American-Eurasian Journal of Toxicological Sciences 1 (2): 69-73, 2009
ISSN 2079-2050

Cellular Response of a Pollution Bioindicator Model (*Ramalina farinacea*) Following Treatment with Fertilizer (NPKs)

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Abstract: Our work concerns the evaluation of toxicity of the NPKs fertilizer (fertilizer widely used in agriculture) on lower plants: lichens (*Ramalina farinacea*) which have different qualities (sensitivity, ability to memorize pollution). NPKs used concentrations are 0, 10, 20, 30, 40 and 50mM. The results show a disturbance of all studied parameters which are the proline rate, the mean levels of total glucids, mean levels of total protein and enzyme activities (CAT) and (APX).

Key words: NPKs · Lichens · *Ramalina farinacea* · Proline · Protein · Soluble glucids · APX and CAT

INTRODUCTION

Since 1970, studies on the ecology of lichens have increased, including applied research in air pollution, due to the particular sensitivity of lichens toward the air quality because of their longevity, their growth, photosynthetic function continuously and their ability to accumulate [1].

Air pollution modifies the development of the lichen flora: depending on the intensity and nature of pollution, lichens exhibit morphological and structural changes and sometimes even disappear completely [2]. On the other hand, the intensive agriculture recommends the application of fertilizer nitrogen-based inorganic source of NOx [3]. The excessive intake of nitrogenous compounds in the environment is causing significant environmental damage [4].

The choice of lichens as bioindicators of pollution based on the structural and physiological properties that distinguish them from other plants. Their sensitivity to pollution is linked to a unique power to accumulate the pollutant, the lack of cuticle, stomata and specialized absorptive organs (root system) makes them highly dependent atmospheric absorption with d water, minerals, nitrogen and dissolved substances by the entire surface of the thallus. In fact, the lack of means to fight against pollution promotes their ability to accumulate [5,6].

In our work, we evaluate the toxicity of a fertilizer widely used in Algeria: the NPKs on biochemical parameters and enzymes involved in cases of stress and toxicity in higher plants, a bio-indicator model: the lichens of the genus Ramalina farinacea.

MATERIALS AND METHODS

Biological Material: The biological model used in our work is a lichen species: *Ramalina farinacea* harvested in an area considered highly polluted little: Séraidi, located 14 km west of Annaba (N-E Algeria) and 1000 meters. This region is characterized by the abundance of Zen oak (*Quercus faginea*) and cork oak (*Quercus suber*). The thalli of *Ramalina farinacea* are taken from the cork oak. The species chosen fruticose lichen is characterized by a thallus developed in length from a single attachment point [7] and is composed of branches narrow, tapering gradually and covered Sorelian marginal. [8]. the lichen thalli located on the trunks of trees are removed and stored in plastic bags tightly closed to limit water losses by evapotranspiration.

Treatment of Lichen: The lichens are treated with an NPK: nitrogen, phosphate and potassium sulfate: NPKs ($NP_2O_5K_2O$), grayish and presentation granular, soluble in water. It comes from the fertilizer company in Algeria called "FERTIAL, it is dissolved in distilled water at concentrations of 10, 20, 30, 40 and 50mM.

Preparation of Culture Medium: Preparation of culture medium was measured by the method of [9]. The different

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solutions corresponding to different concentrations NPKs are prepared and used for soaking samples of lichens. Approximately 4 g of thalli were soaked in 400ml of the solution for 07 days.

Biochemical Assays: Proline was determined according to the technique of [10]. The proteins were assayed by the method of [11] using BSA as standard. The soluble sugars were assayed by the method [12] using the anthrone in sulfuric acid medium.

Enzymatic Assays

Determination of Catalase Activity (Cat): We use for measuring the activity of catalase (CAT) the method of [13] and for the ascorbate peroxidase activity (APX) the method of [14].

RESULTS

Effect of treatment with NPKs on the rate of proline Figure 1 shows the results of treatment effects of lichens in different concentrations NPKs.

Figure 01 shows a dose-dependent NPKs on variations of the average rate of proline (highly significant correlation, r = 0.953, p = 0.003), we think that in samples treated with the highest concentration (50 mM) the rate proline (112.03 µg/100mg DM) (± 12.82) is almost twice as large as that observed in samples treated with concentrations: 30 mM and 40 which are respectively 63.27 (± 13.61) and 67, 14 (± 5.17) µg/100mg PF.

Effect of Treatment with NPKs on the Average Total Protein: Table 1 summarizes the results of changes in average total protein recorded in Ramalina farinacea subjected to treatment with NPKs.

