Nitrogen fertilization: Effect on Cd-phytoextraction by the halophytic plant quail bush [Atriplex lentiformis (Torr.) S. Wats]

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ABSTRACT
Remediation of metal polluted sites by traditional, physical and chemical methods demands large investments of economic and technological resources compared to green remediation. Halophytic plants have been suggested to be more effective in the phytoextraction of metals from the contaminated soils compared to salt-sensitive crop plants. Pot experiment was conducted to study the accumulation of cadmium (Cd) by the Atriplex lentiformis plants when treated with different rates of nitrogen fertilizer. Nitrogen was applied to the soil at rate of 0, 100, 200, 300 and 400 mg kg⁻¹. Increasing the level of nitrogen from 0 to 400 mg N kg⁻¹ increased the dry biomass of roots and shoots of the studied plant by 75 and 27.5%, respectively. The application of N increased the chlorophyll by 100% and leaf area index by 50% and this led to increase in the photosynthesis and plant growth. The A. lentiformis plants tolerate the high levels of Cd in the soil and plant tissues. Under metal stress conditions, the studied plant contained large amount of organic compounds e.g., oxalic acid, proline and phenols. These organic compounds had negative effect on the plant growth and Cd accumulation in the aboveground parts of the plant. When 400 mg N kg⁻¹ was added, the chlorophyll increased by 100% and the proline, phenols and oxalic acid decreased by 33, 50 and 30%, respectively compared to the control treatment. The fertilization of A. lentiformis plants with the highest rate of nitrogen enabled the plants to remove 7.93% of the total soil Cd during a period of 105 days. Nitrogen mitigated the effect of metal stress and increased the accumulation of Cd in the aboveground parts of A. lentiformis plants. The fertilization of A. lentiformis with nitrogen could be an effective tool to enhance Cd-phytoextraction from polluted sites.

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1. Introduction

Trace elements are an important part of the soil ecosystem. However, the increase of these metals in water and agriculture land may be unsafe to all organisms (Dembitsky, 2003). Cadmium is a highly toxic metal and is used in many industries. The main sources of Cd in soils are human activities and agriculture management practices as the application of phosphatic fertilizers and pesticides (Chaney et al., 1997). Remediation technologies such as the washing, vitrification and solidification are not effective in large areas and not accepted by the general public due to the high cost and the disturbances caused to the soil (Chaney et al., 1997). Phytoremediation (green remediation) is a more practical and eco-friendly method (Eissa, 2016). The harvest of plant after the uptake of metal in their aboveground part is called phytoextraction (Palmer et al., 2001; Butcher, 2009). The use of plant to remediate contaminated soils is a safe method for environmental ecosystems and can be conduct by low costs (Butcher, 2009).

Halophytic plants survive in high salt concentrations of water and soil using different physiological mechanisms (Eissa, 2017). Shoot tissues of these plants contain high concentrations of oxalic acid as one of the most important tolerance mechanisms for metal stress (Sayer and Gadd, 2001). These species produce high yield of dry biomass and have a strong deep root system (Eissa, 2017). Under metal stress some halophytic plants reduced their total leaf area and photosynthesis rate and increased the oxalic acid, proline and phenols in the shoot tissues (Sayer and Gadd, 2001; Ali et al., 2013; Eissa, 2017).

The use of some halophyte plants in phytoremediation was reported by Manousaki and Kalogerakis (2009), Nedjimia and Daoudb (2009) and Eissa (2014, 2016). Atriplex is one of the most important halophytic plants with a genus having between 100 and 200 species, known by the common name of saltbush. The genus is widely distributed in different deserts worldwide. A few numbers of these species have been studied for phytoremediation (Lutts et al., 2004; Eissa, 2014, 2017).

Nitrogen is a macronutrient and plants consume a large amount of N compared to other nutrients. Nitrogen is the main organic component of most plant biochemical compounds and plays an important role in plant photosynthesis by improving leaf area index (Marschner, 1995). There is limited information about the response of Atriplex plants to the

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nitrogen fertilization in the heavy metal contaminated soils. Therefore this research paper aims to investigate the accumulation of Cd by *Atriplex lentiformis* and to ascertain the effect of nitrogen levels in Cd-phytorextraction.

