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RESEARCH

# Effects of *Ascophyllum nodosum*-based Biostimulants on Improving Phytoextraction of Cadmium and Lead in Contaminated Soils

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## Abstract
The aim of this study was to improve phytoextraction of cadmium (Cd) and lead (Pb) from contaminated soils using *Ascophillum nodosum* extract (ANE). The experiment was designed in different groups: A-Planting of sorghum within 6 and 12 weeks in unpolluted soil; B-Planting of sorghum within 6 and 12 weeks at three concentrations, as follows: 10 mg $kg^{-1}$ Cd+250 mg $kg^{-1}$ Pb; 30 mg $kg^{-1}$ Cd+500 mg $kg^{-1}$ Pb; and 100 mg $kg^{-1}$ Cd+1000 mg $kg^{-1}$ Pb along with ANE; C-Planting of sorghum within 6 and 12 weeks at the above three concentrations of Cd and Pb without ANE. The results showed that the use of ANE significantly increases the root and shoot biomass, which subsequently improves the uptake of heavy metals from the soil ($p<0.05$). After 12 weeks of using seaweed extract (ANE), the root and shoot biomass of sorghum at the three concentrations of Cd+Pb, i.e., 10+250, 30+500, and 100+1000 (Cd+Pb) mg $kg^{-1}$ increased by 40.7% and 22.4%, 40% and 33.6%, and 100% and 31.8%, respectively ($p<0.05$). Planting sorghum along with ANE significantly improved the uptake of heavy metals from the soil. Therefore, *Ascophyllum nodosum*-based biostimulants can be considered as a green technology to improve the extraction of heavy metals from soil.

## Highlights
- High concentration of heavy metals reduces root and shoot biomass.
- Sorghum biomass is an important factor for the uptake of heavy metals.
- The presence of sorghum improves the bioavailability of heavy metals in the soil.
- *Ascophyllum nodosum* extract promotes plant growth and phytoextraction.

**Keywords** Phytoextraction · Soil pollution · Sorghum · *Ascophyllum nodosum* extract · Heavy metal

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## 1 Introduction

Today, environmental pollution due to heavy metals is considered as a serious concern. These metals enter the environment mainly due to industrial, agricultural, mining and military activities (Tauqeer et al. 2021; Liu et al. 2022). These metals accumulate in products and vegetables and enter the human body through the food chain, thus endangering human health (Hu et al. 2020; Amjad et al. 2022). The U.S. Environmental Protection Agency (USEPA) has classified some of these metals in the priority group. Some of them, such as Cd and Pb, are very dangerous for humans and other organisms (Shah et al. 2021). Cd has a long half-life and accumulates in plants, invertebrates and vertebrates. According to the USEPA, the critical concentration of total Cd in soil is 0.43 mg $kg^{-1}$ (Albarrcín et al. 2019). Cd results in the production of reactive oxygen species (ROS), DNA damage and inhibition of DNA repair (Zhang et al. 2019). European Union Directive 1986/278/EEC limits the concentration of Pb in agricultural soil in the range of 50 to 300 mg $kg^{-1}$ (Nag et al. 2022). Given the harmful effects of the presence of heavy metals in the soil, it is necessary to treat contaminated soils. Soil treatment strategies include physical, chemical and biological methods. Typically, physical and chemical methods for heavy metal removal are relatively extensive. Although these techniques seem appropriate for removing heavy metals from the environment, they are difficult and expensive to apply and can cause negative changes in soil properties (Gavrilescu 2022). For example, EDTA is an appropriate choice to increase the efficiency of phytoextraction. Nevertheless, due to its negative impacts such as toxicity to plants and microorganisms, its non-degradability and reduction in plant biomass, the research on more appropriate and safer methods to improve phytoextraction continues (Liu et al. 2022).

Considering that the methods of removing heavy metals from the soil are often expensive and time-consuming or cause adverse effects on the soil environment, the tendency to use plant-based methods is increasing. Phytoremediation is an environmentally-friendly and cost-effective method for soil pollution that has been considered by many researchers (Ullah et al. 2021). On the other hand, the growth of plants is reduced under the influence of the toxic effects of pollutants, and subsequently their ability to remove pollutants is reduced. Recently, some studies have proposed biostimulants as a new option to increase the efficiency of phytoremediation (Arthur et al. 2016; Ullah et al. 2019).

