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Biochemical Response of a Leguminous Plant (*Phaseolus vulgaris* L.) to Accumulating of Heavy Metal Subjected to Metal Stress in Soils Culture in Souk-Ahras City (North-East Algeria)

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ABSTRACT

Background: The present study was devoted to examine the biochemical responses of a leguminous plant species, *Phaseolus vulgaris* L., to oxidative stress induced in heavy metals contaminated soils culture. Herein, plant samples were collected from the stations of Souk-Ahras city (Northeast Algeria) presenting heavy-metals contaminated lands of culture.

Methods: The sampling of plants and soils was carried out in June 2017 randomly from separate points (approximately 10 meters) at each site. Soil and plant samples from the study environments were subjected to analysis at the Common Services Unit for Research-Atomic Absorption Spectrometer, Faculty of Sciences of Bizerte, University of Cartage -Tunisia-, for the purpose of acquiring the dosage of metallic traces in them, Following that, the measurements enzymatic bioindicators were carried out at the level of the Laboratory of Sciences and Techniques of Water and Environnment at the University Mohamed Cherif Messaadia -Souk Ahras-.

Result: From the analysis of the results obtained adecreasing concentrations of Mn, Zn, Cr, Pb and Cu in the studied soils are showed, a twice much higher concentration of Zn than that of Cu in the different parts of the sampled plants and noteworthy, both Zn and Cu reached the tissues of stems, leaves and even fruits. Moreover, the *Phaseolus vulgaris* L. collected from different sites of Hanancha of Souk-Ahras city showed marked variations in the antioxidant enzymatic activities of ascorbate peroxidase and catalase, reflecting a strong photosynthetic activity. These observations were, especially noticed in the sampling sites of Aïn Guerima and ElFadj Labiadh, known as the high heavy metals contaminated sites due to road traffic and irrigation water pollution.

Key words: Bioaccumulator, Bioindicator, Heavy-metals, Oxidative stress, Pollution.

INTRODUCTION

Nowadays and globally, under conditions of high technogenic load, there is significant pollution of soil and air with heavy metals, particularly in regions with large industrial centers and a high concentration of vehicles. In these areas, biogeochemical provinces form, exhibiting elevated heavy metal levels in soil, water and plants. This situation reduces the biological value of plant products and leads to the entry of heavy metals into the food chain (Butsiak *et al*., 2021; Vinogradov *et al*., 2024). The pollution from heavy metals, which has sharply increased since 1900, has emerged as a significant threat to both the biosphere and human health (Ansari *et al*., 2024). In nature, heavy metals, non-destroyed and highly ecotoxic elements (Adriano, 2001), can have natural origins, resulting from geological processes such as erosion and volcanic activity. However, advancements in industrial metal extraction have also contributed to their dispersion. These metals can be transported over long distances by air currents, affecting areas far from their source. This contamination of soil and water bodies disrupts not only the nutritional quality of crops but also their germination and seedling growth (Kulshrestha *et al*., 2024). Nevertheless, the uncontrolled waste disposal of the industrial discharges, various transformation processes including the non-ferrous production of metals (As, Cd, Cu and Zn), the combustion of coal (Ni and Pb), agricultural activities and traffic transportation result in the increased levels of environmental and soil pollution by heavy metals (Kabata-Pendias, 2000). As a result, plants mobilize their enzymatic and non-enzymatic antioxidant defense system involving different molecules able to scavenge free radicals due to oxidative

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stress induction (Soughir, 2009). In addition, the understanding of the plant response mechanisms to their external environmental conditions is of great interest for basic research and as a way to improve stress tolerance. As a sessile organism, the research interest in the physiological, biochemical and molecular aspects of the plant stress response is highly justifiable and thus can provide helpful knowledge that can be applied on a practical level for the development of agricultural species tolerant to different abiotic stresses (Ferfar, 2017). In this regard, this study was undertaken to analyze the physicochemical properties of the heavy metals contaminated soils of various sites of Souk-Ahras city, to assess the response of a bean species *Phaseolus vulgaris* L. to metals stress and the effects of heavy metals on the enzymatic activity.

