Studii şi Cercetări Ştiinţifice 2019, **20** (2), pp. 237 – 252
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

BIOCHEMICAL AND ENZYMATIC CHARACTERIZATION OF MACROPHYTE PLANT *PHRAGMITES AUSTRALIS* AFFECTED BY ZINC OXIDE

Fadila Khaldi[1,2*], Khaoula Menaiaia[1,2], Nourhene Ouartane[2], Nedjoud Grara[3]

[1]Mohamed Cherif Messaadia University of Souk-Ahras, Laboratory of Sciences and Technology of Water and Environment, PO Box 1553, 41000, Souk Ahras, Algeria
[2]Mohamed Cherif Messaadia University of Souk-Ahras, Faculty of Nature and Life Sciences, Department of Biology, PO Box 1553, 41000, Souk-Ahras, Algeria
[3]Université 8 Mai 1945 Guelma, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers, BP 401, Guelma 24000, Algeria

*Corresponding author: khaldifad@yahoo.fr

Received: September, 19, 2018
Accepted: June, 03, 2019

Abstract: The research aimed to understand the behavior of aquatic plants subjected to xenobiotics, a macrophyte plant *Phragmites australis* from the region of Souk-Ahras, is treated with three concentrations based on zinc oxide (ZnO) (3, 6 and 12 nmol·mL^{-1}) for 7, 14 and 21 days. A measurement of certain biochemical and enzymatic parameters characteristic of oxidative stress allowed us to evaluate not only the effect of zinc oxide but also the behavior of *Phragmites australis* subjected to this nanometric molecule. The statistical analysis of the results obtained showed no significant differences in all the parameters measured, between leaves and roots. The results of the analysis of heavy metals in wastewater, revealed values lower than the Algerian norm with the exception of Zn. On the other hand, we noticed a high concentration of Zn and a low concentration of Fe and Pb. This result makes it possible to conclude that the wastewater is characterized by a pollution of the metallic type loaded with partially degradable effluents.

Keywords: *enzymatic activity, heavy metals, oxidative stress, Phragmites australis, phytoremediation, wastewater, ZnO*

INTRODUCTION

Dynamic development of industrial, urban, and agricultural aspects of human activities in the 20th century resulted in chemical pollution of the environment [1]. Heavy metal contamination in soils has been a serious problem, including decreased crop yields, biomass accumulation, and inhibited plant physiological metabolism in many areas around the world [2].
The major concern is heavy metal stress in different types of terrestrial ecosystems.
Currently, extensive industrializations have affected soil, terrestrial life and agricultural production due to accumulation and hazard of heavy metals [3], the presence of different elements and heavy metals accumulated in the soil adversely affect the physiological and molecular functions of the plant which leads to the reduction of growth [4]. Heavy metals at high concentrations all become toxic in plants affecting these metabolic processes and its functioning including dislodgment of amino acids [5].
Heavy metals such as Zn, Cu, Mo, Mn, Co, and Ni are essential for crucial biological processes and developmental pathways [3]. Toxic metal ions at cellular level evoke oxidative stress by generating reactive oxygen species (ROS) [6]. They promote deoxyribonucleic acid alterations in the chemical structure and repair mechanisms, protein functional perturbation and nutrient homeostasis and also affect the functional activities of membranes and proteins [7].
On the other hand, heavy metal toxicity causes metabolic perturbations and morphological alterations of cells, this basically affects the yield plants [8]. Several studies have depicted a negative impact of nanoparticles on plants in the form of a decrease in plant growth, productivity and pigments [9].
In addition, plants are able to protect themselves by preventing the metal ions with some adaptive strategies such as cysteine-rich oligomers called phytochelatins (PCs) [10]. Notably, PC has considered as one of the important for heavy metals detoxification which enhances the tolerance capacity of the plant against stress [11]. Metals negatively affect photosynthesis, respiration and inhibit the activities of various enzymes [12].
Chlorophyll content is often used to evaluate the impact of many environmental stresses. Proline is considered part of a general adaptation syndrome to adverse environmental conditions. Different functions are attributed to the accumulation of this compatible solute: osmoregulation, chelation, and detoxification of metals, protection of enzymes, regulation of cytosolic acidity, stabilization of protein synthesis and trapping of ROS (superoxide anion and hydroxyl radicals) [13].
Water quality issues are a major challenge faced by mankind in the 21st century [14].
The lack of treatment of wastewater from discharges translates into high levels of these parameters.
The origin of the contamination of these waters can be related to different contributions:

- discharges of domestic wastewater;
- industrial wastewater contains significant amounts of metals;
- nitrogen and phosphorus materials promote the development of aquatic plants and under certain conditions, they may develop a risk of toxicity to other living beings (microorganisms);
- fertilizers use in agriculture [15].

It may be preferable to select appropriate and useful species for successful and efficient phytoremediation [16]. Recently, like any new technology, phytoremediation has raised doubts and worries in the soil remediation [17].
Similarly, common reed (*P. australis* (Cav.) Trin. ex Steud.) and giant reed (*A. donax* L.) are tolerant species to trace elements, and are used in constructed wetlands and for phytoremediation of contaminated soil and sludge [18].
The aim of this study was to elucidate the effects of oxidative stress induced by a nanoparticulate molecule (ZnO) on a macrophyte plant *Phragmites australis* by studying biochemical parameters and enzymatic biomarkers on the different compartments (leaves and roots).

MATERIALS AND METHODS

Plant material

The research was conducted using as biologic material the common macrophyte plant (*Phragmites australis*), whose organs chosen to carry out this study are the leaves and the roots. The species of *Phragmites australis* is given respectively in Figure 1.

Figure 1. *Phragmites australis*

Chemicals and reagents

For study, the chemical material used is a nanoparticle-based on ZnO at concentrations of 3, 6 and 12 nmol·mL^{-1} [19]. This molecule was supplied by the chemistry laboratory of the 8 May 1945 University of Guelma, Algeria.
The reagents used to extract chlorophyll content are: calcium carbonate and acetone (80 %). The following reagents and chemicals were used for the determination of proline content: methanol (40 %), acetic acid, phosphoric acid, ninhydrin, toluene, sodium sulfate. The reagents and solutions used in ascorbate peroxidase activity (APX) and catalase activity (CAT) determination are: hydrogen peroxide, phosphate buffer NaK-ascorbate (*p*H = 7.2), phosphate buffer (*p*H = 7.2) and Bradford reagent. High purity water was used in all experiments. All the reagent and solvents were obtained commercially from Sinal and Equilab-suppliers (Algeria) and used as received, without further purification.

Study site

The study was conducted in Oued Medjerda which is located in the region of Souk Ahras (Algeria). This area from which the samples of *Phragmites australis* were collected is indicated in Figure 2.

