Study of Atmospheric Pollution emitted rated A plant of Fertilizers (Algeria) by the use of bioindicator plants: lichens

Khaldi Fadila, Berrebbah Houria, & Djebar Mohammed- Réda

Cellular Toxicology Laboratory, Biology Department, Annaba University, P.BOX :12, 23000, Algeria

ABSTRACT

In this study, we applied the method of transplantation that involves exposing in a polluted environment of branches covered in lichen thalli after collection in its natural environment of the control area (Séraidi). Subsequently, we transferred at different sites chosen previously. The lichen species was chosen for this transplant is: Ramalina farinacea. To better estimate the levels of air pollution in the Annaba region, it is important to correctly choose 5 sites distributed near the main source of NOx pollution (industrial source: Complex of fertilizer company in Algeria). We also noted variations of some parameters: rate of proline, FW / DW, levels of soluble sugars, total protein content. The experimental study also shows disruption of chlorophyll content (a, b and a + b). The use of antioxidant enzymes, namely glutathione-S-transferase (GST), glutathione reductase (GR) as biomarkers of air pollution has been shown using the species Ramalina farinacea (lichens) transplanted at 5 sites. Dosages of these biomarkers confirmed the disruption of lichens by the air pollutant (NOx) emitted by the industrial complex. The photosynthetic and respiratory metabolism following fluctuations at different sites.

Key words: Air pollution, NOx, lichens, Chlorophyll, Proline, FW / DW, total protein, GSH and GST, respiratory and photosynthetic metabolism.

Introduction

The air pollution by emissions of gas, dust, odor is a nuisance forms to which the opinion is, rightly, the most sensitive. To protect air quality, it is necessary to know the nature of pollutants namely dosing and treatment. This action is fundamental because it is that it relies on the development strategies of reductions in the amount of pollutants released into the atmosphere [8].

Research applied to air pollution using lichens as bioindicators have multiplied. Initial estimates of air pollution, by such plants were initially concerned acid pollution [24]. Then came the first work on the accumulation of radionuclides and heavy metals by lichens [22,33].

The problem is whether lichens, already likely to detect a fluoride pollution [51] or an acid cleanup can attest to the reduction of NOx abatement. The phenomenon of exsorption already demonstrated in favor of the retransplantation of samples in their original, unpolluted, suggests that lichens respond rapidly to an air pollution control [18].

The objectif of complex Annaba is part of the promotion of Algerian agriculture represents an indispensable tool for the country's independence in terms of food self-sufficiency.

This plant is located on the coast east of the city of Annaba. Therefore, it contributes to the relatively large levels of dust, NOx and NH3. It is interesting to use of indicator plants in order to estimate the impact of these pollutants on the environment.

Study refers to bio estimate of overall pollution after an incineration plant with lichens [38]. Bioaccumulation potential of lichens appeared to us a sensible approach to assess the impact of plant fertilizers on the environment [39].

I. Transplantation technique lichens:

I-History:

The first lichen transplants were performed there a hundred years in the city of Munich (ARNOLD 1891 to 1901). Since both techniques have been used extensively for transplants of epiphytic lichens.

The first technique was developed by Brodo (1961). It involves grafting a hard bark supporting a lichen on a phorophyte of the same species. In absence of trees, Schönbeck (1969) proposes to set hard bark on boards.
The second technique involves exposing in polluted thalli branches covered with epiphytes. The one we selected in our work.

2- Duration of transplantation:

Transplants were performed twice, on January 22 and February 21 (2008 and 2009) on five sites. The maximum duration of transplantation did not exceed 1 month. The first sample is taken on the day of the second transplant, and the latter the sample was performed on 22 March (2008 and 2009).

Transplanted thalli were always taken on the same day for each site and samples were harvested from donor sites (Séraïdi: located at 850 m above the sea (Annaba) the same day as the transplanted lichens. Our choice was made on this area because it is a zone considered as not polluted.