Biostimulants are substances widely used in agriculture to increase crop production and resistance to various types of stress (Yakhin et al. 2017). This approach can be used to increase the resistance of plant species against toxic substances (Bartucca et al. 2022). Algal biostimulants are natural polysaccharide polymers from brown algae. The extracts of these seaweeds contain many natural substances that are useful for the growth of plants. Algal biostimulants can increase root growth in plants and improve plant survival (Hu et al. 2020).

Seaweeds produce many substances and secondary metabolites that can be used in various environments. Recently, there has been a growing global interest in knowing more about the potential of seaweed. Research has shown that seaweed has many beneficial compounds for plant growth. Adding seaweed to the soil environment provides plant growth regulators, organic matter and amino acids, and other nutrients (Silva et al. 2019). The effects of using seaweed to extract heavy metals from the soil have shown that the use of aqueous extracts of *F. spiralis* and *C. ericoides* improve the resistance of the plant against the stress caused by heavy metals (El Khattabi et al. 2023). ANE is widely used as a biostimulant in agriculture.

The advantages of using this seaweed on plants include increasing the rate of germination, increasing root growth, increasing shoot biomass, improving the efficiency of nutrient use, delaying aging, increasing the content of chlorophyll, flavonoid and nutrients, and improving tolerance to abiotic and biotic stresses (Wally et al. 2013; Ertani et al. 2018; De Saeger et al. 2020).

In many studies, the effect of using ANE to improve physiological and biochemical parameters and reduce the negative effects of stress on plants was effectively expressed (Ali et al. 2022). Although the positive effects of ANE on improving plant growth have been proven, there is still limited information on the effects of ANE on improving phytoextraction of heavy metals. In order to achieve a cost-effective method with minimal negative effects on the environment, in this experiment, ANE was used to reduce the negative effects of pollutants on plant growth. Therefore, the aim of this study was to investigate the phytoextraction of Cd and Pb using sorghum and the use of ANE to increase plant growth and improve the uptake of heavy metals from the soil.

## 2 Materials and methods

### 2.1 Soil Preparation

The soil sample was prepared from the depth of 0–20 cm. After drying in the open air and passing through a 4 mm sieve, it was uniformly mixed and some physical and chemical properties of the soil including soil texture, electrical conductivity, pH, phosphorus, potassium, nitrogen, cation exchange capacity, percentage of organic matter were determined (Table 1).

### 2.2 Experimental Design

The concentrations of cadmium and lead in the soil were as follows: (10 mg $kg^{-1}$ Cd + 250 mg $kg^{-1}$ Pb, 30 mg $kg^{-1}$ Cd + 500 mg $kg^{-1}$ Pb and 100 mg $kg^{-1}$ Cd + 1000 mg $kg^{-1}$ Pb). In order to add heavy metals to the soil, $Pb(NO_3)_2$ and $Cd(NO3)_2$ were used. Before planting the seeds, the pots were kept inside the greenhouse for 1 week (at 60% of field capacity). The seeds were planted in 2-kg pots. 10 sorghum seeds were planted in each pot. After 4 weeks of planting sorghum, 500 mg $L^{-1}$ of ANE was applied as a foliar spray at 3 times (once every four days). Then, the effect of different parameters such as different pollutant concentrations

**Table 1** Physical and chemical properties of the soil used in the experiment

| Characteristics | Quantity |
|---|---|
| pH | 7.85 |
| Electrical conductivity (dS $m^{-1}$) | 1.53 |
| Cation exchange capacity (cmol $kg^{-1}$) | 24.5 |
| OC (%) | 1.229 |
| Total Nitrogen (%) | 0.139 |
| Potassium ( mg $kg^{-1}$) | 394 |
| Phosphorus ( mg $kg^{-1}$) | 47.8 |
| Soil Texture | Clay loam |
| Sand | 23% |
| Silt | 43% |
| Clay | 34% |

and time were investigated. The experiment lasted 12 weeks and the uptake of heavy metals in the pots was measured at the end of the sixth and twelfth weeks.

## 2.3 Plant Biomass

At the end of the sixth and twelfth weeks, the plants were removed from the soil and the roots and shoots of each plant were separated and washed separately with tap water, and then drained with distilled water. The dry weight of the plant after drying at 105 $^{o}$C was determined within 24 h.

## 2.4 Experimental Procedure

The experimental groups are as follows:

A. Planting sorghum within 6 and 12 weeks in unpolluted soil (Ck).
B. Planting sorghum within 6 weeks at three concentrations of Cd and Pb (10+250, 30+500, and 100+1000 (Cd+Pb) mg $kg^{-1}$).
C. Planting sorghum within 6 weeks at three concentrations of Cd and Pb along with ANE.
D. Planting sorghum within 12 weeks at three concentrations of Cd and Pb.
E. Planting sorghum within 12 weeks at three concentrations of Cd and Pb along with ANE.