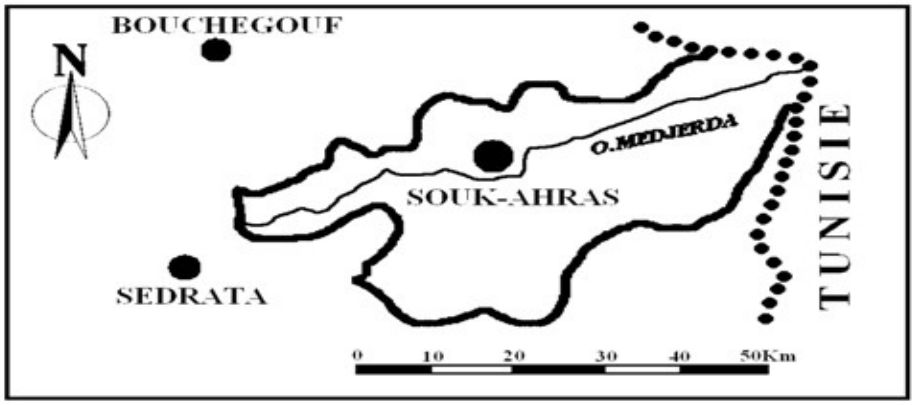

Figure 2. *Sampling site* [20]

Plant culture and experimental set-up

The experimental pilot consists of nine aquariums (41.5 cm long, 20.5 cm wide and 27.5 cm high), filled by a succession of three layers: two composed of gravel of decreasing diameter and the third which is the thickest, consists of sand. These aquariums with 8 liters of wastewater are planted with reeds (*Phragmites australis*) - 3 samples/aquarium, under the conditions of the laboratory with three treatments, at the rate of 3 aquariums for each concentration for three periods (7, 14 and 21 days), with an *in vivo* control. The experimental design as can be observed in Figure 3.

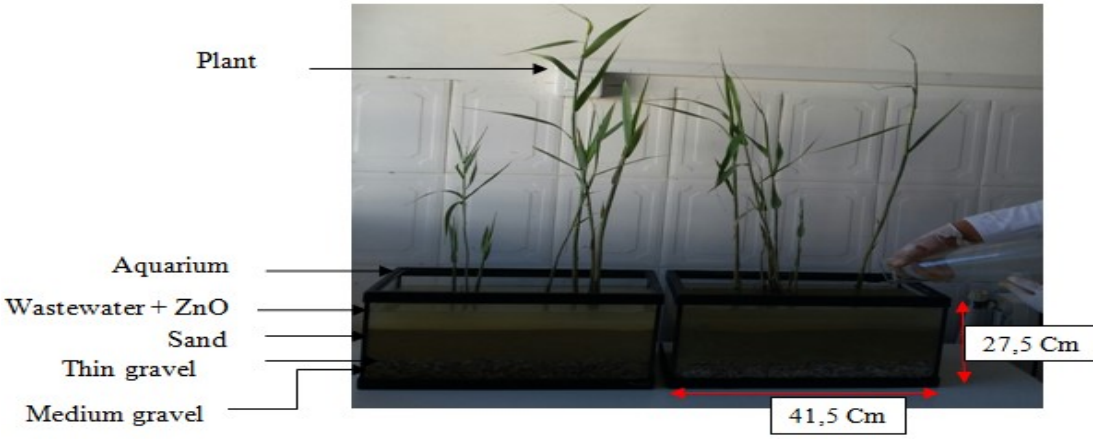

Figure 3. *Experimental design*

The schematic presentation of the experimental set-up is presented in Figure 4 and the diagram in Figure 5 shows the experimental procedure steps.

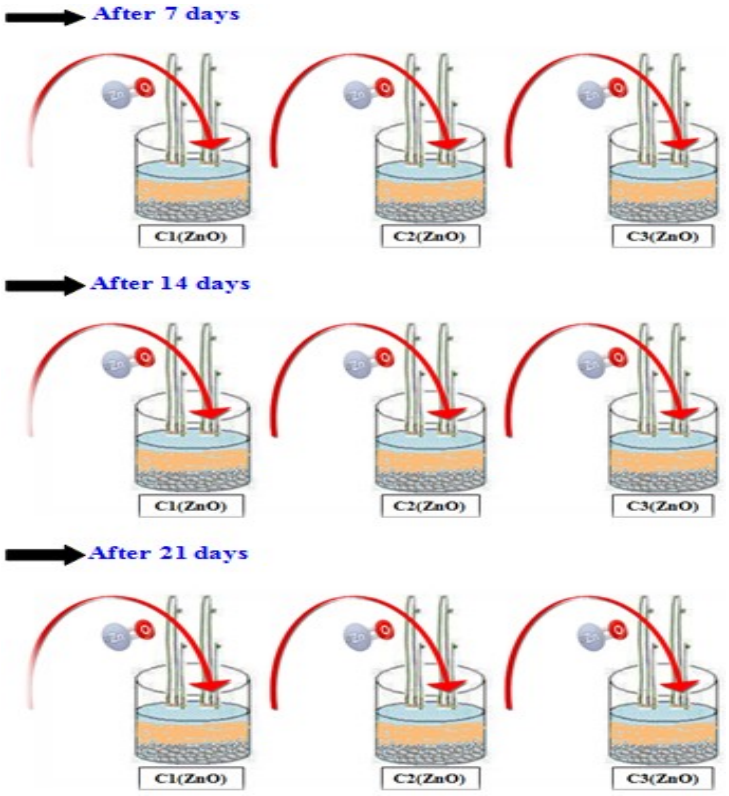

Figure 4. *Schematic overview of the experimental set-up (the macrophyte of the genus Phragmites australis are treated under metal stress by 3, 6 and 12 nmol·mL^{-1} of ZnO for 7, 14 and 21 days)*