3- Strategy and places of transplantation:

The sampling strategy implementation for collecting lichens and the establishment of transplants is based on the distance of the industrial complex and the prevailing wind direction [25] (compass provided by the station of Annaba located approximately 5 km from the plant). (Figure 01).

Fig. 1: The Wind Rose (according to the meteorological Airport, Annaba).

Five transplant sites were selected: 400m, 800m, 1200m, 1600m and 2000m from the industrial complex (Figure 02).

4- Harvest of lichen species in situ:

A census carried out on lichen Greater Séraïdi allowed to choose the lichen species Ramalina farinacea. It is indeed well-developed and abundant and fruticose thallus is easily taking. Several thalli of Ramalina farinacea were harvested from the bark of trees of several stations under standardized conditions (height of 1.50 to 2 m in samples of soil) (Sémadi and Deruelle, 1993).

Sampling was conducted in January and February 2008 and 2009 in the town according to the sampling strategy defined above.

II. Methodes:

1. Determination of chlorophyll:

The method used for extraction of chlorophyll is the traditional method established by Holden,( 1975) which is a maceration of the plant in acetone.

2. Determination of proline:

For the determination of proline, the technique used is that of Monneveux and Nemmar, (1986).

3. Determination of total protein:

The proteins are assayed by the Bradford method (1976) using BSA as standard.

4. Determination of total sugars:

Soluble sugars were determined by the method of Schields and Burnett (1960) using anthrone in sulfuric acid.

5. Determination of report FW / DW:

Having collected fresh samples of thalli of Ramalina farinacea we weighed the samples before and after oven drying samples at 105 ° C for 48 hours. This report is established to obtain the value of the pollution index.

6. Determination biomarkers:

6.1-Determination of Glutathione (GSH):

The glutathione was assayed by the method of Weckberker & Cory (1988), 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.
6.2-Determination of activity Glutathione S-transferase (GST):

The glutathione S-transferase activity is performed by the method of Habig et al., [26]. It is based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a cofactor: glutathione (GSH). This activity is measured at a wavelength of 340nm by a spectrophotometer visible / UV (Jenway 63000).

7. Study of respiratory and photosynthetic metabolism:

The apparatus used is an oxygen electrode, HANSATECH type, which allows the measurement of the production or consumption of oxygen. The intensity of photosynthesis of lichens transplanted is measured by the oxygen electrode as for the respiration rate when the sample is hidden by a black box to speed up the metabolic process [15].

8. Statistical study:

The statistical analysis is performed by the Student “t” test that compares the averages of two populations using data from two independent samples, conducted using a data analysis software: Minitab (Version 16.0) [12].

Results:

The figure (03), highlights the changes in proline content, which increase with the distance from the complex, this corresponds to concentrations of pollutants (the most polluted area). The highest values are recorded at Site 5, although other sites have higher values than those of the control.

In 2008, no significant difference between the proline content in control samples and samples transplanted at the (site 1) (p> 0.05), while very highly significant differences were found for samples transplanted at other sites (2,3,4 and 5) (P <0.001).

For 2009, this analysis reveals significant differences between the rate of proline in the control samples and samples transplanted at the (site 1) (p <0.05), and very highly significant differences for samples transplanted at other sites (2,3,4 and 5) (P <0.001).

The figure (04), highlights the changes in total protein content in Ramalina farinacea, according to the transplant sites (2008 and 2009). We find that in transplanted samples at selected sites to farthest from the pollution source, the total protein tends to increase site-dependent manner. In 2008, the highest value is recorded at site 5 is: 28.39 µg / mg of FW compared to a site 1 which is: 16.22 µg / mg of FW. While, in 2009, the maximum value at the same site (5) is: 35.69 µg / mg of FW.