## 2.5 Translocation Factor of Heavy Metal

Transfer of heavy metals from roots to shoots was calculated according to the translocation factor based on the equation:

$$TF = Cshoot/Croot$$

where $C_{shoot}$ indicates the concentration of Cd and Pb (mg $kg^{-1}$) in the plant shoot, and $C_{root}$ indicates the concentration of Cd and Pb (mg $kg^{-1}$) in the plant roots (Ullah et al. 2020). $TF > 1$ indicates the effective translocation of heavy metals from the root to the shoot of the plant.

## 2.6 Analysis of Cd and Pb

The plant samples were digested using nitric acid according to EPA 3050B (USEPA 1996). Heavy metals were measured using ICP-MS (Agilent 7800, USA).

## 2.7 Statistical Analysis

In this experiment, the rate of Cd and Pb uptake and biomass of root and shoot at different concentrations were investigated with three repetitions. SPSS ver. 22 and ANOVA statistical test were used for the difference of means between different treatments. LSD was used for post hoc. Significance level of $p < 0.05$ was considered.

# 3 Results and Discussion

## 3.1 Effect of Heavy Metals on Root and Shoot Biomass

Since heavy metals are not biodegradable, the optimum mechanism for removal of heavy metal-contaminated soil is mainly phytoextraction. Plants affect the removal of heavy metals by improving the rhizosphere and root secretions, increasing the activity of rhizosphere microbes and the bioavailability of metals, thereby increasing their uptake (Njoku et al. 2022). Li et al. (2021) stated that planting *Sedum alfredii* has increased the bioavailability of Cd, Pb and Zn in the soil, which may have a special relationship with plant root secretions. Due to the stress caused by heavy metals, plant roots secrete a large amount of organic acids with low molecular weight. These organic acids affect the soil environment and reduce the pH of the soil. The heavy metal undergoes a chelation reaction that changes its chemical form, thereby affecting its bioavailability. The high concentration of heavy metals in the soil causes severe damage to the plant metabolic activities. Therefore, the proper performance of the plant during exposure to heavy metals seems very necessary (Ullah et al. 2023). On the other hand, plant biomass is an important factor for extracting heavy metals from the soil (Alaboudi et al. 2018; Wang et al. 2021). Exposure of plants to high concentrations of heavy metals leads to a reduction in growth and subsequently biomass of plants (Duan et al. 2018; Wang et al. 2020; Zhao et al. 2021). The results of this study showed that the presence of Cd and Pb, as well as increasing their concentration, decreased the biomass of sorghum.

Figure 1 shows that after 6 weeks in Root6 group, the root biomass at the concentrations of 10+250 and 30+500 and 100+1000 mg $kg^{-1}$ (Cd+Pb) was reduced by 15.8%, 26.3% and 61.6%, respectively, compared to the control. In general, the presence of heavy metals in the soil causes adverse conditions for the growth of plants. Dubey et al. (2018) stated that toxicity of heavy metals affects the structural properties and permeability of internal membranes, and causes a reduction in enzyme activities, an imbalance of nutrients and a reduction in photosynthesis. The presence of heavy metals in the soil stimulates the formation of ROS, which leads to oxidative stress, and reduces germination and plant growth. Zhang et al. (2018) investigated the effects of heavy metals on the physiological characteristics of *Suaeda glauca and Arabidopsis thaliana*. Similarly to the results obtained in our study, biomass, height and root length decreased with the increase of heavy metal concentrations in soil. The results of the present study show that the use of *Ascophyllum nodosum*-based biostimulant significantly reduce the effects of heavy metals on plant growth.

The application of ANE by providing plant nutrients improves the growth and tolerance of the plant against environmental stresses (Shukla et al. 2019). As shown in Fig. 1, the use of seaweed in Root6+ANE group at the concentrations of 10+250 and 30+500 and 100+1000 (Cd+Pb) mg $kg^{-1}$ increased root biomass by 50%, 64.3% and 132.9% compared to Root6 group, respectively ($p<0.05$). Also, after 12 weeks of using seaweed, root biomass in Root12+ANE group at the concentrations of 10+250 and 30+500 and 100+1000 (Cd+Pb) mg $kg^{-1}$ increased by 40.7%, 40% and 100% compared to Root12 group, respectively ($p<0.05$).

