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RESEARCH ARTICLE

# Evaluation of some chelating agents on phytoremediation efficiency of *Amaranthus caudatus* L. *and Tagetes patula* L. in soils contaminated with lead

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## Abstract

**Purpose** This study was designed to evaluate the possible effects of some chelating agents on phytoremediation efficiency and plant growth parameters of *Amaranthus caudatus* L. and *Tagetes patula* L. in soils contaminated with lead.

**Method** The plant species were grown in pots and treated with lead nitrate and in combination with 2.5, 2.0 and 2.5 mmol/kg of EDTA, SA and CA, respectively.

**Results** The results showed that the highest accumulations of Pb (mg/kg) with 0.74 and 0.13 were found in the roots and stems of *A. caudatus* exposed to 400 mg/kg Pb containing EDTA and SA, respectively. Moreover, the highest accumulation of Pb in the roots and stems of *T. patula* with 0.87 and 1.5 mg/kg were observed in 400 mg/kg Pb- contaminated soil containing SA.

**Conclusions** Although the results obtained showed that *T. patula* would have a better phytoextraction potential than *A. caudatus*, it should be noted that due to the Pb behavior in the soil and/or leaching of Pb from the soil columns during the irrigation period the low amounts of Pb absorption by the root and aerial parts of the plants compared to the added doses of $Pb(NO_3)_2$ solution to the soil samples, imply the studied plants haven't the adequate potential for phytoextraction of Pb from contaminated soils.

**Keywords** Chelating agent · Pb-contaminated soil · Ornamental plants · Phytoremediation

## Introduction

In recent decades, the accumulation of toxic and trace elements and their impacts on both human and environmental health are known as serious concerns around the world [19, 50].

Among the heavy metals, lead is considered as one of the most frequently encountered heavy metals of environmental concern [61]. Lead is released into the environment from burning of gas for heating, the combustion of gasoline containing Pb, the vehicle tires abrasion and spills from batteries [44].

Liver and renal failure, reduced reproductive capacity, impaired organ functioning, disorders in brain functions and tumors are the most adverse effects of Pb on human health. Meanwhile, exposure to this element has been associated with learning disabilities, reduced IQ, slow growth, antisocial behaviors, hyperactivity and impaired hearing [13].

It has been proved that soil pH, organic matter (OM) content, texture, mineralogy, and source and quantity of Pb in the soil are the important factors that can affect the nature of Pb chemical distribution, degree and strength of retention of this element within the soil and may influence the Pb availability to biological organisms [35, 45]. The literature review shows that soils tend to bind the various types of lead to some degree and, therefore, not all soil Pb is equally mobile or bioavailable In this regard, bioavailability of lead varies under some factors such as form and solubility of Pb present in the soil and site-specific soil chemistry [47].

Since the contaminated soil with toxic elements can cause severe damage on the plant, animal and human health, nowadays, the cleanup of heavy metal-contaminated soils is necessary and the development of appropriate in situ and ex situ technologies to remediate contaminated sites has attracted

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great interest [31, 46]. In this regard, it should be noted that some soil factors such as pH, soil cation exchange capacity (CEC), or organic matter content play important roles in successful soil remediation processes [30]. There are several methods such as performing some chemical treatments, introducing organic substances to the soil, or even liming that can limit the uptake of the toxic elements by the plants [33]. The removal of heavy metals by traditional methods is very expensive; thus their application in wide areas is not affordable from the economic point of view. Therefore, some chemical and mechanical cleanup techniques have been developed [37]. Among them, phytoremediation as an emerging biotechnology, which uses green plants to assimilate, degrade, detoxify, extract or metabolize organic, inorganic or nuclear pollutants from environmental media, especially soil, seems eco-friendly, cost-effective, non-invasive and technically applicable in situ. Therefore, phytoremediation has attracted more attention compared to conventional ex-situ clean-up technologies [9, 18, 57, 61]. It appears that the technique might become a viable alternative to chemical and mechanical approaches in the purification of metal-polluted sites [51, 53]; however, the success of the phytoremediation process is dependent on an adequate yield of plants, the bioavailability of metals to plant roots and the efficient transfer of elements from the roots of the plants into their shoots [24]. Phytoremediation techniques include phytostabilization, phytoextraction, phytodegradation, phytovolatilization, and phytofiltration. Among these techniques, phytostabilization and phytoextraction are suitable for remediating inorganic-contaminated soil, especially soil contaminated by toxic heavy metals [18, 27].

A plant's ability to uptake environmental contaminants is directly related to the bioavailability of the toxic pollutants [25] although just a fraction of soil metal content is readily available for plant uptake [61]. In this regard, some plants naturally uptake high amounts of contaminants, while others can uptake the contaminants through the use of chelating agents. Therefore, at present, the usage of chelating agents to raise element uptake by plants from contaminated soils is one of the strategies of phytoextraction. However, the use of hyperaccumulator species is another strategy for this purpose [2, 52].

As hyperaccumulator species have low biomass production and slow growth rate, the accumulation rate of heavy metals by their tissues is low. Therefore, to compensate for this problem and enhance the bioavailability and mobility of toxic elements in contaminated soils using natural and synthesized chelators, such as salicylic acid (SA), citric acid (CA) and ethylenediaminetetraacetic acid (EDTA), has been recommended in many previous studies [11, 17]. It should be noted that although synthetic chelators at high amounts can also be toxic to plants or prove disadvantageous, adding these compounds can increase the solubility of elements in soil solution, facilitate the transport of elements into xylem and also increase the translocation of elements from the roots to shoots (stem and leaves) of plants [30]. However, in soils contaminated with multiple elements, the application of these agents can reduce both the total amount of the metals removed and the biomass of the plant because the high contents of other elements in the soil solution may be toxic to the plant [10, 29]. The review of literature shows that EDTA and EDTA–heavy metal complexes can be toxic to soil microorganisms and plants and they can also persist in the environment due to their low biodegradability. Therefore, increase in the potential off-site migration of metals, either by the leaching of metals into groundwater or through surface runoff is not unexpected [12, 22, 31].

Based on the results of previous studies, the ideal herbaceous species for phytoremediation exhibit a rapid growth rate, high biomass and also the ability to tolerate and accumulate high amounts of elements in their above-ground tissues [40, 54, 65]. However, it should be noted that most of the previous research has focused only on non-economic crops (wild species) and little studies have been conducted on ornamental plants.