The presence of NPKs stimulation induces dose-dependent average rate of total protein. (Positively correlated very highly significant r = 0975, p = 0.001). Our results show that there is a highly significant positive correlation between the average rate of proline and total protein (r = 0915, p = 0.01).

Effect of Treatment with Npks on the Average Total Sugars: Changes in average rates of total sugars obtained after processing samples NPKs are shown in Fig 2:

In Figure 2 we see that the average rates tend to increase with concentrations NPKs (highly significant positive correlation r = 0.95, p = 0.004). This increase is pronounced in samples treated with the highest concentration (50 mM) where the average rate of sugars obtained reaches µg/100mg FP 57.33 (± 2.67), whereas in control samples that rate does not exceed 14.7 mg / 100mg FP (± 3.24).

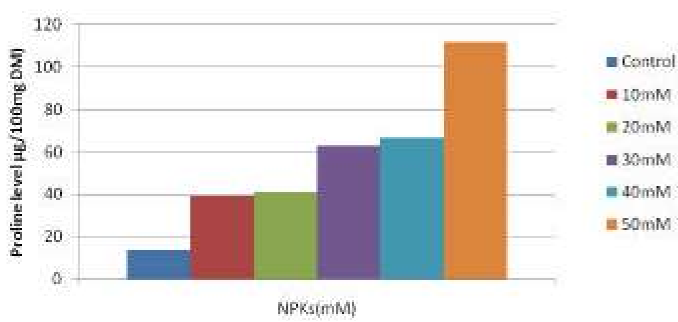

Fig. 1: Variation of the average rate of proline in *Ramalina farinacea* treated with NPKs (p <0.001), DM: dry matter

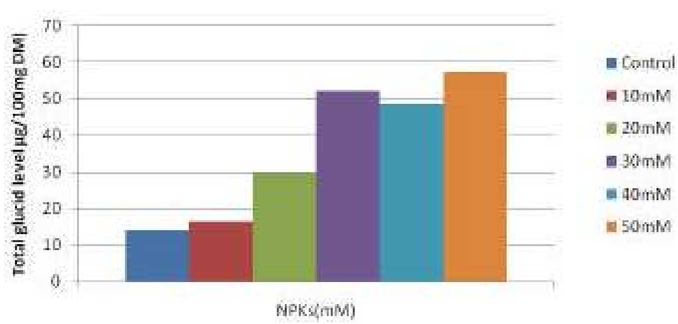

Fig. 2: Effect of NPKs on variations of the average rate of total sugars in *Ramalina farinacea* (p> 0.05)

Tab1e 1: Changes in mean total protein in Ramalina farinacea treated with different concentrations NPKs (P <0.001) (DM: Dry Matter).

NPKs (mM)	0	10	20	30	40	50
Proteins (μg/100mg DM)	28,62±0,65	30,85±0,65	31,51±0,40	33,05±0,96	37,04±0,72	37,57±0,017
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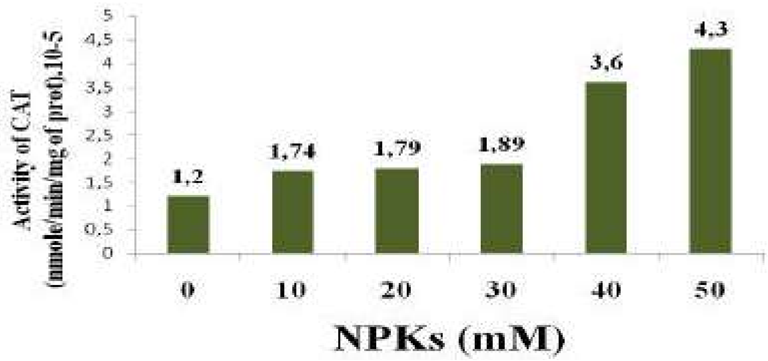

Fig. 3: Activity of Catalase in *Ramalina farinacea* treated with different concentrations of NPKs (p <0.001)

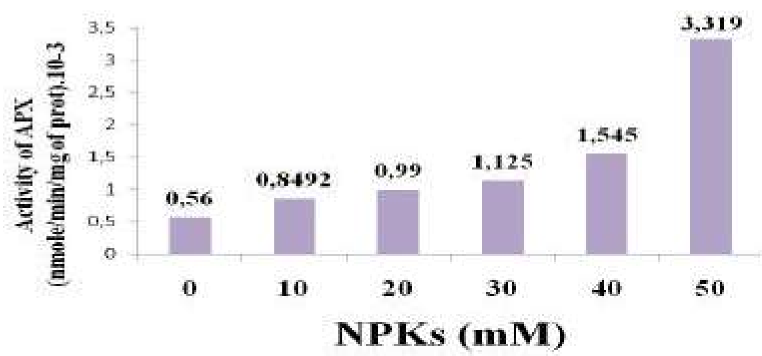

Fig. 4: Ascorbate peroxidase activity in *Ramalina farinacea* treated with different concentrations of NPKs (p <0.001).

