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BIOCHEMICAL RESPONSES OF TWO VARIETIES OF FABA-BEANS (SIDI AÏCH AND SUPER AGUADULCE) TO LEAD STRESS

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Abstract

Lead is the most important toxic element in the environment that affects the life of living organisms. In plants, lead had no physiological role; its presence causes multiple morphological, physiological and biochemical disturbances. In order to study the effect of lead on the physiological and biochemical behaviour of the bean (*Vicia faba* L.), two varieties of bean (Sidi Aïch and super Aguadulce) are exposed to different doses (0, 50, 100, 200, 500, 1000 and 2000 ppm) of lead as $Pb(NO_3)_2$, lead was added to the culture substrate which composed of sand and compost (3v/1v). The accumulation of lead in the treated plants is accompanied by a reduction in protein concentration on the one hand, and on the other hand, presence of lead is accompanied with an increase in soluble sugars and proline. The presence of lead in the medium even at low doses causes significant physiological and biochemical disturbances in whole the plant.
Keywords: Lead, stress, faba-bean, proline, proteins, sugars

Introduction

Heavy metals are those metals with a density greater than 5 and an atomic number greater than 20 (Ali and Khan, 2018). These metals can be classified into essential (Fe, Mn, Cu and Zn) and non-essential (Cd Cr and Pb) elements (Barakat, 2011). Among the non-essential heavy metals, lead is the most important toxic element affecting living organisms in the environment (Shahid *et al.*, 2011). Due to its important physico-chemical properties, lead is widely used in everyday life, in pipes, soldering, painting, pesticides, as an antiknock agent in gasoline and in the manufacture of batteries.

Physiologically, lead has no role and remains toxic even at low doses (Beak *et al.*, 2006). Pb is a non-biodegradable element, it persists in the environment and can be transmitted to plants and humans via soil, air and water (Natasha *et al.*, 2020). Lead is an abundant, important but dangerous chemical element.

Exposure to lead and its compounds occurs mainly in professional environment, industrial processes such as lead smelting and combustion, from pottery, boat building, lead-based paint, lead-containing pipes and piping, battery recycling, the arms industry, pigments and book printing, and also from the use of leaded gasoline (Nas and Ali, 2018).

Exposure to lead occurs primarily either through inhalation of lead-contaminated dust particles or aerosols or through ingestion of lead-contaminated food, water and paint (Tchounwou *et al.*, 2012). Lead poisoning affects several organs in the body, including the kidneys, liver, central nervous system, hematopoietic system, endocrine system and reproductive system (ATSDR, 1999).

The Effect of Lead on Plants can first be manifested on the cell walls, so the adsorption of Pb bound to the cell wall or to the cell membrane constituents leads to variations in tis plasticity (Wierzbicka *et al.*2007) which negatively affects the potential of the membrane by inducing lipid peroxidation (Yan *et al.,* 2010). This peroxidation contributes to the disruption of cell division (Antosiewicz and Wierzbicka, 1999).

Inside the cells lead can affect the enzymatic activity revealing a dysfunction of a very large number of enzymes involved in various metabolic processes (Mitra *et al.*, 2020). The inhibition of enzyme activity resulting from the interaction of Pb with the (-SH) group of the enzyme (Sharma and Dubey, 2005) and the substitution of bivalent cations essential for metalloenzymes (Pourrut *et al.*, 2013), is a typical sign of lead contamination.

Lead also affects seed germination even at low doses (Kopittke *et al.* 2007), and the growth of the root and aerial parts of plants (Islam *et al.* 2007; Kopittke *et al.* 2007; Gupta *et al.,* 2010). These effects can lead to reduced growth and biomass production. Photosynthesis is also affected by the presence of Pb in the medium (Singh *et al.,* 2010; Cenkci *et al.,* 2010), by inhibition of chlorophyll synthesis due to blockade of essential cation (Mn Fe) uptake (Patra *et al.,* 2004; Chatterjee *et al.,* 2004; Gopal and Rizvi 2008), and by increasing chlorophyllase activity (Liu *et al.*, 2008).

