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The Oxidative Stress and the Antioxidant Defense of the Lower Vegetables towards an Environmental Pollution

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***Abstract*—**The use of bioindicators plants (lichens, bryophytes and Sphagnum....) in monitoring pollution by heavy metals has been the subject of several works. However, few studies have addressed the impact of specific type's pollutants (fertilizers, pesticides.) on these organisms.

We propose in this work to make the highlighting effect of NPKs (NPK: nitrogen-phosphate-potassium-sulfate ($NP_2O_5K_2O$) (15,15,15), at concentrations of 10, 20, 30 , 40 and 50mM/L) on the activity of detoxification enzymes (GSH/GST, CAT, APX and MDA) of plant bioindicators (mosses and lichens) after treatment for 3 and 7 days. This study shows the important role of the defense system in the accumulation and tolerance to chemical pollutants through the activation of enzymatic (GST (glutathione-S-transferase, APX (ascorbat peroxidase), CAT (catalase)) and nonenzymatic biomarkers (GSH (glutathione), MDA (malondialdehyde)) against oxidative stress generated by the NPKs.

***Keywords*—**NPKs, Bioindicators, lower plants, GSH / GST, CAT, APX and MDA.

I. Introduction

THE abiotic environmental stresses, such as pollution, drought, salinity and low temperatures are conditions that affect the growth and yield of plants. Unlike animals, which can move when living conditions are more favorable, plants have developed adaptation strategies to respond to environmental changes by monitoring and adjusting their metabolic systems [1].

The cellular and molecular responses of plants to stress conditions have been studied. The mechanisms by which they perceive environmental signals and transmit them to the cellular machinery to activate mechanisms determine appropriate responses every day survival. Knowledge of these answers, based on the transmission (transduction) of stress signals is very important to improve the response of crop plants to different environmental stresses [2].

Thus, a signal transduction pathway of a signal begins with the perception of the signal level of the membrane, followed by the production of second messengers and transcription factors. These factors control the expression of genes involved in stress response including changes in morphological, biochemical and physiological [2]. We can consider the concept of stress, on the one hand, a deviation of more or less abrupt relative to the normal conditions of the plant or the animal, and on the other hand a sensitive reaction of the individual in the different aspects of the physiology which significantly changes with or adaptations to the new situation, or to limit degradation leading to a fatal outcome. We, therefore, two aspects of the concept of stress: external constraints and its result on the individual, and the stress response or stress. It is therefore clearly distinguish the stressor or constraint or state of stress (the response) which, in time, following more or less rapidly and is followed by more or less well adapted [3]. This confirms our results thereafter.

According to the definition proposed by Van Garrec and Haluwyn [4], plant biomonitoring of air quality is the use of responses at all levels of biological organization of one or more organisms to predict and / or be an alteration of the environment. We propose in this study to make the highlighting effect of NPKs on mosses and lichens after treatment for 3 and 7 days.

II. Material and Methods

A. Chemical Material

Our material is a chemical NPK: nitrogen-phosphate-potassium sulfated "NPKs" ($NP_2O_5K_2O$) (15,15,15), greyish and presentation granule, soluble in water. It comes from the fertilizer company in Algeria "FERTIAL"; it is dissolved in distilled water at concentrations of 10, 20, 30, 40 and 50mM. The NPK granulated white colored gray or brown and smell Inodore. Its pH is usually >4.5 in aqueous solution (100g/l). It decomposes at temperatures >130°C depending on the composition, non-explosive as claimed test A14 (67/548/EEC), not classified as an oxidizing material according to Directive 88/379/EEC, its bulk density between 900 and 1100 Normally kg/m^3.Il is soluble in water, depends for composition, the most formulas are hygroscopic [5].

B. Biological Material

The biological model used in our work is a lichen species: *Ramalina farinacea* and a mosses species: *Leucodon sciuroides* harvested in an area considered highly polluted little: Séraidi, located 14km west of Annaba (N-E Algeria) and 850 meters of altitude. This region is characterized by the

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abundance of Zen oak (*Quercus faginea*) and cork oak (*Quercus suber*). The thalli of *Ramalina farinacea* and *Leucodon sciuroides* are taken from the cork oak. The species chosen fruticose lichen is characterized by a thallus developed in length from a single attachment point [6] and is composed of branches narrow, tapering gradually and covered Sorelian marginal [7]. The species thalli located on the trunks of trees are removed and stored in plastic bags tightly closed to limit water losses by evapotranspiration. Mosses are Chlorophyllous plants, small, less than 70cm, the largest being the water foams. This size is quite low due to the lack of supporting tissues lignified conducting tissue and advanced [7].

