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# The effect of air pollution on leaves and pollen of *Senecio bicolor* in the region of Annaba, Algeria

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## Abstract

The Annaba city (North-East of Algeria) is characterized by a very polluted atmosphere due of the road traffic and two big industrial complexes, which have become a danger for the fauna and flora. The present work consists of using the leaves and pollen of *Senecio bicolor* of *Asteraceae* family as a bioindicators to estimate the degree of air pollution in the Annaba region. We measured the pH, MF/MS ratio, and assayed chlorophyll and proline, from leaves taken from four sites, which three are in the vicinity of the three air quality control network stations installed in Annaba. The fourth is a control site in Seraïdi. At the same sites we exposed to air, also, 1g of pollen for 24 hours. The analysis of air quality control network showed the existence of pollution caused especially by the dust notably at station 2 (80.14 µg/m$^3$), far exceeds the WHO threshold (50 µg / m3). Our results showed that the amount of dust deposited on the leaf surfaces notably at station 2, causing significant disturbances in their metabolisms especially at the level of the chlorophyll content (40.22 – 20.52 µg/g), the content of the proline (24.38 – 98.89 µg/g) and viability of pollen (68.66 – 31.33 %) which can easily be observed compared to the control zone, which is far from the source of emission. In conclusion the bioindication by the leaves and the pollen seems to be effective to estimate the quality of the air.

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## Introduction

The pollution is an unfavorable modification of the natural environment, which can affect man and plants. It can also affect it by altering the physical environment, its recreational possibilities, or by undermining nature (Semadi, 1989).

Since 2002, the Ministry of Territorial Planning and the Environment has set up an urban air quality control network in the city of Annaba (North-East of Algeria). This control baptized SAMA SAFIA, is generally carried out by networks of sensors with physico-chemical characteristics, which measure the concentrations of various atmospheric pollutants.

The city of Annaba is one of the most polluted cities in Algeria because of the existence of big industrial complexes such as: The El Hadjar steel complex (Arcelor Mittal) and the fertilizer complex of phosphate (Fertial). In addition, it is known for its dense road traffic and overcrowding.

This prompted researchers to study the effects of pollution and air quality in this region (Semadi and De cormis, 1986).

Several techniques have been applied for the detection and evaluation of air pollution. Among these techniques, the reisphysico-chemical techniques which continuously measure the concentrations of the various pollutants "the case of "SAMA SAFIA" network" and biological techniques which use the plant or a part of the plant as a bioindicator: example lichens (Semadi, 1989), pollen grains (Wolters and Martens, 1987; Hasnaoui, 2000) and leaves (Soumen *et al.*, 1997; Gupta and Mishra, 1994).

The objective of this study is to estimate air pollution in the Annaba region using the leaves and pollen grains of *Senecio bicolor* species (Asteraceae family) as bioindicators of atmospheric pollution in order to characterize the impact of urban pollution on the environment and, on the other hand, the relation of the data recorded by the physico-chemical sensors of the SAMA SAFIA network during the period 2002-2004 with our biological results.

## Materials and methods

*The study area*

The study area is located in Annaba city (Northeast Algeria), between latitudes (36° 30) North and (37° 30) North and longitudes (07° 20) East and (08° 40) East. It covers an area of 1411.98 km² (Tarfaya, 2005). The sites of exposure correspond to the three stations of SAMA SAFIA, and a control site located in the *Seraïdi commune* (Fig. 1).

*Climatic conditions*

To present the climate of the study area, we used the climate data of the period (1996-2005) corresponds to the period of our study (Meteorological station - Airport - RabahBittat - Annaba) in the table 1.

The air quality monitoring network (SAMA SAFIA)

In 2002, the Wilaya of Annaba established an air quality monitoring network.

It provides an accurate knowledge of air quality and concentrations of major pollutants in the atmosphere. The objectives of the SAMASAFIA network are: To monitor continuously the ambient air quality, to inform and to sensitize the population and the authorities concerned.

*Vegetable material*

The plant material used is *Senecio bicolor* (Asteraceae family), it is an ornamental species, velvety-whitish, which grows in the Mediterranean littoral.

*Harvesting leaves and pollen grains*

From each station, we took about ten leaves at three different levels of the plant (the apical part, the middle part and the basal part).

The pollen grains arecollected by shaking the flowers with the fingers. About 1 g of pollen in powder is put in a bag of canvas, intended for exposure.

*The hydrogen potential of the leaves (pH)*

The used method is (Grodzinska, 1982) which consists to add 1 g of leaves in 10 ml of warm distilled water.

After stirring and filtration, the pH is determined by reading with the pH-meter apparatus previously calibrated, with three replicates in an Erlen Meyer of 150 ml.

