Research Article

Effect of Metal Dust in *Phaseolus vulgaris*: Physiological, Enzymatic and Respiration Parameter

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ABSTRACT

Since the industrial revolution, metallurgical industries have been a major source of heavy metals input into the environment. Indeed, beside emissions of fumes and dust contaminating surrounding areas, the production of steels and alloys is generating large amounts of foundry wastes containing high levels of toxic metals. Physiological experiments on plant roots exposed to metal dust were conducted on common bean "Phaseolus vulgaris"; using a metal contaminated dust medium (a single treatment by different increasing doses), at a rate of 10 seeds per box, for 10 days. The measurement of germination parameters is performed during the first 96 hours that follow the germination; Activities of three enzymes (catalase, ascorbate peroxidase, gaïacol peroxidase), the concentration of malonaldehyde in the roots of the young plant were investigated and the study of respiratory metabolism. The result showed that the germination speed and rate of this plants were decreased at the concentration of 1g of metal dust, exposition of this concentration procure activation of defense system enzymatic and non-enzymatic biomarkers. And perturbation in consumption of O2 is detected. This reaction of the plant to the presence of metal dust determine the toxicity of this xenobiotic and all results obtain confirm this.

Keywords: Metal dust, *Phaseolus vulgaris*, Germination, Toxicity, Antioxidant system, Respiration.

INTRODUCTION

Agricultural human activities, urban and industrial, ever-increasing, are the major source of contamination of the environment with heavy metals[1,2]. While many organic molecules may be degraded, heavy metals cannot, and the concentration increased steadily in the soil and surface water and groundwater[3,4]. The retention of high concentrations of heavy metals in the environment exerts toxic effects on fauna and flora[5]. This exposes the plants to increasing concentrations of heavy metals, and the latter presents a toxic hazard for humans because crops are the point of entry into the food chain[4].

In plants, some heavy metals are not essential such as As, Cd, Hg, Pb or Se, since they do not perform any known physiological function in plants; but others are essential to major physiological processes, particularly germination, respiration, photosynthesis, or assimilation of macronutrients, such as Co, Cu, Fe, Mn, Mo, Ni, and Zn[4-8].

Some of these metals are also involved in the molecular processes such as control of gene expression, protein biosynthesis, nucleic acids, growth substances, chlorophyll and secondary metabolites, lipid metabolism or tolerance stress, and control[9].

In addition, heavy metals may be in different oxidation states ($Cu^{2+} + e^- \rightarrow Cu^+$). They play an acceptor or electron donor, a very important role in multiple enzyme systems involving redox reactions[10]. However, heavy metals do not all have a function known to date in the metabolism of the plant and some are considered toxic elements (Hg, Cr, Ni, Pb, and Cd)[7,11-14], and all heavy metals may, from a threshold concentration, induce toxicity in plants[4,11,12].

Plants exhibit a series of physiologic, and nutritional disorders, when submitted to environments contaminated by heavy metals related to absorption, translocation, and function of nutrients[15-17]; which affect the normal growing and development of plants[18,4]. Even though seed germination represents an initial and crucial phase in the life cycle of angiosperms, virtually no information is available on the impact of this heavy metal on the metabolism of germinating seeds. Seed germination is a highly complex process during which, on imbibition under favorable conditions, an inert quiescent seed is transformed into a vigorously metabolizing system. This transition entails development of various biochemical capabilities in a program, finely controlled and coordinated manner[18,19].

Bioaccumulation of toxic heavy metals by various crop plants has been reported by a number of workers and is a matter of serious health hazard[12,14,20,21].

Heavy metal stress in all living organisms often results in the production of reactive oxygen species (ROS), which are relativity reactive compared to molecular oxygen and thus potentially toxic[14,22,23]. A regular balance between oxygen radical production and destruction is achieved by the plant anti-oxidative system that includes enzymatic and non-enzymatic molecules such ascorbate, MDA….. Catalase, APX, GPX… etc.[6,24].

The antioxidant properties of plants exposed to various stress factors have been studied earlier[12,14,25,26], plants have evolved antioxidant pathway that is usually sufficient to protect them from oxidative damage during periods of normal growth and moderate stress[27]. When severely stressed, however, the production of reactive oxygen species (ROS) can exceed the capacity of the antioxidant system to neutralize them and oxidative damage can occur. Heavy metals are known to induce free radical formation[28].

Another effect of heavy metals is observed, the disturbance of respiration activities, and it studies by many reasercher[12,29-31].

The main objective of present study is to assess the damage observed at levels of the roots of beans following their exposure to metal dust released from stacks of Iron and Steel Complex located in the east of Algeria; and the possibility of using this plant common bean (*Phaseolus vulgaris*) as a model of bio-monitoring and evaluation of the toxicity of metal elements.

MATERIALS AND METHODS

Chemical material

The chemical used is metal dust generated by the steel complex (northeast of Algeria). Dust is collected stacks of AC1 and AC2 steel complex and is passed through a sieve to recover the fine particles to facilitate their absorption by the roots.

Experimental design

The experiments are realized at the Laboratory of Cellular Toxicology and pedagogical Laboratories of Department of Biology, Annaba, University, Algeria.

The biological model used in this work is a vascular plant, common bean (*Phaseolus vulgaris L.);* this plant has been extensively studied by many scientific views that it has several Interests, economic[32], nutritional[33], medicinal[34],... environmental[35] (In this test we have chosen to work on the roots).

The bean seeds are meticulously chosen before use (No cracks or obvious signs of disease and of the same size). Then they are disinfected with sodium hypochlorite for a few seconds, and then rinsed thoroughly with distilled water, and placed in boxes of languor 7cm, 4cm wide and 3.5 cm in height placed on cotton (10 seeds per box).

