Toxic Effect of Fertilizers on Inferior Plants Resed as Biological Models

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Abstract

The inopportune throws out of diverse substances in the atmosphere, constitutes without any doubt the obvious of environmental pollution by man. Among these substances, we are interested in the NPK (nitrate –phosphate-potassique).Nitrate fertilizers widely used in farming in our region - Annaba located in the eastern part of Algeria – and manufactured in the same region. In fact, the excessive fertilization, the intensive spreading of animal faeces and the industrial pollution are the accumulation sources of nitrate in vegetables, drilling and the underground waters.

The treatment by NPK affect the respiratory metabolism of mosses as well as the measure of the consumption of the oxygen shous the abviousness contrasted with a dampening of respiration but also of the photosynthesis.The perturbation of the respiration and photosynthesis of mosses can explain the degradation of the plant material and the disappearance of certain species from our ecosystem.

The effect of NPK indicate also the perturbation of enzymes antioxidants fonctions : GSH and GST.

Keywords: NPK, mosses, Cytotoxicity tests, respiratory and photosynthetic metabolism, Biomarkers; Antioxidant enzymes ,GSH,GST.

1. Introduction

Bryophytes are particularly suitable organisms for the study of metal and organic pollutants. They owe this to their anatomical efficiency (high ratio surface / volume or surface area / mass, no waxy cuticle, of conducting vessels and real root system, easy to identify the annual growth) and physiological (photosynthetic activity continues at year round). They are therefore subject to the impact of pollutants in both dry depositions. Bioaccumulation of pollutants in plant species is an indicator of exposure. Indicators of effects of these pollutants can also be measured; they may be more defined, especially in the form of various biochemicals or physiological parameters (biomarkers) [1].

2. Methods

2.1. Sampling procedure of the lower plants

The samples of Mousses (species *Leucodon sciuroides)* were taken in the area of Séraïdi, located at 850m above the sea (Annaba,Algeria). Our choice was made on this area because it is a zone considered as not polluted.

2.2. Tests of cytotoxicity for the moss

NPK fertilizer was tested with four concentrations:10 ,20 ,30 and 40 mM. The solutions prepared with the various concentrations of NPKs are used for the imbibition of the samples of mousses. Approximately 1g of thallus was soaked in 100 ml of solution during 3 days [2].

2.3. Determination of Glutathione (GSH) and activity Glutathione S-transferase (GST)

The glutathione was assayed by the method of [3], based on measuring the absorbance of the 2-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 [4]. It is based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene).The GSH and GST biomarkers are expressed in μm/mg of protein. The protein level was measured according the method of [5].

2.4. Proportioning of chlorophyls

The extraction of chlorophyls at summer was carried out according to the method of [6]. The formula related to solvent, enables us to calculate the values of chlorophylls [7].

2.5. Polarographic study

The apparatus used is an electrode with oxygen (HANSATECH) which allows the measurement of the production of the oxygen uptake during a reaction. Its sensitivity makes it possible to detect concentrations of about 10μM [8].

2.6. 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 [9].

3. Results

After 3 days of treatment, we found that glutathione-S-transferase tends to increase a dose-dependent manner. This increase was highest in the treaties with 40 mM concentration where the rate is: (0.103 (± 0.003)) μmole / min / mg of protein)(Fig.1).According to Fig.2, we find that glutathione levels decreased dose-dependent manner. Thus at 40 mM concentration, the GSH level is low (16.09 (± 0.49)) μmole / mg of protein) compared to the control of which is: (31.27 (± 0.21)) μmole / mg of protein).

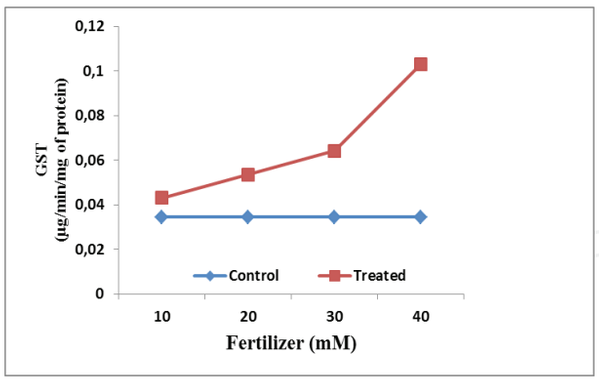

Figure 1. Evolution of GST activity according to the fertilizer concentrations (P ≤ 0,001).