Although several studies have been carried out for evaluating the effects of complexing chelating agents, usually with a similar structure and specificity, on the uptake of the Pb by ornamental plant species, the objective of the current study is to assess the effects of some chelating agents with different structures and specificity (i.e. EDTA, SA and CA) on the phytoremediation capability of *Amaranthus caudatus* L. and *Tagetes patula* L. in artificially Pb-contaminated soils. Also, the efficacy of EDTA, SA and CA on the uptake of Pb by the above-mentioned plants and the effect of the Pb toxicity, chelating agents and interaction between them on some plant growth parameters are also evaluated.

## Materials and methods

### Soil sampling and soil characterization

Soil specimens were collected in a single batch for the study from a depth of 0–20 cm from a clean site (an abandoned agricultural land) in Hamedan Province, west of Iran with the aim of artificially contaminating soil samples with lead nitrate [30, 55]. The sampling site was divided into four parts and specimens were collected from each of the parts. These were then mixed thoroughly to make the samples homogeneous for the experiment [8, 9, 54]. In the laboratory, the air-dried specimens were prepared for soil characterization and pot experiments. In so doing, after homogenizing and sieving all the soil specimens for the removal of foreign materials and coarse particles like wood particles, plastic pots (20.0 cm × 15.0 cm) were filled with about 3.5 kg soil [49]. In this work, based on the results and suggestions of the previous related studies, the experimental treatments were

artificially contaminated with 200 and 400 mg/kg of lead nitrate [Pb $(NO_3)_2$] solution to significantly improve the phytoremediation efficiency of Pb-contaminated soils [1, 21]. Also, to reproduce the process of Pb sorption by the soil, the soil specimens were left to equilibrate for a period of 14 days before they were remixed and used for the experiments [16].

Then, the pH [3], CEC [60] and OM [64] as basic physicochemical properties of the soil specimens were determined. Based on the data, the pH of the studied soil was 7.95; the sand, silt, and clay of the studied soil were 21.14%, 39.22%, and 28.49%, respectively; CEC content was 10.06 cmol/kg; total nitrogen, available phosphorus and available potassium were 0.94 g/kg, 8.2 mg/kg and 13.5 mg/kg, respectively; the content of OM was 0.21%, moisture content was 0.72% and the content of total Pb was 0.38 mg/kg.

## Greenhouse experiments

Greenhouse studies were performed in a natural light condition having a daily temperature of 21–26 °C, relative humidity from 30 to 40% and 12.5 h photoperiod for surveying Pb uptake by *A. caudatus* and *T. patula* and its effect on their growth characteristics [34]. In so doing, plant seeds were purchased from Agriculture and Natural Resources Research and Education Center of Hamedan Province. Then, to eliminate the contaminants before the beginning of the actual experiment the seeds were surface sterilized according to the methods described by Bardiya-Bhurat et al. (2017) [6]. In the next step, seeds were sown in trays containing the collected soil and allowed to grow for 1 week. After this period of time, 15 seedlings of similar sizes to the cultured plants were selected to initiate the greenhouse experiment (i.e. experimental and control treatments) with three replicates in a completely randomized block design [34, 66] (Table 1). Then, chelating agents were initially applied to each pot at rates of 0 for control, 2.5 mmol/kg for EDTA treatment, 2.0 mmol/kg for SA treatment and 2.5 mmol/kg for CA treatment at the same time as shown in Table 1. Finally, all the experimental and control pots were placed in plastic trays to prevent any leachate loss, and the plants were harvested 60 days after the first application of each chelating agent [31].

**Table 1** Experimental treatments and control used in the study

| Treatment | The added dose of lead nitrate solution (mg/kg) | Cultured seedlings | Chelator concentration (mmol/kg) | | |
|---|---|---|---|---|---|
| | | | EDTA | SA | CA |
| Control (CT) | 200 | *A. caudatus* | – | – | – |
| | 400 | and | – | – | – |
| EDTA treatment | 200 | *T. patula* | 2.5 | 2.5 | 2.5 |
| | 400 | | 2.5 | 2.5 | 2.5 |
| Salicylic acid | 200 | | 2.0 | 2.0 | 2.0 |
| treatment (SAT) | 400 | | 2.0 | 2.0 | 2.0 |
| Citric acid treatment | 200 | | 2.5 | 2.5 | 2.5 |
| (CAT) | 400 | | 2.5 | 2.5 | 2.5 |

Experimental species were treated with the chelating agent solutions while control plants were grown in soil samples without added chelating agent solutions. After 30 days, the cultivated plants were harvested to determine the rate of Pb accumulation. Also, dry weight and length of root and shoot parts and metal accumulation in the roots and stems were evaluated separately. In the current study, the deionized water was used to keep the soil moist [66].

## Analysis of soil specimens

For the analysis of total content of Pb in soil samples, 1 g of each samples was digested by triacid attack i.e., mixture of 2.5, 5 and 7.5 mL HF, $HClO_4$ and $HNO_3$, respectively, at 180 °C for 10 min in a microwave oven. Then, the prepared solutions were diluted to 50 mL using double distilled water, and finally, the Pb content was determined using ICP-OES (ES-710, Varian, Australia) [5].

## Plant samples preparation and chemical analysis

In so doing, at the first, plant samples were divided into the root and stem parts [66]. Then each part was prepared for elemental analysis according to the methods described by Pavlović et al. (2017) [38]. At the end, the concentration Pb in the prepared specimens was measured with three replicates using ICP-OES (710-ES, Varian, Australia). Also, according to Davodpour et al. (2019) [14], the precision of the analysis was measured by triplicate analyses of the same specimen and white clover as certified reference materials. The results showed that the recovery rates for Pb were between 93.7% and 102.3%.

