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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.

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-phytoextraction.

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 HF–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 Folin 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
Growth parameters of *A. lentiformis* when treated with different rates of nitrogen fertilizer.

N rates	Roots (g/pot)	Shoots (g/pot)	Leaf number/plant	Leaf area/plant (cm^2)	Plant length (cm)
C	20 ± 1.52 d	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*