A single treatment is carried out for each test with different doses of metal dust at the start of the experiment (for each concentration of metal dust, three repetitions are performed for each treatment).

Germination is conducted in the light and medium temperature of 25 ± 1 °C [36]. The duration of the experiment was 10 days (two leaf stage), watering is performed with distilled water only.

Table 1: Treatment distribution of different doses

Treatment	Metal dust doses
T	Untreated
C1	Beans treated with 0.50g of metal dust.
C2	Beans treated with 1.00g of metal dust.
C3	Beans treated with 1.50g of metal dust.
C4	Beans treated with 2.00g of metal dust.

(**T**: control, **C1**: dose 1, **C2**: dose 2, **C3**: dose 3, **C4** : dose 4).

Composition of dust metal

Laboratory analysis of metallic elements requires preparation phase of the sample (crushing, sieving), an extraction phase of the solid matrix, and finally a phase of the assay of the contaminant itself.

A dosage of this dust is carried out to identify their composition of trace metals by the technique of "X-ray fluorescence spectrometry," the unit used is fluorescence spectrometer X type Thermo Fisher Scientific model FXL-950.

Germination parameters

Germination is a process whose boundaries are the beginning of the hydration of the seed, and the beginning of the growth of the radical[37].

Speed germination

The measurement of speed germination was carried out after 48h, 72h, and 96h in the presence or not of metal dust [38].

Rate of germination

The germination medium rate represents the average percentage of bean seeds germinated relative to the total number of seeds per box of each treatment condition. The seeds are considered to have germinated when their roots reach 2mm in length[38].

Determination of Lipid Peroxidation

Lipid peroxidation was estimated by the changing the content of malondialdehyde (MDA) determined according to the method described by Alia and *al.*(1995)[39]. Homogenizing the plant tissue in trichloroacetic acid (TCA) 5% in an amount of 5 ml to 0.5 g fresh tissue and followed by a 15 min centrifugation at 12000 g. The supernatant was added an equal volume of thiobarbituric acid (TBA) 0.5% in the TCA 20%.

The mixture is incubated at 100 °C for 25 min. Then read at 532nm and the value for non-specific absorption at 600 nm was subtracted.

MDA content was calculated using an extinction coefficient 155$mM^{-1}.cm^{-1}$ and expressed as nmol g^{-1} FW.

Antioxidant enzyme activity determinations

The measure of CAT, APX and GPX is conducted with the method of Loggini and *al.* (1999)[40]. The roots of bean were collected for enzyme analysis. Fresh samples (1.0 g) were homogenized in ice-cold 50mM phosphate buffer (pH 7.5). The homogenate was centrifuged at 12 000 g for 20 min at 4°C, and the supernatants were used for the various enzymatic assays.

Catalase (CAT) activity was determined according to Cakmak and Horst (1991)[41]. The reaction mixture for catalase in a total volume of 3mL contained 2850µl Na-phosphate buffer (50mM, pH 7.2), 50µl H_2O_2 (300mM) and 100 µl of crude enzyme extract. The reaction was started by adding enzyme extract and the activity was determined by monitoring the initial rate of H_2O_2 disappearance at 240 nm in 1mn (**ε=** 39400 M^1cm^1).

Ascorbate peroxidase (APX) activity was according by method of Nakano and Assada (1987)[42] determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min in 3 ml of a reaction mixture containing 50 mM potassium phosphate buffer pH 7.2, 0.5 mM ascorbic acid, 50µl H_2O_2 and 100 µl of crude enzyme extract (**ε=** 2800$M^{-1}.cm^{-1}$).

Guaiacol Peroxidase (GPX) activity was measured according to the method of Fielding and *al*. (1978)[43] .The reaction mixture (3 mL) consisted of100µL enzyme extract, 2850µL of potassium phosphate-gaïacol buffer (50mM NaK, 8mM of gaïacol, pH 7.2) and 50 µL H_2O_2 (0.03%). Any increase in the absorbance due to oxidation of guaiacol was measured spectrophotometrically at 470nm in 1min, with a coefficient of molar extinction linear**ε** = 2470$M^{-1}.cm^{-1}$

Study of the respiratory metabolism

This analysis is performed using an oxygen electrode, of Hansatech-type, which enables measurement of the production or consumption of oxygen[29].

Statistical analysis

Data were calculated as Mean ± SD and analyzed using Student's test followed by the analysis of variance (ANOVA) in one way of classification. The probability of 0.05 or less was considered significant. All statistical analysis was done according to the software MINITAB of analysis and data processing version 17 Ink[44].

RESULTS

Heavy metal concentrations in metal dust

The detection limit is between 1 and 10 µg / g according to the metallic elements.

According to the results obtained in table 1, concerning the content of elements specifically heavy metal elements, the most significant values are those of Fe, Mn, and Mg; in parallel calcium, as a mineral salt is present with a value is half that of iron.