## Bioconcentration, bioaccumulation, translocation, accumulation factors and translocation efficiency

In this study, the BCF, BAF, TF, TE, and AF were used to study a plant's capability to accumulate Pb from soils, to examine its efficiency in up-taking Pb from the soil, to measure its ability to accumulate them in its tissues, and to estimate the transportation of this element through the plant, respectively. These indexes are defined in accordance with eqs. 1 to 5 [36, 42, 43]:

$$\mathrm{BCF} = \frac{Cr}{Cm} \quad (1)$$

, where $C_r$ (mg/kg) and $C_m$ (mg/kg) are element contents in the underground (root) part of plants and in the medium (soil), respectively.

$$\mathrm{BAF} = \frac{Cs}{Cm} \tag{2}$$

, where $C_s$ and $C_m$ are element contents (mg/kg) in the stem part of plants and in the medium (soil), respectively.

$$\mathrm{TF} = \frac{Cs}{Cr} \tag{3}$$

, where $C_s$ and $C_r$ are element contents (mg/kg) in the stem or other aerial parts (i.e., leave, and flower) and root parts of plants, respectively.

$$\mathrm{TE}\ (\%) = \frac{Cs}{Cs + r} \times 100 \tag{4}$$

, where $C_s$ and $C_{s+r}$ stand for element contents (mg/kg) in the stems and in the whole plant, respectively.

$$\mathrm{AF} = \frac{Cr + s}{Cm} \tag{5}$$

, where $C_{r+s}$ (mg/kg) and $C_m$ (mg/kg) are the element concentrations in the plant tissues (root and stem) and in the medium (soil), respectively.

Here, when TF is higher than 1, it could be concluded that the plant has the potential to translocate the elements effectively from root to the stem. Also, BFC > 1 indicates that the plants are suitable for the phytoextraction process [14].

### Statistical analysis

For statistical analyses, SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used. In so doing, the means and standard deviations of Pb contents were computed for each plot. The normality of the data was assessed using the Shapiro–Wilk test. The results obtained were evaluated using ANOVA, followed by Duncan's multiple range test (DMRT). A $p$ value ≤0.05 indicated a significant difference. Also, correlations between each level of Pb for experimental pots were computed running Pearson's correlation coefficient.

## Results and discussion

Nowadays, chemically enhanced phytoextraction is known as an effective approach for removing toxic and trace elements from contaminated soils using plants. Therefore, so far some compounds such as EDTA, SA and CA as chelating agents have been used to increase the accumulation and translocation of elements, notably Pb, from the root into aboveground biomass [11, 26, 28].

The contents of Pb in the analyzed soil specimens and also root and stem parts of *A. caudatus* and *T. patula* in different treatment conditions after 60 days of pots irrigation are presented in Tables 2 and 3, respectively. As can be seen, the content of Pb in the root samples increased with an increase in Pb contents in soils. Also, the content of Pb in the soils, roots, and stems of the studied plants was significantly varied, and the difference was remarkable in Pb accumulation and transport from soils to the different organs of *A. caudatus* and *T. patula*.

As shown in the Table 2, the application of EDTA and CA to the soil led to a significant increase in the content of Pb in the root of *A. caudatus* compared to the control (CT), while the application of SA resulted in the accumulation of Pb in the stem of *A. caudatus*. In this regard, the highest accumulation of lead in the roots of *A. caudatus* with 0.74 mg/kg (almost 1.3-fold compared to the CT) was found in 400 mg/kg Pb-contaminated soil containing EDTA; whereas, the highest accumulation of Pb in the stems of *A. caudatus* with 0.13 mg/kg was found in 400 mg/kg Pb- contaminated soil containing SA. Therefore, SA appeared more effective at increasing the content of Pb in stems than EDTA and CA (Table 2). However, in *T. patula* the accumulation of Pb increased only in the roots that were grown in the SA and CA treatment conditions compared to the control. Also, like *A. caudatus*, the application of SA to the soil led to a significant increase in the content of Pb in the stem of *T. patula* compared to the control. Here, SA was also more effective at increasing the content of Pb in stems than EDTA and CA. Accordingly, the highest accumulation of Pb (mg/kg) in the roots and stems of *T. patula* with 0.87 (almost 2.3-fold in comparison with those in the control) and 1.5 (7.5-fold compared to the CT) both were found in 400 mg/kg Pb- contaminated soil containing SA (Table 3). The literature review indicated that EDTA and CA, known as chelating agent, have widespreadly been used to enhance bio-availability and subsequent uptake and translocation of the element in aerial parts of different plant species [23, 48, 59, 62, 67]. According to the previous studies, the application of EDTA solution can notably increase the exchangeable or soluble fraction of elements which is accessible to plants [7, 10, 32, 64] and the biodegradation of chelate-metal complexes strongly depends on the type of element involved [56]. For example, Lai and Chen (2004) reported that EDTA solution would enhance the removal of Pb from contaminated soil and also the accumulation of this element in the shoots. Also, they indicated that the Pb concentration in the soil samples collected from *Vetiver zizanioides* L. and *Dianthus chinensis* L. considerably increased by adding EDTA solution [27]. Also, Ebrahimi (2015) reported similar findings [16]. Furthermore, Baghaie and Aghilizefreei (2020) reported that the application of EDTA would significantly increase the phytoremediation efficiency in the Pb-polluted soil [4]. Moreover, Sinhal et al. (2010) reported that despite the fact that all treatments with

**Table 2** Lead contents (mg/kg) in the soil specimens and roots and stems of *A. caudatus* in the different treatments after 60 days of pots irrigation

| Medium / Tissue | The added dose of lead nitrate solution (mg/kg) | Control | Experimental treatments | | |
|---|---|---|---|---|---|
| | | | EDTA | SA | CA |
| Soil | 200 | 4.35±0.03c[a] | 4.04±0.03b | 4.46±0.07d | 3.06±0.04a |
| | 400 | 7.71±0.01c | 6.00±0.03a | 8.63±0.06d | 7.12±0.05b |
| Root | 200 | 0.29±0.01a | 0.50±0.01b | 0.24±0.01a | 0.58±0.02b |
| | 400 | 0.58±0.01b | 0.74±0.02d | 0.36±0.01a | 0.64±0.03c |
| Stem | 200 | 0.08±0.01b | 0.02±0.00a | 0.12±0.00c | 0.03±0.01a |
| | 400 | 0.21±0.01c | 0.03±0.01a | 0.13±0.01b | 0.02±0.01a |

[a] The letters (a, b, c, …) in each row show the considerable difference between the control and experimental treatments in terms of mean content of Pb in soil, root and stem samples of *A. caudatus* that was computed by One-way ANOVA and DMRT ($p = 0.05$)

different doses of EDTA added were shown to significantly increase the accumulation of Pb by roots and stems of *Tagetes erecta* L. as compared to the control condition, the increases in the content of CA showed reduced accumulation of Pb by the stem of this plant [46].