C. Preparation of Culture Medium

NPK fertilizer was tested with five concentrations: 10, 20, 30, 40 and 50mM. The solutions prepared with the various concentrations of NPKs are used for the imbibition of the samples of lichens and mosses. Approximately 1g of thallus was soaked in 100ml of solution during 3 and 7 days [8].

D. Enzymatic Assays

1. Determination of Catalase (CAT) and Ascorbat-peroxydase Activity (APx)

We use for measuring the activity of catalase (CAT) the method of [9] and for the ascorbat-peroxidase activity (APx) the method of [10].

2. Determination of Glutathione (GSH) and Activity Glutathione S-transferase (GST)

The glutathione was assayed by the method of [11], based on measuring the absorbance of the2-nitro-5 mercapturic resulting from the reduction of the acid 5-5 'thiol-bis-2-nitrobenzoic acid (DTNB) by the thiol groups (-SH) glutathione. The glutathione S-transferase activity is performed by the method of [12]. It is based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene).The GSH is expressed in (µMole/mg of protein) and the GST is expressed in in (µMole/mn//mg of protein). The protein level was measured according the method of [13].

3. Determination of Malondialdehyde (MDA)

Lipid peroxidation was estimated by changing the content of malondialdehyde (MDA) determined according to the method described by [14]. The homogenization of the plant tissue in trichloroacetic acid (TCA) 5% in an amount of 10ml per 1g of plant tissue is followed by centrifugation for 15min at 12 000g. The supernatant was added an equal volume of thiobarbituric acid (TBA) in 0.5% (TCA) 20%. The mixture is incubated at 100°C for 25min. The absorbance of the supernatant obtained after centrifugation at 10 000g for 5min, is read at 532nm. The concentration of MDA was calculated using the extinction coefficient of $155mM^{-1}cm^{-1}$.

E. Statistical Study

The statistical analysis was performed by Student t test used to compare between two samples (control and treated). This test is performed using the analysis software statistical processing of data: Minitab version 16.1.0. , n = 5 [15].

III. Results

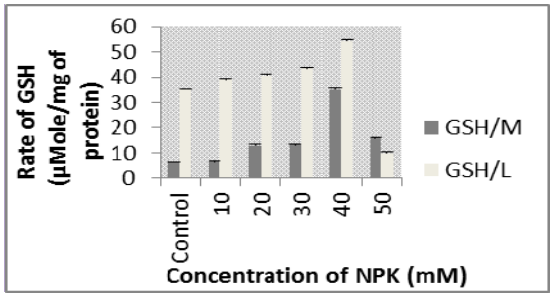

Fig. 1 NPK effects on glutathione levels in *Leucodon sciuroides* and *Ramalina farinacea* (Treatment 7 days) (M: Mosses and L: Lichens)

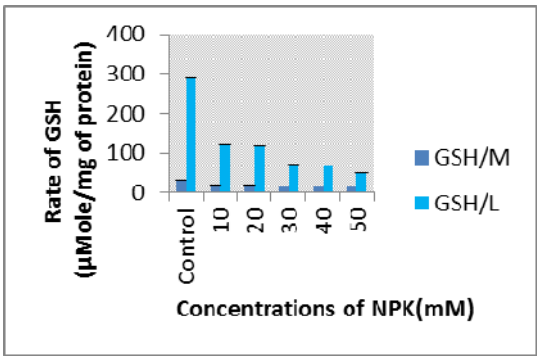

Fig. 2 NPK effects on glutathione levels in *Leucodon sciuroides* and *Ramalina farinacea* (Treatment 3 days) (M: Mosses and L: Lichens)

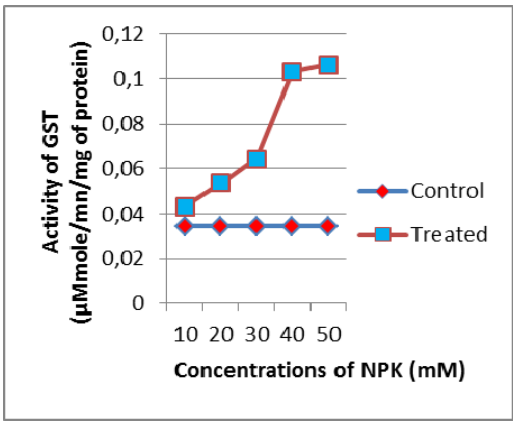

Fig. 3 NPK effects on changes in the activity of glutathione S transferase in Mosses (Treatment 3 days)