2. Materials and methods

2.1. Soil characterization

Surface soil sample (0–30 cm) was collected from a soil irrigated by sewage water for more than half a century. The texture of the studied soil is sandy loam (80% sand, 15% silt and 5% clay) and the total and available Cd concentrations are 45 and 2.5 mg kg$^{-1}$. This soil also contains 3 g of organic carbon/kg of soil and 100 mg of total nitrogen. The studied soil has a pH of 7.88 and EC of 2 dS/m. Soil texture was measured by the pipit method (Burt, 2004). A digital pH meter was used to assess the soil pH. Organic matter content was determined by the dichromate oxidation method as described by Wakley and Black (Burt, 2004). Soil salinity (EC) was measured by an electric conductivity meter (Burt, 2004). The Kjeldahl method was used to determine total nitrogen (Burt, 2004). DTPA-extractable metal Cd was extracted from the studied soil samples using a 0.005 M DTPA (diethylenetriaminepentaacetic acid) described by Lindsay and Norvell (1969). Soil samples were digested using a mixture of H$\text{F}$–HNO$_3$–HClO$_4$ (1:1:1, v/v) in Teflon beakers to extract Cd.

2.2. Pot experiment

The pot experiment was conducted out in a greenhouse (25 °C and 12 h light) to study Cd uptake by *A. lentiformis* plants to accumulate Cd. Soil was put in black plastic pots (5 kg) and two seedlings (30 days old) of *A. lentiformis* were transplanted in each pot. The seedlings were obtained from the Center of Desert Agriculture in Assiut University, Egypt. The cultivated plants were irrigated regularly to near field capacity and fertilized with 1 g/pot of phosphorus and potassium. Nitrogen levels (0, 100, 200, 300 and 400 mg kg$^{-1}$ soil) were added to the pots of each treatment in the form of NH$_4$ NO$_3$ (33% N). Each level was replicated four times and N was added to the soil in the form of solution. Nitrogen fertilization treatments were added at three equal doses after two, five and eight weeks of transplanting. The plants were harvested after 1 5 weeks of transplanting.

The Duncan test and one-way ANOVA were analyzed with SPSS version 15.

2.3. Chemical analysis of plant samples

Plants were left in the pots for 15 weeks after transplanting. The plant samples were collected, washed with tap water, then with 0.1 HCl and Tween 80 to remove inorganic wastes. Plant samples were then washed with distilled water and oven-dried at 70 °C to a constant weight. Plant samples were ground and subjected to acid-digestion by a 2:1 HNO$_3$–HClO$_4$ acid mixture. Cadmium concentrations were measured by an atomic absorption spectrophotometer (AAS). A certificated standard material was analyzed during soil and plant analysis for quality control and assurance. Proline in dried leaves was measured with the sulfosalicylic acid method (Bates et al., 1973) and chlorophyll in fresh leaves was determined with the acetone extraction method (Arnon, 1949). Total phenolic content was extracted from fresh leaves of *A. lentiformis* plants and was estimated using the colorimetric method of Folini and Denis (1915). Oxalic acid was extracted from oven dried *A. lentiformis* plant leaves and determined by the traditional method described by Naik et al. (2014)

### Table 1

| N rates (g/pot) | Roots (g/pot) | Shoots (g/pot) | Leaf number/plant | Leaf area/plant (cm$^2$) | Plant length (cm) |
|----------------|--------------|----------------|-------------------|--------------------------|------------------|
| C 20 ± 1.52 c | 80 ± 3.2 c | 30 ± 1.82 d | 80 ± 3.52 d | 100 ± 5.25 c |
| 100 25 ± 1.54 c | 90 ± 3.0 b | 35 ± 1.94 c | 85 ± 4.33 d | 115 ± 5.22 b |
| 200 28 ± 1.45 b | 95 ± 4.0 b | 38 ± 2.00 b | 92 ± 5.00 c | 120 ± 5.12 b |
| 300 33 ± 1.43 a | 100 ± 4.5 a | 40 ± 2.40 a | 100 ± 5.46 b | 130 ± 5.55 a |
| 400 35 ± 1.48 a | 102 ± 3.1 a | 42 ± 2.40 a | 120 ± 5.44 a | 133 ± 5.62 a |

Means (± standard deviation, n = 4) denoted by different letters are significantly different at P < 0.05.