It has been shown that the induced hyperaccumulation of elements in plant stems can be performed as a two-step process [46]. In the first step, plants accumulate elements in their roots. Then an inducing agent is used to enhance the transfer of the elements from roots to the stems [30]. The results of this research indicated that the distribution of lead in the roots and stems of *A. caudatus* was also significantly affected by the application of chelates. As shown in Table 2, in the control condition, the content of Pb in the root of the *A. caudatus* was significantly higher than that in the stem specimens. In other words, most of the Pb absorbed by the *A. caudatus* concentrated in the root. In this study, SA was found to be more effective than EDTA and CA in stimulating the translocation of Pb from roots to stems of *A. caudatus*. In other studies, it was found that the application of EDTA via destroying the physiological barrier(s) in roots could normally control uptake and translocation of solutes and considerably increase the root-to-shoot ratios of Pb, and this chelating agent would have a more effective role in stimulating the translocation of Pb from roots to stems. Therefore, in the xylem, solutes such as Pb-EDTA would follow the transpiration stream and accumulate to a high content in stems [58, 63]; however, competition between Fe and Pb on EDTA binding sites (i.e. Fe-EDTA) would reduce the formation of EDTA complexes with the target metal (Pb-EDTA) and consequently its translocation [39], while CA would have a less effective potential in increasing the translocation of Pb between the plant's tissues [30, 52]. This may be related to the fact that CA would improve the elements solubility and plant uptake through the formation of soluble citrate-metal complexes and therefore, TF values of Pb in plant species decreased after CA was added into the soil. Thus, application of CA caused that Pb would be retained in the cation exchange sites of the vessel walls of xylem parenchyma cells in roots and would get immobilized in the vacuoles of the root cells [41]. In the case of SA, it should be noted that environmental pollution with heavy metals induces the elevation of its biosynthesis in comparison with other compounds with chelation properties. Here, the rate of SA translocation from roots/rhizomes to aerial parts of plant species may have been related to the binding of this phenolic compound with glucose. However, the presence of Pb could

**Table 3** Lead contents (mg/kg) in the soil specimens and roots and stems of *T. patula* in the different treatments after 60 days of pots irrigation

| Medium / Tissue | The added dose of lead nitrate solution (mg/kg) | Control | Experimental treatments | | |
|---|---|---|---|---|---|
| | | | EDTA | SA | CA |
| Soil | 200 | 3.87±0.00b[a] | 3.75±0.05a | 3.88±0.02b | 4.05±0.05c |
| | 400 | 8.43±0.04d | 6.79±0.06a | 8.04±0.09b | 8.32±0.03c |
| Root | 200 | 0.07±0.00b | 0.01±0.00a | 0.02±0.01a | 0.01±0.00a |
| | 400 | 0.38±0.03b | 0.03±0.01a | 0.87±0.03d | 0.52±0.02c |
| Stem | 200 | 0.40±0.19b | 0.03±0.01a | 1.20±0.00c | 0.06±0.01a |
| | 400 | 0.20±0.02b | 1.17±0.02c | 1.50±0.01d | 0.13±0.00a |

[a] The letters (a, b, c, …) in each row show the considerable difference between the control and experimental treatments in terms of mean content of Pb in soil, root and stem samples of *T. patula* that was computed by One-way ANOVA and DMRT ($p = 0.05$)

**Table 4** Bioconcentration, bioaccumulation, translocation and accumulation factors of Pb in *A. caudatus*

| Treatment | The added dose of lead nitrate solution (mg/kg) | BCF of root | BAF of stem | TF | TE (%) | AF |
|---|---|---|---|---|---|---|
| Control | 200 | 0.07a[a] | 0.02c | 0.27b | 21.62c | 0.08a |
| | 400 | 0.07B | 0.03D | 0.36B | 26.58B | 0.10C |
| EDTA | 200 | 0.12b | 0.005a | 0.04a | 3.85a | 0.13b |
| | 400 | 0.12D | 0.005B | 0.04A | 3.90A | 0.13D |
| SA | 200 | 0.05a | 0.03c | 0.50c | 33.33d | 0.08a |
| | 400 | 0.04A | 0.01C | 0.36B | 26.53B | 0.06A |
| CA | 200 | 0.19c | 0.01b | 0.05a | 4.92b | 0.20c |
| | 400 | 0.09C | 0.003A | 0.03A | 3.03A | 0.09B |

[a] The letters (a, b, c, … and A, B, C, …) in each column show the considerable difference between the calculated values of BCF, BAF, TF, TE, and AF of *A. caudatus* that was computed by One-way ANOVA and DMRT ($p = 0.05$)

probably diminish the level of the metabolite in photosynthetic tissue and stimulated its binding with glucose [15].

In comparison with *A. caudatus* as indicated in Table 3, the content of Pb in the stem of the *T. patula* especially in the EDTA and SA treatments was significantly higher than that in the root specimens. In other words, the stems of *T. patula* have the acceptable efficiency for the accumulation of Pb. Here, although the results obtained showed that T. patula would have a better phytoextraction potential than *A. caudatus*, it should be noted that due to the Pb behavior in the soil and/or leaching of Pb from the soil columns during the irrigation period the low amounts of Pb absorption by the root and aerial parts of the plants compared to the added doses of $Pb(NO_3)_2$ solution to the soil samples, imply the studied plants haven't the adequate potential for phytoextraction of Pb from contaminated soils. Similarly, according to the study of Sinhal et al. (2010) the addition of EDTA and CA would lead to an increase in the transfer of lead from the roots to stems of *T. erecta* [46]. Also, the findings of Ghosh and Manchanda (2019) showed that *T. patula* could efficiently accumulate some heavy metals more in the aerial parts (stem and leaves) than in underground tissues i.e. root [20]. These findings may be related to forming metal complexes from free protonated EDTA and/or to increases in the metal availability due to the application of CA that caused could enhance the Pb uptake and translocation to aerial parts of the *T. patula* [27, 62]. On the contrary, based on the results of a study by Sun et al. (2011) a higher amount of Pb accumulation was recorded in the roots of *T. patula* [53].