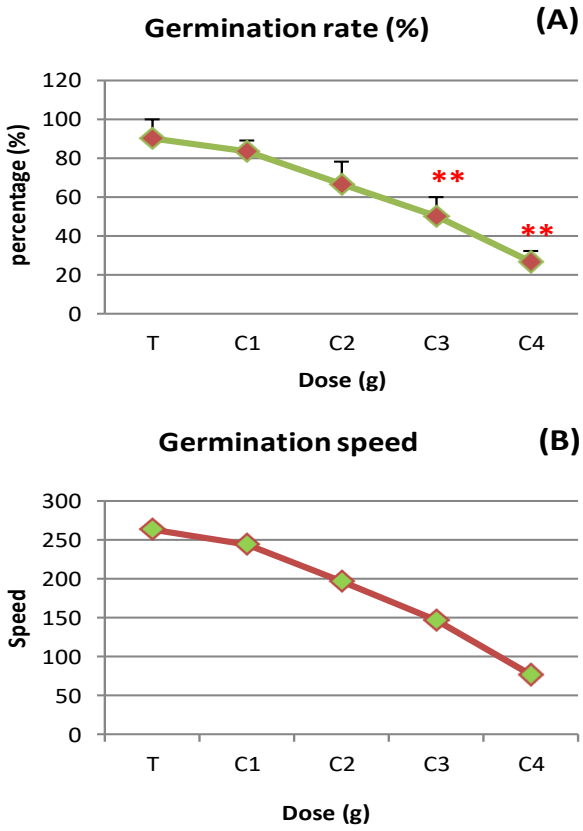

Figure 1: germination parameters of bean seeds exposure to 0, 0.5, 1, 1.5 and 2 g of metal dust. **(A)**Germination rate, **(B)** Germination speed. Results are expressed as mean ± standard deviations (SD).

* : Significant difference compared to the control (P ≤0,05).
** : Highly significant difference compared to the control (P ≤0,01).
***: Very highly significant difference compared to the control (P ≤0,001).

Table 1: Heavy metal concentrations in the metal dust (Rb, Sn, Cd, Pd, Ag, Co, W, Au, Se, Hg, Ga, Mo, Nb, Th, Y, U, Bi < limit of detection)**.**

Content	Fe	Ca	Si	Mn	Sb	Sr	Zr	As	Pb	Zn	Cu	Ni	Cr	V	Ti	K	Ba	Al	P	Cl	S	Mg
in %	31.032	15.054	2.426	1.705	0.002	0.023	0.003	0.006	0,113	0,063	0,002	0,045	0,016	0,008	0,109	0,095	0,118	0,287	0,22	0,128	0,415	0,403
in µg/g of M.D.	310320	150540	24260	17050	20	230	30	60	1130	630	20	450	160	80	1090	950	1180	2870	2200	1280	4150	4030

Germination parameters

The germination rate was affected by 1.5 g of metal dust, as compared with control, it was lowered about 50%. And the effect was distinctly more pronounced with 2g with ≈ 26% (figure 1).

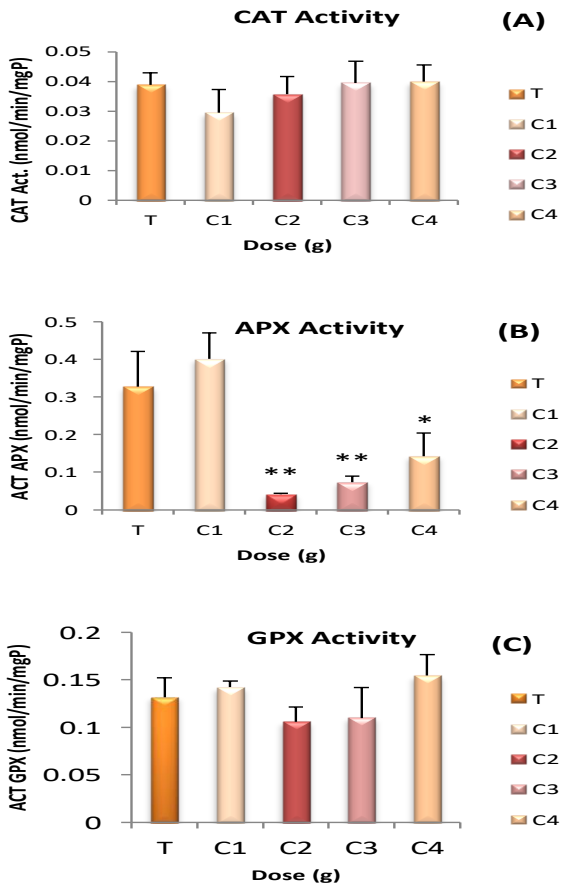

Figure 2: enzymatic parameters of bean seeds exposure to 0, 0.5, 1, 1.5 and 2 g of metal dust. **(A)**CAT activity, **(B)** APX activity and, **(C)** GPX activity. Results are expressed as mean ± standard deviations.

Figure 2, shows the effect of metal dust on the antioxidant activity of three enzymes (CAT, APX, and GPX) in the roots of *Phaseolus vulgaris*.

Our results show that catalase, no significant difference between the treated and the control group, only a slight decrease was observed for the dose of 0.5g metal dust.

For the APX, we see a highly significant decrease for 1g, and 1.5g doses from 0.32 nmol/mg protein for the control to 0.041 and 0.073 nmol/mg protein, respectively, and a significant decrease in the dose of 2g metal dust compared the control.

Finally, as regards the GPX a very slight increase in the 0.5g and 2g dose, followed by a non-significant decrease for both 1g, and 1.5g doses of metallic dust comparing to the control.

Figure 3, illustrates the variations of the MDA levels, in the roots of young seedlings of common bean, due to their exposure to metal dust, a significant increase in the production of MDA in the treated groups, compared to control groups was recorded. Indeed, the dose 2 g marks a very highly significant increase, in the synthesis of MDA from 2.018 µmol/mg protein for the witness to 23.271 µmol.

