Accumulation of polyphenols and flavonoids in *Atriplex canescens* (Pursh) Nutt stressed by heavy metals (zinc, lead and cadmium)

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**Abstract**

Pollution of the environment and soils by heavy metals is one of the major problems of our time. Our study was to determine the effect of five doses for: zinc, lead and cadmium (0, 2500, 5000, 7500, and 10000 ppm) applied to the *Atriplex canescens* after 60 days for two weeks. The results showed an increase in the total polyphenols and flavonoids, depending on the increasing concentration of heavy metals at the leaf and root levels. The leaves have total polyphenols and flavonoids higher than those of the roots. The highest levels of total polyphenols and flavonoids in the leaves (3.47 mg EAG/g dry weight of total polyphenols and 3.70 mg EQ/g dry weight of flavonoids) were obtained at a dose of 10000 ppm (cadmium, lead) respectively. On the other hand, the highest levels of total polyphenols and flavonoids in the roots were obtained at the metallic dose of 10000 ppm for cadmium (3.38 mg EAG/g dry weight of total polyphenols and 0.61 mg EQ/g dry weight of flavonoids). These results also underlined the important role of secondary metabolites (total polyphenols and flavonoids) in the defense against oxidative stress caused by metallic stress.

**Keywords:** Heavy metals, *Atriplex canescens* (Pursh) Nutt, total polyphenols, flavonoids.

**INTRODUCTION**

The increasingly growing of human agricultural, urban and industrial activities has caused the contamination of our environment by heavy metals. Heavy metals are not able to degrade and their concentration increases steadily in soils. This exposes plants to increasing concentrations of heavy metals. Phytoremediation is one of the biological methods based on the use of plants to eliminate or degrade heavy metals in contaminated soil [1]. In response to various environmental stresses, plants have storage or detoxification systems that allow them to reduce the harmful effects of heavy metals [2]. One of the main traits of tolerance to environmental stresses is the synthesis of the secondary metabolite such as total polyphenols and flavonoids, which plays a crucial role in the detoxification of reactive oxygen species [3]. The effectiveness of these substances to stop the free radical reactions by neutralizing free radicals is mainly due to their phenolic structures with the presence of the hydroxyl groups [3].

Several species belong to the genus Atriplex are well adapted to harsh environmental conditions and characterized by their great diversity with more than 400 species [4]. It is a useful material for the identification of physiological mechanisms involved in resistance to abiotic stresses [5]. *Atriplex canescens* (Pursh) Nutt is a halophyte being a part of the family of chenopodiaceae, come from North America [6].

In this context, our work was designed to evaluate the effect of heavy metals zinc, lead and cadmium on the contents of total polyphenols and flavonoids of *Atriplex canescens* (Pursh) Nutt.

**EXPERIMENTAL**

**Materials**

**Plant material**

The plant material that was the subject of this study involved only the seeds of the *Atriplex canescens* (Pursh) Nutt of the chénopodiaceae family. The seeds of the *Atriplex canescens* originated in the El Bayedh region and were collected in December (2015).

**Seed Preparation**

The seeds were manually peeled, disinfected with bleach for 5 minutes and then rinsed abundantly with distilled water to remove all traces of chlorine. Lastly they were dried before germination.

**Germination**

Seeds were sown in alveoli to obtain seedlings. Each cell contained a seed. These seeds were irrigated with distilled water once every two days.

**Transplant**

After one month of germination, seedlings were transplanted into cylinders (height 50 cm and diameter 20 cm) that bottom lined with a
layer of gravel to ensure drainage and filled with a sand/potting mixture (2V/V).

Watering
Watering was carried out three times a week by distilled waters and substituted once in three by a nutrient solution of [7] until a sufficient plant material was obtained for the analyses.

Application of stress
Metal stress was applied to the plant after 60 days for two weeks. Five metal doses (0, 2500, 5000, 7500, and 10000 ppm) were selected for Zn, Pb, and Cd Metals.

Collection and preparation of plant material for analysis
After two weeks of the stress, plants were removed for obtaining leaves and roots that were dried at 80 °C for 24h.

The parameters analyzed
Total polyphenol Content
The total polyphenol content was determined by the folinic-Cicalteu reagent method [8] by measuring the absorbance at 760 nm.