In the current study, the BCF, BAF, TF, and AF were used to evaluate the plant's ability to accumulate, uptake and translocate Pb from soil. As shown in Tables 4 and 5, by comparing the experimental treatments together, in the case of *A. caudatus*, a considerable increase in bioconcentration factor was recorded for Pb after EDTA and CA application, while in the case of *T. patula* only a considerable increase in this factor was recorded for Pb on 400 mg/kg Pb-contaminated soil with the application of the SA solutions. Moreover, the maximum BAF value of *A. caudatus* with 0.03 was achieved in 200 mg/kg Pb-contaminated soil containing SA. Likewise,

**Table 5** Bioconcentration, bioaccumulation, translocation and accumulation factors of Pb in *T. patula*

| Treatment | The added dose of lead nitrate solution (mg/kg) | BCF of root | BAF of stem | TF | TE (%) | AF |
|---|---|---|---|---|---|---|
| Control | 200 | 0.02c | 0.10c | 5.71b | 85.11b | 0.12b |
| | 400 | 0.04B | 0.02A | 0.53B | 34.48B | 0.07A |
| EDTA | 200 | 0.003a | 0.008a | 3.00a | 75.00a | 0.01a |
| | 400 | 0.004A | 0.17B | 39.00D | 97.50D | 0.18B |
| SA | 200 | 0.005b | 0.31d | 60.00c | 98.36c | 0.31c |
| | 400 | 0.11D | 0.19C | 1.72C | 63.29C | 0.29C |
| CA | 200 | 0.002a | 0.01b | 6.00b | 85.71b | 0.02a |
| | 400 | 0.06C | 0.01A | 0.25A | 20.00A | 0.08A |

* The letters (a, b, c, … and A, B, C, …) in each column show the considerable difference between the calculated values of BCF, BAF, TF, TE, and AF of *T. patula* that was computed by One-way ANOVA and DMRT ($p = 0.05$)

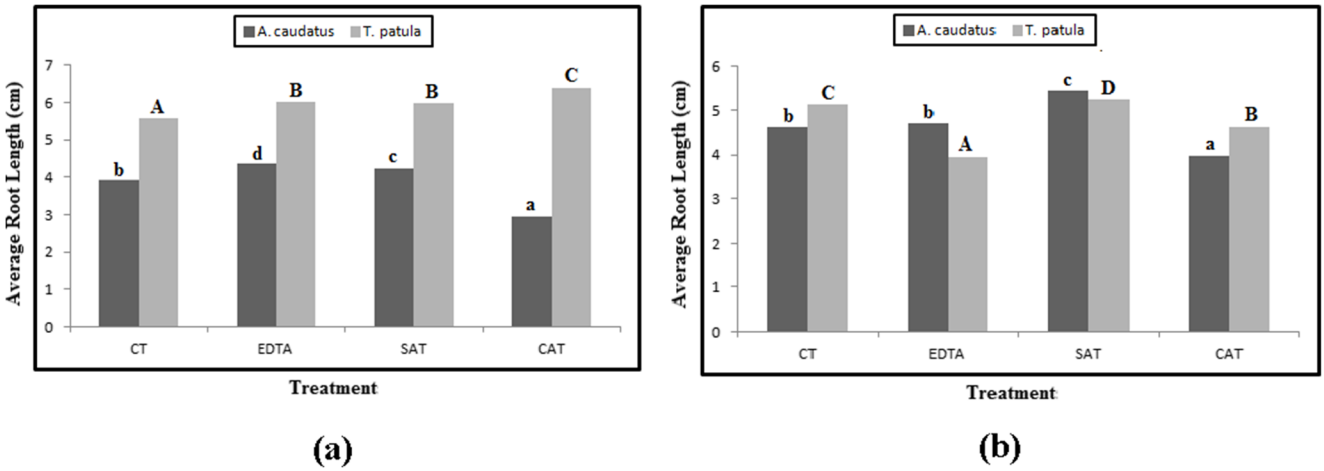


**Fig. 1** **a** Average root length (cm) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb-contaminated soil

in the case of *A. caudatus*, a significant increase in TF was observed for Pb after application of SA in 200 mg/kg Pb-contaminated soil, while in the case of *T. patula* a significant increase in TF was observed on 200 and 400 mg/kg Pb-contaminated soil with the application of the SA and EDTA solutions, respectively. Besides, the maximum AF value of *A. caudatus* with 0.20 was achieved in 200 mg/kg Pb-contaminated soil contain the CA. However, as listed in Table 5, the maximum BAF value of *T. patula* with 0.31 was achieved in 200 mg/kg Pb-contaminated soil containing SA. Moreover, the maximum AF value of *T. patula* with 0.31 was also achieved in 200 mg/kg Pb-contaminated soil containing SA. As observed in the Tables 4 and 5, in most cases uptake and transport of Pb in studied plants were increased with the application of SA compared to other experimental conditions.

Based on the results of linear regression analysis, a considerably positive correlation was observed between the lead content in the root tissue of *A. caudatus* and 200 mg/kg Pb-contaminated soil ($R^2 = 0.79$, $n = 12$). Conversely, a considerably positive correlation was observed between the lead content in the root tissue of *A. caudatus* and 400 mg/kg Pb-contaminated soil ($R^2 = 0.92$, $n = 12$). While no significant correlation was found between the Pb content in the root tissue of *T. patula* and 200 and 400 mg/kg Pb-contaminated soil. Besides, there was no significant correlation between the Pb content in the stem tissue of *A. caudatus* and *T. patula* and 200 and 400 mg/kg Pb-contaminated soil.

The lengths of the roots and stems of *A. caudatus* and *T. patula* was measured in two levels of Pb-contaminated soil (200 and 400 mg/kg) as shown in Figs. 1 (a and b) and 2 (a and b). Based on the results, the average root lengths (cm) of *A. caudatus* were found to be 3.94, 4.38, 4.25, and 2.96 for the CT, EDTA, SAT, and CAT (200 mg/kg) respectively; whereas, the average root lengths of *T. patula* in 200 mg/kg Pb-contaminated soil were 5.59, 6.01, 6.00 and 6.38 for the CT, EDTA, SAT and CAT respectively (Fig. 1a). Besides, the average root lengths (cm) of *A. caudatus* in 400 mg/kg Pb-