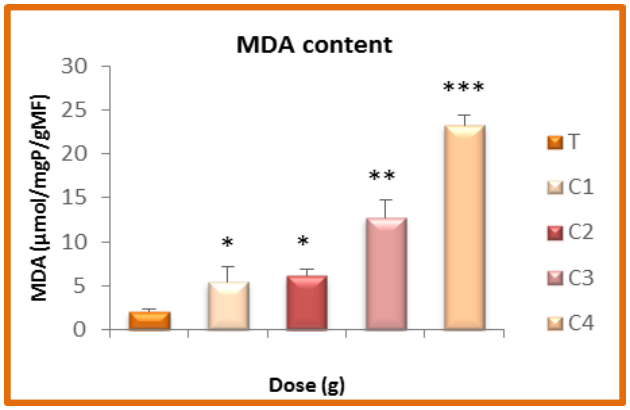

Figure 3: MDA content of roots bean seeds exposure to 0, 0.5, 1, 1.5 and 2 g of metal dust. Results are expressed as mean ± standard deviations (SD)

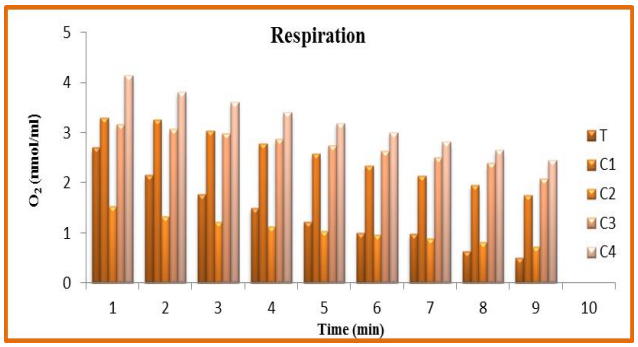

Figure 4: Respiration of roots bean seeds exposure to 0, 0.5, 1, 1.5 and 2 g of metal dust.

Figure 4, illustrates the respiration in the roots of the treated and control group's lots. The consumption of oxygen is rather regular, and continuing to the witness, unlike treaties in which it is strongly inhibited at all doses.

DISCUSSION

Heavy metal pollution is one of the important topics in plant stress physiology[45], more toxicity that presents a threat to human health via the food chain [46,47,48].

Some of the metals are essential and necessary for the proper metabolism of plants to ensure good growth.[49]

Depending on the needs of a plant can be distinguished the following different types of nutrients: the side (As, Cd, Hg), the major elements (N, P, K), the secondary elements (Ca, Mg, S) and finally the minor elements (Fe, Cu, Mn, Zn, Pb, Si, ...), the latter at low concentrations contribute enormously to different physiological and metabolic functions organisme[50]; others such as silicon (Si) has not been recognized as an essential element for plant growth[51], but several Experiments have shown its contribution the improvement of yield[52], we assume an active role in plant defense by activating or speeding up

pathogen defense responses[53]. Silicon alleviated Cu toxicity symptoms in *Arabidopsis thaliana*[54], thus extenuating the effect of chromium and cadmium that observed in three grass species (*Durum wheat, Soft wheat*, and Barley)[55], and the beneficial effects of Si have been observed in a wide variety of plant species, expressed more clearly in Si-accumulating plants under various abiotic and biotic stress conditions[51].

Even though seed through seed germination represents an initial and crucial phase in the life cycle of angiosperms, virtually no information is available on the real impact of heavy metal on the metabolism of germinating seed[19]. But Germination ability of seed is a useful parameter for the decision of tolerance level as it is the first interface for material exchange between plant development cycle and his environment[56,57]. Some previous studies have proved that higher levels of essential or non-essential heavy metals imposed an adverse effect on seed germination resulted in retarded plant growth[58,59,60]; This is in perfect agreement with our results, where the germination rate of bean seed, is significantly lowered in the presence of metallic dust. This reduction is observed in germination percent of French bean, treated by different concentration of lead[61]; in other tests on three cultivars of bean (Atendaba, Argene, and Nassier) treated by different concentration of copper sulfate, the germination percentage revealed no significant difference between cultivars, and between concentrations used in this study, and demonstrating a good tolerance for this metal[47], while higher concentrations have germination inhibitory effect[62], which confirms the results of Soughir and *al.*, when the exposure of two Fabaceae: *Vicia faba* and *Pisum sativum* to higher concentration of copper-induced genotoxicity effect[63]; It was also observed that the seed germination of Sunflower (*Helianthus annuus*) is noticeably reduced by Cd toxicity[58]. We can explain this decrease that interference of metal dust with metabolic processes, which loss of viability decrease of energy generation for on embryo. Energy generation is very important for seed germination and its blockage affects protein, nucleic acids production, as well as mitosis[64].

Enzyme biomarkers appear to be good tools to detect tolerance or not living organisms vis-a-vis abiotic limiting factors or pollutants that cause them all sorts of nuisances; and the proper functioning of the enzyme system shows a good tolerance of the individual in question, contrary the converse situation[65].

unlike the results presented in the works of Grara et *al*., the effect of this metallic dust used in our experiments on *Helix aspersa* show a remarkable decrease in the catalase activity at the hepatopancreas and kidneys, with a dose-dependent effect[66]; in our results previously presented in Figure 2 it shows the good operation of the activity of catalase and that of GPX, and there is no significant difference with the control, This is concord with the results obtained by Khaldi F. and *al.* (2013)[26], having said that the activity of APX shows a significant decrease from the 1g dose of metallic dust in the middle of culture. In the presence of abiotic stress, plant cell uses different mechanisms including antioxidant enzymes like CAT (catalase), and peroxidases (POD: APX, GPX), and the good function of latter is a synonym of a good tolerance of heavy metal in organism[67].