The toxic effects of Pb on plants are manifested genetically through DNA damage (Pourrut *et al.,* 2011; Shahid *et al.,* 2011; Kumar *et al.,* 2017), and chromosomal abnormalities (Rodriguez *et al.* 2013).

In order to evaluate the impact of bioavailable lead and the assessment of its effects on the development of the plant and risks related to its presence in the substrate, our work is based on the study of the effect generated by Pb stress on the physiological and biochemical behaviour of the faba-bean. The present study consists to applicated different doses of lead as Pb-nitrates $Pb(NO_3)_2$, in the substrate, during the experimental period of the cultivation of two varieties of the faba-bean. The reactions of the plants resulting from this variability, measuring the impact and manifestation of adaptation mechanisms, are represented by physiological and biochemical parameters.

Materials and Methods

The present experiment was carried out in the experimental greenhouse of the National Centre for Biotechnology Research (CRBT) Constantine, Algeria.

Plant material

The plant material used in the experiment is composed by two varieties of broad bean (*Vicia faba* L.) which are derived from seeds carefully supplied by the National Centre for control and certification of seeds and plants (C.N.C.C) Constantine, Algeria. The first variety or V1 (Sidi Aïch) is a local variety, the second or V2 is a variety introduced from Spain (Super Aguadulce).

Substrate preparation

The substrate used in the experiment consists of a mixture of sand and compost in the proportions 3v/ 1v. The sand was sieved, washed several times with water, then dried in the open air and mixed with compost, the mixture homogenized and transferred to plastic pots, which will receive the plants.

Experimental device

The pots used for cultivation are made of plastic with a volume of three liters (3l) drained by a gravel, these pots are filled with the substrate at a rate of 2.5 Kg /pot, the pots are installed on two tables in the greenhouse until the end of the experiment.

Germination

The seeds of *Vicia faba*, of both varieties are sterilized in a 6% sodium hypochlorite solution, rinsed with distilled water, then placed in food-grade plastic boxes, soaked in water and kept in the dark to germinate. After four days, the germinated seeds are transplanted into the culture pots installed in the greenhouse where they are kept until the end of the experiment. In order to ensure a good water supply, the plants are watered periodically, keeping the substrate at maximum retention capacity. Irrigation water was replaced every three days by a nutrient solution (Table 1).

Table 1 : Composition of mineral nutrient solutions.

Element	Quantity
N	20%
P_2O_5	20%
K_2O	20%
S	0.8%
MgO	0.4%
Fe EDTA	650 ppm
Mn	650 ppm
Mo	50 ppm
SO_3	0.8%
Cu	60 ppm
Zn	300 ppm
Bore (B)	300 ppm

Lead Application

Lead was added five days after transplanting the young plants as a solution of lead nitrate $Pb(NO_3)_2$, the different doses used are 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm and 2000 ppm.

Lead content determination

The determination of lead was carried out by atomic absorption spectrometry (AAS) according to the method described by Vogel-Milkus *et al*. (2005).

Biochemical analysis

After 30 days of cultivation, the plants were separated from their substrate and the plant material was prepared for biochemical analysis.

a. Proline content determination

Proline concentration was determined by using Troll and Lindsley (1955) method, simplified and developed by Dreir and Goring (1974). The concentration was estimated in mg/g of fresh material after conversion of the optical density read on a UV-Visible spectrophotometer at wavelength of 528ηm.

b. Soluble sugars content determination

The determination of the quantity of soluble sugars was carried out by the Anthrone method of Shields and Burnett (1960). Concentrations are expressed in mg/g of fresh material by UV-Visible spectrophotometer at wavelength of 585ηm.

c. Protein content determination

The protein content was determined according to the Bradford method (1976) using bovine serum albumin (BSA) as the standard. Concentrations are calculated from the UV-Visible spectrophotometer reading at 595ηm, and expressed in mg/g of fresh material.