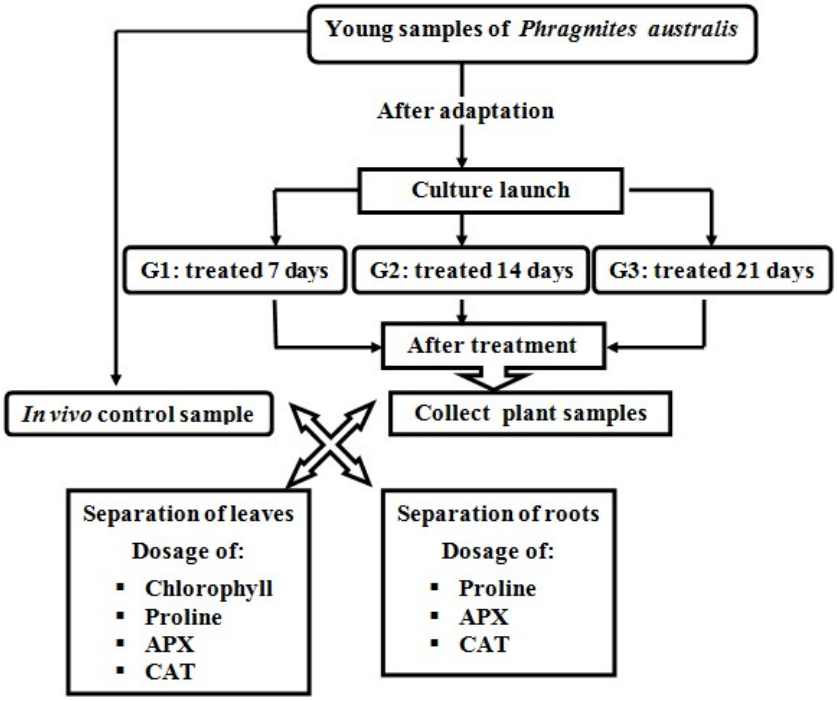

Figure 5. *Experimental procedure steps (G1:group 1; G2: group 2; G3: group 3)*

Methods of analysis

Water analysis

The rejection of Oued Medjerda site is the source of the wastewater samples, from which the *Phragmites australis* were collected. The water samples were taken to a laboratory specialized in the analysis of water in a cooler sheltered from light at a temperature of 4 °C. For the analysis of heavy metals in wastewater, atomic absorption spectrometry (SHIMADZU-AA-6200) was used. This analysis was carried out in the Laboratory of Sciences and Technology of Water and Environment of the Mohamed Cherif Messaadia University of Souk Ahras.

Determination of pigment contents, proline and enzymatic antioxidants (ascorbate peroxydase (APX) and catalase (CAT))

The method used for the extraction of chlorophyll is the traditional method established by Holden [21], which consists of maceration of the plant in acetone.

Sample processing is as follows:

- 1 g of the plant, cut into small pieces and crushed in a mortar with 20 mL of 80 % acetone with 100 mg of calcium carbonate.
- After total grinding, the solution is then filtered and placed in black boxes, in order to avoid the oxidation of chlorophyll by the light.
- The reading is done at the two wavelengths of 645 and 663 nm, after calibration of the apparatus with the 80 % acetone control solution.

The proline assay technique used is that of Monneveux and Nemmar [22] using 100 mg of the samples, cut into small pieces and introduced into a test tube, to which 2 mL of 40 % methanol are added, the whole is then heated in a water bath at 80 °C for 60 min, the tubes are covered of aluminum foil to prevent the volatilization of alcohol. After cooling, 1 mL of the solution is removed, to which 1 mL of acetic acid and 1 mL of a modified mixture containing: 120 mL of distilled water + 300 mL of acetic acid + 80 mL of orthophosphoric acid and 25 mg ninhydrin.

The solutions were brought to boiling for 30 minutes; after cooling, 5 mL of toluene was added and two phases were separated after agitation:

- the lower phase without proline.
- the upper phase which contains proline. This phase is then recovered and dehydrated by the addition of sodium sulfate.

Finally, the optical densities of the samples are determined at a wavelength of 528 nm, after calibration of the apparatus with the mixture (acetic acid + distilled water + orthophosphoric acid + ninhydrin).

The APX is carried out according to the protocol adopted by Nakano and Azada [23]. The final reaction volume of 3 mL contains: 100 µL of enzymatic extract, 50 µL of 0.3 % hydrogen peroxide (H_2O_2) and 2850 µL of NaK-Ascorbate phosphate buffer (50 mM NaK, 0.5 mM ascorbate, *p*H = 7.2). The calibration of the apparatus is done in the absence of the enzyme extract. The reading is carried out at 290 nm (Spectrophotometer JENWAY 63000) for 1 min and for a molar extinction coefficient ε = 2800 $M^{-1} \cdot cm^{-1}$, the APX activity is expressed in nmol / min / mg of proteins.

The CAT is performed according to the method of Cakmak and Horst [24]. The decrease in absorbance is recorded for three minutes by a spectrophotometer (JENWAY 6300) for a wavelength of 240 nm and a molar extinction coefficient

ε = 39400 $M^{-1} \cdot cm^{-1} \cdot L$. For a final volume of 3 mL, the reaction mixture contains 100 µL of the crude enzyme extract, 50 µL of 0.3 % H_2O_2 and 2850 µL of phosphate buffer (50 mM, *p*H = 7.2). The calibration of the apparatus is done in the absence of the enzyme extract. The reaction is triggered by the addition of H_2O_2. The catalase activity is expressed in nmol / min / mg of protein.

Statistical data analysis

The processing and data analysis were performed using specialized statistical software: Statistica ® 8.0 (Statsoft: www.statsoft.com) where a statistical description was given for each studied variable (physiological, biochemical and enzymatic). Our statistical analysis is based on the following points:

- Comparison of several groups: the non-parametric *Kruskal Wallis* test was used to test the effect "dose" of ZnO (nanoparticles) for each of the three periods of exposure compared to control batches;

The application conditions of the statistical tests were verified and respected according to the recommendations of Scherrer [25].

RESULTS AND DISCUSSIONS

Water quality

After the analysis of the heavy metals in wastewater, the results obtained show a high concentration of Zn and a low concentration of Fe and Pb, while the Cu concentration is below the limit of detection (Table 1).

Table 1. *Average concentrations of metallic elements in wastewater*

Metallic dusts	Concentrations [mg·L^{-1}]	Algerian norms
Fe	1.3539	≤ 3
Cu	0.0000	≤ 0.5
Zn	38.1845	≤ 3
Pb	0.0078	≤ 0.5

From the analysis of the results of heavy metals in wastewater from the culture medium of *Phragmites australis* obtained on all sampling campaigns. These observations reveal an anthropogenic factor to the contamination which explains the presence of certain heavy metals in the environment of samples of *Phragmites australis*.

Pigment contents, metabolites and enzymatic antioxidants in the plant parts (leaves and roots)

Chlorophyll content

The determination of chlorophyll pigments indicates no significant differences throughout the treatment periods with ($P > 0.05$), after a 7-days exposure, chlorophylls *a*, *b* and *a* + *b* have the same variation profile of which the samples treated with the

doses: C1: 3, C2: 6, C3: 12 nmol·mL^{-1} of ZnO, have higher levels than controls (Figure 6).