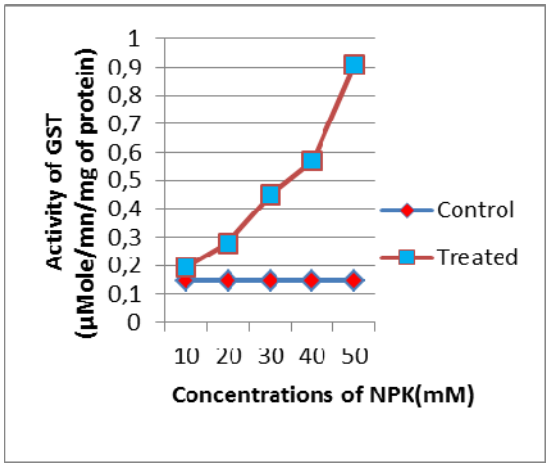

Fig. 4 NPK effects on changes in the activity of glutathione S-transferase in Mosses (Treatment 7 days)

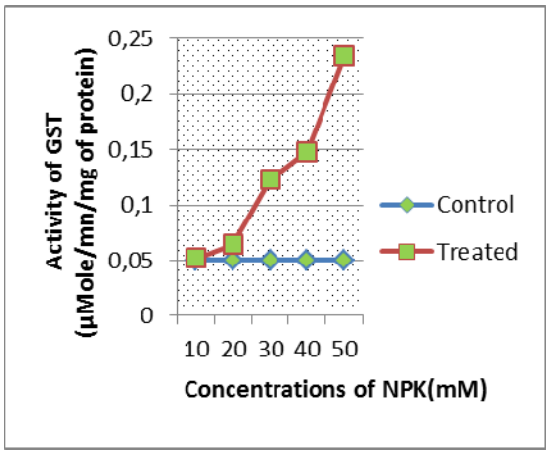

Fig. 5 NPK effects on changes in the activity of glutathione S-transferase in Lichens (Treatment 3 days)

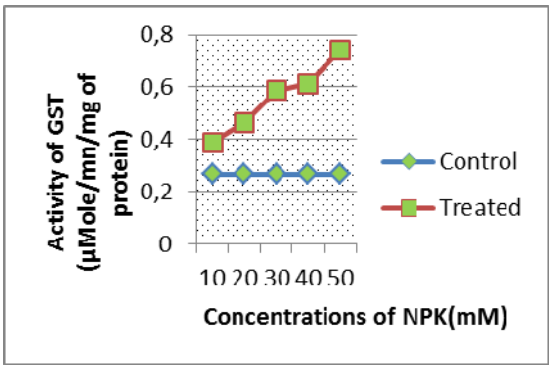

Fig. 6 NPK effects on changes in the activity of glutathione S-transferase in Mosses (Treatment 3 days)

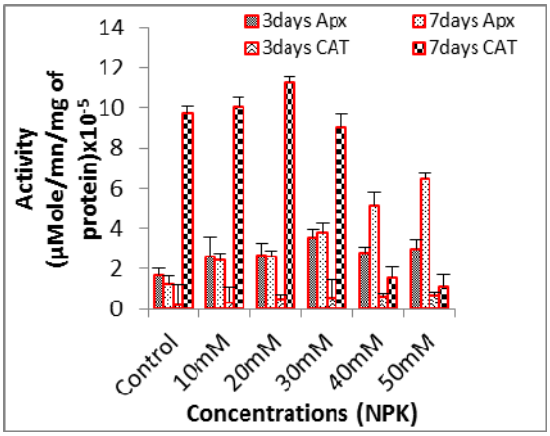

Fig. 7 Changes in catalase activity and ascorbat-peroxidase in *Leucodon sciuroides* treated with increasing concentrations of NPK