MATERIALS AND METHODS

Biological material

The study was conducted on a leguminous plant (*Phaseolus vulgaris* L.) of the Fabaceae family, an efficient heavy metals-bioindicating and bioaccumulating plant, that is globally used due to its agro-economic importance. The plant material was harvested during the full vegetative growth stage in June 2017 from three heavy-metals polluted sub-humid stations in North-East Algeria (Hanancha of Souk Ahras city) as shown in Fig 1.

In this study, approximately ten plants from each of the three separate points (approximately 10 meters apart) at each station sampled and sent to the Laboratory for Metallic Trace Elements Analysis and Biochemical Evaluation (Enzymatic activities).

Methods

Analysis of soil heavy metals

The five soil samples were taken from random dispersed points at each site, each collected at a depth of 20 cm were subjected to the physicochemical analysis method consisting of the steps of drying in an oven at 60°C until obtaining a constant weight, grinding and sieving (140 µm) to obtain fine soil powder. The obtained samples were analyzed by X-ray fluorescence (XRF) of the research unit of materials, nanomaterials and ecosystems of the Sciences Faculty of Bizerte (Tunisia). Herein, 8 grams of soil sample were used for the test, or boric acid to provide a sufficient amount of the pellet under the sample. Accordingly, the sample can be prepared as a pellet by centrifugation (40 rpm) and then placed in an XRF spectrometer autosampler (Thermo Scientific Model Niton FXL 950). In the XRF method, samples must be placed under an X-ray beam and consequently, the energy spectrum of fluorescent X-rays can reveal the characteristic peaks of the elements present in the samples, where the height of the peaks reflects the number of elements (Guessasma *et al.*, 2020).

Determination of the contents of metallic trace elements in plant

The dry plant material was reduced to a fine powder and then digested with a mixture of HNO_3 and $HClO_4$ (4:1) acids (Barthwal *et al.*, 2008). The mineralization was gradually undertaken from 100 to 350°C for 2h and the samples were afterward, taken in 20 ml of 0.5% nitric acid solution. After that, the extracts were filtered and the concentrations of metallic trace elements in the plant tissues were determined by atomic absorption spectrometry (Perkin Elmer PinAAcle™ 900T, USA).

Determination of antioxidant enzymatic activity

The plant enzymatic extract was obtained according to the method of Loggini *et al.* (1999). The catalase activity (CAT) and the ascorbate-peroxidase activity (APX) were determined, respectively according to the method of Cakmak and Horst (1991) and Nakano and Azada (1987). Themeasurement of the catalase activity is based on the spectrophotometric recording (JANEWAY 6705) of the absorbance decrease for three minutes at 240 nm and a molar extinction coefficient ε=39400 $M^{-1}.cm^{-1}.L$. The reaction is triggered by the addition of hydrogen peroxide. The reading is taken at 290 nm (JANEWAY 6705 Spectrophotometer) for 1 min, using a molar extinction coefficient ε= 2800M^{-1}. cm^{-1}.



S_1: AïnGuerima, S_2: El FadjLabiadh, S_3:Near the Hananchabridge.

Fig 1: Geographical location and coordinates of the sampling stations (Google Maps, 2022).

Statistical analysis

The effect of dose relative to the trace metallic elements detected in the sampling sites was tested by the non-parametric test of *Kruskal Wallis* applied to test the effect relating to the metallic trace elements detected at the sampling sites. The sharpness of linear relationships between all quantitative variables used in this work was analyzed by Spearman's correlation coefficient. The statistical tests were applied using Statistica 8. The application conditions of the statistical tests met the recommendations reported in several statistical works (Dagnelie, 2007 and Scherrer, 2007).