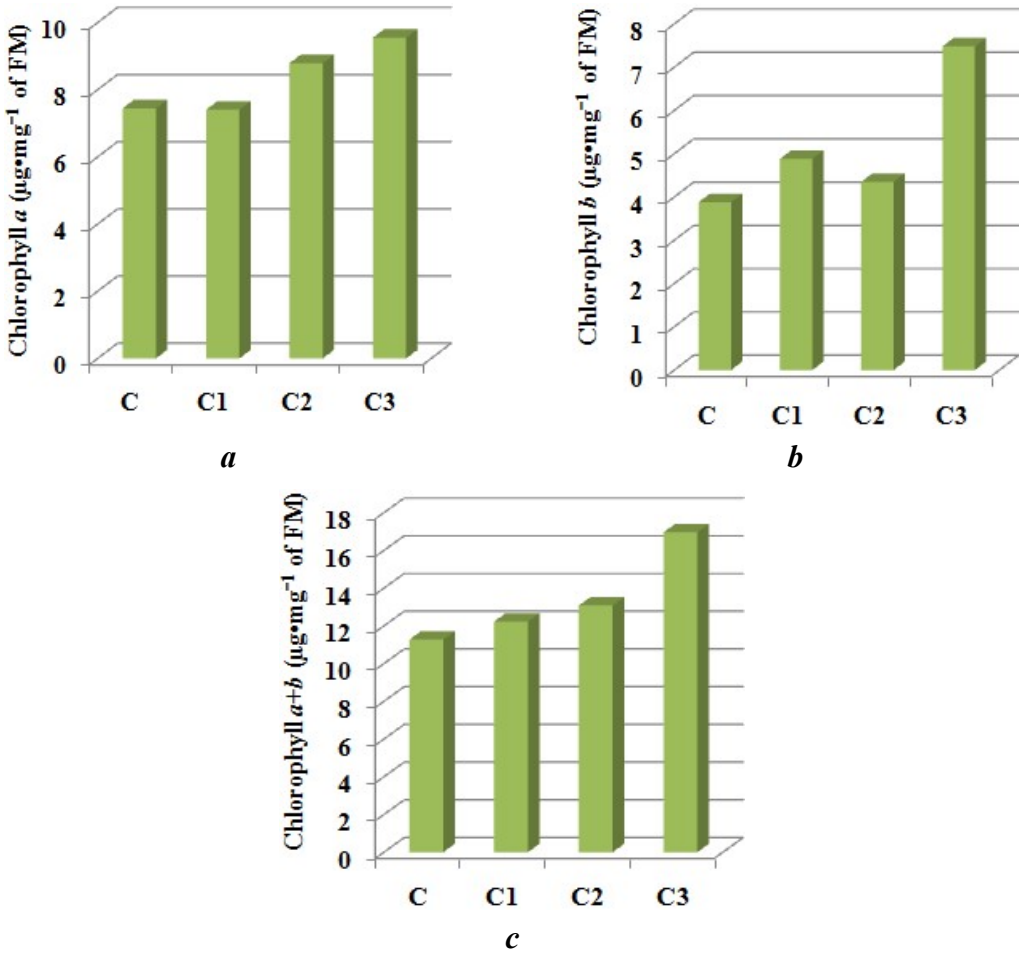

Figure 6. *Effect of ZnO on the content of chlorophyllian pigment of Phragmites australis after 7 days of exposure (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) chlorophyllian pigment a; (b) chlorophyllian pigment b; (c) chlorophyllian pigment a+b*

After 14 days of treatment, the results obtained show that the samples treated with the doses 3, 6 and 12 nmol·mL^{-1} have higher levels than controls (Figure 7).

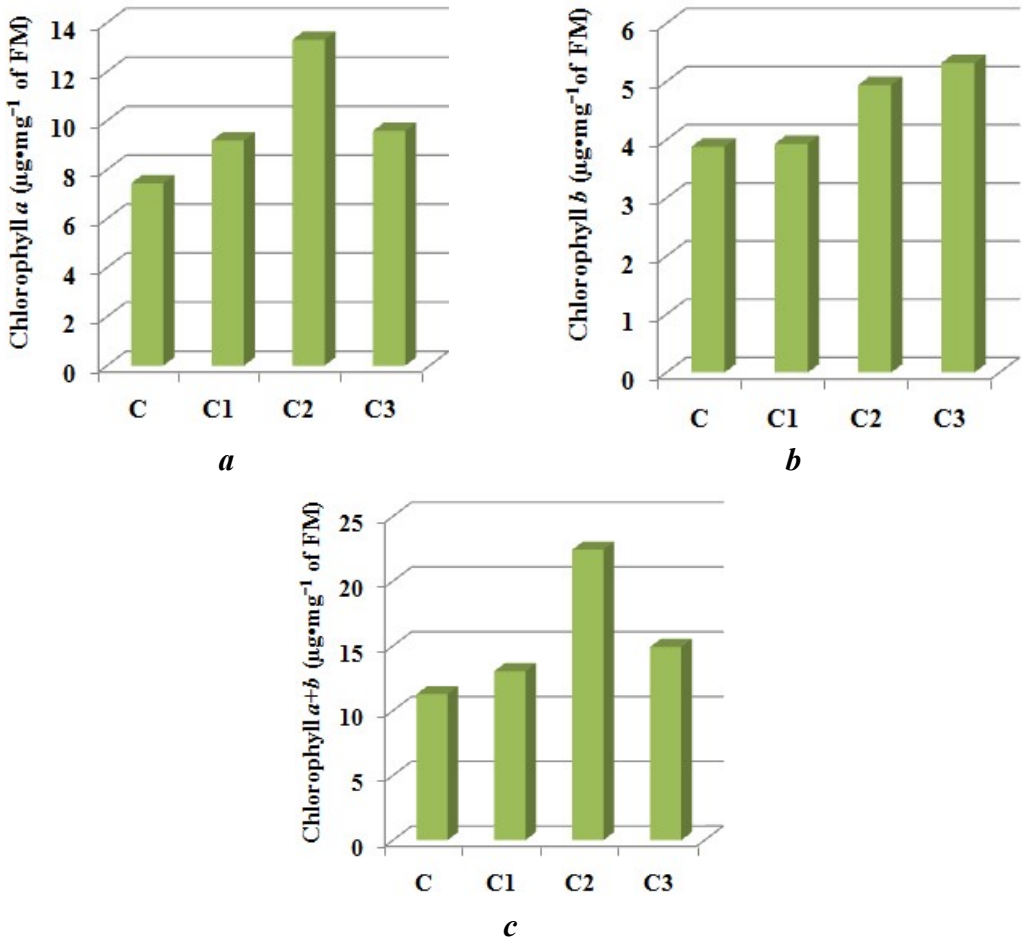

Figure 7. *Effect of ZnO on the content of chlorophyllian pigment of Phragmites australis after 14 days of exposure (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) chlorophyllian pigment a; (b) chlorophyllian pigment b; (c) chlorophyllian pigment a+b*

In the last treatment period (21 days), there is a progressive increase ($P > 0.05$), where the application of the dose 6 and 12 nmol·mL^{-1} of ZnO records the highest levels of chlorophylls (*a*, *b* and *a* + *b*) (Figure 8), while those treated with the 3 nmol·mL^{-1} dose give higher levels.