Effect of Treatment on Npks Enzyme Activities Catalase (CAT) and Ascorbate Peroxidase (APX): The results NPKs effects on enzyme activity catalase (CAT) are shown in Figure 3 and ascorbate peroxidase (APX) in Fig. 4.

After 7 days of exposure Ramalina farinaceax to NPKs different concentrations, enzyme activities CAT and APX have significant disturbance (P <0.001). The CAT activity (Fig. 3) reached its peak with 50 mM concentration: (4.3±0187) x10-5; It is the same for the APX activity (3.31±0.26) x10-3. The correlations of CAT and APX activities with the concentrations used were respectively: (r = 0921, p = 0.009) and (r = 0861, p = 0.028). One notes from these results that there is a positive correlation between the activities APX, CAT and concentrations tested. Between both CAT and APX activities, there is a positive correlation very highly significant: (r = 0913, p = 0.000).

DISCUSSION

Lichens because many biological and structural features (no root, stem, leaf and cuticle, revival, growth rates very low) are widely used bioindicators in the detection and assessment of pollution including air [15-19]. However, few studies have addressed the impact of specific types of pollutants (fertilizers, pesticides... etc.) on these organisms. [9,20-24]. The bioaccumulation potential of lichens appeared to us a sensible approach to evaluate the toxicity of a fertilizer NPKs.

Our research aims to highlight the impact of pollution related to the actual use of this fertilizer on a species of lichen (*Ramalina farinacea*). The determination of proline in lichens is an effective way to detect a possible stress phenomenon. Proline is an amino acid known for its accumulation in a wide variety of organisms from yeast to higher plants exposed to abiotic stress, such as stress obtained by heavy metals [25] and by fertilizers [9]. Proline is considered a biomarker of metabolic stress in plants [26].

Lichens (*Ramalina farinacea*) metabolism is disturbed by the treatment NPKs This is highlighted by the accumulation of proline. Factors influencing the accumulation of proline, inhibition of oxidation due to mitochondrial dysfunction [27].

The results obtained dan our work are in perfect agreement with those of [28], who finds a significant increase of amino acids and proteins in bean (*Phaselous vulgaris* L. cv. Strike) Treaty with 24mm of NH_4NO_3. These results were supported by the work of [9], who found an increased rate of proline and total protein foams (*Leucodon sciuroides*) and in lichens (*Ramalina farinacea*) treated with different concentrations of NH_4NO_3 fertilizer and those of [29] on macrophyte Potamogeton crispus treated with different concentrations of ammonium.

Alongside this, our results show a high disturbance rates of total sugars obtained, this confirms the results of [30], showing that the process of concentration of soluble sugars in the leaf tissue of plants under stress reflects a characteristic phenomenon of adaptation of plants subjected to oxidative stress.

Finally, induction of antioxidant enzymes of plants under stress conditions is often reported [31,32]. The majority of plants treated with different concentrations of heavy metals (Cu, Cd, Pb, Zn.... Etc.) show an increase of enzyme activities: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate (APX) and guaiacol (GPX) peroxidases recorded compared to control samples [33]. Such work would support our results show that enzymatic activity called defense strongly stimulated following treatment with the NPKs. These enzymes are known to be involved in detoxification and in particular the elimination of ROS (Reactive Oxygen Species) generated during oxidative stress generated by the NPKs thereby protecting cells against these xenobiotics [34]. Indeed, recent work [35] conducted on aquatic moss Fontinalis antipyretica highlight of peak activity of CAT and APX in the presence of 1mM Cu.

Finally, our results confirm the sensitivity of lichens to pollutants (air) and also highlight the fact that they are also very dependent on the nature of the substrate in particular (NPKs) [1]. They also highlight the response to oxidative stress of a species of lichen Ramalina farinacea subjected to treatment with fertilizer NPKs and the possibility of using antioxidant enzymes of this species as biomarkers of air quality.

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