After 6 weeks, the shoot biomass in Shoot6+ANE group increased by 27.9%, 39.3% and 47.8% at the concentrations of 10+250, 30+500 and 100+1000 (Cd+Pb) mg $kg^{-1}$ compared to Shoot6 group, respectively ($p<0.05$). At the end of week 12, the shoot biomass in Shoot12+ANE group at the concentrations of 10+250, 30+500 and 100+1000

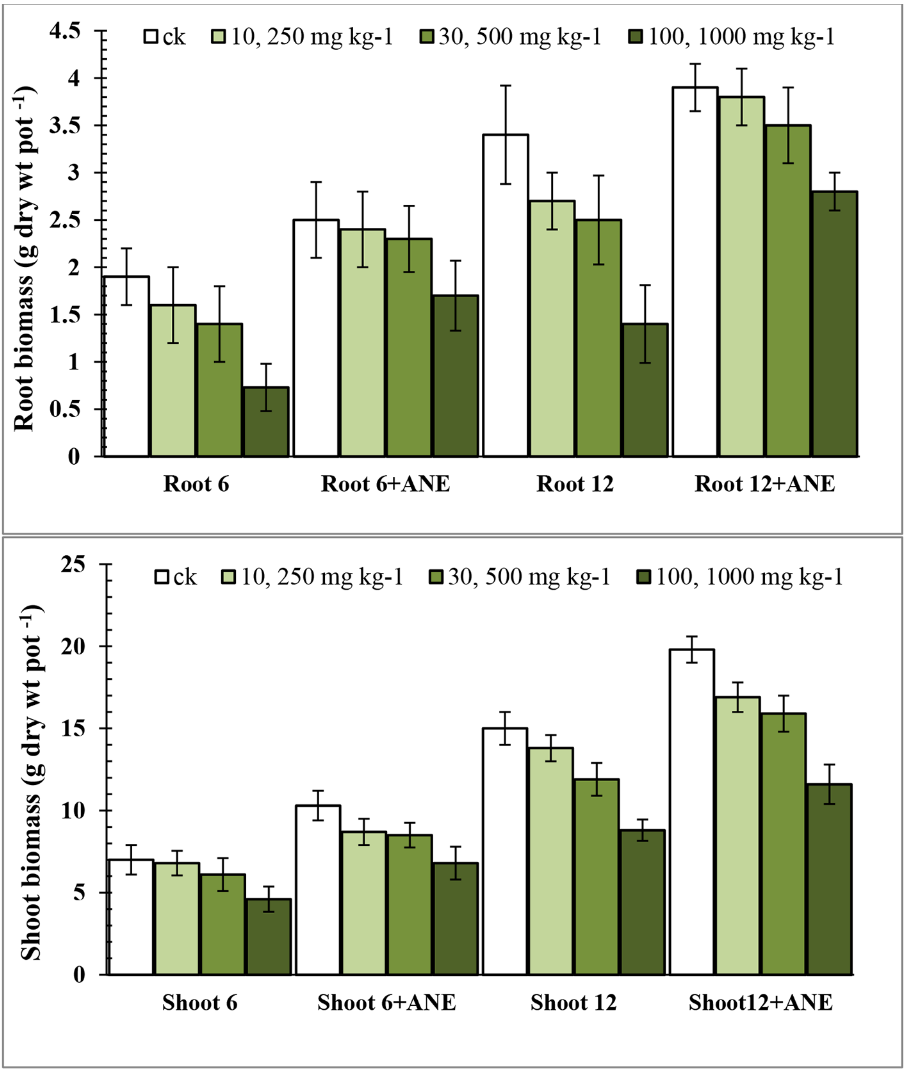


**Fig. 1** Root and shoot biomass of sorghum at three concentrations. **(Root6 and Shoot6)**: root and shoot biomass after 6 weeks; **(Root6 + ANE and Shoot6 + ANE)**: root and shoot biomass after 6 weeks along with *Ascophyllum nodosum* extract; **(Root12 and Shoot12)**: root and shoot biomass after 12 weeks; **(Root12 + ANE and Shoot12 + ANE)**: root and shoot biomass after 12 weeks along with *Ascophyllum nodosum* extract. All results are expressed as means ± standard deviation, n = 3

(Cd + Pb) mg $kg^{-1}$ increased by 22.4, 33.6 and 31.8% compared to Shoot12 group, respectively ($p < 0.05$).