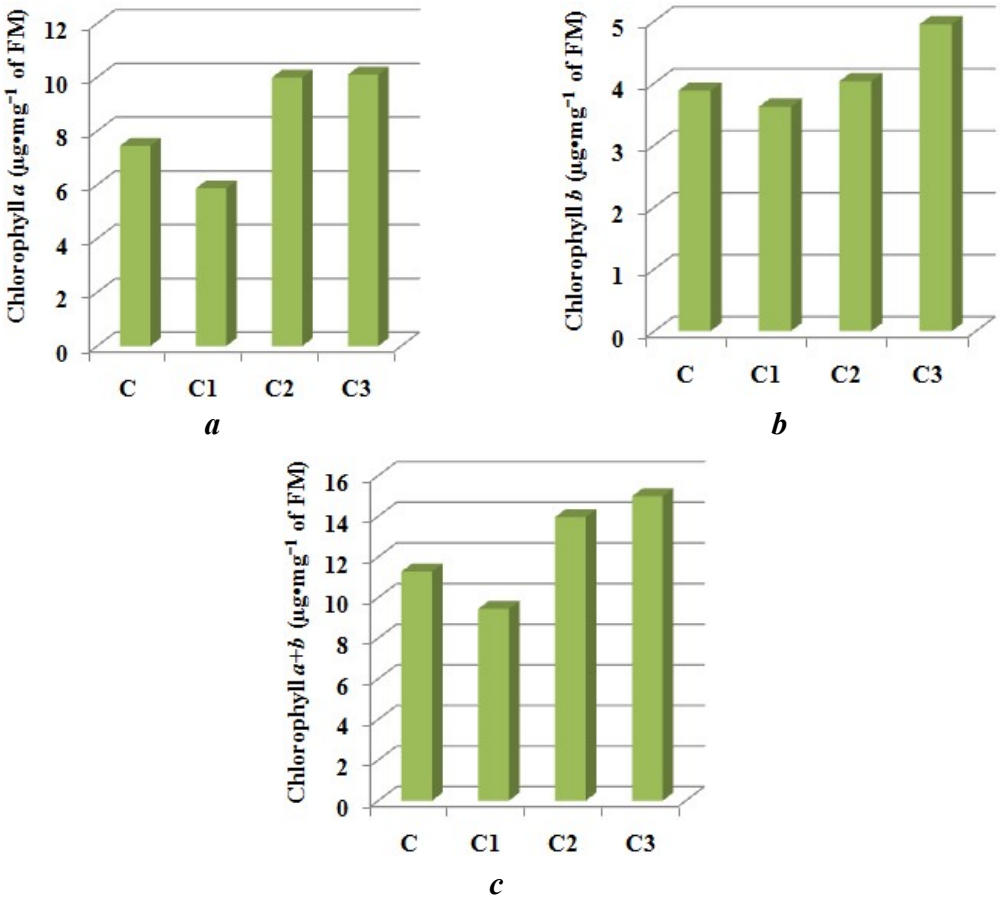

Figure 8. *Effect of ZnO on the content of chlorophyllian pigment of Phragmites australis after 21 days of exposure (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) chlorophyllian pigment a; (b) chlorophyllian pigment b; (c) chlorophyllian pigment a+b*

The exposure of *Phragmites australis*, during all treatment periods 7, 14 and 21 days to this xenobiotic (ZnO), shows that there is no significant difference in chlorophylls (*a*, *b* and *a* + *b*). This effect agrees with previous work [26].

Proline content

At the level of the foliar and root system, it is interesting to note also that the metabolites produced by *Phragmites australis* show no significant differences with ($P > 0.05$).

After 7 days of exposure, a decrease affects doses 6 and 12 nmol·mL^{-1}, after 14 and 21 days, the figures show a depletion of the proline level for the 6 nmol·mL^{-1} dose and thus a remarkable increase for doses 3 and 12 nmol·mL^{-1} were observed (Figure 9).

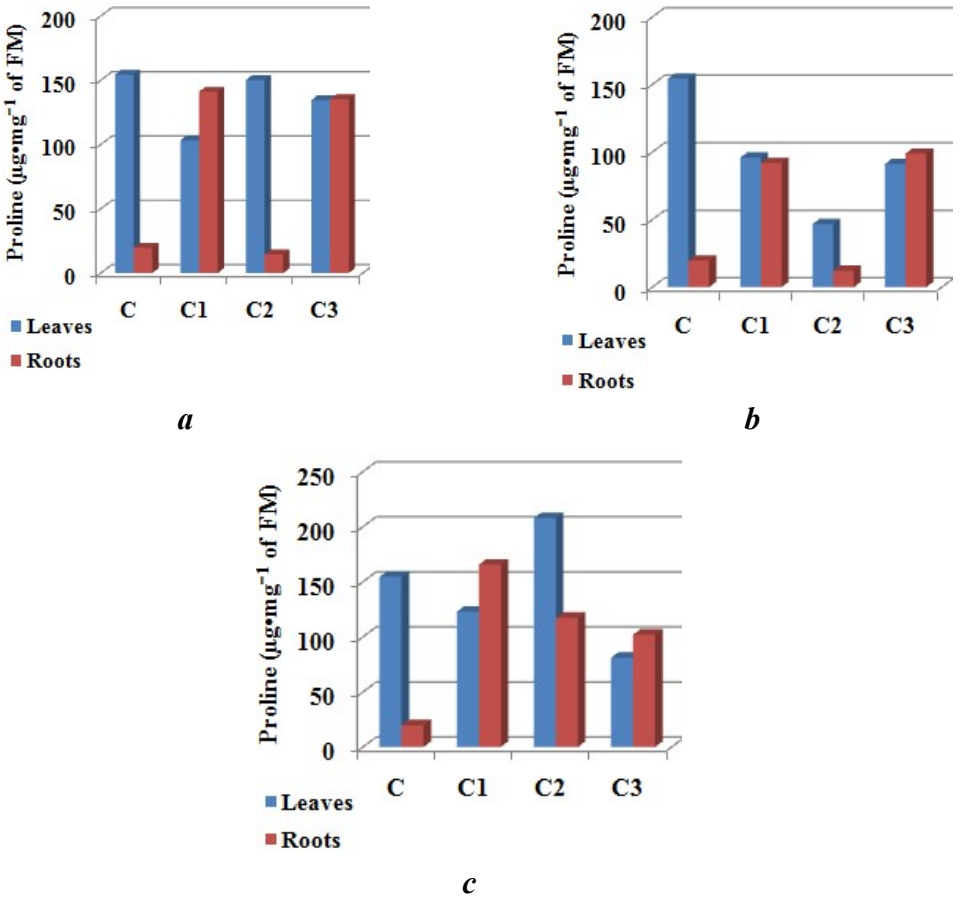

Figure 9. *Effect of ZnO on the proline level of Phragmites australis (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) after 7 days; (b) after 14 days; (c) after 21 days of exposure*

In this work, we have also investigated the proline content of *Phragmites australis* exposed to increasing concentrations of ZnO; we thus highlighted a high increase in this parameter known as a biomarker of stress [27].
Our results are consistent with Meloni *et al.* [28], who demonstrated that proline accumulates in the plant under adverse conditions.