In plants catalase is mainly localized level has peroxisome and mitochondria[68], while peroxidases are present everywhere in the cell level: They have secreted an enzyme, produced by genes that encode also a signal peptide which mediates the entry of the nascent peroxidase peptide into the endoplasmic reticulum. Thus, they were found in the Golgi apparatus, the endoplasmic reticulum, and transport vesicles[69], was detected in the mitochondria, nuclei, and plasma membrane[70]; also are mainly found in apoplast and vacuoles[71,72]. But the vacuole is a principal compartment of accumulation of heavy metal in plant[73], and the higher concentration of this latter affect the good function of peroxidase[74] such as activity of APX when it decreases in our essay; peroxidases are involved in numerous cellular processes such as stress responses, and development (germination and senescence)[75]; which explains in our case the decrease in germination rate following the addition of metallic dust.

Estimation of lipid peroxidation analysis based malondialdehyde (MDA) which is a marker of oxidative lipid injury which changes in response to environmental factors lead to stress in plants[76]. The level of MDA content has been considered as in indicator of oxidative stress[77,78]. Our results concern the levels of MDA are increase with addition of metal dust, and is in perfect accord with the work of Zengin F. (2013) when the young plants of 7 days of *Phaseolus vulgaris* were exposed to various concentrations of nickel ($NiCl_2.6H_2O$), cobalt ($CoCl_2.6H_2O$), chromium ($CrCl_3.6H_2O$), and zinc ($ZnCl_2.6H_2O$) separately[77]. Other research on the same plant model and other plants support this increase of MDA content, following the addition of different heavy metals, such as lead, cadmium, iron, copper, etc.[78-81].

To confirm again the state of stress in the Bean a respiratory metabolism study was done on the roots of this plant, the results show an almost complete inhibition of respiration in treated plants by 2g of metal dust, and a disturbance to other treatment versus control. This anomaly was observed in other work and in several biological models exposed to abiotic stress such as heavy metals [82].

Its resorts in this results about the effect of metal dust on the bean (*Phaseolus vulgaris*), even if these metal dust used in our essay are composed of the majority of essentials element for the development and growth of plants such as iron, copper, magnesium, manganese [50] etc., their presence in great quantities the make toxic[83].

the testing of our plant model common bean confirms via the decrease of the rate and speed of germination, of APX activity, and of the respiratory metabolism, and the increase in the levels of MDA, the extreme toxicity of this metal dust and installation of oxidative stress as a result release of ROS, but other factors such as the presence of calcium[84], phosphorus[85], and the silicon[86,87]in the same metal cocktail attenuated this negative effect and it was observed at the level of CAT activity as GPX activity; other factors such as polyphenols[88] and vitamin A, C, and E[77] helps also to acquire him tolerance and power to accumulate heavy metals and have strong antioxidant potential.

CONCLUSION

In the light of this experiment, it can be concluded that:

This explosive cocktail that represents these metal dust is a source of disturbance for different eukaryotic organisms including humans. Our results regarding *Phaseolus vulgaris* show firstly an increase in MDA levels, alongside a decrease in speed and germination rates, and a decline of APX activity and dose-dependent inhibition of the respiratory metabolism that confirms the toxicity of this xenobiotic.

Besides using the *Phaseolus vulgaris* plant seems interesting, because it can be listed as a biological model to serve the bio-monitoring of contaminated habitats program.

On the other hand, the steel complex where dust was collected is near areas of agriculture where we cultivate all kinds of plants for consumption including beans, which may present a risk of direct contamination to humans and grazing animals.