RESULTS AND DISCUSSION

As indicated in Table 1, the antioxidant enzymatic markers were found to be varied depending on the content of metal trades in the site. The average concentrations of the major important heavy metals (Mn, Zn, Cu, Cr, Pb) measured in the soil samples of each site were decreased in the order; of Mn>Zn>Cr>Pb>Cu. Additionally, the concentrations of each heavy metal were found to be varied between the sites, in particular, site 1 showing an increased concentration of manganese (448.3 ppm), site 2 near the bridge characterized by an urban wastewater discharge showing the highest level of the Zn (171 ppm) and site 3 showing the highest threshold (91.3 ppm) of the chrome. Whilst, a very low selenium concentration (1.1 ppm) was observed in site 2. The obtained results showed also a non-significant difference in the contents of metal trace elements in the three study sites (U=9, p>0.001).

As shown in Fig 2, the heavy metal content in the underground part of the sampled plants from sites 1 and 2 revealed the presence of three metals whose contents are distributed in increasing order: [Zn]>[Cu]>[Cr] and thus, plants (*Phaseolus vulgaris* L.) concentrate zinc twice as much higher as copper and chromium.

The plant roots of the different study sites present only Zinc, Copper and Chromium, which are absent in the site3. In addition, Zn reaches the tissues of the stems and leaves, as well as the fruits where the highest content was noted in site 2 with a threshold of 49.4ppm followed by that of site 1 (36.4 ppm) then site 3 (34.6 ppm). Meanwhile, Copper was detected in the stems of the plants of sites 1 and 2 and the leaves of site 2 with concentrations lower than those of Zinc which hence, is absent in the fruits of the three study sites (1, 2 and 3). No significant difference was

Table 1: Heavy metal rates detected in the studied soils.

	Site 1	Site 2	Site 3
As	9,2	11,0	4,1
Cd		**< LOD**	
Cr	42,1	62,8	91,3
Mn	448,3	331,0	213,2
Cu	26,1	25,0	23,6
Ni	36,1	46,7	49,9
Hg	2,2	2,4	2,5
Zn	51,2	171,0	65,0
Pb	34,8	47,7	12,3
Se	< LOD	1,1	< LOD

Note: <LOD=Lower than the detection limit (<à 1 ppm).