Many studies have reported that ANE improves plant growth and reduces biotic and abiotic stresses (Goñi et al. 2018; Frioni et al. 2021; Drygaś et al. 2021) reported that ANE improved agricultural productivity by regulating the uptake of some nutrients and plant performance under adverse conditions. In the study by Hasanuzzaman et al. (2023) that was conducted in order to reduce arsenic toxicity for plants using ANE, the results showed that ANE contains a wide range of minerals that promoted plant growth and improved plant tolerance against abiotic stresses. The results of this study similarly showed that the application of ANE reduces the negative effects of arsenic and increases the height and biomass of the plant compared to the control.

### 3.2 Uptake of Heavy Metals by Sorghum

Figure 2 shows the concentration of Cd and Pb in sorghum roots. After 12 weeks, the use of *Ascophyllum nodosum*-based biostimulant increased the concentration of Cd in the root by 81.8%, 28.4% and 19.3% at the levels of 10, 30 and 100 mg $kg^{-1}$, respectively ($p<0.05$). Also, after 12 weeks, the concentration of Pb in the root increased by 60.6%, 24.3% and 15.8% at the levels of 250, 500 and 1000 mg $kg^{-1}$, respectively ($p<0.05$).

Figure 3 shows the concentration of Cd and Pb in sorghum shoots. In the groups with ANE, the concentration of Cd in the shoot after 12 weeks increased by 77.5%, 36.9% and 26.8% at the levels of 10, 30 and 100 mg $kg^{-1}$, respectively ($p<0.05$). In addition, after 12 weeks, the use of ANE increased the Pb concentration in the shoot by 72%, 29.9% and 16.8% at the levels of 250, 500, and 1000 mg $kg^{-1}$, respectively ($p<0.05$). Figure 4 shows the translocation of Cd and Pb from the root to the shoot of sorghum at different concentrations after 12 weeks. The results of various studies showed that the use of biostimulants could significantly increase the efficiency of plants and their biomass under adverse conditions (Mrid et al. 2021; Ahmed et al. 2021) investigated the effect of *Ulva fasciata* and *Sargassum lacerifolium* seaweeds to reduce heavy metals in soil and improve plant growth. Seaweed mixture reduced the content of Pb, Cu, Zn and Ni in soil samples. Seaweed inoculation increased plant growth and improved germination rate and morphological and biochemical growth parameters. They also stated that the reason for the increase in plant growth is the presence of nutrients and plant growth regulators (gibberellin, indole acetic acid and cytokinin) in seaweed.

El Khattabi et al. (2023) investigated the effects of three seaweeds *Fucus spiralis*, *Cystoseira ericoides* (Phaeophyceae) and *Ulva lactuca* (Chlorophyceae) on the tolerance and accumulation of Pb in plants. The results showed that the extracts of *F. spiralis* and *C. ericoides* increased the biomass of the plant compared to the control. Due to the presence of Pb in the soil, these seaweed extracts increased plant tolerance by reducing anthocyanin and proline content. Finally, similar to the results obtained from our study, the use of *F. spiralis* extract improved phytoextraction of Pb.

Increasing plant biomass improves the physical and chemical conditions of the soil, which improves the uptake of heavy metals from the soil. The results of our study indicated that sorghum along with ANE had a significant effect on increasing root and shoot biomass (Fig. 5). Hussain et al. (2021) stated that the application of ANE has a positive effect on plant performance. This study showed that plant amplification with ANE improves the uptake of heavy metals from the soil. In this process, root biomass is considered as one of the important factors so that the higher the biomass, the higher the uptake rate of heavy metals. Similarly, Kubátová et al. (2016) reported that increasing biomass improves the uptake of heavy metals from soil.