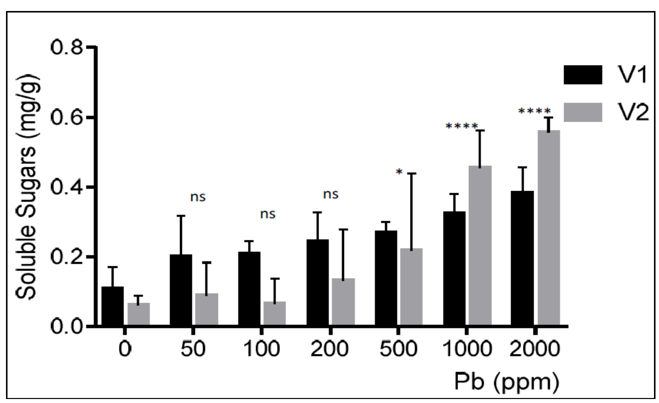
Statistical analysis

All data obtained was treated by statistical study based on a two-factor ANOVA analysis of variance, plus a means comparison according to Dunnett test at $p < 0.05$.

Results

Soluble sugars contents

The presence of lead caused a high increase in the soluble sugar content (Fig. 01), the variations in this parameter are strongly influenced by lead stress ($p<0.0001$). According to the Fig. 01, the increase in sugar content is only significant from the 500 ppm, where it is more than 149% for V1 and more than 259% for V2 compared to the controls (Fig. 01). Then the increase become very significant at 1000 ppm (200% for V1 against 653% for V2) and at 2000 ppm (256% for V1 and 821% for V2).

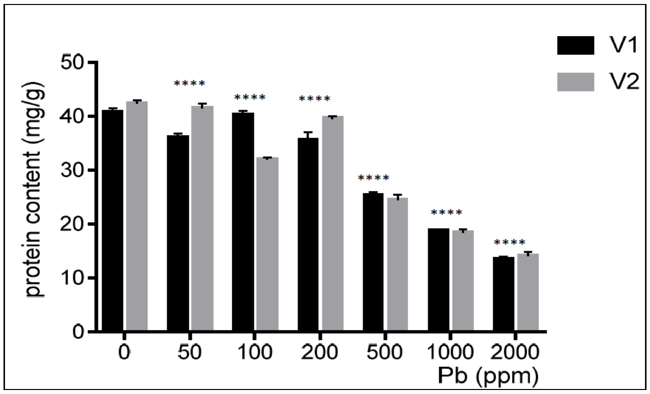


ns: not significant, *: significant and ****: very significant difference at 0.05 level

Fig. 1 : Effects of different Pb concentrations (ppm) on soluble sugar content (mg/g) of two varieties of *Vicia faba,* Sidi Aïch (V1) and Super Aguadulce (V2).

Protein contents

The results showed that the protein contents decrease with the presence of lead in the substrate, this decrease is very highly significant (p<0.0001). Protein contents registered a very significant decrease at 2000 ppm, where it was more than 66% for both varieties (Fig. 2), while it was only 11.46% for V1 and 1.84% for V2 at 50 ppm dose in comparison with the control plants.

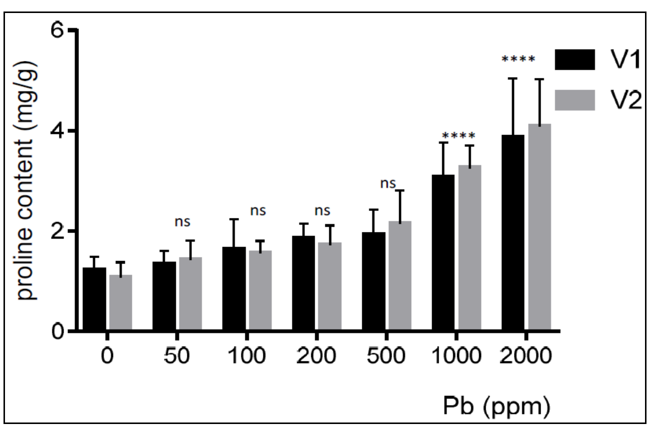


****: very significant difference at 0.05 level

Fig. 2 : Effects of different Pb concentrations (ppm) on protein content (mg/g) of two varieties of *Vicia faba,* Sidi Aïch (V1) and Super Aguadulce (V2).