APX activity levels
As it can be seen from Figure 10, the enzymatic activities analysis involved in the oxidative stress management process APX, throughout the treatment, shows no significant differences ($P > 0.05$) in the leaves and roots.
At the level of the foliar and root system of *Phragmites australis*, the results of enzymatic biomarkers showed variable profiles as a function of exposure time. The APX activity dosage in the roots showed no significant differences during the 7 days treatment with an increasing profile in the samples treated with doses 3, 6 and 12 nmol·mL^{-1} of ZnO (Figure 10).

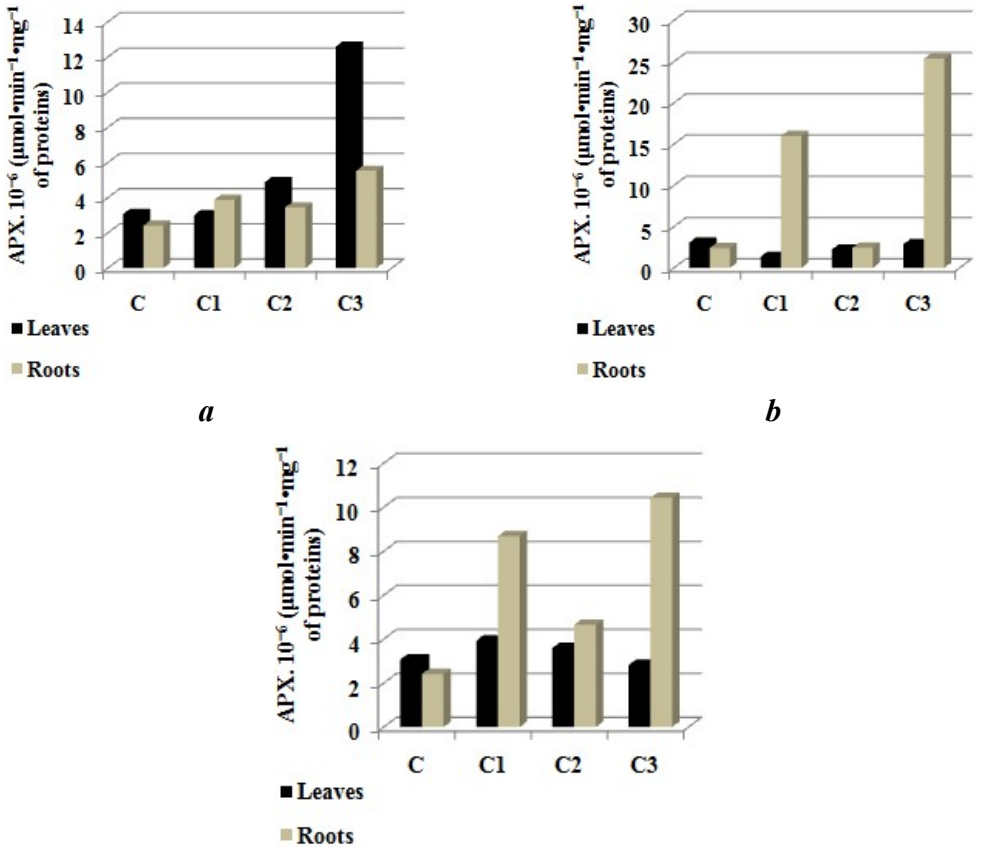

Figure 10. *Effect of ZnO on the enzymatic activity of APX of Phragmites australis (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) after 7 days; (b) after 14 days; (c) after 21 days of exposure*

We also noted an increase in APX activity in both parts of *Phragmites australis*. These results are consistent with those obtained by Gallogo *et al.* [29], which showed an increase in the APX level under the effect of chemical stress in sunflower.

CAT activity levels

Figure 11 indicate that the enzymatic activities analysis involved in the CAT oxidative stress management process throughout the treatment shows no significant differences ($P > 0.05$) in the leaves and roots. At the level of the foliar and root system, the results of enzymatic biomarkers of *Phragmites australis* show variable profiles as a function of exposure time. The dosage of CAT activity in the roots showed no significant differences during the 7-day treatment with increasing profile in the samples treated with the doses 3, 6 and 12 nmol·mL^{-1} of ZnO (Figure 11).

We have demonstrated an increase in CAT activity in both compartments of the plant; roots and leaves. This could be due to the triggering of detoxification systems, which allows tolerance and adaptation to xenobiotic thus resulting in an increase in these enzymes (CAT) that play a role in regulating the concentrations of ROS [30]. Catalase is an enzyme that catalyzes the dismutation of hydrogen peroxide into a molecule of water and oxygen [31].