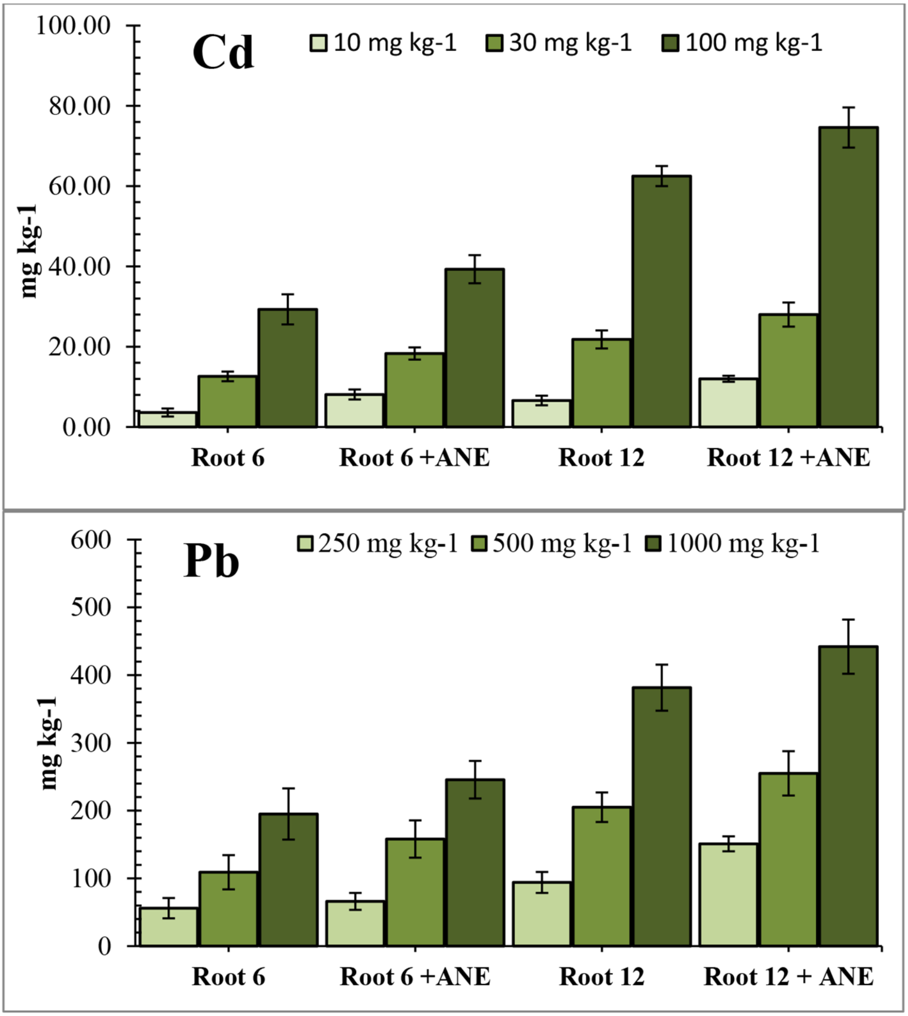


**Fig. 2** Uptake of Cd and Pb by sorghum roots at three concentrations. **(Root6)**: uptake of Cd and Pb by sorghum roots after 6 weeks; **(Root6+ANE)**: uptake of Cd and Pb by sorghum roots after 6 weeks along with *Ascophyllum nodosum* extract; **(Root12)**: uptake of Cd and Pb by sorghum roots after 12 weeks; **(Root12+ANE)**: uptake of Cd and Pb by sorghum roots after 12 weeks along with *Ascophyllum nodosum* extract. All results are expressed as means ± standard deviation, n = 3

## 4 Conclusions

The effects of *Ascophyllum nodosum*-based biostimulant on improving the growth of sorghum and extracting Cd and Pb from the soil were investigated in this experiment. The study results showed that ANE significantly increased the growth of sorghum at all concentrations ($p < 0.05$). After 12 weeks of ANE use, the root and shoot biomass at concentrations of 10+250, 30+500 and 100+1000 (Cd+Pb) mg $kg^{-1}$ increased by 40.7% and 22.4%, 40% and 33.6%, and 100% and 31.8%, respectively ($p < 0.05$). The use of ANE led to an increase