3. Results

3.1. Effect of nitrogen rates on the growth of *A. lentiformis* plants

There were significant effects for the nitrogen fertilization levels in the recorded growth parameters of *A. lentiformis* as shown in Table 1. Raising the level of nitrogen from 0 to 400 mg N kg$^{-1}$ enhanced the dry biomass of roots and shoots of the studied plant by 75 and 27%, respectively. Number and area of leaves were increased by 40 and 50% when N was raised from 0 to 400 mg kg$^{-1}$. The application of 400 mg of N increased the plant height and caused a 33% increase compared to the unfertilized treatment. In general, N fertilization increased the recorded growth parameters of *A. lentiformis* plants.

3.2. Effect of nitrogen rates on the uptake of Cd by *A. lentiformis* plants

Treating *A. lentiformis* with the nitrogen fertilizers had a significant effect on the concentration of Cd in both root and shoot of *A. lentiformis* as shown in Fig. 1. The highest rate of nitrogen fertilization (400 mg) caused a 64 and 52% increase in Cd concentration in the root and shoot respectively, in comparison with the unfertilized treatments. Root Cd concentration in the studied plant ranged between 170 and 280 mg kg$^{-1}$, while in the shoots it ranged between 115 and 175 mg kg$^{-1}$. The roots of *A. lentiformis* contained higher concentrations of Cd than the shoots. Increasing the level of nitrogen raised Cd concentration in the root and shoot of *A. lentiformis*.

3.3. Effect of nitrogen fertilization on the concentrations of some organic compounds in the leaves of *A. lentiformis* plants

Figs. 2 and 3 show the effect of nitrogen fertilization rates on chlorophyll, proline, total phenolic content and oxalic acid in leaves of *A. lentiformis* plants. The application of nitrogen significantly increased the chlorophyll and decreased the proline in leaves of *A. lentiformis* plants. When 400 mg N kg$^{-1}$ was added, the chlorophyll increased by 100% and the proline, total phenolic content and oxalic acid decreased by 33, 50 and 30%, respectively in comparison to the unfertilized soil. Fig. 4 shows the correlation between chlorophyll and Cd concentrations beside the effect of proline in the shoot Cd concentrations. Plant uptake of Cd had a positive significant correlation with the chlorophyll content in the leaves of *A. lentiformis* plants and negative significant correlation with the proline content. Fig. 5 illustrates the correlation between total phenolic acid and shoot Cd concentrations, in addition it shows the correlation between oxalic acid and shoot Cd concentrations. It is clear that oxalic acid and total phenolic content reduced the accumulation of Cd in shoot tissue. Proline, total phenolic content and oxalic acid had negative correlation with the Cd concentrations in the shoots of *A. lentiformis* plants.

3.4. Cd-phytoextraction capacity of *A. lentiformis* as affected by nitrogen levels

The effect of nitrogen fertilization rates on transfer of Cd from soil to root and from root to shoot as well as the removed Cd by *A. lentiformis* plants...
Fig. 1. Cadmium concentrations in the roots and shoots of *A. lentiformis* plants. Means (± standard deviation, n = 4) denoted by different letters are significantly different at \( P < 0.05 \).

Fig. 2. Total chlorophyll and proline content in leaves of *A. lentiformis* plants. Means (± standard deviation, n = 4) denoted by different letters are significantly different at \( P < 0.05 \).

Fig. 3. Total phenolic and oxalic acid content in leaves of *A. lentiformis* plants. Means (± standard deviation, n = 4) denoted by different letters are significantly different at \( P < 0.05 \).
plants are shown in Table 2. The application of nitrogen fertilizer to the *A. lentiformis* plants grown in a Cd-contaminated soil affected the transfer of Cd from soil to the roots significantly. Moreover, the removed Cd from soil increased from 4.09% to 7.93% when the rate of N was increased from 0 to 400 mg N kg\(^{-1}\). The plants of *A. lentiformis* fertilized by the highest rate of nitrogen (400 mg) were able to remove 7.93% from the total soil Cd during a period of 105 days. The soil–root transfer of Cd was increased by 105% when N was added at a rate of 400 mg in comparison with the control soil. Root–shoot transfer of Cd ranged from 0.60 to 0.68 and no significant differences between treatments were found. The values of root–shoot transfer of Cd were less than one indicating that *A. lentiformis* plants absorbed more Cd in roots and transferred less concentrations to the shoots (Eissa and Ahmed, 2016).