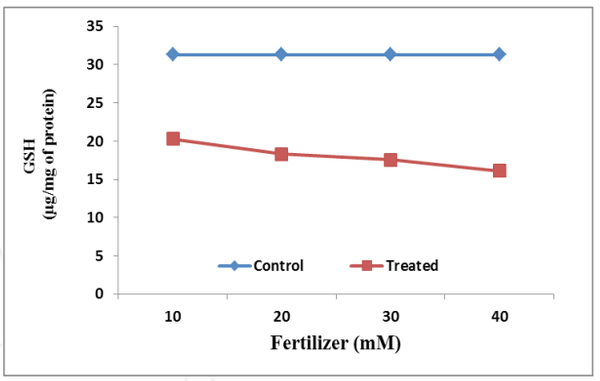

Figure 2. Evolution of GSH based on fertilizer concentrations (P ≤ 0,001).

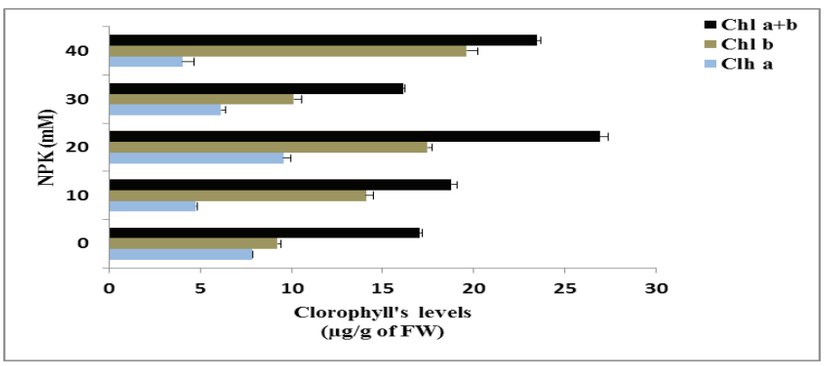

Figure 3. Changes in chlorophyll (a, b, a + b) in Leucodon sciuroides treated by different concentrations of NPK.

Fig. (3), highlights the changes in rates of chlorophyll *a, b* and (*a* + *b*) as a function of increasing concentrations NPK.Statistical analysis revealed a significant difference between control and treated with the concentration (30 mM) for (Chl *b*) (P ≤ 0.05), while very highly significant differences for all treated and all concentrations (10,20, 30.40 mM) (Chlorophyll *a, b* and *a* + *b*) (P ≤ 0.001) compared with controls always.

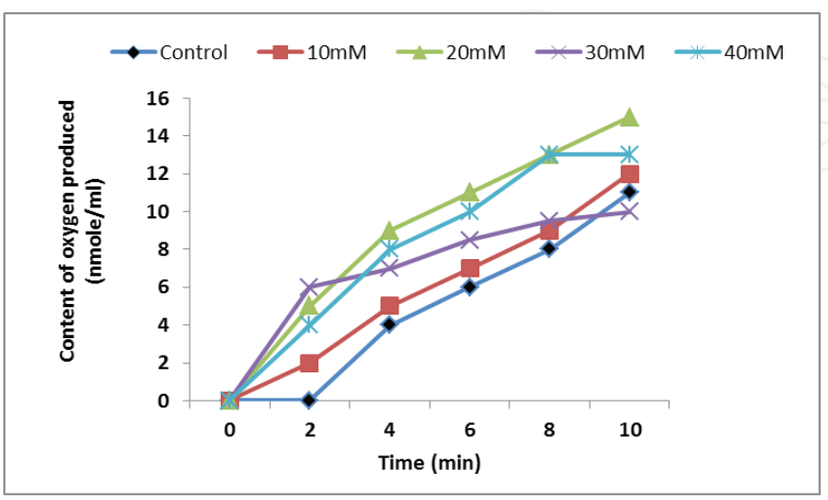

Figure 4. Effects of NPK on photosynthetic metabolism of Mousses (Leucodon sciuroides) (P ≤ 0,001).