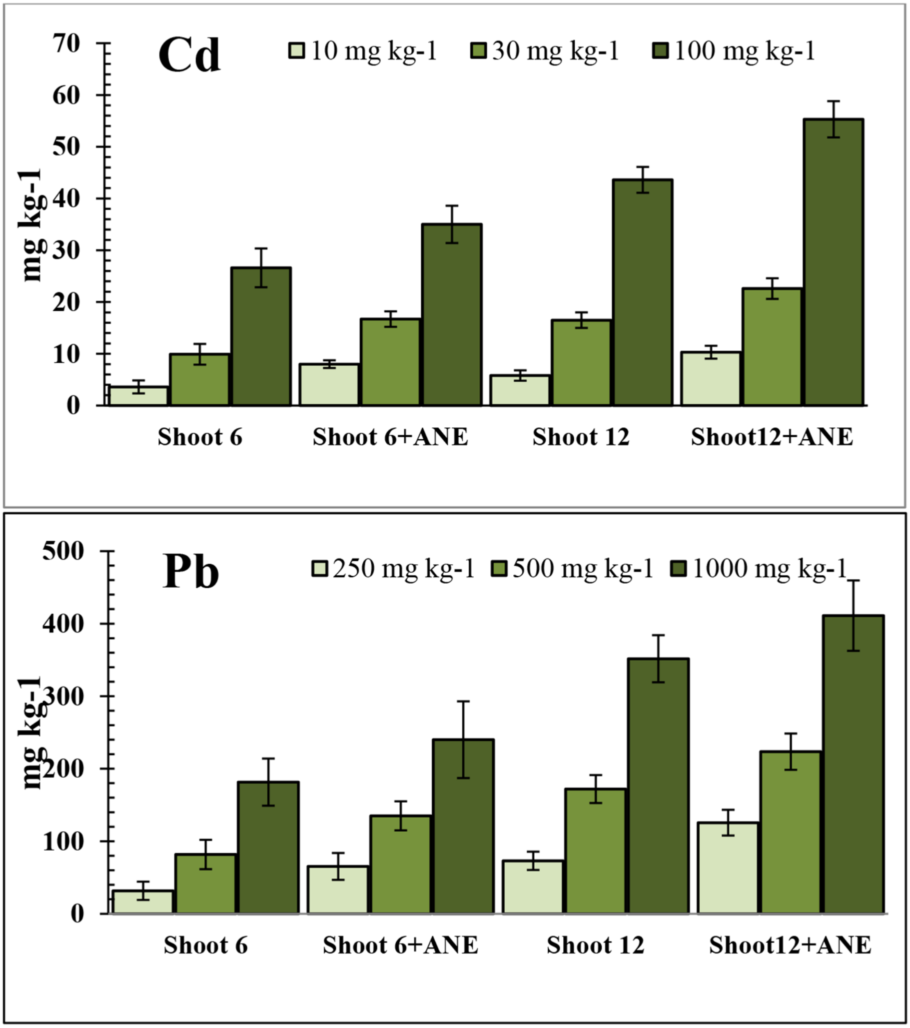


**Fig. 3** Uptake of Cd and Pb by sorghum shoots at three concentrations. **(Shoot6)**: uptake of Cd and Pb by sorghum shoots after 6 weeks; **(Shoot6+ANE)**: uptake of Cd and Pb by sorghum shoots after 6 weeks along with *Ascophyllum nodosum* extract; **(Shoot12)**: uptake of Cd and Pb by sorghum shoots after 12 weeks; **(Shoot12+ANE)**: uptake of Cd and Pb by sorghum shoots after 12 weeks along with *Ascophyllum nodosum* extract. All results are expressed as means ± standard deviation, n = 3

in plant growth and an increase in the plant tolerance to environmental stress, which subsequently improved the uptake of heavy metals from the soil. Therefore, the use of *Ascophyllum nodosum*-based biostimulant can be considered as a cost-effective strategy with high efficiency for the extraction of heavy metals.