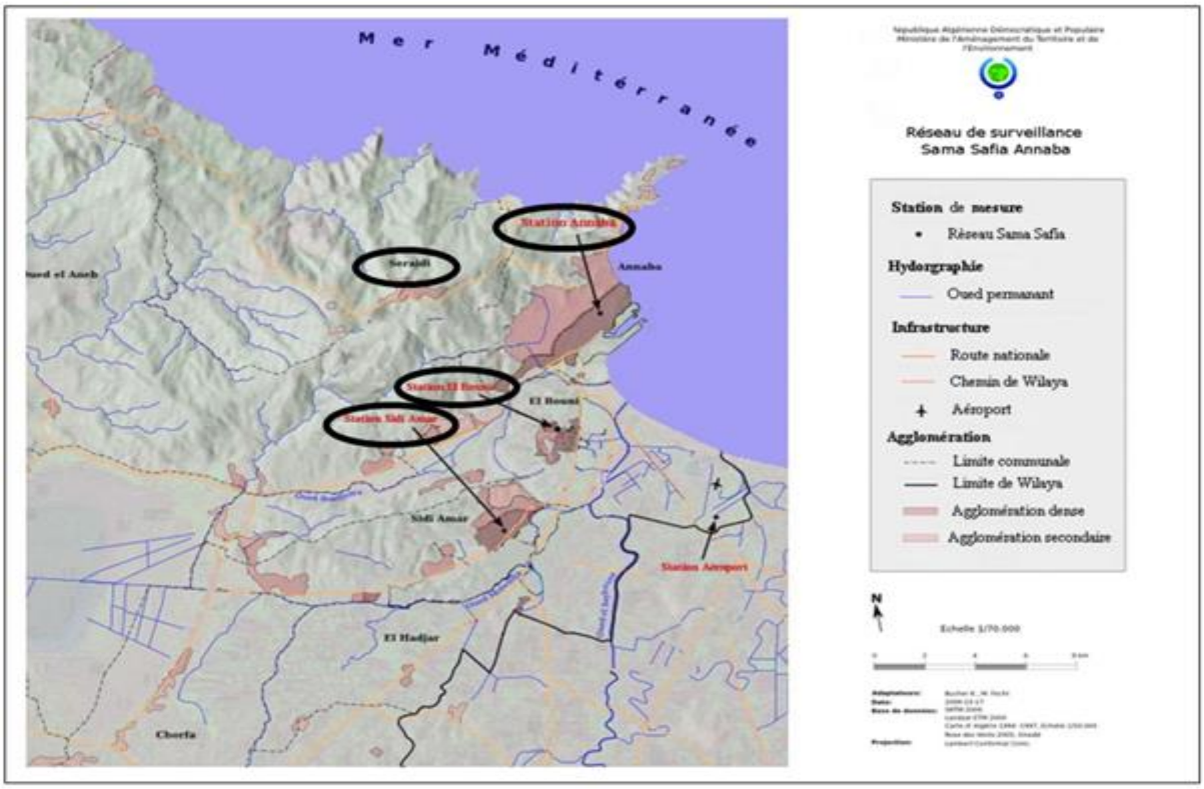


**Fig. 1.** Location of the measurement stations of the SAMASAFIA network and the control zone (Seraidi), (Source: SAMA SAFIA, Annaba).

Reference station (ST.0): located in a courtyard of a school in the town center of Seraïdi. It is considered unpolluted, located at an altitude of about 1000 m.

Station 1 (ST.1) in Annaba downtown.

Station 2 (ST.2) at the level of the municipality of El Bouni, distant about 6 km from Annaba downtown, near the fertilizer complex of Asmidal (Fertial).

Station 3 (ST.3) at the commune of Sidi Amar, located at 12 km from the city of Annaba center near the El Hadjar steel complex (Arcelor Mittal).

*The MF/MS ratio*

After drying in the oven at 105 ° C for 48 hours. The fresh material (M.F.) and the ratio MF/MS are calculated after a series of weighing using a precision balance.

*Leaf content of chlorophyll*

The extraction of chlorophyll from the foliar tissues is carried out according to the method used byHolden, 1975and is expressed in μg/g of fresh matter (M.F.) using a spectrophotometer.

*The proline dosage*

The proportion of proline expressed in μg/g of the fresh matter (M.F.) is determined by the method of (Monneveux and Nemmar, 1986).

*Study of pollen viability by staining test before and after exposure*

Pollen viability is tested on 100 pollen grains placed on slide in a drop of acetic carmine solution.

The pollen grains are observed under an optical microscope after 20 minutes with three replicates. The red-colored grains are considered viable and the non-colored grains considered as non-viable.

This is done before and after exposure to air at the four study stations. Pollen exposure is done in small canvas sachets with 1g of pollen on trees at 2 m of height in the direction of the prevailing winds for 24 hours.

