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Effect of lead stress on polyphenols, flavonoids, and proline contents in radish (*Raphanus sativus* L.)

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Abstract

Radish (*Raphanus sativus* L.) is a suitable modal plant for eco-toxicological studies due to its tolerance to various stresses, such as metallic and saline stress. Lead is a toxic and exogenous metal to plants, and its presence causes several disturbances in plant. This work consists to determine the effect of lead stress applied during two weeks (0, 500, 1000, 2000 ppm) after 45 days of the plant's growth. The parameters analyzed in the plant are polyphenols, flavonoids, and proline. The assay is performed using a JENWAY 6505 UV-Visible Spectrophotometer. The results obtained show a decrease in proline, but polyphenols and flavonoids show a marked increase. The high levels of proline (0.117 mg/g dry matter in the control) and polyphenols (0.248 mg/g dry matter at the 2000 ppm dose of lead) are recorded in the root system. However, the highest content of flavonoid (8,586 mg/g dry matter in the control) is recorded in the aerial part of the plant. Radish (*Raphanus sativus* L.) is a tolerant plant to lead stress and has a phytoremediator power for lead.

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Introduction

The increase of heavy metals levels in the soil presents a risk for the environment, mainly due to human activities of over exploitation of the soil. Lead can be found in the environment, such as through discharges around lead treatment sites or through automobile pollution, which contaminate soil, vegetation, animals and humans (Ben Ghanya *et al.,* 2006). Lead is a non-essential element for plants, and its phytotoxicity can inhibit the enzymatic activity, disrupt the mineral nutrition, unbalance the water, modify the hormonal status and alter the membrane permeability (Sharma and Dubey, 2005). Thus, these ions can intensify the production processes of reactive oxygen species (ROS) leading to oxidative stress (Prasad *et al.,* 1999; Cuypers *et al.,* 1999). In the face of this disturbance, plants develop several defense mechanisms, including the use of biochemical compounds as stress regulators such as amino acids and phenolic compounds. Proline is an amino acid known as osmolyte (Ashraf and Foolad, 2007), it is involved in the adaptation of plants to environmental constraints by stabilizing proteins, free radical scavenging and regulation of cellular redox potential (Kilani *et al.,* 2012). Polyphenols are among the metabolites that have important antioxidant capacities (Gomez-Caravaca *et al.,* 2006; Xiuzhen *et al.,* 2010), and a high chelating power of metals which represents an important biological interest (Ravichandran *et al.,* 2014; Selvaraj *et al.,* 2014; Kasprzak *et al.,* 2015). According to Symonowicz and Kolanek (2012) flavonoids have an ability to detoxify free radicals, and their ability to chelate heavy metals has been demonstrated by (Brown *et al.,* 1998).

These antioxidant mechanisms have been exhaustively described (Gill and Tuteja, 2010; Dat *et al.,* 2000; Apel and Hirt, 2004).

The plant material used in this study is radish (*Raphanus sativus* L.) from the Brassicaceae family, which was selected for its use in the laboratory as a model plant for eco-toxicology studies of various pollutants (Sun *et al.,* 2010), and for its improved germination rate, rapid growth and high biomass (Bitour, 2012).

The objective of this study is to evaluate the effects of increased lead levels in the soil on the antioxidant activity of radish (*Raphanus sativus* L.) by the determination of antioxidants such as total polyphenols, flavonoids, and proline synthesis.

Materials and methods

Plant material

The plant material used in this study is the radish (*Raphanus sativus*L.), the variety “National”.

Methods of culture

The radish seeds (*Raphanus sativus* L.) are disinfected with 2% sodium hypochlorite for 10 min, then washed several times with distilled water and germinated in alveoli containing compost for 15 days. The seedlings are transplanted into the cylinders (30 cm high and 20 cm in diameter) containing a mixture of sand and compost (2V/V) for a month and a half (45 days). Watering was done 3 times a week, twice with distilled water and once with a nutrient solution of Hoagland (1938). The plants are harvested after a period of 60 days of cultivation, and transported to the laboratory in order to be cut in two aerial and root parts, then they were dried at 80°C during 48 hours.