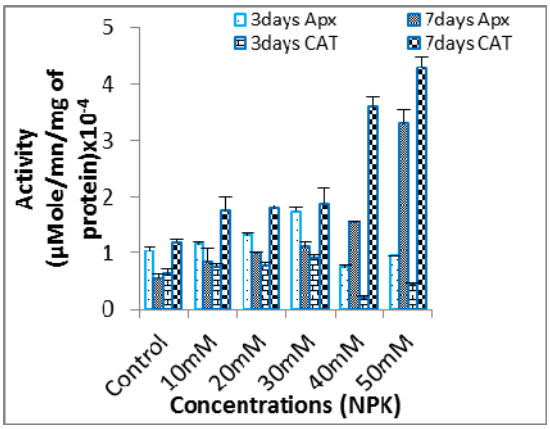

Fig. 8 Changes in catalase activity and ascorbat-peroxidase in *Ramalina farinacea* treated with increasing concentrations of NPK

IV. Discussion

In the current studies of atmospheric chemical bioaccumulation of pollutants, three major types of organisms are used: lichens, mosses and higher plants. There are two approaches: passive and active. The first is to collect individuals naturally present in the study area, the second exhibit on selected sites of individuals previously cultivated under standardized conditions or harvested or uncontaminated environments [16]. Depending on environmental problems, it is possible to combine different types of vegetable matrices for the purpose of integrated surveillance. Our work has been to measure biomarkers enzyme (GST, CAT and APX) and nonenzymatic (GSH and MDA) that tell us the state of stress due to the presence of a xenobiotic (pollutant) .The induction of antioxidant enzymes of plants under stress conditions is often reported [17], [18].

TABLE I
EFFECTS OF INCREASING CONCENTRATIONS OF NPK ON CHANGES IN MALONDIALDEHYDE IN "*LEUCODON SCIUROIDES*" AND "*RAMALINA FARINACEA*"

MDA Rate of Malondialdehyde **(µMole/mg of protein)**	3	7	3	7	**(Days)**
	Mosses		**Lichens**		
Control	0.245±0.004	3.434±0.110	0.176±0.014	0.192±0.019	
10mM(NPK)	0.285±0.013	3.510±0.178	0.350±0.030	0.477±0.041	
20mM(NPK)	0.514±0.018	4.110±0.075	1.97±0.320	0.758±0.038	
30mM(NPK)	0.892±0.040	1.860±0.030	2.16±0.670	0.901±0.093	
40mM(NPK)	1.469±0.147	1.360±0.020	4.09±0.040	2.450±0.065	
50mM(NPK)	0.814±0.018	6.440±0.075	0.651±0.110	1.430±0.080	

Assay of GSH in mosses and lichens treated with NPKs suggests the involvement of this element in the detoxification of reactive oxygen species (ROS) generated by this xenobiotic (Figs. 1 and 2). This result is confirmed the observations reported by [19]. A decrease in glutathione content is mainly related to a decrease in the activity of the glutamyl cysteine synthetase, the latter involved in the biosynthesis of GSH. Our results are also consistent with those of [20]-[22] in which the GSH level is decreased with increased tolerance to the accumulation of pollutant for low concentrations and also with those observed by [23]-[25] where the level of GSH decreases in response to stress induced by heavy concentrations of the pollutant.

Our results show a significant increase in the rate of GST, as well as in mosses in lichens (treatment 3 and 7 days) in the presence of NPKs (Figs. 3-6), this increase is a response to oxidative stress caused by the presence of xenobiotic in the plant cell. Biotransformation enzymes are among the first to respond to the presence of a xenobiotic in a living organism [26]. This increase indicates a high rate of conjugation of atoms of NPKs with glutathione. The GST is induced by many compounds, some of which are also responsible for the induction of cytochromes P450. The monoxygenases of cytochrome P450 can metabolize certain organic contaminants [27]. Our hypothesis is that induction of GST enzyme system can be explained by the entry of Xenobiotics (NPKs) in plant cells (mosses and lichens) and induction of detoxification system. Plant cells are able to protect their lives through the use of enzymatic and nonenzymatic mechanisms: SOD, CAT, Peroxidases (APX and GPX) and glutathione [28]. The assumption of Modenesi [29], explains the relationship between oxidative stress and the activity of the peroxidase ascorbat in plants (lichens), by increasing this activity as first line of defense [30].