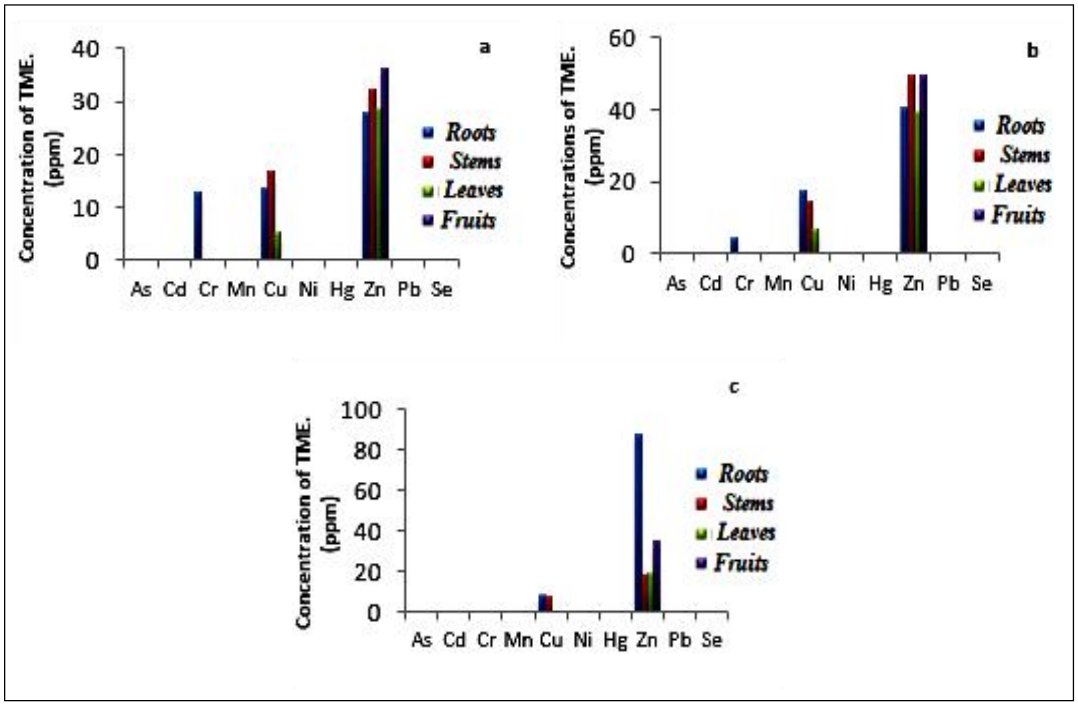


Fig 2: Average levels (ppm) of heavy metals in the leaves, stems, roots and fruits of *Phaseolus vulgaris* L. from the three studied sites: Site 1 (a), site 2 (b) and site 3 (c).

found between the concentrations of heavy metals accumulated in the different parts of the plants at the study sites (U=9, P>0.1).

The transfer of polluting elements, in particular, heavy metals causing mainly serious public health concerns has received great interest from several environmental researchers. Heavy metals contaminated soil and environment can be impacted by the long gone or operative industries and overall, the levels of heavy metals in the environment are closely linked to natural and anthropogenic factors. The anthropogenic processes are the principal sources of soil contamination by heavy metals, as well as the use of chemicals including fertilizers and pesticides, in addition to road traffic and surface runoff produced by atmospheric pollutants (Guessasma *et al*., 2020). Our results are similar to those obtained by Cuypers (2000) reporting high copper concentration in the plant roots known as the primary and the first copper target plant organ (Paschke and Redente, 2002). Further, the transfer of heavy metals from roots to shoots is of great concern to those interested in the phytoremediation process. Most of the absorbed Cu can be retained in the roots (Arduini *et al*., 1996) and thus the translocation from roots to aerial parts was suggested to be decreased (BES, 2008). Previous studies conducted on *Triticum durum* Desf., showed an accumulation of Cu and Cd in the plant biomass (Azizi, 2017). In addition, the differences in quantities exported by the plant organs could be explained by the quantities of dry biomass harvested from each organ. The Zn content in the leaves was found to be above the level of toxic concentration in the plant (Dudka *et al*., 1995).

The antioxidant enzymatic activities (CAT and APX) differed significantly in the three studied sites (Fig 3). The highest enzymatic activity of CAT and APX respectively, 0.386 µmol/min/mg of protein and 7.23×10^{-4} µmol/min/mg of protein was noticed in the plant of site 3, followed by those from site 1 (0.3 and 0.36 µmol/min/mg protein), but the lowest values were observed in site 2 (0.114×10^{-4} µmol/min/mg of CAT proteins and 2.54×10^{-4} µmol/min/mg of APX proteins).

As displayed in Table 2, the studied antioxidant markers (CAT and APX) statistically differed non-significantly between the plants sampled from the three study sites (P>0.001).

Table 3 illustrates that there is a reasonable correlation between the levels of CAT and APX.