*Statistical analysis methods of data*

The description of different variables studied for the Species *Senecio bicolor* is made by calculating the average (m), the standard deviation (s), the minimal (Xmin) and maximal (Xmax) values for each station. The variance analysis (ANOVA) of the general linear model (GLM) of Minitab software for data statistical analysis (Minitab Inc, 2016) is used to compare the averages among the four stations for each studied characteristic (Dagnelie, 2009).

It is considered that there are significant differences between the averages of the four stations when the probability value (p) is less than or equal to the risk $\alpha = 0.05$ ($p \leq \alpha = 0.05$); highly significant differences when $(p) \leq \alpha = 0.01$ and, very highly significant differences when $(p) \leq \alpha = 0.001$ (Dagnelie, 2009).The TUKEY test (Dagnelie, 2009) made possible to determine the stations of homogeneous groups by considered characteristic (Minitab Inc, 2016).

The STUDENT t test (Dagnelie, 2009) was used to compare the pollen grains viability averages of the species studied, before and after exposure to air for four stations (Minitab Inc, 2016).

## Results and discussion

*The hydrogen potential (pH)*

Tables 2 and 3 show that there are very highly significant differences between the pH averages of the stations. Fig. 2 of the TUKEY test shows an overlap of 3 groups of homogeneous stations.

**Table 1.** Climate Data for (1996-2005).

| Temperature (°C) | | | Rainfall (mm) | | | Moisture (%) | | | The prevailing winds (m/s) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mini. | Max. | M. | Mini. | Max. | M. | Mini. | Max. | M. | Mini. | Max. | M. |
| 11.22 | 26.12 | 17.92 | 3.55 | 127.3 | 59.78 | 70.36 | 77.85 | 74.57 | 3.5 | 4.21 | 3.86 |

Mini.: Minimum; Max.: Maximum; M.: Mean.

**Table 2.**The values of basic statistical parameters calculated on physiological and biochemical parameters of the species *Senecio bicolor*. The number of simples (n), the mean (m), standard deviation (s), minimum values (Xmin) and maximum values (Xmax).

| Variable | | Stations | n | m | s | Xmin | Xmax |
|---|---|---|---|---|---|---|---|
| pH | | ST.0 | 3 | 6,6800 | 0,0917 | 6,5800 | 6,7600 |
| | | ST. 1 | 3 | 5,910 | 0,392 | 5,470 | 6,220 |
| | | ST. 2 | 3 | 4,287 | 0,656 | 3,710 | 5,000 |
| | | St. 3 | 3 | 5,027 | 0,335 | 4,790 | 5,410 |
| MF/MS | | ST.0 | 3 | 2,720 | 0,324 | 2,450 | 3,080 |
| | | ST. 1 | 3 | 1,6000 | 0,1493 | 1,4300 | 1,7100 |
| | | ST. 2 | 3 | 0,757 | 0,253 | 0,530 | 1,030 |
| | | ST. 3 | 3 | 1,1867 | 0,1601 | 1,0300 | 1,3500 |
| Chlorophyll (a+b) | | ST.0 | 3 | 40,22 | 2,66 | 37,87 | 43,11 |
| | | ST. 1 | 3 | 33,04 | 3,14 | 31,19 | 36,67 |
| | | ST. 2 | 3 | 20,52 | 3,43 | 18,20 | 24,46 |
| | | ST. 3 | 3 | 25,01 | 4,52 | 20,87 | 29,37 |
| Proline | | ST.0 | 3 | 24.380 | 0,156 | 24,200 | 24,470 |
| | | ST. 1 | 3 | 72,22 | 9,48 | 65,97 | 83,13 |
| | | ST. 2 | 3 | 98.89 | 13,40 | 85,63 | 112,43 |
| | | ST. 3 | 3 | 79,86 | 8,57 | 74,10 | 89,70 |
| The percentageviability of pollen grains per color | Beforeexposure | ST.0 | 3 | 69,333 | 1,528 | 68,000 | 71,000 |
| | | ST. 1 | 3 | 52,67 | 5,03 | 48,00 | 58,00 |
| | | ST. 2 | 3 | 39,33 | 3,06 | 36,00 | 42,00 |
| | | ST. 3 | 3 | 44,33 | 2,08 | 42,00 | 46,00 |
| | Afterexposure | ST.0 | 3 | 68,667 | 1,528 | 67,000 | 70,000 |
| | | ST. 1 | 3 | 48,667 | 1,528 | 47,000 | 50,000 |
| | | ST. 2 | 3 | 31,33 | 3,06 | 28,00 | 34,00 |
| | | ST. 3 | 3 | 40,67 | 2,08 | 39,00 | 43,00 |

The first group consists of stations ST.0 (control) and ST.1, the second group consists of stations ST.1 and ST.3 and the third group consists of stations ST.2 and ST.3.