4. Discussion

4.1. Growth of *A. lentiformis* in Cd-contaminated soils

The studied soil contained 45 mg Cd per kg of soil. Based on the maximum permissible limits of heavy metals in soils (USEPA, 1997; EU, 2002), the studied soil is highly Cd-contaminated soil. The two mentioned references reported that the maximum permissible Cd level is less than 3 mg Cd kg\(^{-1}\). The high concentrations of toxic metals pose primary problems to plant growth in the contaminated soils. Cadmium is a highly toxic metal and inhibits plant growth (Fernandes and Henriques, 1991). In the current pot experiment, the tested plant grows normal and without the appearance of any effect for the toxicity of Cd. The normal growth of *A. lentiformis* in the contaminated soils is well known because of its high tolerance to metal stress (Lutts et al., 2004; Eissa, 2017). The halophyte plant can tolerate the high levels of metal stress by linking the metal to some organic compounds e.g., glutathione, oxalate malate and citrate (Salt et al., 1998). Moreover, Cd concentrations in the shoots of *A. lentiformis* ranged between 115 and 175 mg kg\(^{-1}\) of dry biomass. According to Eissa (2016, 2017), *A. lentiformis* plants are Cd-hyperaccumulators. Shoot Cd concentrations in Cd-hyperaccumulators must be more than 100 mg Cd per kg of dry weight (Baker et al., 2000).

Table 2

| N rates | Soil–root transfer | Root–shoot transfer | % Removed Cd |
|---------|--------------------|---------------------|-------------|
| C       | 85 ± 4.25 e        | 0.68 ± 0.02 a       | 4.09 ± 1.22 d |
| 100     | 122 ± 5.46 d       | 0.64 ± 0.04 a       | 5.60 ± 1.44 c |
| 200     | 144 ± 6.78 c       | 0.60 ± 0.05 a       | 6.33 ± 1.75 b |
| 300     | 165 ± 5.06 b       | 0.63 ± 0.06 a       | 7.56 ± 2.00 a |
| 400     | 175 ± 6.12 a       | 0.63 ± 0.04 a       | 7.93 ± 2.02 a |

Root–shoot transfer = Cd concentrations in shoot / Cd concentrations in roots.

Soil–root transfer = Cd concentrations in roots / available soil Cd (mg kg\(^{-1}\)).

% Removed Cd = Cd uptake by shoot / available soil Cd.

Means (±standard deviation, n = 4) denoted by different letters are significantly different at *P* < 0.05.
4.2. Growth of A. lentiformis as affected by nitrogen rates

In general, heavy metals reduce nutrient uptake and inhibited the growth of plants (Bibi et al., 2006). Eissa and Ahmed (2016) found that the addition of N fertilizer significantly decrease the toxic effect of Cd and Zn and improved plant growth. In the current study the application of N fertilizer enhanced all growth parameters of A. lentiformis. Increasing the level of N fertilization enhanced plant growth through increasing the leaf area and the number of leaves which led to an increase of the dry matter production (Noaman, 2004; Arif et al., 2010). Increasing levels of nitrogen fertilization in the current study enhanced the growth of A. lentiformis. Nutrients affect not only the growth status of plants, but also the toxicity of metals and conditions of soil. An et al. (2002) presented that amending soil with N, P, K and Fe could alleviate the toxicity of Cd, Pb, Ni, and Hg. For halophyte plants, added nitrogen fertilizer may improve the production of dry matter of halophytes under high levels of salinity and increase their tolerance to salinity (Noaman, 2004). Under metal stress conditions, the leaves of A. lentiformis contained large amount of organic compound e.g., oxalic acid, proline and phenols. These organic compounds have a negative effect on the plant growth and Cd accumulation in the aboveground parts of the plant (Eissa, 2017). The application of 400 mg of N kg−1 reduced the concentrations of proline, total phenolic content and oxalic acid by 33, 50 and 30%, respectively and increased chlorophyll by 100% compared to the control treatment. Nitrogen mitigated the effect of metal stress and increased the growth of A. lentiformis plants. These results were similar to the findings of Wang et al. (2004), Rodriguez-Ortiz et al. (2006) and Eissa (2017).