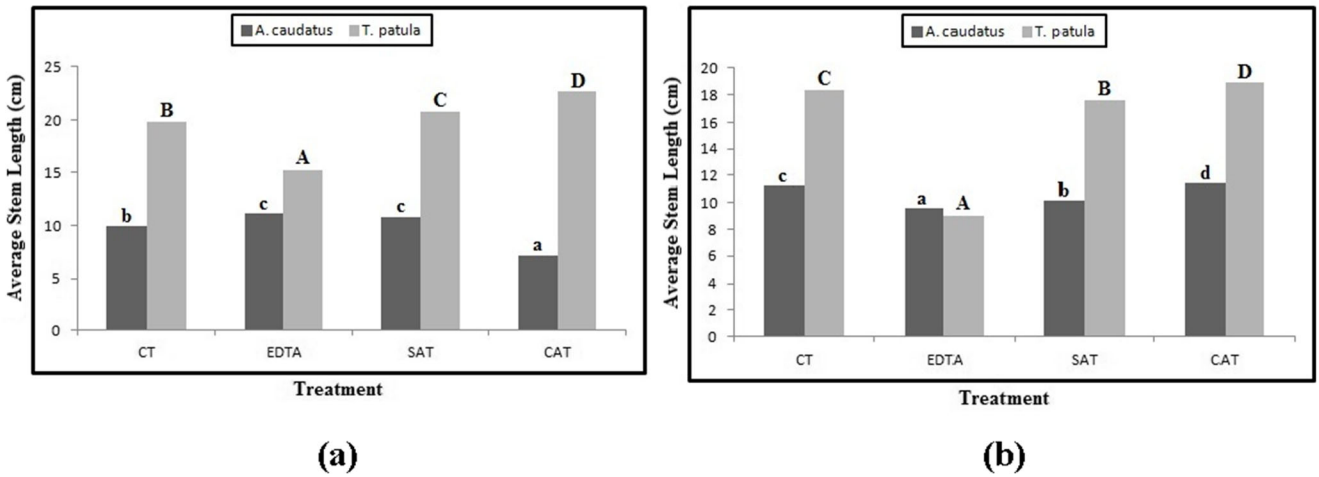


**Fig. 2** **a** Average stem length (cm) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb- contaminated soil

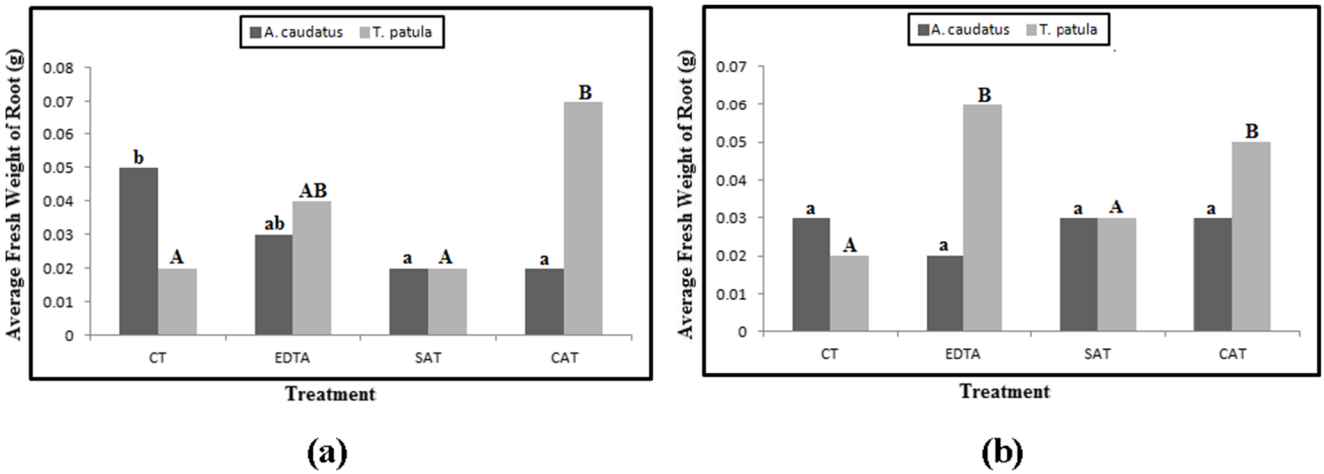


**Fig. 3** **a** Average fresh weight of root (g) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb-contaminated soil

contaminated soil were 4.60 for CT, 4.69 for EDTA, 5.44 for SAT and 3.98 for CAT, while the average root lengths (cm) of *T. patula* were found to be 5.13, 3.94, 5.25 and 4.60 for the CT, EDTA, SAT and CAT (400 mg/kg) respectively (Fig. 1b). Also, the average stem lengths of *A. caudatus* in 200 mg/kg Pb-contaminated soil were found to be 10.00 cm, 11.19 cm, 10.81 cm and 7.16 cm for CT, EDTA, SAT and CAT, respectively. Furthermore, the average stem lengths (cm) of *T. patula* were 19.82 for CT, 15.20 for EDTA, 20.75 for SAT and 22.73 for CAT (200 mg/kg) (Fig. 2a). Moreover, the average stem lengths (cm) of *A. caudatus* in 400 mg/kg Pb-contaminated soil were found to be 11.21, 9.58, 10.19 and 11.40 for CT, EDTA, SAT and CAT, respectively. On the other hand, the average stem lengths of *T. patula* in 400 mg/kg Pb-contaminated soil were 18.31 cm for CT, 9.05 cm for EDTA, 17.56 cm for SAT and 18.93 cm for CAT (Fig. 2b). As shown, in most experimental treatments the average root and stem lengths of *A. caudatus* and *T. patula* increased compared to the CT. Also, the average root and stem lengths of *T. patula* decreased with an increase in the added dose of Pb.