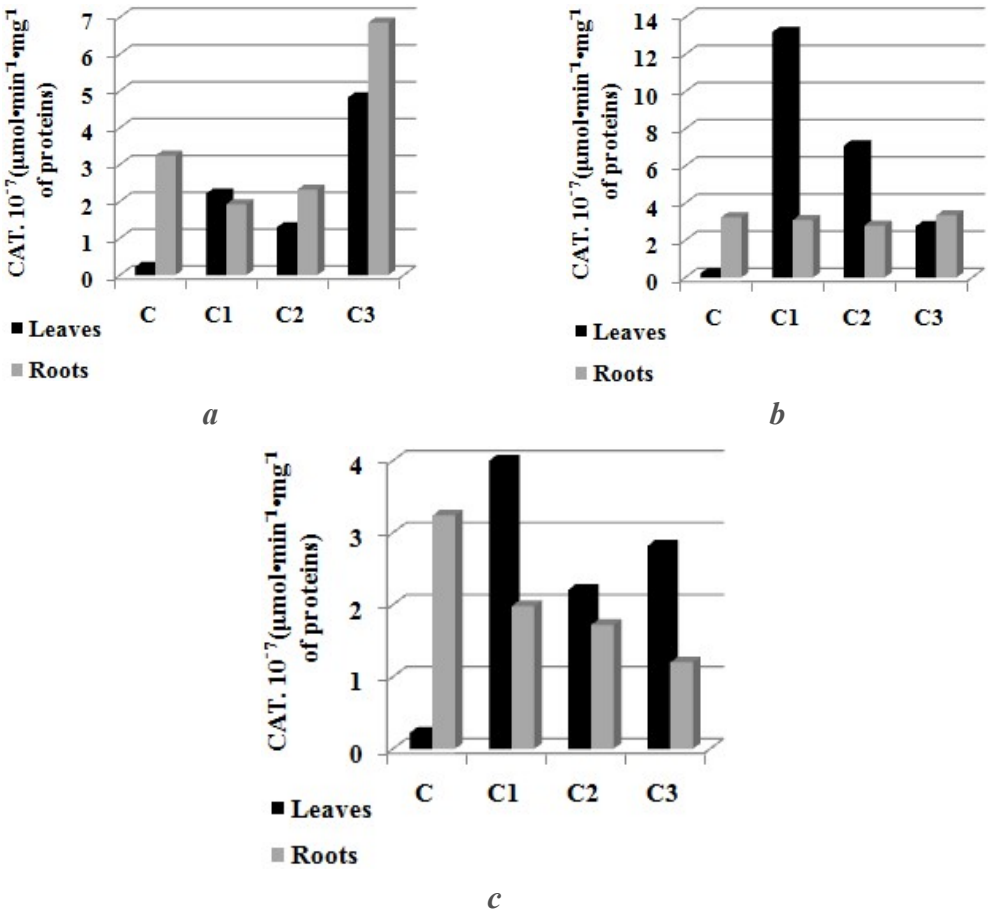

Figure 11. *Effect of ZnO on the enzymatic activity of CAT of Phragmites australis (Kruskal Wallis test, ddl = 2 and n = 12). C: control, C1: 3, C2: 6, C3: 12 nmol·mL^{-1}: (a) after 7 days; (b) after 14 days; (c) after 21 days of exposure*

These results abound in the same direction as those of Humă *et al.* [32], which show a stimulation of catalase synthesis in the presence of nitrates and nitrites in wheat. These results allow us to assign a role to the CAT in the responses to environmental stress defenses, especially salinity. Some studies have shown an increase in catalytic activity after exposure to different pollutants such as cadmium, nitrogen, phosphorus and potassium (NPK) [33], sodium chloride and silicone [34] and uranium [35].

Indeed, CAT and APX have complementary roles in the detoxification of hydrogen peroxide [36]. APX reduces hydrogen peroxide (H_2O_2) to water using ascorbate as an electron donor from dehydroascorbate [37], subsequently producing the radicals (monodehydroascorbate MDHA) [38, 39].

The results obtained show that ZnO caused in *Phragmites australis*, a high peroxide activity which results in an increase in the activities of (APX and CAT). However, it should be noted, that these enzyme activities are higher in stressed roots than in leaves. These high activities of APX and CAT appear to be due to oxidative stress induced by ZnO.

CONCLUSIONS

In conclusion, the results obtained seem to converge towards the demonstration of the oxidative toxic character of ZnO, through the study of metabolites and antioxidant activities of enzymes such as CAT and APX. The retention of *Phragmites australis* with ZnO stimulates the accumulation of proline. The results of enzymatic activities: APX and CAT indicate a high antioxidative capacity of *Phragmites australis*. This activity may represent a response of this plant to oxidative stress probably caused by the accumulation of this xenobiotic at the cellular level.
Water contributes to the protection of the environment, the preservation of natural resources and components, and above all it offers the possibility of reusing wastewater in agricultural and industrial fields. The analysis of the wastewater (environment of the *Phragmites australis*) of the region of Souk-Ahras notably the site of Oued Medjerda allowed us to release a set of information at the level of its quality as a direct rejection in the medium receiver. The different parameters measured in the raw wastewater obviously indicate a significant metallic pollution by Zn.
The use of *Phragmites australis* in the treatment of wastewater, where the removal of pollutants provides good quality water that can be reused in the industrial sector and in irrigation and could solve one of the problems of water scarcity in our country. Indeed, Scientists are always looking for the best way to treat wastewater with the minimum of expense and maximum efficiency.

NOTATIONS

FM - fresh matter
ddl - data definition language
P - probability value
n - sample size
NaK - sodium-potassium

REFERENCES

1. Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., Fava, F.: Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, *New Biotechnology*, **2015**, **32** (1), 147-156;
2. Jiang, W., Cai, Q., Xu, W., Yang, M., Cai, Y., Dionysiou, D.D., O'Shea, K.E.: Cr (VI) adsorption and reduction by humic acid coated on magnetite, *Environmental Science and Technology*, **2014**, **48** (14), 8078-8085;
3. Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., Aslam, M., Dumat, C.: Heavy Metal Stress and Crop Productivity, in: *Crop Production and Global Environmental Issues* (editor: Hakeem, K.R.), Springer, Cham, **2015**, 1-25;
4. Hassan, T.U., Bano, A., Naz, I.: Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field, *International Journal of Phytoremediation*, **2017**, **19** (6), 522-529;
5. Krumova, E., Kostadinova, N., Miteva-Staleva, J., Gryshko, V., Angelova, M.: Cellular response to Cu- and Zn-induced oxidative stress in *Aspergillus fumigatus* isolated from polluted soils in Bulgaria, *CLEAN Soil Air Water*, **2016**, **44** (6), 657-666;