Flavonoid content
The flavonoid content was determined by the aluminum trichloride method [9]. The contents were expressed in (mg EQ/g) dry weight after reading the D.O at 430 nm on a spectrophotometer JENWAY 6505UV/Vis.

Statistical analyzes
The results obtained were treated statistically using the STATBOX software. Version 6.4, an analysis of the variance calling to the test of Newman-Keuls P = 5%.

RESULTS
Total Polyphenols
Total polyphenol content of the leaves and roots of Atriplex canescens stressed to zinc
The content of total polyphenols in the aerial and root parts of Atriplex canescens was increased with increasing of zinc concentration (Fig. 1).

For the same concentrations of zinc, accumulation of total polyphenols in the leaves was higher than that recorded in the roots of the plant. The total polyphenols were spread out evenly in the leaves 3.22, 3.29, 3.30 and 3.31 mg EAG / g dry weight of total polyphenols stress doses 2500, 5000, 7500 and 10000 ppm of zinc, respectively.

The application of zinc caused an increase of polyphenols levels in the roots at 2500, 5000, 7500 and 10000 ppm (3.09, 3.22, 3.30 and 3.30 mg EAG / g dry weight of total polyphenols, respectively) compared with the control (2.90 mg EAG / g dry weight of total polyphenols).

Total polyphenol content of the leaves and roots of Atriplex canescens stressed to lead
The results found in the stressed plants led to different concentrations (2500, 5000, 7500 and 10000 ppm) and revealed an increase of total polyphenols in the leaves and roots of Atriplex canescens (Fig. 2). The contents obtained in total polyphenols were elevated in the leaves of the plant (3.23, 3.28, 3.31 and 3.34 mg EAG / g dry weight of total polyphenols) than the roots (3.18, 3.18, 3.30 and 3.32 mg EAG / g dry weight of total polyphenols).

Total polyphenol content of the leaves and roots of Atriplex canescens stressed to cadmium
The results showed that the accumulation of total polyphenols was higher in the leaves than in the roots of the plant Atriplex canescens. The accumulation of total polyphenols in the leaves and roots of the plant was increased progressively with increasing doses of cadmium (Fig. 3).

In the leaves, the highest total polyphenols content (3.47 mg EAG / g dry weight of total polyphenols) was obtained for treatment at 10000 ppm of cadmium while the total polyphenol content (3.25, 3.30 and 3.42 mg EAG / g dry weight of total polyphenols) was obtained for treatments at 2500, 5000 and 7500 ppm of cadmium, respectively.

In the roots, the highest total polyphenols content (3.38 mg EAG/ g dry weight) was obtained for treatment at 10000 ppm of cadmium.

Statistical analysis using the Newman-Keuls test at P = 5 % showed insignificant effect of zinc treatment on the accumulation of total polyphenols in the stressed leaves compared with the control leaves. Thus, the values of the total polyphenols content under all treatments were significantly varied (P = 0.00) for the roots.

Total polyphenols content of the leaves and roots of Atriplex canescens stressed to lead
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In the roots, the highest total polyphenols content (3.38 mg EAG/ g dry weight) was obtained for treatment at 10000 ppm of cadmium.
Flavonoids
Flavonoid content of the leaves and roots of Atriplex canescens stressed to zinc

The results showed an increase in the flavonoid content in the leaves and roots in the plant Atriplex canescens.

In the leaves, there was a significant increase in the flavonoids of plants subjected to stress by zinc compared to the control, because in the leaves of plants subjected to 7500 ppm flavonoid content decreases slightly (Fig. 4).

The application of zinc resulted in a decreased content of flavonoids in the roots at 2500, 5000 and 7500 ppm (0.33, 0.43 and 0.46 mg EQ / g dry weight of flavonoids) successively relative to the control (0.53 mg EQ / g weight dry of flavonoids). While in the roots of the plants subjected to 10000 ppm of zinc, flavonoid content was increased slightly compared to the control.

Flavonoid content of the leaves and roots of Atriplex canescens stressed to lead

The level of flavonoids in the leaves and roots of Atriplex canescens was increased progressively with increasing doses of lead applied to the plant. The flavonoid content was greater in the leaves than in the roots of the plant (Fig. 5).

In the leaves, the higher content of flavonoids (3.70 mg EQ / g dry weight of flavonoid) was obtained for the treatment of 10000 ppm of lead. A slight decrease was noted for flavonoid stressful dose of 2500 ppm of lead (2.57 mg EQ / g dry weight of flavonoid) compared to control (2.58 mg EQ / g dry weight of flavonoid).