Application of stress

Radish stress is applied after 45 days of cultivation twice for two weeks, the lead doses used are 0, 500, 1000 and 2000 ppm, with three repetitions for each dose.

Analyzed parameters

Preparation of methanolic extracts

The plant material used in the preparation of the extracts was macerated in a methanol/water mixture (7:3 V/V) at a ratio of 1/10 (W/V), under gentle agitation overnight at room temperature (Talbi *et al.,* 2015).

Content of polyphenols

The determination of total polyphenols is performed by a colorimetric method of (Folin-Ciocalteu) (Singlton *et al.,* 1999; Heilerova *et al.,* 2003). The absorbances are read from the UV-Visible Spectrophotometer at 760 nm.

Content of flavonoids

The estimation of the total flavonoid content is made by the method of (Bahorun *et al.,* 1996) the absorbance is read at 415 nm using a UV-Visible Spectrophotometer.

Content of proline

The method used to measure proline is that of (Troll and Lindsley, 1955) modified by (Dreier and Goring, 1974) and then by (Monneveux and Nemmar, 1986). The assay was performed by a UV-Visible Spectrophotometer JENWAY 6505 at a wavelength of 528 nm. The content is expressed in mg/g dry matter.

Statistical analyzes

The results obtained were analyzed statistically using STATBOX software. Version 6.4. The analyzes performed are the variance (ANOVA) and the NEWMAN-KEULS test with a significance level (P = 5%).

Results

Polyphenols content of the arial and root part of Radish stressed by the lead

The total polyphenols content increases in both aerial and root parts of the radish. This content is higher in the root part (between 0.202 mg/g dry matter in the control and 0.248 mg/g dry matter in the 2000 ppm dose of lead) compared to the aerial part (between 0.140 mg/g dry matter in the control and 0.091 mg/g dry matter in the 2000 ppm dose of lead). The level of total polyphenols recorded in plants stressed with lead is higher than that of the control in the root part and lower than the control in the aerial part (Fig.1). The variation of the total polyphenols content in both the aerial and root parts of radish under lead stress is not significant (P>0.05).

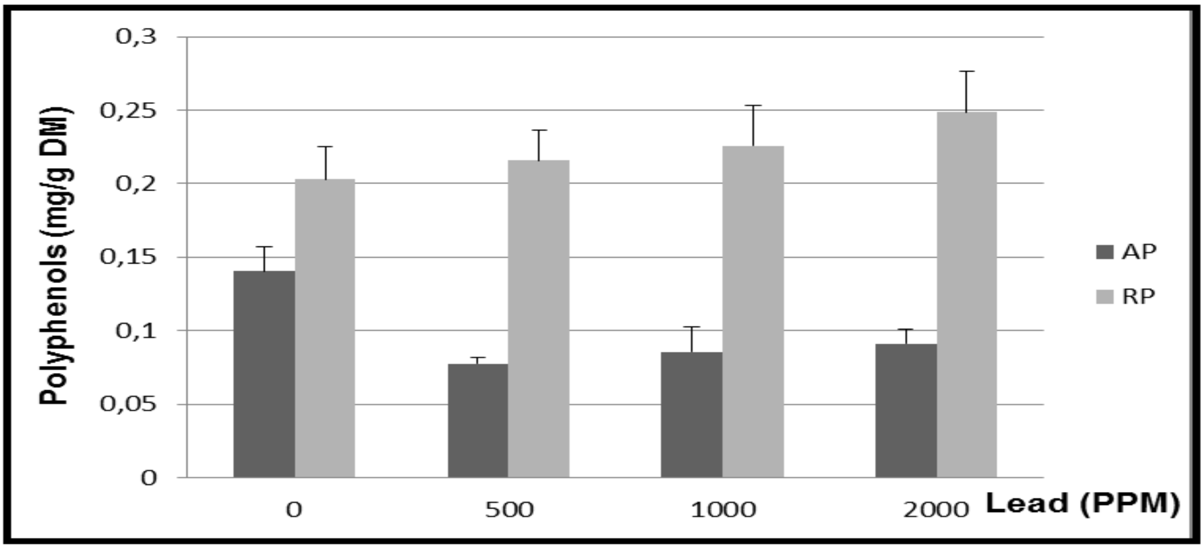

Fig.1. The polyphenols content (mg/g dry matter) in the aerial (AP) and root part (RP) of radish (*Raphanus sativus* L.) under lead stress.