It is found that the station ST.1 is similar to the stations ST.0 and ST.3, but different from the station ST.2, and that the station ST.3 is also identical to the stations ST.1 and ST.2, but different from the station ST.0.

The station ST.0 has the highest mean pH (6.68) compared to other stations, and ST.2 has the lowest mean pH (4.28).

**Table 3.**Results of the analysis of variance (ANOVA) to criteria for species *Senecio bicolor*. The number of degrees of freedom (DF), the sum of squared differences (Seq SS), the middle square (MS), the observed value of the variable F Fisher (F) and the probability (p).

| Variables | | Source of variation | DF | Seq SS | MS | F | P |
|---|---|---|---|---|---|---|---|
| pH | | stations | 3 | 9.7632 | 3.2544 | 18.49 | 0.001*** |
| MF/MS | | stations | 3 | 6.3954 | 2.1318 | 39.23 | 0.000*** |
| Chlorophyll (a+b) | | Stations | 3 | 658.58 | 219.53 | 17.85 | 0.001*** |
| Proline | | Stations | 3 | 9036.9 | 3012.3 | 35.14 | 0.000*** |
| The percentage viability of pollen grains | Before exposure | Stations | 3 | 1556.25 | 518.75 | 50.20 | 0.000*** |
| | After exposure | stations | 3 | 2272.00 | 757.33 | 165.24 | 0.000*** |

*** : There are very highly significant differences.

**Table 4.**STUDENT t test results. Comparison of pollen grain viability averages, before and after exposure, for the four stations.

| Stations | Means | | $t_{obs}$ | P |
|---|---|---|---|---|
| | Before exposure | After exposure | | |
| ST.0 | 69.33 | 68.67 | 0.53 | 0.621 N. S. |
| ST.1 | 52.67 | 48.67 | 1.32 | 0.258 N. S. |
| ST.2 | 39.33 | 31.33 | 3.21 | 0.033* |
| ST.3 | 44.33 | 40.67 | 2.16 | 0.097 N. S. |

tobs: the observed value of the variable t of student, P: probability to evidence of significant differences, N.S: there are no significant differences between the two averages, *: there are significant differences between the two averages.

This station ST.2 (El Bouni) is known for its high amount of dust coming from numerous construction sites and the Fertial complex.

The decrease in pH can be explained by the dissolution of pollutants in the surface water of the cells, affecting the cellular pH (Chakhparonia, 1995).However, the deposition of dusts containing the heavy metals may, after the time of its dissolution, enter the plant. Thus, the cuticle can pass through the mineral elements (Ward, 1990).

*The MF/MS ratio*

According to Tables 2 and 3 there are very highly significant differences between the station averages, and Fig. 3 of the TUKEY test for the MF/MS ratio shows that the control station ST.0 constitutes a separate group with the highest value (2.720), followed by the stations ST.1 and ST.3 forming a single homogeneous group which overlaps with the third group composed of stations ST.2 (El Bouni) and ST.3 (Sidi Amar).

The latter two stations recorded the lowest values of (0.757) and (1.1867), respectively, due to the presence of pollutants from the Fertial and Arcelormittal complexes.

The decrease in the MF/MS ratio may be due to mesophyll tissue damage, leading to leaf wilting and drying or loss of water (Sharma, 1987). Air pollution can cause damage to plants and implies a reduction in fresh and dry weight (Braun and fluckiger, 1985, Woodbury and Hudler, 1994).

**Table 5.**Air Quality Monitoring Network Data (2002 - 2004) for the three stations (ST.1, ST.2 and ST.3).

| Stations | Station ST.1 (Town center of Annaba) | Station ST.2 (El Bouni) | Station ST.3 (Sidi Amar) |
|---|---|---|---|
| Dust (µg/m3) | 63.22 | 80.14 | 68.08 |
| NO (µg/m3) | 7.72 | 9.14 | 4.10 |
| CO (mg/m3) | 1.08 | 0.85 | 0.56 |

*The chlorophyll (a + b) contents*

According to Tables 2 and 3 there are very highly significant differences between station averages for chlorophyll (a + b), and Fig. 4 of the TUKEY test shows an overlap of three groups of homogeneous stations. The first group includes stations ST.0 and ST.1.The second group consists of stations ST.1 and ST.3 and the third group consists of stations ST.2 and ST.3.It is observed that the station ST.1 is similar to the stations ST.0 and ST. 3 but different from the station ST. 2 and, that the station ST.3 is also identical to the stations ST. 1 and ST. 2 but different from the station ST.0.This station has the highest mean chlorophyll (a + b) (40.22 µg/g MF) and is considered to be less polluted, while station ST.2 has the lowest mean chlorophyll (a + b) (20.52 µg/g of MF). This degradation of chlorophyll is manifested by the accumulation of pollutants at the level of ST.2 (El Bouni).