Statistical analysis revealed no significant difference between the levels of total protein in control samples and samples transplanted at the (site 5) (2008), and between control samples and samples transplanted at the (site 4) (2009) (p> 0.05), while very highly significant differences were revealed between control samples and samples transplanted at other sites (p <0.001).
Fig. 3: Variations in the rate of proline in *Ramalina farinacea* at different sites.

Fig. 4: Changes in levels of total protein in *Ramalina farinacea* at different sites.

Fig. 5: Changes in levels of total sugar in *Ramalina farinacea* at different sites.

The figure (05) highlights the changes in total sugar content in *Ramalina farinacea* according to the transplant sites (2008 and 2009). We find that in transplanted samples at selected sites to the further from the pollution source the rate of total sugar tends to increase site-dependent manner. The highest value was recorded at site 5 (2009), is: 193.99 µg / mg of FW compared to the control site which is: 148.32 µg / mg of FW.

In 2008, statistical analysis showed significant differences between the content of total sugars in control samples and samples transplanted at site 1 (p <0.05), while highly significant differences were revealed for samples transplanted at the (site 2) (p <0.01). As for the other sites (3, 4 and 5), the differences are very highly significant (p <0.001).

In 2009, this analysis reveals some very highly significant between the control samples and samples transplanted at 4 sites (2, 3, 4 and 5) compared to control (p <0.001). However, insignificant differences were found between controls and samples (site 1) (p> 0.05).

The figure (06) shows the ratio FW / DW, at 5 sites is small compared to the control. In 2008, statistical analysis revealed highly significant differences transplanted samples at site 1 compared to control (p <0.01). However, these differences are very highly significant between controls and samples sites (2,3,4 and 5) (P <0.001).While this analysis (2009), reveals very high significant differences between the rate FW / DW in controls and samples transplanted at 5 sites (P <0.001).
Fig. 6: Changes in the rate FW / DW in *Ramalina farinacea* at different sites.

Fig. 7: Changes in GSH levels in *Ramalina farinacea* at different sites.

The figure (07), shows variations in the GSH levels at 5 selected sites where you can see a decrease in the GSH samples transplanted at 5 sites compared to control. During the two years (2008 and 2009), site 5 shows the lowest rate is: (0.099 µM / mg of protein) compared to control that is: (0.706 µM / mg of protein) (2008) and this rate is: 0.602 µM / mg of protein compared to control (2009).

During the two years (2008 and 2009), statistical analysis revealed very highly significant differences in the rate of GSH between the control samples and samples transplanted at 5 sites (p <0.001).

Fig. 8: Changes in GST activity in *Ramalina farinacea* at different sites.

Figure (08), represents the variations of the activity of GST at 5 selected sites (2008 and 2009). Our results show that the GST activity of transplanted samples at different sites increases compared to control. There is also a marked decrease in the activity at site 4 and 5 compared to control (0.292 and 0.213) x10^{-4} µM/min/mg of protein (2008); (0.216 and 0.14) x10^{-4} µM / min / mg of protein (2009)).

In 2008, statistical analysis reveals some very highly significant between control and transplanted samples at 5 sites (p <0.001). While in 2009, this
the chlorophyll \( a + b \) increased at all sites compared to the control that have the lowest content (5.17 (2008) and 7.27 mg / g FW (2009)). The content of \( C_{h} a + b \) is maximum: 12.90± 0.03 (2008) and 16.90 ± 0.02 mg / g of FW (2009) at site 3.

Statistical analysis showed very highly significant differences between control samples and those transplanted at different sites and on (\( C_{h} a, b \) and \( a + b \)) (P <0.001). However, \( C_{h} b \) site 2 stores differences were not significant compared to control (p> 0.05). \( C_{h} a + b \) of the same site presents significant differences (p <0.05).

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Statistical analysis showed very highly significant differences between control samples and those transplanted at different sites and those for \( C_{h} a + b \) and also for \( C_{h} a \) sites 1,2,3 and 5 and \( C_{h} b \) site 3 (P <0.001). However, highly significant differences were recorded for \( C_{h} a \) site 4 and \( C_{h} b \) sites 2 and 5 (p <0.01). Statistical analysis also revealed significant differences of \( C_{h} b \) in site samples 1 and 4 (p <0.05).