Proline contents

The results (Fig. 3) showed that the presence of lead in the medium had a very highly significant effect (p<0.0001) on the proline content. The increase in the lead concentration in the medium was accompanied by an increase in the proline content in the bean leaves. This increase was significant only from the 1000 ppm dose where it was more than 153% recorded for V1, and more than 201% for V2 (Fig. 03). Proline content recorded an increase at 2000 ppm over 217% for V1 and more than 278% for V2 compared to the controls.

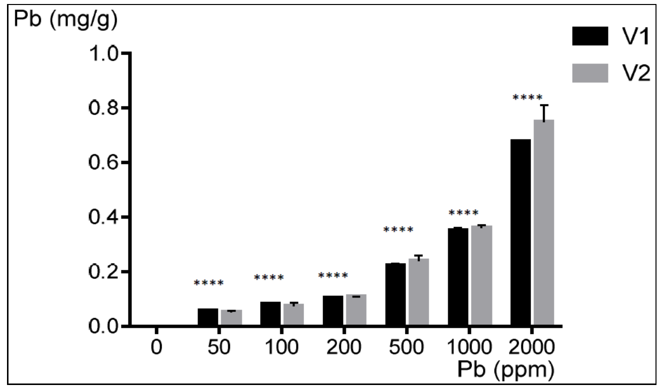


ns: not significant, ****: very significant difference at 0.05 level

Fig. 3 : Effects of different Pb concentrations (ppm) on proline content (mg/g) of two varieties of *Vicia faba,* Sidi Aïch (V1) and Super Aguadulce (V2).

Lead content

The results (Fig. 4) showed that the presence of lead in the medium is closely related to the accumulation of this element in plant tissues, this accumulation is strongly influenced by the increase of the applied lead dose (p<0.0001). The accumulation of Pb in the tissues reached its maximum at 2000 ppm with more than 0.6 mg/g for V1 (Sidi Aïch) and more than 0.7 mg/g for V2 (Super Aguadulce), while it was only 0.056 mg/g for V1 and 0.053 mg/g for V2 at 50 ppm dose in comparison with the values recorded for the controls (Fig. 04).



****: very significant difference at 0.05 level

Fig. 4 : Effects of different Pb concentrations (ppm) on Pb accumulation in tissues (mg/g) of two varieties of *Vicia faba,* Sidi Aïch (V1) and Super Aguadulce (V2).

Discussion

The results obtained showed a significant regression in soluble sugar content as a function of the intensity of lead stress. The sugar mechanism is strongly affected by lead contamination (Govindjee, 1976; Gawęda, 2007; Sofi *et al.*, 2014). Changes in the levels of soluble sugars in the tissues of plants growing in lead-polluted environments are not determined with certainty (Gawęda, 2007). Some authors (Huang *et al.*, 1974; Soheir *et al.*, 2002) have detected a decrease in soluble sugars, others (Azouz *et al.*, 2011) have instead reported an increase depending on the intensity of the stress applied.

Soluble sugars may play a role in protecting chlorophylls and carotenoids from the aggressiveness of metal stress (Azouz *et al.*, 2011). Verma and Dubey (2001) and Gawęda (2007) attribute this accumulation to a very possible adaptation of the plant to adjust and maintain a favourable osmotic potential. These hypotheses explain the results obtained in this study, which show that lead application is accompanied by a clear accumulation of soluble sugars. However, this accumulation remains conditioned by the dose of lead and the time of its application.