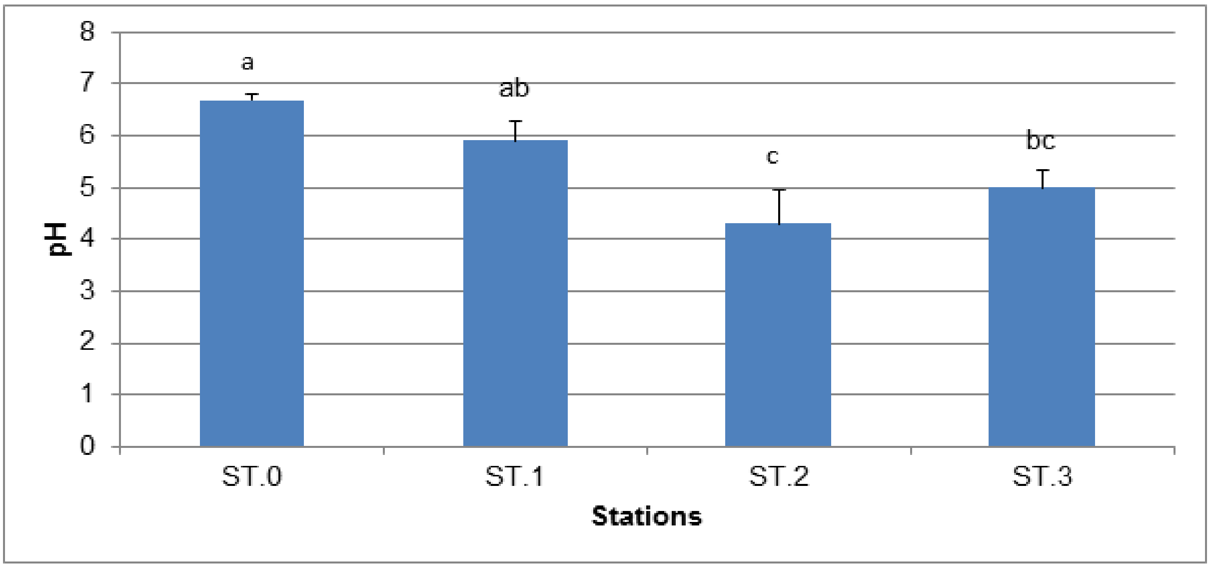


**Fig. 2.**Hydrogen potential in the different stations (stations with the same alphabetical letter constitute a homogeneous group according to the TUKEY test).

This is in line with our previous study of the *Rosa odorata* species, which showed a decrease in chlorophyll (a + b) at the same station (El Bouni) (Tlili, 2010).

Air pollution causes changes in photosynthetic activity and stomata's conductance (Garrec and Haluwyn, 2002).

It is known that all gaseous pollutants penetrate through the stomata to the intercellular spaces. They can change stomatal conductance directly by affecting guard cells, which alter photosynthesis (Chakhparonia, 1995). The results of the experiments carried out by Renaud *et al.*, 1998 have shown that plants subjected to atmospheric pollution show decreases in chlorophyll levels.