Peroxidases are hemoproteins with heme prosthetic group: proto porphyrin IX ferro, 3 [31]. These are glycoprotein oxidoreductases that catalyze the oxidation of many organic and inorganic compounds by hydrogen peroxide (H_2O_2). APX activity, an enzyme important in the defense system, induces a response to different concentrations of NPKs among mosses and lichens and for the two treatments (3 and 7 days) (Fig. 7 and 8). The APX protects the cell against oxidative damage by removing toxic H_2O_2, released in chloroplasts, cytosol, mitochondria and peroxisomes of plant cells [32]. The catalase is localized mainly in peroxisomes and mitochondria, is also involved in the degradation of H_2O_2 generated by the xenobiotic [33]. In our work, we find that in lichens, catalase activity increases at low concentrations NPKs (10, 20 and 30mM) and then dropped at high concentrations (40 and 50mM). The same results were found, as well as in mosses lichens in the activity of APx (treatment 3 days). This could be explained by the fact that at low concentrations NPKs there trigger detoxification systems that for the most part consist of mainly the enzymes catalase. This allows the plant to tolerate and adapt to xenobiotic thus resulting in an increase of these enzymes (CAT). NPKs to high concentrations of the systems in question are outdated and enzymes are completely inhibited this is in perfect agreement with the work of [34], [35]. However, the activity of both enzymes (CAT and APx) increased in a dose-dependent in mosses (treatment 3 days) (Fig. 7) and in both species during treatment (7 days) (Fig. 7, 8), which can be explained by the fact that detoxification systems are sensitive to the highest concentrations of NPKs. These results are consistent with those obtained [36] where they exposed for 2 and 7 days an aquatic moss *Fontinalis antipyretica* five different concentrations of heavy metals (Cu and Zn). The results show maximal activities of CAT and APx with the highest concentration (1000µM).

MDA is a potent alkylating agent capable of reacting with biological macromolecules. The dosage of this compound has therefore some interest in plants subjected to multiple infections [37].

On our results, the values for the levels of the substances: MDA reacts with thiobarbituric acid (TBA); indicate that they increase with the addition of NPKs in the control medium. Indeed, the content of Mosses and lichens in these substances is higher in both plants and with both treatments (3 and 7 days) in the presence of (NPKs) (Table I). This content increases with increasing concentration NPKs. Except for the maximum concentration 50mM which has low levels of these substances compared with controls.

Comparing these results, we note that the mosses and lichens accumulate less of these substances in the presence of the maximum concentration of NPKs. The dosage of substances MDA level mosses and lichens grown in the presence of various concentrations NPKs and control shows that, on the latter, the sample accumulates less of these substances, unlike the treated samples accumulated many of these substances indicating a strong membrane lipid peroxidation. This strong peroxidation is due to a large dismutation of O_2, one of the active forms of oxygen [38]. Decreased lipid peroxidation in control due to the absence of NPKs translated by [39] a decrease oxidative effect EAOx (reactive oxygen species). This is evidence that the plant has

benefited from NPKs to avoid or reduce the damage caused by the accumulation of EAOx. This confirms the genesis of a state of oxidative stress in thalli (lichens and mosses) processed by the NPKs. Indeed, the fertilizer can cause membrane lipid peroxidation by catalyzing the formation of free radicals or reactive oxygen species ($1O_2$, O_2-, OH, H_2O_2, HOO.) Which attack the unsaturated lipids leading to the release of hydroperoxides (Rom., ROO., RCOO., etc ...) very harmful to cellular components [40]-[42].

V. Conclusion

To explain these physiological differences (favoring the development of mosses and lichens, we hypothesized that they were related to oxidative stress, and we analyzed the activity of some antioxidant enzymes in related to the amount of NPKs added to the culture medium. Among the mineral elements, nitrogen plays a very important role in its availability and its chemical formulation. Indeed, the presence of nitrogen (reduced or oxidized) affects development, morphogenesis, anatomical structure, the chemical composition of tissue, cellular and molecular machinery plant.

Any imbalance of nitrogen the plant can submit to a situation of nutritional stress responsible for changes in the internal hormonal balance. Plant metabolism, growth and development can be affected by the genesis of active forms of oxygen (FAOx) [Forms active oxygen] such as superoxide ion-O_2, the singular form of the oxygen O_2-, hydrogen peroxide (H_2O_2) or the hydroxyl radicals OH. The accumulation of FAOx alters cellular components, causing either a direct inhibition of enzymes or reactions of protein oxidation and peroxidation of membrane lipids. To cope with such damage, the plant can use its systems antioxidant defense type enzyme (SOD, CAT, POD, APX, etc..) and nonenzymatic (ASC, GSH, vitamin E, carotenoids, flavonoids).

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2. 2.Advances in Environmental Biology, 6(5): 1823-1833, 2012 ISSN 1995-0756.
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