In contrast, in the roots of the plant Atriplex canescens, flavonoids were accumulated very slowly when the lead concentration was increased.

Statistical analysis indicated that flavonoids were accumulated in a highly significant way in leaves and roots with all lead treatments.

Fig. 5: Content of flavonoids (mg EQ / g dry weight) of the leaves and roots of Atriplex canescens (Pursh) Nutt stressed to lead.

Flavonoid content of the leaves and roots of Atriplex canescens stressed to cadmium

The results showed that the applied cadmium caused an increase in flavonoid levels in the leaves of the plant 2500, 5000, 7500 and 10000 ppm (2.72, 3.30, 3.51 and 3.39 mg EQ / g dry weight of flavonoids respectively) compared to control (2.58 mg EQ / g dry weight of flavonoids), which was against by the roots, the accumulation of flavonoids was decreased progressively with increasing doses of cadmium. While in the roots of the plants subjected to 10000 ppm of cadmium, flavonoid content was increased slightly compared to the control (Fig. 6).

Fig. 6: Content of flavonoids (mg EQ / g dry weight) of the leaves and roots of Atriplex canescens (Pursh) Nutt stressed to cadmium.

The statistical study highlighted a highly significant response to the effect of cadmium on the accumulation of flavonoids in leaves and roots by contribution to the control plants of the Atriplex canescens.

DISCUSSION

The main obtained results showed that:

The levels of polyphenols were elevated at the level of the leaves and roots of the Atriplex canescens under metallic stress compared to the control plant. These results were consistent with those of [10] or observed an increase in polyphenols under the effect of metallic stress in Vicia faba L. Phenolic compounds intervene in many mechanisms to allow the plant to adapt to its medium [11].

Polyphenols are capable of preventing membrane lipid peroxidation and capturing hydroxyl, superoxide and peroxyl radicals [12,13].

The large accumulation of polyphenols in the stressed plant with heavy metals (zinc, cadmium and lead) was a response to metallic stress unlike the unstressed plant or the rate of polyphenols was low, because according to [11], the accumulation of polyphenols could play an essential role in the balance and adaptation of the plant. Polyphenols are capable of acting as antioxidants that can trap free radicals, resulting in the suppression of the formation of ERO by inhibition of some enzymes or by chelation of the metal ions, involved in their production, and the protection of antioxidant defense systems of the organism [14].

Indeed, polyphenols possessed hydroxyphenolic groups in their structures and the antioxidant properties were attributed in part, to the ability of these natural compounds to trap free radicals such as hydroxyl radicals (OH·) and Superoxides (O2·−)[15].

This accumulation was more important in the leaves than in the roots, in this regard several authors [16,17,18] showed the richness of the leaves of cereals in polyphenols which possessed with great antioxidant power.

The accumulation of phenolic compounds, especially flavonoids, has been demonstrated in many species and in different situations of biotic stress [19], also under abiotic conditions [20].

The flavonoid content in the leaves and roots in the Atriplex canescens was increased proportionately with the concentration of heavy metals, because according to [21], flavonoids are found in large quantities in the different parts of the Plant under the influence of different stresses.
For the concentrations of 2500, 5000 and 7500 of (zinc, lead and cadmium), a decrease in the rate of flavonoids was observed at the root parts level, this could be explained by the resistance of the plants to these concentrations. The response of plants to metal stresses varies from one concentration to another. The results obtained on the significant accumulation of the flavonoid in the stressed plant were corroborated with those of [22] that bound the accumulated amounts of flavonoids to stress tolerance. Consistent with a study done by [23] showing those flavonoids as good chelators of heavy metals.

Indeed, phenolic compounds and especially flavonoids are recognized as potentially antioxidant substances with the ability to trap radical species and reactive forms of oxygen. These compounds participate very effectively in the tolerance of plants to various stresses [11].

CONCLUSION

The response of Atriplex canescens to metal stress has resulted in an increase in antioxidants (polyphenols, flavonoids) as a function of the increasing concentration of heavy metals, this increase was more important in the leaves than roots.

Accumulation of total polyphenols and flavonoids was very important in stressed Atriplex compared with the unstressed plant.

According to the results obtained it could be concluded that the plant Atriplex canescens could be exploited for use for the decontamination of the soils by the approaches of the phytoremediation.

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