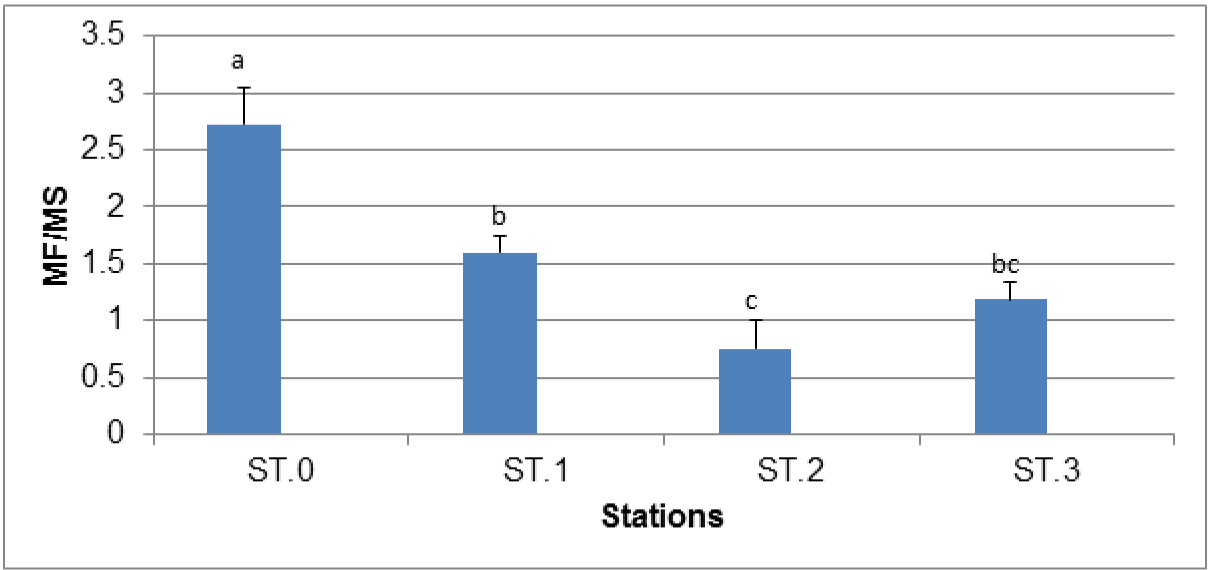


**Fig. 3.**TheMF/MS ratio in the different stations (stations with the same alphabetical letter constitute a homogeneous group according to the TUKEY test).

*The proline contents*

Tables 2 and 3 show that there are very highly significant differences between the station averages for proline, and Fig. 5 of the TUKEY test for the proline shows that there are three groups of stations obtained from TUKEY test. The first group is formed by station ST.0 with a very low value (24.38 µg / g of MF).The second group consists of stations ST.1 and ST.3.The third group comprises stations ST.2 and ST.3 which give the highest averages, respectively 98.89 and 79.86 µg/g of MF. Therefore, the stations ST.2 (El Bouni) and ST.3 (Sidi Amar) are the most affected stations.

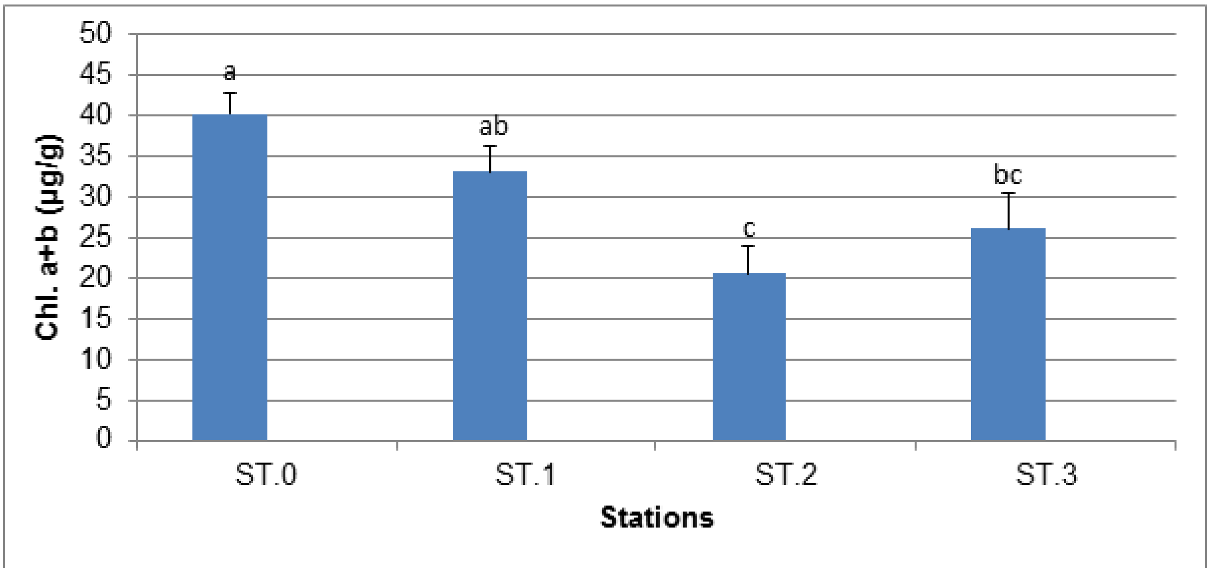


**Fig. 4.**Chlorophyll (a+b) content in the different stations (stations that have the same alphabetical letter constitute a homogeneous group according to the TUKEY test).

Lagadie *et al*., 1997 affirm that an increase in proline can be observed if plants are subjected to the oxidative stress created by atmospheric pollution. The increase of this parameter known as a biomarker stress (Panda, 2003; Ben Khaled *et al*., 2003; Abdul, 2004).

*The percentage of viability of pollen grains before and after exposure*

According to Tables 2 and 3 there are also very highly significant differences between the station averages for the percentage of viability before and after exposure. While Fig. 6A relating to the percentage of viability before exposure shows three groups of stations. Station ST.0 is the first group with the highest percentage (69.33%). The stations ST.1 and ST.3 represent the second group and the stations ST.2 and ST.3 form a third homogeneous group overlapping with the preceding group. The viability of pollen grains by staining was low before the exposure due to air pollutants from the three stations (ST.1, ST.3 and ST.2).