The average fresh and dry weight of the root and stem samples of the studied plants in different treatments of Pb-contaminated soil (200 and 400 mg/kg) are presented in Figs. 3 (a and b) and 4 (a and b). In 200 mg/kg Pb-contaminated soil, the average fresh weights of root samples (g) in CT, EDTA, SAT and CAT of *A. caudatus* were 0.05, 0.03, 0.02 and 0.02, respectively. Also, the average fresh weights of root samples (g) of *T. patula* in 200 mg/kg Pb-contaminated soil were 0.02 for CT, 0.04 for EDTA, 0.02 for SAT and 0.07 for CAT (Fig. 3a). In 400 mg/kg Pb-contaminated soil the average fresh weights of root samples (g) in CT, EDTA, SAT, and CAT of *A. caudatus* were 0.03, 0.02, 0.03 and 0.03, respectively. Also, the average fresh weights of root samples (g) of *T. patula* in 400 mg/kg Pb-contaminated soil were 0.02 for CT, 0.06 for EDTA, 0.03

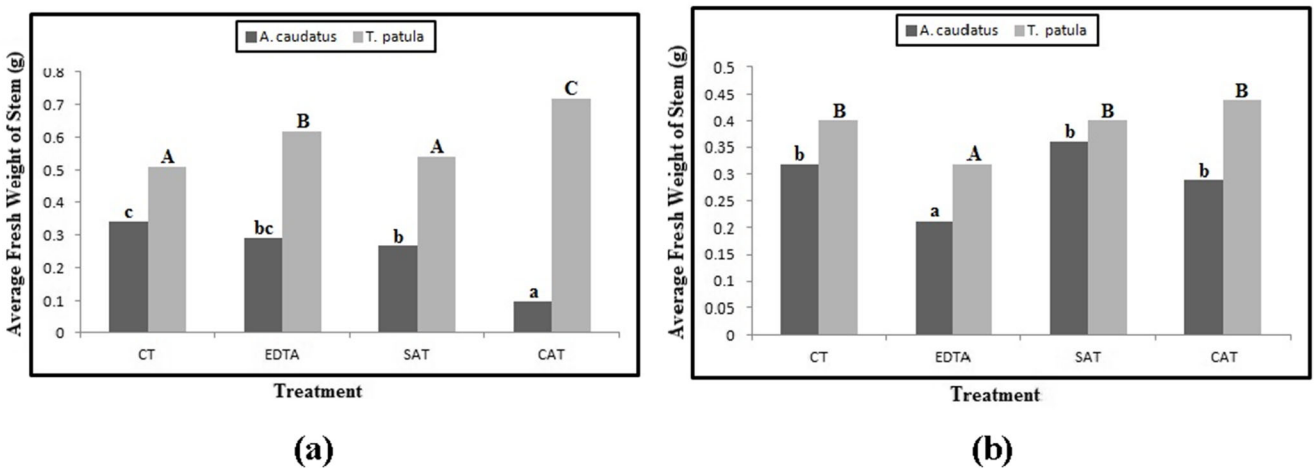


**Fig. 4** **a** Average fresh weight of stem (g) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb-contaminated soil

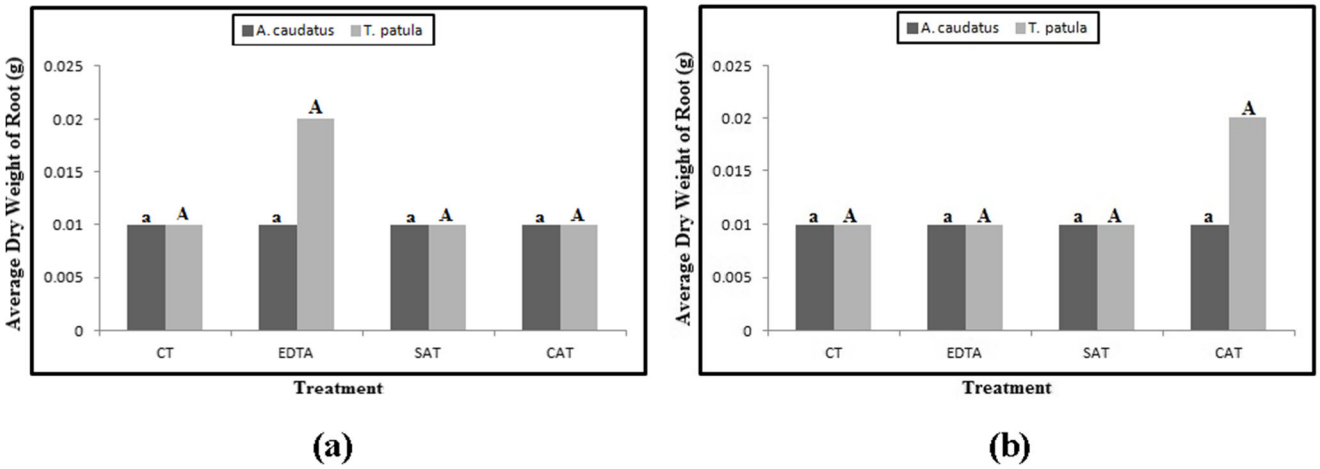


**Fig. 5** **a** Average dry weight of root (g) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb-contaminated soil

for SAT and 0.05 for CAT (Fig. 3b). However, In 200 mg/kg Pb-contaminated soil, a regular decrease was found in the average fresh weights of stem samples (g) of *A. caudatus* (0.34 for CT, 0.29 for EDTA, 0.27 for SAT and 0.10 for CAT), while an irregular trend in average fresh weight changes of stem samples of *T. patula* in 200 mg/kg Pb-contaminated soil (0.51 g for CT, 0.62 g for EDTA, 0.54 g for SAT and 0.72 g for CAT) was noticed (Fig. 4a). Moreover, in 400 mg/kg Pb-contaminated soil, the average fresh weights of stem samples in CT, EDTA, SAT and CAT of *A. caudatus* were 0.32 g, 0.21 g, 0.36 g and 0.29 g, respectively. Whereas, the average fresh weights of stem samples (g) of *T. patula* in 400 mg/kg Pb-contaminated soil were 0.40 for CT, 0.32 for EDTA, 0.40 for SAT and 0.44 for CAT (Fig. 4b). The average dry weights of root samples of *A. caudatus* in 200 mg/kg Pb-contaminated soil were 0.01 g for all treatments, while the average dry weights of root samples (g) of *T. patula* in 200 mg/kg Pb-contaminated soil were 0.01 for CT, 0.02 for EDTA, 0.01 for SAT and 0.01 for CAT (Fig. 5a). In 400 mg/kg Pb-contaminated soil, the average dry weight of root samples in all treatments (CT, EDTA, SAT and CAT) of *A. caudatus* was 0.01 g; however, for *T. patula* the average dry weights of root samples were 0.01, 0.01, 0.01 and 0.02 g for CT, EDTA, SAT and CAT, respectively. Here, no significant effects were observed for the application of chelating agents on the dry weights of the roots of the two plant species which were exposed to 200 and 400 mg/kg Pb (Fig. 5b). Besides, in 200 mg/kg Pb-contaminated soil, the average dry weights of stem samples (g) of *A. caudatus* were 0.05 for CT, 0.06 for EDTA, 0.06 for SAT and 0.03 for CAT, while for *T. patula* they were 0.14, 0.17, 0.18 and 0.17 for CT, EDTA, SAT and CAT, respectively. Here again, no significant effects were found for the application of EDTA and SA on the dry weight of stem of *A. caudatus* and for the application of all