This figure (4), illustrates the effect of different concentrations on the photosynthetic metabolism NPK foams where there has been a release of O_2 in the medium for control samples and for samples treated with the four selected concentrations. 0n a marked increase in the amount of O_2 produced in the middle as the fourth minute of recording for control samples. The minimum of this amount was recorded at the time (10min) for the sample treated with 30 mM concentration, which reached 10 nmol O_2 / ml (Lower than the control: 11 nmol O_2 / ml). While the sample treated with 20 mM concentration shows the highest amount produced until the time (10 min) or 15 nmol O_2/ml.On samples treated with other concentrations (10 and 40), have produced higher amounts of oxygen to those of the control resulting in stimulation of photosynthesis.

Time (min) Mousses	NPK treatments (mM)				
	0	10	20	30	40
0	340.00	340.00	340.00	340.00	340.00
2	340.00	332.00	328.00	324.00	320.00
4	355.00	324.00	316.00	308.00	300.00
6	330.00	316.00	304.00	292.00	280.00
8	325.00	308.00	292.00	276.00	260.00
10	320.00	300.00	280.00	260.00	240.00

Table 1. Oxygen consumption (nmole/ml) in moss response to the NPK treatment (mM) ($P \leq 0{,}001$).

The observation of the table (1) shows that the foams have a witness who starts breathing 340nmole O_2 and reached 320 nmol O_2 after 10min, the oxidation rate is an average of 2 nmol O_2 / min.Ce treatment causes an acceleration observed from the 2nd minute especially in samples treated with 40 mM concentration where the rate of oxidation is 10 nmol of O_2 / min). Indeed, this speed is about 4, 6 and 8 nmol of O_2 / min, respectively, in samples treated with 10, 20 and 30 mM of NPK.

4. Discussion

We propose in this work to proceed with the demonstration of the effect of NPK on foams, where we found a decrease in dose-dependent manner in the presence of GSH NPK. This condition can be explained by the direct connection of glutathione to the atoms of xenobiotic (NPK) as glutathione has a carboxylic acid group, an amine group, a group sulfhydryl (-SH) and two bypass likely peptide to be involved in reactions with other atoms. Its functional group-SH would then play an important role in binding to the xenobiotic [10].Our results agree with those of [11] and [12] in which the GSH level is decreased with increasing tolerance the accumulation of the pollutant for low concentrations .Our results show a significant increase of GST, in mosses in the presence of NPK; this increase is a response to oxidative stress caused by the presence of xenobiotics in the plant cell. The biotransformation enzymes are among the first to respond to the presence of a pollutant in a living organism [13]. This increase indicates a high rate of conjugation of atoms

NPK with glutathione. Our hypothesis is that induction of GST enzyme system can be explained by the entry of Xenobiotics (NPK) in plant cells (foam) and induction of detoxification system.

The other aspect of our work was to measure the mean levels of chlorophylls *a, b* and (*a* + *b*), parameters that can tell us a possible state of stress due to the presence of a pollutant in mosses. In general, chlorophyll appears to be affected by the xenobiotic (NPK). This perturbation in the mean levels of chlorophyll *a, b* and (*a* + *b),* in these plants, explains the attenuation of photosynthetic activity. Our results agree with our previous work [2], which have demonstrated a disruptive effect of nitrate of ammonium on the biosynthesis of chlorophylls. Our results are quite revealing, and the NPK causes a stimulation of photosynthesis in mosses, is excessive production of oxygen in the culture medium with a clear stimulation of respiratory metabolism.

Air pollution exposes plants to various forms of nitrogen that can be highly toxic (nitrogen dioxide, ammonia and ammonium). Among the reactions to the toxic effects of these compounds include: defoliation, training of larger cells thin-walled, yellowing, the lesions on some organelles of the plant and the reduction of drought resistance [14].The most important direct effect on vegetation results from the interaction of these various forms of nitrogen with other pollutants and impaired balance with other nutrients.

5. Summary and conclusion

We can conclude that the NPK disrupts the photosynthetic metabolism and respiratory mousses. Our results are in perfect agreement with our previous work [2] and [15] which have demonstrated a stimulation of photosynthesis and respiration in these plants. But in higher plants one of the mechanisms of defense against air pollution is indeed a decrease in respiration and photosynthesis.

6. References

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