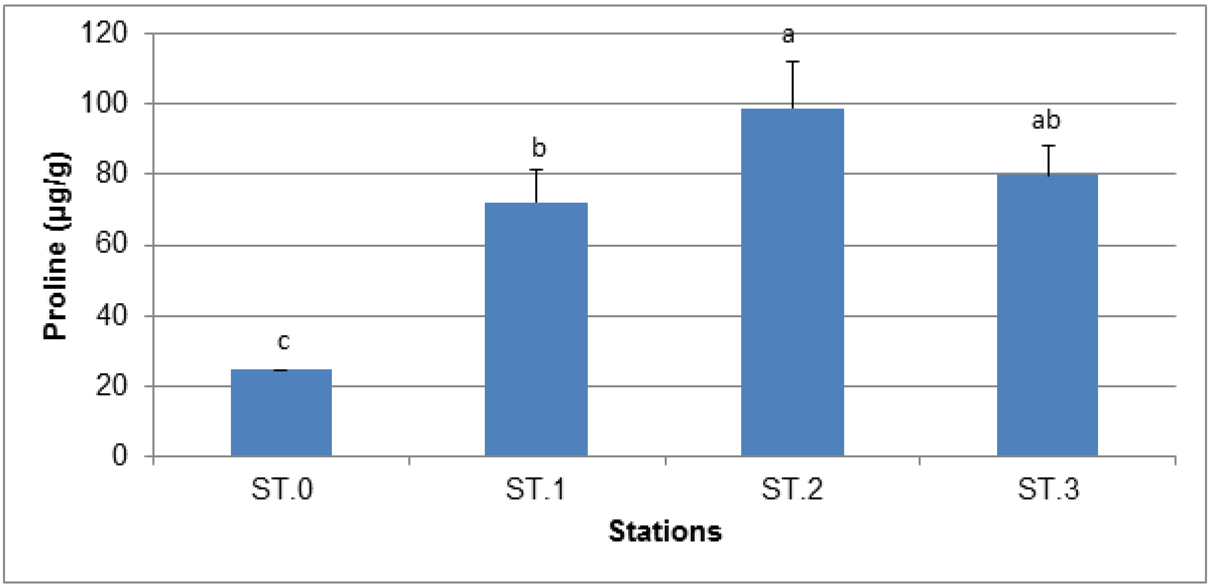


**Fig. 5.** Theproline contents in the different stations (the stations that have the same alphabetical letter constitute a homogeneous group according to the TUKEY test).

Fig. 6Brelating to the percentage of pollen grains viability after the exposure shows that each station forms a separate group. Station ST.2 has the lowest percentage (31.33%), and station ST.0 gives the highest percentage (68.667%). Thus, the percentage of viability decreased further due to pollen exposure to air.

Table 4of STUDENT t test results for independent samples, applied to pollen grain viability data before and after exposure, shows that there are just significant differences for station ST.2, while for the other stations no significant difference was recorded, therefore the ST.2 station represents the most polluted site because of its proximity to the Asmidal complex of phosphate fertilizers and many construction sites.

This is in agreement with our results of a study carried out on the species *Rosa odorata* (Tlili, 2010). It seems that the pollutants were fixed on the wall and the micro-channels of the pollens passing through the exine, which disrupts the osmotic exchanges (Cerceau-Larrival and Derout, 1988).

From the tables (2, 3, 4, and 5) and the figures (2, 3, 4, 5 and 6), we have found that all the parameters vary from one station to another.

The comparison of SAMA SAFIA network data with our results shows that the ST.2 (El Bouni) presents the maximum values for dust (80.14 g/m$^3$) and NO (09.14 ug/m$^3$), while ST. 1 (town center of Annaba) gives the highest CO value (1.08 µg/m$^3$).

The high value of NO at El Bouni (ST.2) can be explained by the exposure of the area to tributaries of the fertilizer complex (Fertial).

For dust, the value of 80.14 µg/m³ far exceeds the World Health Organization (WHO) threshold (50 µg/m³) (AIRFOBEP, 1999 - 2000). The same area is located next to a major road network (National Road 44) and characterized by many construction sites.