Flavonoids content of the arial and root part of Radish stressed by the lead

The total flavonoid content in radish decreases in the aerial part and increases in the root part gradually and proportionally with increasing lead dose. The level of flavonoids recorded in the aerial part (between 8.586 mg/g dry matter in the control and 6.287 mg/g dry matter in the 2000 ppm dose of lead) is higher than that recorded in the root part (between 2.490 mg/g dry matter in the control and 4.751 mg/g dry matter in the 2000 ppm dose of lead). The 500 and 1000 ppm doses of lead mark almost equal levels of flavonoids in both parts of the plant (Fig.2). The ANOVA statistical analysis shows that the difference in flavonoids content recorded in the aerial and root part of radish under the lead effect is not significant (p>0.05).

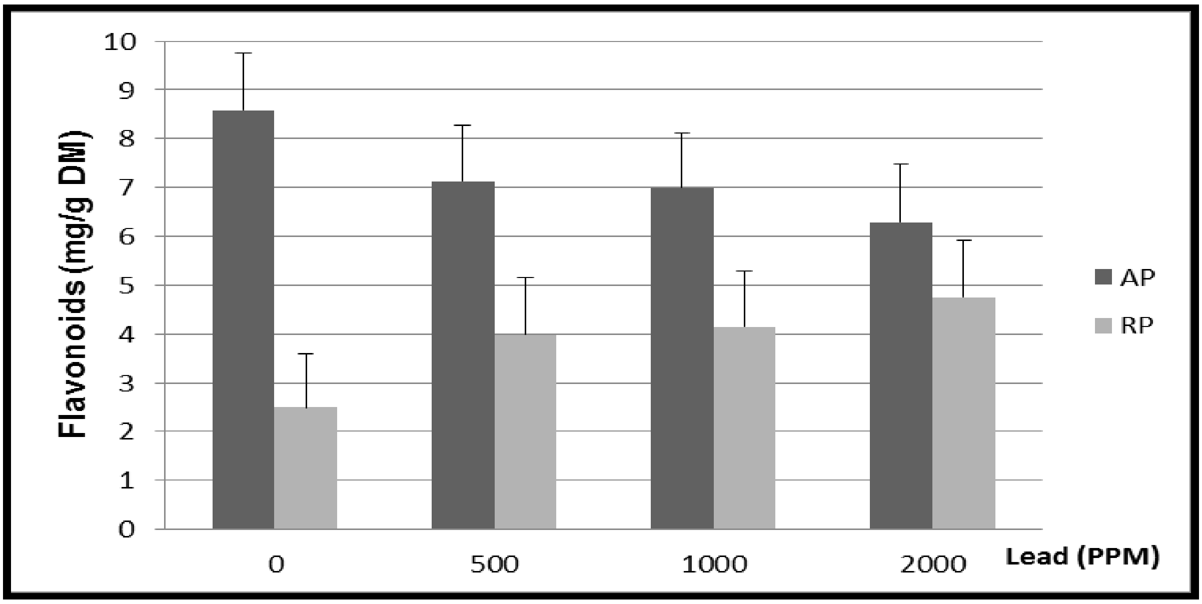

Fig. 2. The flavonoids content (mg/g dry matter) in the aerial (PA) and root part (RP) of radish (*Raphanus sativus* L.) under lead stress.

Proline content of the arial and root part of Radish stressed by the lead

The proline content decreases proportionally with increasing lead dose in both aerial and root parts of Radish. The proline level is higher in the root part compared to the aerial part, and the difference in proline content between these two parts is 0.025, 0.041, 0.048 and 0.049 mg/g dry matter for the control, for 500, 1000 and 2000 ppm doses of lead respectively. The highest proline content is recorded in the control plant (0.117 and 0.092 mg/g dry matter) in the root and aerial parts respectively. On the other hand, the lowest proline content is recorded in the 2000 ppm dose of lead (0.080 and 0.031 mg/g dry matter) in the root and aerial parts respectively (Fig.3). Statistical analysis of variance shows a non-significant effect (P>0.05) of the lead dose increase on proline content in both aerial and root parts of radish.