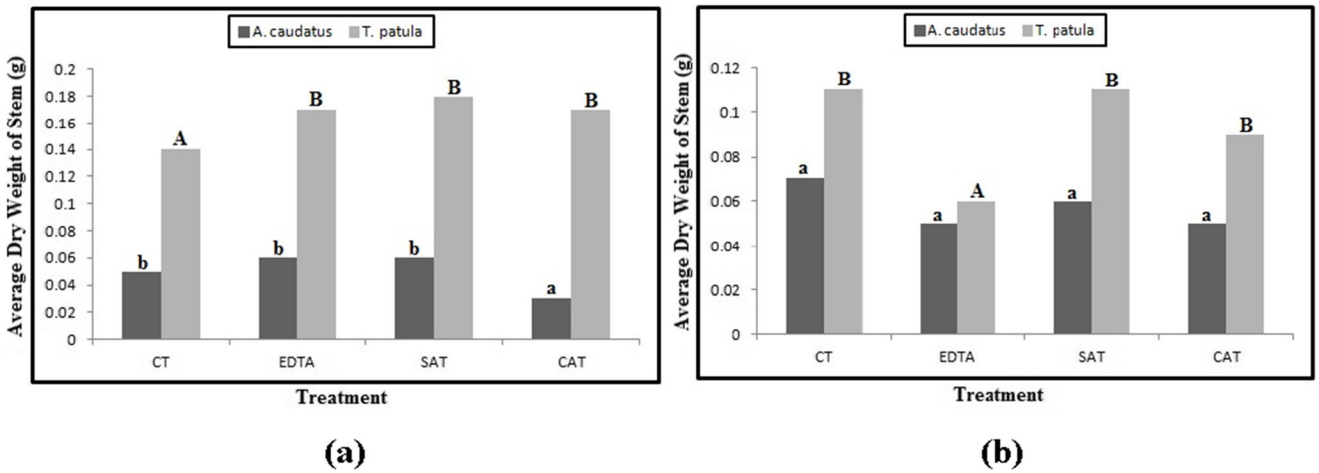


**Fig. 6** **a** Average dry weight of stem (g) of *A. caudatus* and *T. patula* in 200 mg/kg and **b** 400 mg/kg Pb-contaminated soil. * The letters (a, b, c, …) in Figs. 1, 2, 3, 4, 5, 6 show the considerable difference between the control and experimental treatments in terms of average root length, average stem length, fresh weight of root, fresh weight of stem, dry weight of root and also dry weight of stem of *A. caudatus* and *T. patula* that was computed by One-way ANOVA and DMRT ($p = 0.05$)

chelating agents of *T. patula* exposed to 200 mg/kg Pb. Also, no significant effects were observed for the application of all chelating agents on the dry weight of stem of *A. caudatus* and for the application of SA and CA of *T. patula* exposed to 400 mg/kg Pb. (Fig. 6a). Moreover, in 400 mg/kg Pb-contaminated soil, the average dry weights of stem samples (g) in CT, EDTA, SAT and CAT of *A. caudatus* were 0.07, 0.05, 0.06 and 0.05, respectively. Whereas, the average dry weight of stem samples (g) of *T. patula* in 400 mg/kg Pb-contaminated soil was 0.11 for CT, decreased to 0.06 for EDTA, remained almost the same for SAT and again decreased to 0.09 for CAT (Fig. 6b). Moreover, it should be noted that the average fresh weight of stem samples of *T. patula* decreased in higher levels of Pb-contaminated soil compared to that of the stems grown in low levels of Pb.

The data in Figs. 1, 2, 3, 4, 5, 6 indicates that except for the average root lengths of *A. caudatus* and *T. patula* in 400 mg/kg Pb-contaminated soil, the treatment with 2.5 mmol/kg soil CA considerably affected the plants growth including root and stem lengths and also fresh and dry weights compared to the other treatments.

## Conclusions

This research was designed to assess the potential of phytoremediation of Pb-contaminated soil with *A. caudatus* and *T. patula* as ornamental species. Small amounts of lead that are absorbed by root and aerial parts of the studied plants compared to the added doses of $Pb(NO_3)_2$ solution to the soil samples, might be related to the fact that not all soil Pb content is equally bioavailable or mobile and this element has a high affinity for soil solids, therefore, it can be argued that the studied plants haven't the adequate potential for phytoextraction of Pb from contaminated soils. The results indicate that to the average root and stem lengths of *A. caudatus* increase as the content of the added dose of lead nitrate solution to the soil increases, while in *T. patula* the average root and stem lengths decrease as the content of the added dose of lead nitrate solution to the soil increases. However, no significant differences are observed in average fresh weights of root samples of *A. caudatus* and *T. patula* between the 200 mg/kg and 400 mg/kg Pb-contaminated soil. Also, in 200 mg/kg Pb-contaminated soil a regular decrease is observed in the average fresh weight of stem samples of *A. caudatus*, while an irregular trend can be noted in *T. patula*. Compared with the control condition, the addition of chelating agents cannot significantly increase the average dry weights of the roots of *A. caudatus* and *T. patula* while, compared with the control condition, the addition of chelating agents shows a significant increase in the average dry weight of the stem of *A. caudatus*, in 200 mg/kg Pb-contaminated soil. In this regard, it should be noted that the application of CA as a biodegradable chelating agent can have a significant effect on some agronomic characteristics of *T. patula* such as average root and stem lengths and average fresh weight of the stem compared to when EDTA is used as a chemical and poorly biodegraded chelating agent. Therefore, based on the results obtained, the application of CA as a synthetic plant growth regulator is recommended.

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**Authors' contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Nastaran Aghelan, Soheil Sobhan Ardakani, Mehrdad Cheraghi, Bahareh Lorestani and Hajar Merrikhpour]. The first draft of the manuscript was written by [Soheil Sobhan Ardakani] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Declaration

**Conflict of interest** The authors declare that they have no conflict of interest.

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