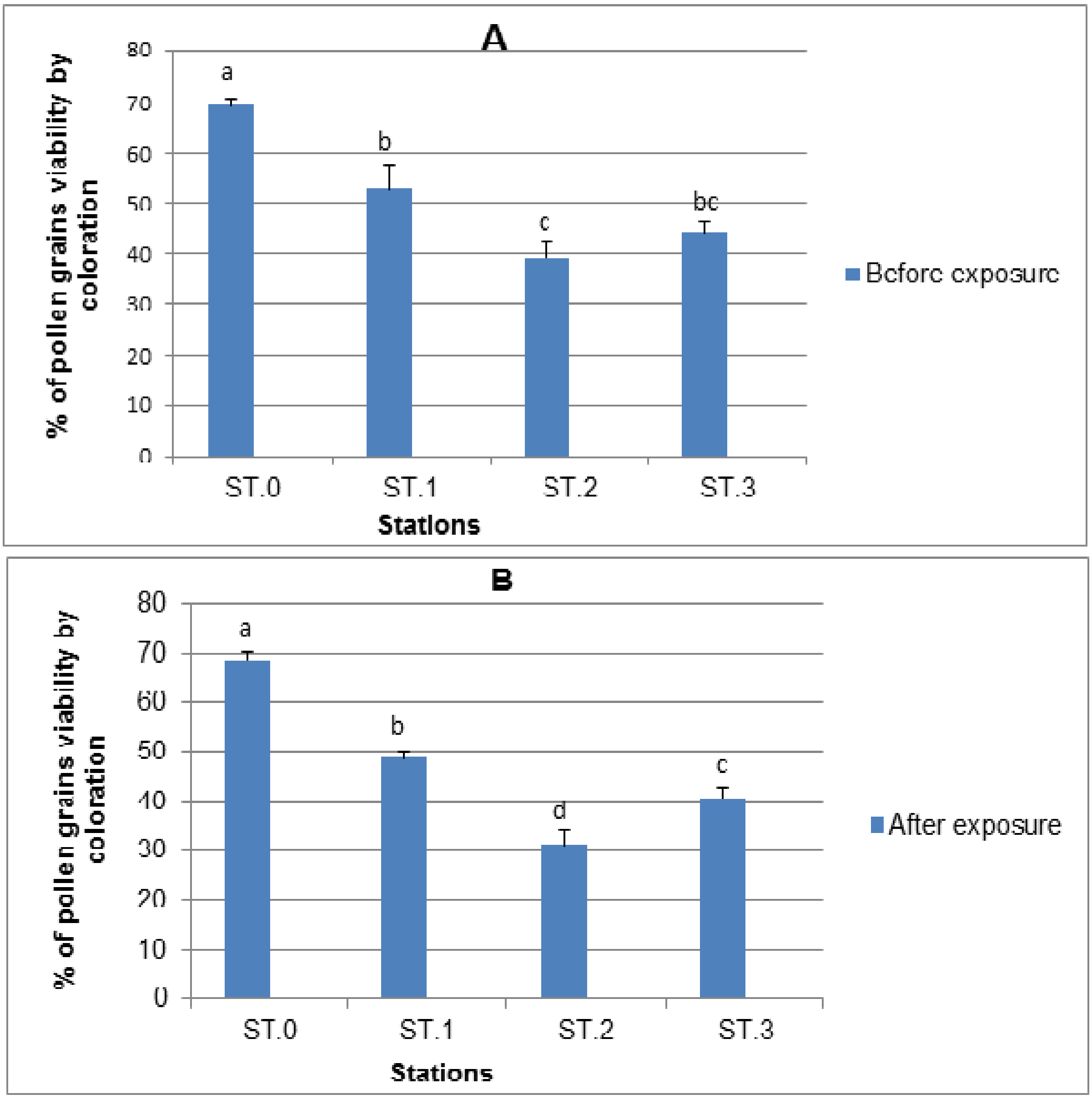


**Fig. 6.** (A and B). The percentage of pollen grains viability before (A) and after exposure (B) to air in the different stations (stations with the same alphabetical letter constitute a homogeneous group according to the TUKEY test).

On the other hand, the high value of CO in downtown Annaba (ST.1) can be simply explained by the wide circulation of vehicles whose number exceeds 15,000 vehicles/10 hours (Tarfaya, 2005).

The comparison of our results with SAMA SAFIA data presents a concordance between the studied parameters and values for pollutants: the more polluting rate increases, the pH levels, the MF/MS ratio, the chlorophyll (a + b) contents, and the percentage of pollen grains decreases, while the proline level increases with increasing pollutants.

The classification of stations according to the degree of growth of pollution would be: the downtown Annaba station (ST.1), the Sidi Amar station (ST.3) and El Bouni station (ST.2).

**Conclusion**

According to the air quality monitoring network data (SAMA SAFIA), our results and WHO thresholds, we distinguish that dust is the most important pollutant that exceeds the threshold of 50 µg/m³in downtown Annaba (ST.1), Sidi Amar (ST.3) and in particular EL Bouni (ST.2), which seems to be the station most affected by pollution.

We have also noticed that atmospheric pollution has adverse effects on the physiological parameters studied in the plant.

The bio-indication by leaves and pollen seems to be effective because of the sensitivity of these to all changes in environmental parameters.

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