Discussion

Polyphenolsandflavonoids

Polyphenols increase in the root and aerial part of the radish proportionally with increasing doses of lead. However, flavonoids increase in the root part and decrease in the aerial part of radish as lead dose increases. Stressed plants accumulate polyphenols and flavonoids more than the control in the root part and less than in the aerial part of the plant. This could be caused by triggered stress levels in the different organs of the plant; which shows that the lead effect is not only dose-dependent, but also organ-dependent. According to Smirnoff, (2005) polyphenols are found in large quantity in different parts of plant under several stresses effects, such as heavy metals (Briat and Lebrum, 1999). A correlation between polyphenol content and antioxidant activity was observed, since phenolic levels contribute directly to antioxidant activity (Duh, 1999). The accumulation of polyphenols can be explained by the stimulation of polyphenol oxidase production which is a key enzyme in the metabolic pathway of polyphenols (Tuna *et al.,* 2008). Flavonoids act as antioxidant molecules that fix ROS (Lovdal *et al.,* 2010). Flavonoids can act in different ways in oxidative stress regulation processes by directly capturing reactive oxygen species, chelating metals and inhibiting the activity of some enzymes responsible for the production of ROS (Morris, 1995).

The results obtained in this work are in conformity with several studies showing an increase in polyphenols and flavonoids under lead stress in *Atriplex halimus* at dose (0.1000, 4000, 8000 ppm) (Belarbi, 2018), in *Vallisneria natans* at different lead concentrations (Chao Wang *et al,* 2011), in *Echuim* *vulgare* L. under metallic stress from lead and zinc (Slowomir Dresher *et al.,* 2017), in *Ginsingpnanax* at a dose of 3000 ppm copper (Ali *et al,* 2006), in *Kandelia obovata* after 7 days of treatment with lead and manganese (Zhongzhenget *al.,* 2011), in *Vaccinium coryombosum* L. under aluminium and cadmium stress (Manquian-cerdaa *et al.,* 2016). An increase in polyphenol content is also marked in *Raphanus sativus* (Tomislav *et al.,* 2008), in *Aeluropus littoralis* under the effect of heavy metals (Pb, Co, Cd, Ag) (Rastgoo *et al.,* 2011), and in *Viciafaba* subjected to different mercury concentrations (Benahmed f. 2010). An increase in flavonoid content is also estimated in *Taraxacum officinal* under stress of (Cr, Pb, Cu, Ni, and Zn) (Bretzel *et al.,* 2014). The decrease in flavonoids and the increase in polyphenols in the aerial part of radish are also recorded in *Brassica juncia*, a species of the same family of radish under cadmium stress (Dhriti K. *et al.,* 2014).