REFERENCES

1. Alloway BJ, Soil processes and the behavior of heavy metals: Heavy metals in soils, Alloway B.J. (Ed.), Chapman & Hall, London, 1995, pp 11-35.
2. Esteban R, Tolérance et accumulation des métaux lourds par la végétation spontanée des friches métallurgiques : vers de nouvelles méthodes de bio-dépollution. Thesis, Vegetal Biology, Jean Monnet-Saint-Etienne University, French, 2006, 166p.
3. He ZL, Yang XE, Stoffella PJ, Trace elements in agroecosystems and impacts on the environment, J Trace Elem Med Biol, 19, 2005, 125-140.
4. Zorrig W. Recherche et caractérisation de déterminants contrôlant l'accumulation de cadmium chez la laitue « Lactuca sativa », Physiologie végétale et biologie moléculaire, Thesis, el Manar University, Tunis, and Montpellier II University, french, 2013, 276p.
5. Mishra VK, Tripathi BD, Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes, Bioresour Technol 99, 2008, 7091-7097.
6. Zengin F, Biochemical and Physiological Effect of Excess Manganese (Mn) in bean (*Phaseolus vulgaris* L. cv. Strike), Proc. Natl. Acad. Sci., 83(4), 2013, 651-657.
7. kabata Pendias A, Pandias H, Trace elements in soils and plants, Boca Raton, FL: CRC press, 2001.
8. Chungh LK, Sawhney S K, Effect of Cadmium on germination, amylases and rate of respiration of germinating Pea Seeds, Environmental Pollution, 92(1), 1995, 1-5.
9. Rengel Z, Heavy Metals as Essential Nutrients. In: Prassad, M.N.V. & Hagemayer, J. (Ed.), Heavy metal stress in plants: From molecules to ecosystems. Springer-Verlag, Berlin, 1999, pp 231-251.
10. Chaignon V, Biodisponibilité du cuivre dans la rhizosphère de différentes plantes cultivées. Cas de sols viticoles contaminés par des fongicides. Thesis : Ecole doctorale, Sciences de l'Environnement : Système Terre, Aix-Marseille University, 2001.
11. Gjorgieva D, Panovska TK, Mitrev S, Kovacevik B, Kostadinovska E, Baceva K, Stafilov T, Assessment of the genotoxicity of heavy metals in *Phaseolus vulgaris* L. as a model plant system by Rando amplified polymorphic DNA (RAPD) analysis, Journal of Environmental Science and Health, part A, 47, 2012, 366-373.
12. Sbartai H, Djebar M R, Rouabhi R, Berrebbah H, Antioxidative response in tomato plants *Lycopersicon esculentum* L, Roots and leaves to zinc, Am-Eur J Toxicol Sci, 3, 2011, 41-46.
13. Gjorgieva D, Panovska TK, Ruskovska T, Bačeva K, Stafilov T, Mineral nutrient imbalance, total antioxidants level and DNA damage in common bean (*Phaseolus vulgaris* L.) exposed to heavy metals, Physiol. Mol. Biol. Plants, 19(4), 2013, 499-507.
14. Alayet A, Souiki L, Grara N, Djebar MR, Boumerdis ZE, Benosmane S, Amamra R, Berrebbah H, Effect of cadmium on water content, soluble protein, proline changes and some antioxidant enzymes in wheat (*Triticum durum* desf.) leaves, Annual Research & Review in Biology, 4(24), 2014, 3835-3847.
15. Mengel K, Kirkby EA, Principles of plant nutrition. Bern, Switzerland: International Potash Institue, 1987.
16. Soares CRFS, Toxidez de zinco, cobre, cadmino e chumbo para o eucalipto em solução nutritiva. M Sc, Thesis, Universidade Federal de Lavras, Lavras, 1999.
17. Paiva HN, Influênca de doses crescentes de chumbo sobre o teor e o conteúdo de nutrientes e Pb em mudas de Ipê-roxo (*Tabebuia impetiginosa* (Mart.) Standl.) Revista Arvore, 27, 2003, 151-158.
18. Cannata MG, Carvalho R, Bertoli AC, Bastos ARR, Carvalho JG, Freitas MP, and Augusto AS, Effect of lead on the content, accumulation, and translocation of nutrients in bean plant cultivated in nutritive solution, Soil Science and Plant Analysis, 44(5), 2013, 939-951.
19. Mayer, poljakoff- mayber A, The germination of seeds, Fourth edition, Pergamon Press, London, 1989.
20. Mishra SN, Singh DB, Accumulation of lead and cadmium in hupper parts of mustard (*Brassica juncea*) seedlings in response to putrescine, J. Expt. Biol., 38, 2000, 814-818.
21. Bahardwaj P, Chaturvedi A K, Prasard P, Effect of inhanced lead and cadmium in soil on physiological and biochemical attributes of *phaseolus vulgaris* L., Nature and Science, 7(8), 2009, 63-75.
22. Cargnelutti D, Tabaldi LA, Spanevello RM, Jucoski GO, Battisti V, Redin M, Linares CEB, Dressler VL, Flores EMM, Nicoloso FT, Morsch VM, Schetinger MRC, Mercury toxicity induce oxidative stress in growing cucumber seedlings, Chemosphere, 65, 2006, 999-1006.
23. Van Assche F, Clijsters H, Effect of metals on enzyme activity in plants, Plant Cell Environ, 13, 1990, 95-206.
24. Prochazkova D, Sairam RK, Srivastava GC, Singh DV, Oxidative stress and antioxidant activity as the basis of senescence in maize leaves, Plant Sci., 161, 2001, 765–771.
25. Belhacene N, Mouaissia W, Zenati N, Djebar MR, Etude de l'effet d'un stress oxydatif causé par un herbicide systématique cossak

sur le blé dur (*Triticum durum* desf.), International Journal of Innovation and Scientific Research, 14, 2015, 104-111.

26. khaldi F, Berrebah H, Djebar MR, Induction de biomarqueurs du stress oxydatif associé au stress de NPKs chez *Leucodon sciuroides* (Hedw.) Schägr., Bulletin de l'Institut Scientifique, Section Science de la Vie, 35, 2013, 67-74.

27. Hauptmann N, Cadenas E, The oxygen paradox: biochemestry of active oxygen.Sandalios JG (Ed), Oxidative stress and molecular biology of antioxydant defence. Cold Spring Harbor Laboratory Pess, Cold Harbor, NY, 1997, 1-20.

28. Aust SD, Morhouse LA, Thomas CE, Role of metals in oxygen radical reactions, J. Free Radic Biol Med, 1, 1985, 3-25.

29. Djebar MR, Djebar H, Bioénergétique, Les mitochondries végétales. Synthèse. Publications of Badji Mokhtar University Annaba, 2000, 103p.

30. Marc D, Caractérisation de la colonisation spontanée par les plantes de sols pollués Rôle des systèmes cellulaires de détoxication, Thesis, Environmental toxicology, METZ–UFR Sci.F.A. University, 2008, 295p.

31. Prasad MNV, Heavy metal stress in plants: from biomolecules to ecosystems. Springer-Verlag, Berlin-Heidelberg, 1999, 462 p.

32. Sarikamis G, Yasar F, Bakir M, Kasan K,Ergül A, Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey, Genetics, and Molecular Research, 8(3), 2009, 880-887.

33. Berber I, Yaşar F, Characterization of bean (*Phaseolus vulgaris* L.) cultivars grown in Turkey by SDS-page of seed proteins, Pak. J. Bot., 43(2), 2011, 1085-1090.