4.3. Effect of nitrogen fertilization rates on Cd uptake by A. lentiformis

The plant uptake of Cd by many species of Atriplex has been reported in several research papers (Lutts et al., 2004; Eissa, 2016, 2017). Lutts et al. (2004) studied the accumulation of Cd in halophyte species Mediterranean saltbush (Atriplex halimus L.) and found that the mean Cd accumulation in aerial parts was 440 mg kg−1, while the roots stored more Cd (3000 mg kg−1). High Cd concentration in the root compared to the shoot of Atriplex plants was reported by Nedjimia and Daoud (2009). Cadmium accumulates in the roots of A. lentiformis plants more than in the shoots (Eissa, 2017). The higher Cd uptake of A. lentiformis plants treated with nitrogen fertilizer may be due to the increasing growth of roots. Increasing the growth will enable roots to explore more area of soil (Wang et al., 2006; Eissa, 2017). Moreover, application of nitrogen can reduced the soil pH and increase the soil available Cd (Eissa, 2016).

4.4. Effect of nitrogen levels on the translocation factor of Cd by studied plants

The values of root–shoot transfer of Cd ranged between 0.60 and 0.68. Moreover, these values were less than one, and this means that A. lentiformis plants absorbed more Cd in roots and transferred low concentrations to the shoots. The root–shoot transfer of Cd as shown in Table 2 is defined as the ratio of the metal concentration in the shoots to that in the roots of plants. It is used to evaluate the capacity of a plant to transfer a metal from the roots to the shoots (Zhao et al., 2003). The studied plants absorbed high amount of Cd but their ability to translocate absorbed Cd to the shoot was low. This result also confirmed the findings of Lutts et al. (2004), Nedjimia and Daoud (2009) and Eissa (2017).

4.5. Mechanism of Cd-phytoextraction by A. lentiformis plants as affected by nitrogen fertilization

The A. lentiformis plants under the current study were able to remove 4.0% from the total soil Cd without any nitrogen fertilization. But when N rate was increase to 400 mg N, the plants were able to remove 7.93% from the total soil Cd. The highest rate of nitrogen fertilization increased the dry matter of shoots by 80% and increased the Cd concentrations in the shoots by 52%. Increasing the produced dry biomass and Cd concentration will lead to an increase of the total Cd removed at the end of phytoextraction process (Prasad and Freitas, 2003; Ruley et al., 2006). Under the toxicity of Cd some halophytic plants increased the oxalic acid, proline and phenols in the shoot tissues (Salt et al., 1998; Sayer and Gadd, 2001; Ali et al., 2013; Eissa, 2017; Shackira et al., 2017). The high concentrations of these organic compounds in leaf tissues reduced the growth and metal uptake (Eissa, 2017). In the current study, the application of nitrogen minimized proline, total phenolic content and oxalic acid by 33, 50 and 30%, respectively and increased the chlorophyll by 100%. Nitrogen fertilization increased chlorophyll and mitigated the proline, phenolic and oxalic acid effects. Proline, phenolic and oxalic acid in leaves of A. lentiformis played an important role in metal stress resistance (Shackira et al., 2017) but had negative effects in metal accumulation in the aboveground parts (Eissa, 2017).

5. Conclusion

A. lentiformis plants, which are C4 perennial native shrubs, have an excellent tolerance to drought and salinity. In addition, it can be cultivated using saline irrigation water, since high-quality irrigation water is not often available for crops in arid regions and thus resort in using brackish waters. The response of A. lentiformis grown in Cd-contaminated soils to nitrogen fertilization rates was studied in a pot experiment. Leaves of these plants contained high concentrations of proline, phenolic and oxalic acid, which may assume positive functions in tolerance mechanisms to metal stress. However, there is a negative correlation between these organic compounds and the accumulation of Cd in the aboveground parts of A. lentiformis plants. In the current study, the application of nitrogen minimized proline, total phenolic content and oxalic acid in the leaf tissues and increased the accumulation of Cd in the harvestable parts. The results obtained in this study suggest that nitrogen fertilization to A. lentiformis may be more effective in the phytoextraction of Cd contaminated soils.

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