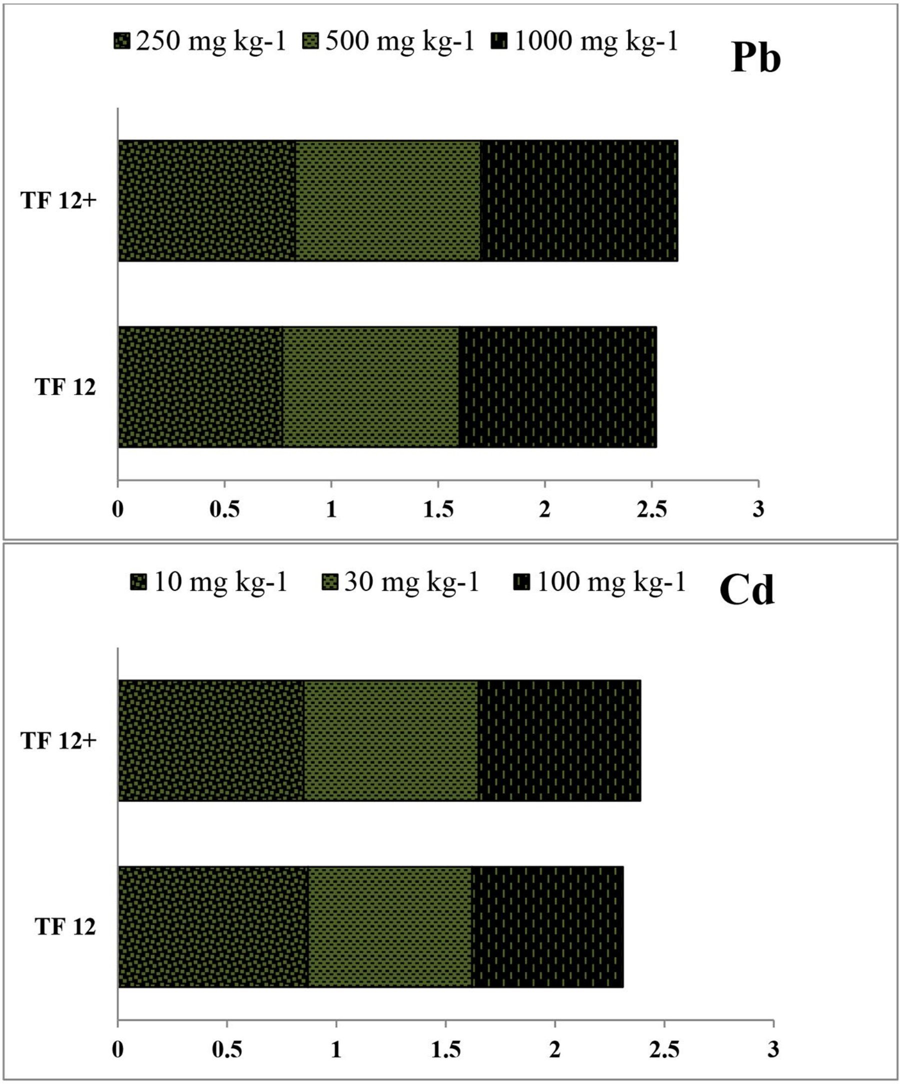


**Fig. 4** Translocation factor for Cd and Pb after 12 weeks at different concentrations (TF12+): Application of ANE; (TF12): Without ANE

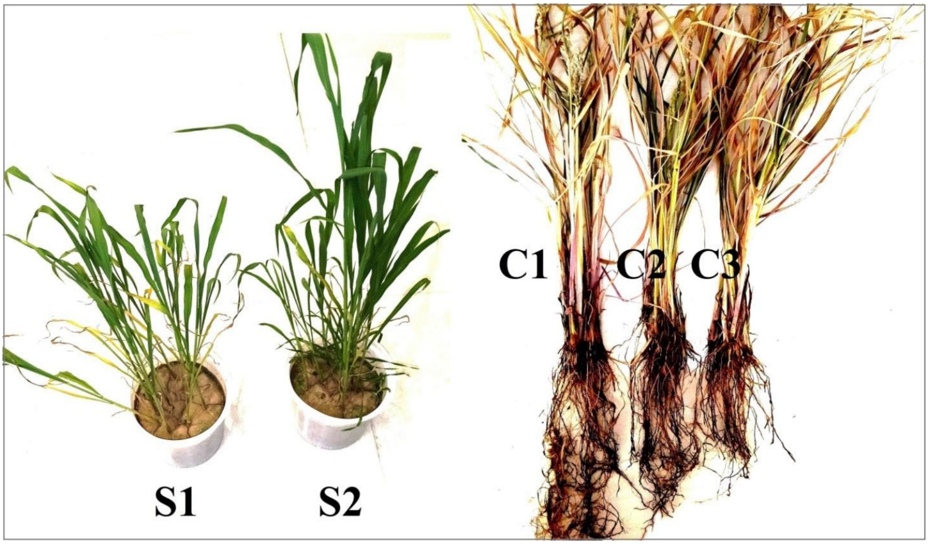


**Fig. 5** Effects of heavy metal concentration on sorghum biomass after 12 weeks: **C1**-Sorghum biomass at a concentration of 10+250 (Cd+Pb) mg kg$^{-1}$; **C2**-Sorghum biomass at a concentration of 30+500 (Cd+Pb) mg kg$^{-1}$; **C3**-Sorghum biomass at a concentration of 100+1000 (Cd+Pb) mg kg$^{-1}$. Effects of ANE on improving plant growth after 12 weeks: **S1**-sorghum at a concentration of 30+500 (Cd+Pb) mg kg$^{-1}$without ANE; **S2**-sorghum at a concentration of 30+500 (Cd+Pb) mg kg$^{-1}$ along with ANE.

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**Author Contributions** Saeid Rostami performed the measurements and wrote the manuscript. Hesam Akbari was involved in planning and supervised the work. Hamed Akbari and Amir Adibzadeh developed the theoretical framework. All authors discussed the results and contributed to the final manuscript.

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**Data Availability** Most of the data are available in the manuscript.

## Declarations

**Competing interests** There are no relevant financial or non-financial competing interests.

**Ethical Approval** Not applicable.

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