34. Reynonoso-Camacho R, Ramoz-Gomez M, Loarca-Pina G, Bioactive components in common beans (*Phaseolus Vulgaris* L.), Research Signpost, 37, 2006, 217-236.

35. Fatma Lazrek-ben friha, Analyse de la diversité génétique et symbiotique des populations naturelles tunisiennes de *Medicago truncatula* et recherche de QTL liés au stress salin, Thesis, Biology, Toulouse III - Paul Sabatier University, 2008, 255p.

36. Salcedo, J.M., Directives pour la régénération: haricot commun. In: Dulloo ME, Thormann I, Jorge MA, Hanson J, editors. Crop specific regeneration guidelines [CDROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy, 2008, 10 p.

37. Nabors M, Biologie végétale: Structure, fonctionnement, écologie et biotechnologies, Nouveaux horizons Ed., Pearson Education France, Paris, 2009, pp 132-133.

38. Mazliak P, Physiologie Végétale II : Croissance et Développement, Méthodes Ed., 1998, pp 185-313.

39. Alia Prasard KVSK, Pardha Saradhi P, Effect of zinc on free radical and proline in *Brassica juncea* and *Cajanus cajan*. Phytochemistry, 39, 1995, 45-47.

40. Loggini B, Scartazza A, Burgnoli E, Navari-Izzo F, Antioxidative defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiology, 119, 1999, 1091-110.

41. Cakmak I, Horst JH, Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max), Physiologia Plantarum, 83, 1991, 463-468.

42. Nakano Y, Asada K, Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical, Plant Cell Physiol., 28, 1987, 131-140.

43. Fielding CJ, Metabolism of cholesterol-rich chylomicrons. Mechanism of binding and uptake of cholesteryl esters by the vascular bed of the perfused rat heart. J Clin Invest., 62, 1978, 141–151.

44. Dagnelie P, Statistiques théoriques et appliquées, T2, références statistiques à une et à deux dimensions. Bruxelles, (Ed.) Univ. De Boeck et Larcier, 659p.

45. Xing W, Huang W, Liu G, Effect of excess iron and copper on physiology of aquatic plant Spirodela polyrrhiza (L.)Schleid, Environmental Toxicology, 25, 2010, 103-112.

46. Kováčik J, Bačkor M., Kaduková J, Physiological responses of *Matricaria chamomilla* to cadmium and copper excess, Environmental Toxicology, 23, 2008, 123–147.

47. Ashagre H, Shelema M, Kedir R, Ebsa Simegn, Seed germination and seedling growth of haricot bean (*Phaseolus vulgaris* L.) cultivars as influenced by copper sulphate, World Journal of Agricultural Sciences, 1(10), 2013, 312-317.

48. Wagner G, Accumulation of cadmium in crop plants and its consequences to human health, Advances in Agronomy, 51, 1993, 173-212.

49. Cannata MG, Carvalho R, Bertoli AC, Augusto AS, Bastos ARR, Carvalho JG, Freitas MP, Effects of cadmium and lead on plant growth and content of heavy metals in Argula cultivated in nutritive solution, Communication in Soil Science and Plant Analysis, 44:5, 2013, 952-961.

50. Esteves B, L'importance des éléments mineurs : des carences à la toxicité. Une préoccupation en agriculture biologique? Agriculture, pêcherie et Alimentation Québec, 2006, pp 2.

51. Jian FM, Role of Silicon in Enhancing the Resistance of Plants to Biotic and Abiotic Stresses, Soil Sci. Plant Nutr., 50 (1), 2004, 11-18.

52. Conner SD, Liming in its relation to injurious inorganic compounds in the soil. J. Amer. Soc. Agron., 13(3), 1921, 113-124.

53. Pozza EA, Alexandre Pozza AA, Dos Santos Botelho DM, Rev. Ceres, Viçosa, 62, 2015, 323-331.

54. Frantz JM, Leisner S, Silicon mediates copper toxicity in *Arabidopsis thaliana* and *Zinnia elegans*, Indo-US Workshop on Silicon in Agriculture, Bangalore, India, 25–27 February 2010.

55. Alayat A, Etude de l'impact toxicologique de certains agents chimiques sur la qualité des céréales (cas du Blé et de l'orge), Thesis, Cell. Toxicology, Université Badji Mokhtar Annaba, Algeria, 2015, 266p.

56. Wang H, Zhong G, Shi G, Pan F, Toxicity of Cu, Pb, and Zn on seed germination and young seedlings of wheat (*Triticum aestivum L.*), IFIP Advances in Information and Communication Technology, 346, 2011, 231–240.

57. Rahoui S, Chaoui A, El Ferjani E, Reserve mobilization disorder in germinating seeds of *Vicia faba* L. exposed to cadmium, J. Plant Nutr., 33, 2010, 809-817.

58. Sadiq R, Hussain M, Ashraf M, Rehman KU, Exogenous application of Ethylenediaminetetraacetic acid and Oxalic acid improve the seed germination and enzymes activities of sunflower (*Helianthus annuus*) under cadmium stress, Int. J. Agric. Biol., 17, 2015, 1117–1124.

59. Pandey SK, Germination and Seedling growth of Field Pea *Pisum sativum* Malviya Matar-15(HUDP-15) and Pusa Prabhat (DDR-23) under varying level of Copper and Chromium, The Journal of American Science, 4(1), 2008, 33-47.

60. Fargašová A, Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young seedlings of *Sinapis alba* L., PLANT SOIL ENVIRON., *50*, 2004 (1), 33–38.