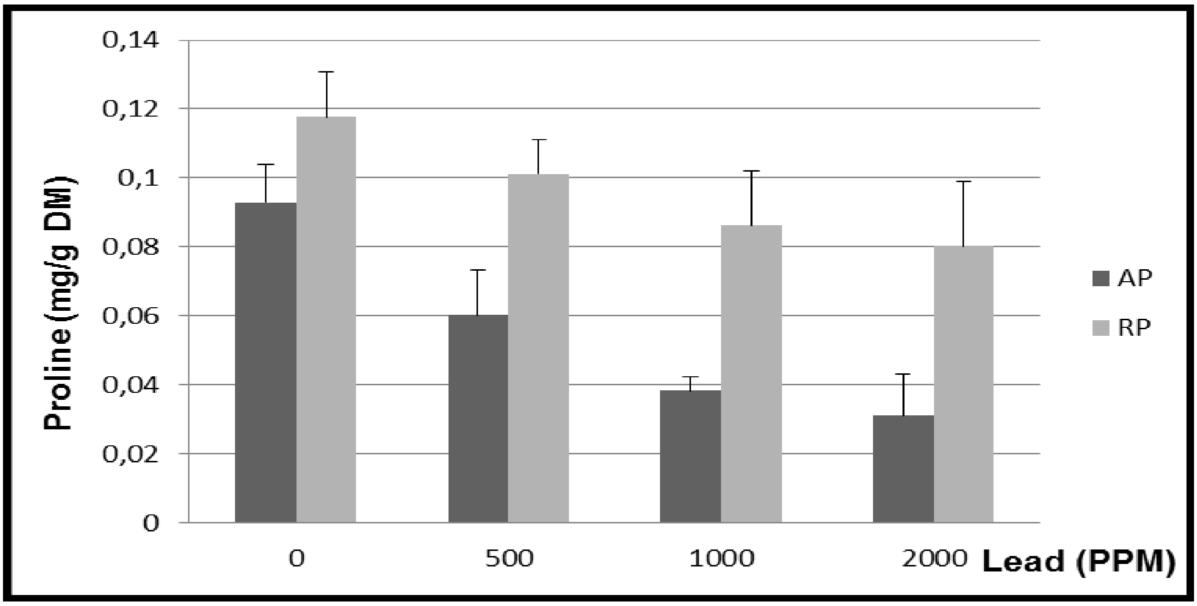

Fig.3. Proline content (mg/g dry matter) in the aerial (AP) and root part (RP) of radish (*Raphanussativus* L.) under lead stress.

Proline

The proline content recorded in *Raphanus sativus*plants subjected to different lead concentrations (0, 500, 1000, 2000 ppm) decreases progressively with increasing of the above doses of lead. According to Lemzeri (2007), the species most sensitive to stress react by accumulating proline more rapidly. In contrast, the tolerant ones show relative stability or low proline accumulation compared to the sensitive ones. This could justify the low proline accumulation in radish under lead stress by the tolerance of this plant to metallic stress. These results are consistent with several researchers demonstrating a decrease of proline in *Raphanus sativus* stressed by zinc at doses of 0 and 100 ppm (Tihana *et al.,* 2008), in *Brassica juncia* subjected to high doses of lead and cadmium (John *et al.,* 2009), and in *Atriplex canescens* stressed by lead at doses of 1000 and 5000 ppm (Babou, 2014). According to Chen, 2004 proline also decreases in rice roots under metallic stress.

The highest levels of proline and polyphenols are superior in the root part of radish, and the flavonoid content increases gradually with the increase of the lead dose in the root part. Proline accumulation is higher in the root part than the aerial part of plant (Fig.3). These results are identical to those obtained by (Tihana *et al.,* 2008; Tomislav *et al.,* 2008), confirming that the proline content is higher in hypocotyl than in leaves of *Raphanus sativus* under lead stress. According to (Biteur, 2012), proline synthesis increases in roots more than leaves after ten weeks of lead stress in radish.

According to (Jones and Clement 1972; Khan and Frankland, 1983), it is generally known that the roots act as a barrier to the movement of toxic heavy metal through the soil-plant system. Several authors confirm the accumulation of lead in radish roots such as Påhlsson (1989) which showed that lead accumulates mainly in the root system of R. sativus, where it binds to the root surface and cell walls. Kristen *et al,* (2015) also confirm that the level of lead in the aerial parts of radish is inferior to the roots. A study conducted by (Asadi Kapourchal *et al.,* 2009) showed that the measured lead accumulated in the roots was 208 ppm whereas it was 27.25 ppm in the radish shoots harvested. These results may justify the estimation of high levels of proline, polyphenols and flavonoids in the root part of radish. Asadi Kapourchal and colleagues (2009) also report that there was no toxicity for radish up to 1000 ppm lead in soil. Consequently, they considered radish as an accumulator plant of lead and that it can be seeded up to five times a year in the same soil and its yield can reach 20 t / ha, so it can be used to remediate the polluted soils by lead.

Conclusion

The increased lead dose causes in radish (*Raphanus sativus* L.) a decrease in proline content and an increase in total polyphenols and flavonoids. The higher levels of proline and total polyphenols are recorded in the root part of the plant, unlike the high levels of flavonoids in the aerial part. The statistical analysis of lead stress's effect on the accumulation of proline, polyphenols and flavonoids in radish is not significant, which shows that *Raphanus sativus* is not affected by the increase in lead dose at the point where stress causes significant differences on the antioxidant (polyphenols, flavonoids) and proline content of this plant. From these results, we can deduce that *Raphanus sativus* is a tolerant plant to the lead stress and it has a phytoremediator power for this metal.

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