The figure (09) below shows a marked increase in the amount of O2 produced in the middle, dice the second minute of recording for samples transplanted at 5 sites. The maximum amount of oxygen produced is recorded at 10min for the sample site that reaches 3: 16.30nmole O2 /ml.

Transplantation samples at 5 sites, causing an acceleration of the oxidation rate is: 14 nmol of O2 / min at site 3.

The maximum amount of oxygen produced was recorded at 10 min for the sample site that reaches 3: 20 nmol O2 /ml. We also note that the oxygen produced stored with the samples from both sites 4 and 5 eventually join after 10 minutes of recording, in contrast to samples from other sites. We can note that the control samples exhibit a respiration quite normal with O2 consumption proportional to the time of measurement, the oxidation rate is 8 nmol O2 / min. The latter is higher in samples from all sites is: 10 nmol of O2 / min (site1), 12.5 nmol of O2 / min (site2), 12 nmol of O2 / min (site 5) and 13 nmol O2 /
min (site4). Site 3 samples exhibit the maximum oxidation rate of 16 nmol O₂/min. Statistical analysis revealed very highly significant differences between photosynthesis of the control samples and those transplanted at five selected sites (2008 and 2009) (p < 0.001).

Discussion and Conclusion:

The Annaba region was particularly affected by air pollution, it was important to address this problem through the bioindication plants and not only by the sensor measurements. The detection and estimation of air pollution with lichens are possible insofar as these plants meet the conditions you would expect of biological indicators [56,46]. Besides the qualities:

- Remarkable sensitivity to pollution linked to an exceptional power to accumulate rapidly from the atmosphere;
samples, we also followed changes in the rate of
To confirm the state of stress induced in our
the environment.
The results obtained showed that the observation
of parameters measured in plant material occurs
naturally or transplanted was entirely appropriate for
a study monitoring the impact of a fertilizer plant on
the environment.
To confirm the state of stress induced in our
samples, we also followed changes in the rate of
proline, known as a marker of stress in plants. Our
results showed, increased levels of proline in
transplanted samples, are consistent with those of [7]
which recorded an increase of proline during stress in
the NH4NO3 in mosses and lichens. This
accumulation has been demonstrated in many
varieties of wheat and several types of stress
(osmotic, water, heat) [6,30,31,44]. Lagadic et al.,
[37] argue that an increase in proline may occur if
plants are subjected to oxidative stress created by air
pollution. For most pollutants (SO2, NOx and O3 ......
etc.), the symptoms of their effects include changes
in concentration of certain compounds (amino acids)
[40,5,19].
The main element of an effective indicator of
stress in the plant is the increase of proline. In our
work, we demonstrated a significant increase of
proline in lichens transplanted. Proline may play a
role osmoprotecteur [14,45], protein stabilizer
[53,36], an inhibitor of metals [20] and inhibitor of
peroxidation [41]. This rate increase can be
explained according proline [44], by an effect of
stress in plants. The synthesis of proline may also
involve a reduction of acidification of the cytoplasm
that maintains the ratio (NADP / NADPH) to a value
compatible with that of metabolism [27]. According
to Monneveux and Nemmar, [42], accumulation of
proline is associated with plant resistance to stress,
which could be one of the factors that best explain
the strategy of plant adaptation.
To confirm this stress in a polluted environment,
we looked to changes in total protein levels in
transplanted samples of lichens. According to
[48,49], in the presence of xenobiotics, the plant
increases protein synthesis of phytochelatins in
particular whose role is the detoxification of
xenobiotics, particularly metals. Stalt et al., [55]
reported that nearly 80% of the xenobiotic is
detoxified by this type of protein.Which are
consistent with our results that show an increased
rate of total protein in lichen thalli of the species
(Ramalina farinacea). According to [60], the
increase of enzymes of detoxification.
The other indicator of a disruptive effect of
stress in plants is the increased levels of total sugars.
According Deraissac [16], the process of
concentration of soluble sugars and/or proline in
leaf tissue of plants under stress is recognized as a
feature adaptation. This stress is due to air pollution.
Our results show that as one moves away from the
industrial complex, the rate of sugar tends to
increase, which implies a subsequent disruption of
the photosynthetic process in the species Ramalina
farinacea at contaminated areas (plus the site is
polluted, the higher the rate of total sugar is high).
This confirms that the rate of total sugar varies with
the distance from the pollution source, the nature of
the plant species, its vegetative stage and
morphology [4].
The report FW / DW is a good indication of the
state of the air quality, the more air is polluted, the
greater the development of the plant is disturbed.
Thus the ratio FW / DW in polluted areas will be
lower than that recorded at the lower pollution zone
(Semadi, 1989).Our results on the relationship fresh
weight / dry weight at different study sites, we can
conclude that the sites representatives a report low
compared to the control, specifically at site 1. The
proportion of fresh weight in relation to dry weight
(FW /DW) decreases with distance from the
pollution source has been determined by several
authors Woodbury and Hudler, [59]. These decreases
may be due to tissue damage plant material in
transplanted samples of lichens, which leads to
wilting and drying of thalli or water loss [56,1].
Air pollution can cause damage to plants and
implies the decrease of fresh and dry weight [10,59].
Indeed, Chakhparonia [11] showed in Arabidopsis
thaliana subjected to air pollution decreased fresh
and dry weight.
The induction of detoxifying enzymes of plants
under stress conditions is often reported [43]. Plant
cells are able to protect their lives through the use of
enzyme mechanisms (GST) and no-enzymatic (GR)
[2]. Contrary to previous results, the site of
transplantation 3 (2008 and 2009) present a
maximum activity of GST biomarquer subsequently
decreases parallel at site 4 and 5. From these results,
we can see that the site 3 is the most polluted site.
Induction of the GST enzyme system can be
explained by the entry of Xenobiotics in plant cells
(lichens) and induction of detoxification system [37].
Our results agree with those obtained with Dazy et
al., [13], where they found maximal activities of
GST and a significant decrease of GSH in samples
exposed to heavy pollution.
The metabolism of chlorophyll is certainly the
most visible biochemical process. Its biosynthesis
leaves appear green color of plants, while its
degradation is manifested by loss of pigment [21].
According to our results, we observed a marked
variation of chlorophyll according to the different
selected sites. These variations may be associated
with changes in the life cycle of the plant [21,23].
The results of experiments conducted by Knudson et
al., [35]; Bechulal and Ambasht [3], Renaud et al.,
From Deruelle and Lallement, [17], disruption of photosynthesis appears to be due to a change of chlorophyll resulting from a displacement of Mg molecules of chlorophyll by a pollutant. Our results are in perfect agreement with this work because they have shown a reduction in photosynthetic activity resulting in a maximum increase of chlorophyll concentrations observed at site 3. The photosynthetic metabolism following fluctuations at different sites. At high air pollution levels and different distances of the sites in relation to sources of pollution, photosynthesis remains active and still higher than the control. Our results are consistent with those obtained by [32]. On respiration, we noticed that the transplantation of lichen samples at different selected sites stimulates the respiratory activity. This increase in respiration of lichens is due to absorption of various substances and pollutants contained in air. The perturbation of the respiration and photosynthesis of lichens transplanted can explain the degradation of the plant material and the disappearance of certain species from our ecosystem.

According to work Bensoltane et al., [7]; Khaldi, [34], the penetration of xenobiotics within the lichen is the cause for triggering the phenomena of detoxification / biodegradation, this phenomenon involves the cytochrome P450 oxygenases, where the activity of oxygen resulted in respiratory drive observed.

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