6. Li, M., Ahammed, G.J., Li, C., Bao, X., Yu, J., Huang, C., Yin, H., Zhou, J.: Brassinosteroid ameliorates zinc oxide nanoparticles-induced oxidative stress by improving antioxidant potential and redox homeostasis in tomato seedling, *Frontiers in Plant Science*, **2016**, **7**, Article ID 615, 13 pages, https://doi.org/10.3389/fpls.2016.00615;
7. Tamás, M.J., Sharma, S.K., Ibstedt, S., Jacobson, T., Christen, P.: Heavy metals and metalloids as a cause for protein misfolding and aggregation, *Biomolecules*, **2014**, **4** (1), 252-267;
8. Amari, T., Ghnaya, T., Abdelly, C.: Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction, *South African Journal of Botany*, **2017**, **111**, 99-110;
9. Tripathi, D.K., Singh, S., Singh, S., Srivastava, P.K., Singh, V.P., Singh, S., Prasad, S.M., Singh, P.K., Dubey, N.K., Pandey, A.C., Chauhan, D.K.: Nitric oxide alleviates silver nanoparticles (AgNps)-induced phytotoxicity in *Pisum sativum* seedlings, *Plant Physiology and Biochemistry*, **2017**, **110**, 167-177;
10. Pochodylo, A.L., Aristilde, L.: Molecular dynamics of stability and structures in phytochelatin complexes with Zn, Cu, Fe, Mg, and Ca: Implications for metal detoxification, *Environmental Chemistry letters*, **2017**, **15** (3), 495-500;
11. Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y.: Heavy metal stress and some mechanisms of plant defense response, *Scientific World Journal*, **2015**, **2015**, Article ID 756120, 18 pages, http://dx.doi.org/10.1155/2015/756120;
12. Mosa, K.A., Kumar, K., Chhikara, S., Mcdermott, J., Liu, Z., Musante, C., White, J.C., Dhankher, O.P.: Members of rice plasma membrane intrinsic proteins subfamily are involved in arsenite permeability and tolerance in plants, *Transgenic Research*, **2012**, **21** (6), 1265-1277;
13. Biteur, N.: *Essais d'utilisation du radis (Raphanus sativus) dans la phytoremédiation (biodépollution) au niveau du sol contaminé par les métaux lourds (plomb): Etude du stress oxydatif et quelques paramètres enzymatiques*, Thèse de Doctorat, Université d'Oran (Algérie), **2012**;
14. Corcoran, E., Nelleman, C., Baker, E., Bos, R., Osborn, D., Savelli, H. (editors): *Sick Water? The Central Role of Wastewater Management in Sustainable Development*, *A Rapid Response Assessment*, United Nations Environment Programme (UNEP), UN-HABITAT, GRID-Arendal, Birkeland Trykkeri AS, Norway **2010**, http://www.grida.no/publications/218;
15. Ngaram, M.N.: *Contribution à l'étude analytique des polluants (en particulier de type métaux lourds) dans les eaux du fleuve Chari lors de sa traversée de la ville de N'Djamena*, Thèse de Doctorat, Université Claude Bernard - Lyon I (France), **2011**;
16. Lacalle, R.G., Gomez-Sagasti, M.T., Artetxe, U., Garbisu, C., Becerril, J.M.: *Brassica napus* has a key role in the recovery of the health of soils contaminated with metals and diesel by rhizoremediation, *Science of The Total Environment*, **2018**, **618**, 347-356;
17. Jagtap, S.S., Woo, S.M., Kim, T.-S., Dhiman, S.S., Kim, D., Lee, J.-K.: Phytoremediation of diesel-contaminated soil and saccharification of the resulting biomass, *Fuel*, **2014**, **116**, 292-298;
18. Sagehashi, M., Liu, C., Fujii, T., Fugjta, H., Sakai, Y., Hu, H.Y., Sakoda, A.: Cadmium removal by the hydroponic culture of giant reed (*Arundo donax*) and its concentration in the plant, *Journal of Water and Environment Technology*, **2011**, **9** (2), 121-127;
19. Ferfar, M.: *Toxicité et bioaccumulation de pesticides sur quelque variétés de blé dur (T. durum Desf)*, Thèse de Doctorat, Université Badji Moktar de Annaba (Algérie), **2017**;
20. Khaldi, F., Smati, H., Grara, N., Smati, N., Maizi, N., Boukehili, K., Gheid A.: Mesure et contrôle des propriétés de quelques eaux souterraines de l'agglomération d'Ouled Driss, Algérie, *Environnement, Risques & Santé*, **2018**, **17** (3), 253-261;
21. Holden, M.: Chlorophylls, in: *Chemistry and Biochemistry of Plant Pigments* (Editor: Goodwin, T.W.), 2nd Edition, Academic Press, New York, **1976**, 133;
22. Monneveux, P., Nemmar, M.: Contribution à l'étude de la résistance à la sécheresse chez le blé tendre (*Triticum aestivum* L.) et chez le blé dur (*Triticum durum* Desf.) : étude de l'accumulation de la proline au cours du cycle de développement, *Agronomie*, **1986**, **6** (6), 583-590;
23. Nakano, Y., Azada, K.: Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical, *Plant and Cell Physiology*, **1987**, **28** (1), 131-140;
24. Cakmak, I., Horst, W.J.: Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*), *Physiologia Plantarum,* **1991**, **83** (3), 463-468;

25. Scherrer, B.: *Biostatistique*, *Volume 2,* 2nd ed., Gaëtan Morin Edition, Montreal, **2009**, 592;
26. Groppa, M.D., Tomaro, M.L., Benavides, M.P.: Polyamines and heavy metal stress: the antioxydant behavior of spermine in cadmium-and copper-treated wheat leaves, *Biometals*, **2007**, **20**, 185-195;
27. Wahid, A.: Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane, *Botanical Bulletin of Academia Sinica*, **2004**, **45**, 133-141;
28. Meloni, D.A., Gulotta, M.R., Martinez C.A., Oliva, M.A.: The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*, *Brazilian Journal of Plant Physiology*, **2004**, **16** (1), 39-46;
29. Gallego, S.M., Benavides, M.P., Tomaro, M.L.: Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of an oxidative stress, *Plant Science*, **2002**, **121**, 151-159;
30. Lin, C.C., Kao, C.H.: Effect of NaCl stress on H_2O_2 metabolism in rice leaves, *Plant Growth Regulation*, **2000**, **30**, 151-155;
31. Arora, A., Sairam, R.K., Srivastava, G.C.: Oxidative stress and antioxidative system in plants, *Current Science*, **2002**, **82** (10), 1227-1238;
32. Humă, A.-M., Toma, O., Ungureanu, E., Negură, L.: L'influence des nitrates et des nitrites sur les activités de la catalase et de la peroxydase au cours de la germination des graines de *Triticum aestivum*, *Analele Ştiinţifice ale Universităţii "Alexandru Ioan Cuza" Iaşi (Serie nouă). Secţiunea IIa. Genetică şi Biologie Moleculară*, **2006**, **7** (1), 65-68;
33. Khaldi, F.: *Mécanismes d'action des polluants chimiques industriels (engrais et NOx) sur des bio-indicateurs de pollution (Mousses et Lichens)*, Thèse de Doctorat, Université de Annaba - Badji Mokhtar (Algerie), **2013**;
34. Zhu, Z., Wei, G., Li, J., Qian, Q., Yu, J.: Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.), *Plant Science*, **2004**, **167** (3), 527-533;
35. Vandenhove, H., Cuypers, A., Van Hees, M., Koppen, G., Wannijn, J.: Oxidative stress reactions induced in beans (*Phaseolus vulgaris*) following exposure to uranium, *Plant Physiology and Biochemistry*, **2006**, **44** (11-12), 795-805;
36. Barata, C., Varo, I., Navarro, J.C., Arun, S., Porte, C.: Antioxidant enzyme activities and lipid peroxidation in the fresh water cladoceran *Daphnia magna* exposed to redox cycling compounds, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **2005**, **140** (2), 175-186;
37. Faburé, J.: *Étude de l'accumulation et des effets des composés organiques volatils (BTEX) chez les bryophytes*, Thèse de Doctorat, Université du Droit et de la Santé - Lille II (France), **2009**;
38. Khaldi, F., Grara, N., Berrebbah, H., Djebar M.R.: The oxidative stress and the antioxidant defense of the lower vegetables towards an environmental pollution, *International Scholarly and Scientific Research & Innovation (World Academy of Science, Engineering and Technology, International Journal of Environmental and Ecological Engineering)*, **2013**, **7** (4), 233-238, https://waset.org/publications/789/the-oxidative-stress-and-the-antioxidant-defense-of-the-lower-vegetables-towards-an-environmental-pollution;
39. Lanez, T., Ben Haoua, K.: The effect of Soxhlet and ultrasonic-assisted extraction on antioxidant components and antioxidant properties of selected south algerian red potatoes cultivars, *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2017**, **4** (18), 435-448.