Table 3 was explained by the stress that results in marked changes in the levels of the antioxidant enzymatic activities (CAT, APX,) (Khaldi *et al., 2019*). In this study, the obtained results are in agreement with those of Melo *andal.* (2011), reportingan increased activity of CAT in barium (Ba) exposed *Glycine max.* L plants, those of Azizi (2017) who reported inhibition of CAT activity in plant roots exposed to cadmium and Anca *et al.* (2006) reporting a stimulation of the synthesis of catalase to nitrates and nitrites in wheat. This could be explained by the strong involvement of this enzyme in the detoxification systems against chemicals-induced oxidative stress, as well as saline stress. Similarly, the catalase activity was decreased in *Brassica juncea* treated with 200, 300 and 500 µM of Ba (Bouslimi *et al*., 2021), while catalytic activity was increased in plants treated with cadmium (Bouchelaghem, 2012), a mixture of sodium chloride and silicone (Zhu *et al*., 2004) and uranium (Vandenhove *et al*., 2006). No antioxidant catalase activity was observed in cells exposed to against high concentration of metallic trace elements-induced ROS production (Rastgoo and Alemzadeh, 2011). Indeed, CAT and APX have complementary roles in the detoxification of

Table 2: *Kruskal Wallis* testing the effect of heavy metals detected in the 3 plots on the enzymatic parameters of *Phaseolus vulgaris* L. (values of *H* and P).

Parameters	CAT	APX
H	7.2000	5.6000
P	0.0273	0.0608

Table 3: Spearman correlation matrix of the different enzymatic parameters in *Phaseolus vulgaris* L.

	CAT	APX
CAT	1,000000	0,750000
APX	0,750000	1,000000

Note: The significant differences are indicated in red color.

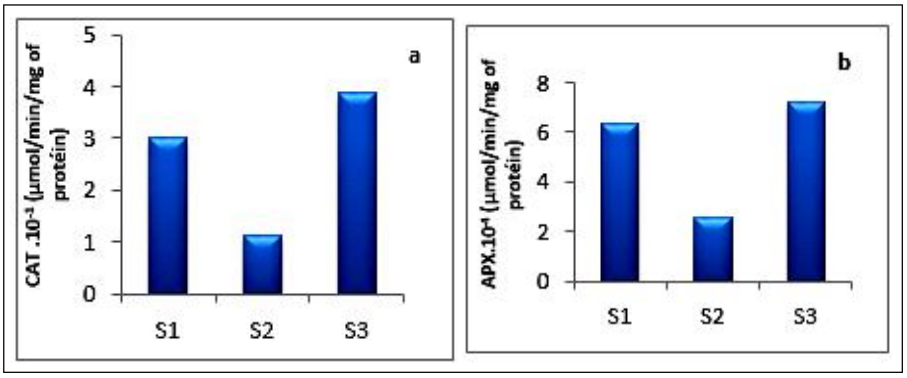


Fig 3: CAT (a) and APX (b) enzymatic activities (median) in *Phaseolus vulgaris* L. tissues.

hydrogen peroxide (Barata *et al*., 2005). APX was reported to reduce H_2O_2 to water using ascorbate as an electron donor from dehydroascorbate (Gill and Tuteja, 2010), resulting in the production of monodehy droascorbate MDHA radicals (Alayat *et al*., 2014) have found a significant increase in APX in *Lemna minor* L. exposed to an herbicide and this was explained by the fact that the expression of APX can protect the biological membranes by forming complexes with iron (II) and the polar head of the phospholipid molecule changing thus, the sensitivity of iron to auto-oxidation. Accordingly, the obtained results showed that the significant presence of metal trace elements MTE in *Phaseolus vulgaris* L. of the study sites caused a strong peroxidation activity resulting in an increase in the activities of APX and CAT.

CONCLUSION

The performed study revealed a positive correlation between the level of metallic trace elements (MTEs) in the soil and their accumulation in the different parts of the *Phaseolus vulgaris* L. plant. On the enzymatic level, the obtained results highlighted the oxidative toxic feature of the heavy metals in the analyzed soils, through the study of the antioxidant enzyme activities (CAT and APX). The study proved the efficient role of these antioxidant enzymes against metals-induced oxidative stress. This activity of these enzymes may represent a response of these plants to oxidative stress probably caused by the accumulation of MTEs at the cellular level.

Conflict of interest

I (we) affirm that there are no financial, personal, or professional conflicts of interest egarding the research presented in this article.

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