient capacity to translocate Cd from the roots to the aerial parts, thereby reinforcing its potential for phytoremediation. Moreover, at this same dose, the plant exhibited high levels of dry biomass, even under conditions of salinity and Cd exposure, which underscores its tolerance to abiotic stress and its capacity to tolerate Cd.

Therefore, *A. nummularia* Lindl. has been identified as a promising species for phytoremediation, particularly in regions with saline and Cd-contaminated soils. It has demonstrated effective Cd accumulation, metal translocation capacity, and the capacity to adapt to saline environments without significant detriment to biomass production. This last aspect also qualifies the species for revegetation of waste piles in mining areas in semi-arid regions.

## Environmental Implication

This research demonstrates the potential of *Atriplex nummularia* Lindl. as a sustainable solution for remediating saline soils contaminated with cadmium (Cd), a dual environmental challenge affecting degraded ecosystems. The findings highlight that salinity enhances Cd translocation to the aerial parts of the plant without disrupting its water balance, making it suitable for phytoextraction under real-world saline conditions. This study addresses critical knowledge gaps in the remediation of salt-affected areas contaminated with heavy metals, providing a practical approach to restoring soil health and reducing environmental hazards. It underscores the relevance of integrating halophyte-based strategies in the management of contaminated saline landscapes.

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## CRediT authorship contribution statement

**Leal Lucas Yago de Carvalho:** Methodology, Formal analysis. **Silva Allef Rodrigues da:** Methodology, Formal analysis. **Bezerra Fabiano Simplicio:** Methodology, Formal analysis. **Nunes José Alfredo:** Methodology, Formal analysis. **Gonçalves Wellinton Julio Ferreira:** Methodology, Formal analysis. **Berto Steffany Daiana da Costa:** Methodology, Formal analysis. **Souza Edivan Rodrigues de:** Writing – review & editing, Conceptualization. **Nascimento Clístenes Williams Araújo do:** Writing – review & editing, Supervision. **Araújo Cicero Aparecido Ferreira:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Costa Diana Honorato Gomes da:** Methodology, Formal analysis. **Paulino Martha Katharinne Silva Souza:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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