61. Choudhury H, Goswami RK, Bora SP, Mahanta, Das J, Effect of lead on germination and early seedling growth of French Bean (*Phaseolus vulgaris* L.), Crop Res., 43, 2012, 98-101.

62. Hema C, Hubramani A, Phytotoxic effect of copper and chromium on seed germination percentage of *Vigna radiate* L., Int. J. Curr. Res. Rev., 5, 2013, 95-100.

63. Souguir DE, Ferjani GL, Gopupil P, Exposure of *Vicia faba* and *Pisum sativum* to copper-induced genotoxicity, Protoplasma, 233, 2008, 203-207.

64. Sedzik M, Smolik B, Krupa-Malkiewicz M, Effect of lead on germination and some morphological and physiological parameters of 10 days old seedling of various species, Environmental protection, and natural resources, 26, 2015, 22-27.

65. Mittler R, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci, 7, 2002, 405-410.

66. Grara N, Boucenna M, Atailia A, Berrebah H, Djebar MR, Stress oxydatif des poussières métalliques du complexe sidérurgique d'Annaba (Nord-Est algérien) chez l'escargot *Helix aspersa*, Environ Risque Sante, 11, 2012, 221-229.

67. Kabel AM, Free Radicals and Antioxidants: Role of Enzymes and Nutrition, World Journal of Nutrition and Health, 2(3), 2014, 35-38.

68. Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, VanMontagu M, Inze D, VanCamp W, Catalase is a sink for H_2O_2 and is indispensable for stress defense in C-3 plants, Embo Journal, 16, 1997, 4806-4816.

69. Mäder M, Compartmentation of peroxidase isoenzymes in plant cells. In: Penel C, Gaspar T, Greppin M (eds) Topic and detailed literature on molecular, biochemical and physiological aspects. University of Geneva, Geneva, 1992, pp 37-46.

70. Chibbar RN, Van Huystee RB, Immunochemical localization of peroxidase in cultured peanut cells. J plant Physiol, 123, 1986, 477–486.

71. Takabe K, Takeuchi T, Sato M, Ito M, Fujita M, Immunocytochemical localization of enzymes involved in lignification of the cell wall, J Plant Res, 114, 2001, 1021–1029.

72. Andrews J, Adams SR, Burton KS, Edmondson RN, Partial purification of tomato fruit peroxidase and its effect on the mechanical properties of tomato fruit skin, J Exp Bot, 53:379,2002, 2393–2399.

73. Manara A, Plants and Heavy Metals, Chapter 2: Plant Responses to Heavy Metal Toxicity, Furini A (ed.), Springer Briefs in Biometals, 2012, 86p.

74. Haji Housseini R, Khanlarian M, Ghorbanly M, Effect of lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L., J Biol Sci, 7(4), 2007, 592-598.

75. Jouili H, Bouazizi H, El Ferjani E, Plant peroxidases: biomarkers of metallic stress, Acta Physiol Plant, 33, 2011, 2075–2082.

76. Aras S, Soydam Aydin S, Aksoy Kope D, Donmez C, Comparative genotoxicity analysis of heavy metal contamination in higher plants, Ecotoxicology, Ed. Dr. Ghousia Begum, in Tech, 2012, 146p.

77. Zengin F, Physiological behavior of bean (*Phaseolus vulgaris* L.) seedlings under metal stress, Biological research, 46, 2013, 79-85.

78. Khaldi F, Grara N, Berrebbah H, Djebar MR, The oxidative stress and the antioxidant defense of the lower vegetables towards an environmental pollution, world academy of science, 7, 2013, 04-20.

79. Priti B, Ashish KC, Prasad P, Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L, Nature and science, 7(8), 2009, 63-75.

80. Xing W, Huang W, Liu G, Effect of excess iron and copper on physiology of aquatic plant Spirodela polyrrhiza (L) Schleid, Environmental toxicology, 25, 2010, 103-112.

81. Zhang FQ, Wang YS, Lou ZP, Dong JD, Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedling (*Kandelia candel* and *Bruguiera gymnorrhiza*), Chemosphere, 67, 2007, 44-50.

82. Sbartai H, Etude des effets du Cadmium sur la Tomate (Lycopersicon esculentum L.): essai in vivo et in vitro, thesis, Badji Mokhtar university, Annaba, 2008, pp 95-96.

83. Hall JL, cellular mechanisms for heavy metal detoxification and tolerance, J. Exp. Bot.,53, 2002, 1-11.

84. Singh RP, Maheshwari R, and Sinha SK, Recovery of lead caused decreased in biomass accumulation of mung bean (*Vigna radiata* L) seedlings of K2HPO4 and Cacl2, Indian J. Exp. Biology, 32, 1994, 507-510.

85. Grant CA, Bailey LD, Effect of phosphorus and zinc fertilizer management on cadmium accumulation in flaxseed, J Sci Food Agric, 73, 1997, 307-314.

86. Baylis AD, Gragopoulou C, Davidson KJ, Birchall. JD, Effects of silicon on the toxicity of aluminum to soybean. Commun. Soil Sci. Plant Anal. 25(5&6), 1994, 537–546.

87. Pavlovic J, Samardzic J, Maksimović V, Timotijevic G, Stevic N, Laursen KH, Hansen TH, Husted S, Schjoerring JK, Liang Y, Nikolic M, Silicon alleviates iron deficiency in cucumber by promoting mobilization of iron in the root apoplast, New Phytol., 198(4), 2013, 1096–1107.

88. Lattanzio V, Kroon PA, Quideau S, Treutter D, Recent advances in polyphenol research Volume 1, ed. Fouad Daayf and Vincenzo Lattanzio, 2008, 437p.

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