Protein contents have also decreased with the presence of lead in the medium, and this decrease is highly correlated with the intensity of lead stress. In the present study, the lead present in the medium was accompanied by a reduction in the protein composition of the leaves of the two varieties tested. This is in agreement with previous work (Mishra *et al.*, 2006; Garcia *et al.* 2006; Piotrowska *et al.*, 2009).

In the presence of high lead concentrations, Pb in the cytoplasm interacts with proteins and the protein pool decreases significantly (Mishra *et al.*, 2006; Garcia *et al.*, 2006; Piotrowska *et al.*, 2009, Shu *et al.*, 2012, Sofi *et al.*, 2014). The effect of lead on total protein concentration is unclear, although high concentrations may decrease the protein pool (Mishra *et al.* 2006; Garcia *et al.* 2006; Piotrowska *et al.* 2009). The decrease in protein content may be due to a altered gene expression (Kovalchuk *et al.*, 2005, Pourrut *et al.* 2011) and a increased ribonuclease activity

(Gopal and Rizvi, 2008); lipid peroxidation and protein fragmentation (Nas and Ali, 2018) as a result of reactive oxygen species (ROS) (John *et al.*, 2008, Azooz *et al.*, 2011) and a stimulation of protease activity (Sharma and Dubey, 2005; Gupta *et al.*, 2009), the decrease in protein is also reported to the decrease in free amino acid content (Xiong *et al.*, 2006; Gupta *et al.*, 2009). However, there was an increase in the concentration of some amino acids such as proline in the presence of lead (Qureshi *et al.*, 2007; Hedaya, 2008; Wang *et al.*, 2010).

In addition to a quantitative change, lead affect the qualitative composition of cellular proteins. It modifies the protein profile of bean root cells exposed to Pb (Beltagi, 2005), which may be correlated with changes in transcriptome profile (Kovalchuk *et al.*, 2005). Pb^{2+} ions also strongly influence the activities of many enzymes involved in different metabolic processes. This strong interactivity with plant enzymes is one of the major causes of the toxicity of this metal (Kovalchuk *et al.*, 2005).

The results found in this study, also show a relative increase in proline levels as a function of increased lead doses applied to the substrate. Accumulation of this amino acid is reported in the presence of high doses of lead on the bean (Qureshi *et al.*, 2007; John, *et al.*, 2008; Hedaya, 2008), or on other species (Jiang Wang *et al*., 2010; Azooz *et al.*, 2011).

Proline is among the amino acids most present in cells under stress conditions, it plays a role in plant tolerance to heavy metal contamination, including Pb (John *et al.*, 2008; Shahid, 2010; Azooz *et al.*, 2011; Rucińska-Sobkowiak *et al.,* 2013). To maintain water status, the plant massively secretes osmolytes, particularly proline (Qureshi *et al.*, 2007; Azooz *et al.*, 2011). According to Hedaya (2008) this amino acid seems to play a vital role in osmotic adjustment. In addition, Proline is considered as a stabilizer of macromolecules (Parys *et al.,* 2014), a metal chelator through thiol groups (-SH) and a protector of the subcellular structure (Azooz *et al.,* 2011).

Proline accumulation in tissues may be due to proteolysis (Pourrut, 2008; Azooz *et al.*, 2011) induced by the presence of Oxygen Reactive Species (ORS) resulting from the presence of lead (Sharma and Dubey, 2005, Wong *et al.*, 2008).

Conclusion

The present study revealed that the presence of lead in the environment even at low doses causes multiple physiological and biochemical disturbances in the bean. These disturbances affect the mechanisms of osmoregulator synthesis. Our results show that proline present a significant accumulation with the intensity of lead stress, specially at the highest doses, these results also show that soluble sugars rate increase when lead doses increase in the medium. The accumulation of these osmoregulators is reported for the two varieties. The presence of lead in the medium also caused a decrease in the protein content of plant cells exposed to this stress for both varieties.

The study of the phytotoxicity of lead showed significant changes in physiological and biochemical behaviour, which requires further study.

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