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Full Length Research Paper

Effects of particulate pollution induced by cement dust on biochemical metabolites in Conifers: *Pinus halepensis* and *Cupressus* sp

Yasmina Dellaa [a,b,c], Chaabane Rahmoune [b], Jacky J. Kister [a], Nathalie Dupuy [a]

[a] Laboratoire des Systèmes Chimiques Complexes, Université Paul Cézanne, UMR 6263, Sce 451, 13397 Marseille cedex 20 (France)
[b] Laboratoire Ecotoxicologie et Stress Abiotiques, Université Mentouri, Faculté des Sciences de la Nature et de la Vie, 25000 Constantine (Algeria)
[c] Institut des Sciences de la Nature et de la Vie, Centre Universitaire de Khenchela, 40000 Khenchela (Algeria)

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ABSTRACT

The subject of this study is around the impact of potential air pollution, possibly attributable to nearby cement factories, on the metabolic mechanisms of trees at five sites in natural medium conditions in the region of Constantine in Algeria. The study focused on the investigation of metabolic changes involved in plants and occurring under a chronic stress caused by dusts. Three biochemical parameters were selected: chlorophylls, proline, and soluble sugars contents. The species selected showed a great sensitivity to continuous exposure. The washing of tree leaves confirmed the existence of deposits thick enough to impair and sometimes prevent exchanges between the plant and the medium, essentially the photosynthesis process. Medium particulate constraints have a determining influence on presented situation of trees, which showed highly significant variations in contents of several metabolites in polluted sites. The plants grown at polluted sites (S2 and S3) showed significant reduction in chlorophylls and soluble sugars. The highest in total Chlorophyll was observed in *Pinus halepensis* (90.84 %) at S2, the lowest reduction (68.53 %) was recorded in *Cupressus macrocarpa* at the same site, but in *Cupressus sempervirens* the reduction was (78.84 %). For proline, plant responses are slightly variable. However, for soluble sugars accumulation, the pollution constraints varied considerably and appeared correlated directly with environmental conditions. The data obtained were further analyzed using one-way Anova and a significant change was recorded in the studied parameters.

Key words: Dust pollution, Biochemical indicators, Cement, Coniferals, Plant stress

1. INTRODUCTION

In Algeria, the notable deterioration of conifers, mainly in the area of Constantine, leads to several assumptions: a) pollution caused by the deposits of cement factory dust localized in the Hamma Bouziane agglomeration; b) the damaging nature of the components of dust; and c) the air pollution caused by the road traffic emiting many pollutants in the atmosphere; associated to deterioration of the leaf areas observed, and the

*Corresponding author: dellaayasmina@yahoo.fr

deterioration of vegetable cover. The deterioration phenomenon of the coniferous plantations was observed in many Algerian forests and applied to up to 20% of the trees. The first senescence was announced approximately 30 years ago, but the deterioration was accelerated since 1985 (Hacène et al., 1995).

In order to use trees to clean polluted air, conifers should be selected for their ability to retain dusts. In this respect, exploiting physiological and morphological traits with an aim to define tolerant species to environmental stress is a prerequisite. Conifers are often considered to be the best temporary biological indicators of a contamination of the environment, because their wood quality reduces the side transfer of the pollutants through the rings (Zayed et al., 1991). The capacity for biochemical defense is an indicator of the potential stress resistance of trees (Tegischer et al., 2002). Of all plant parts, the leaf is the most sensitive part to the air pollutants and several other such external factors (Lalmann and Singh, 1990).

Enhanced accumulation of organic metabolites is a common feature of plants undergoing various biotic and/or abiotic stresses (Khan et al., 2000). Chlorophylls (Chl) are the dominant photosynthetic pigments in green plants and assessment of their concentrations in foliage can provide an estimate of potential photosynthetic capability (Gitelson and Merzlyak, 1996; Carter, 1998). The total Chl. content is a potential indicator of vegetation stress because of its direct role in the photosynthetic processes and its responsiveness to a range of stresses (Gitelson and Merzlyak, 1996, Zarco-Tejada et al., 2002, Rejskova et al., 2007). All the atmospheric pollutants retained by leaves are transformed inside the plant and affect its photosynthesis and respiration. The caused damage appears by chloroses and necrotic lesions at leaves level (Landis and Yu, 1995). Analysis of photosynthetic pigments may therefore provide insight into the physiological status of vegetation (Moran et al., 2000). Therefore, the changes in carbohydrate content as a consequence of exposure to stress may be caused by impaired photosynthesis (Rejskova et al., 2007).

Proline (Pro) accumulation in plants may be part of a general adaptation to adverse environmental conditions (Delauney and Verma, 1993); and has been often reported as a consequence of a wide range of abiotic stresses (Hare et al., 1999; Hong et al., 2000; Klotke et al., 2004; Kishore et al., 2005; Srinivas and Balasubramanian, 1995; Rejskova et al., 2007).

In higher plants, Pro. is synthesized in cytosol either from L–glutamic acid or from L–ornithine (Pavlicova, 2008). Glutamyl kinase and glutamyl phosphate reductase have been proposed to convert glutamate to proline–5–carboxylate. A chloroplast localization of Pyrroline–5–carboxylate reductase enzyme has been reported in pea (Rayapati et al, 1989).

The physiological effect of Pro. accumulation may be expressed in sustained photosynthesis and/or prevention of proteins and enzymes, from degradation (Wang et al., 2007). It can also serve as a rapidly available source of nitrogen, carbon, and reduction equivalents during the recovery from stress (Hellmann et al., 2000). In addition to the stabilizing effects of Prl, stabilizing effects of carbohydrates on membranes and proteins are also accepted (Vereyken et al., 2003).

Carbohydrates are a major category of compatible solutes that include hexoses, disaccharides, sugar alcohols, and complex sugars, all of which are accumulated during stress (Jouve et al., 2004). Sugars play a central role in plant life (Loreti et al., 2001). Sugar alcohols, straight chain polyhydric alcohols constitute an important group of compatible solutes. Mannitol, as polyols is an osmoprotectant and serves as storage compounds and redox agents (Loescher, 1987).

In this work, three spread conifers in the area are selected to study the impact of the particulate air pollution: *Cupressus sempervirens*, *Cupressus macrocarpa* and *Pinus halepensis*. The three biochemical metabolites (Pro., Chl. and sugars) were analyzed according to the chemical species and in various sites with different amount of pollution.

2. MATERIALS AND METHODS

2.1. Study sites

The study was conducted in five sites (S1–S5) selected at various distances from a cement factory of natural medium in the periphery of Constantine in east Algeria (36°30’N, 6°45’E), located at 900 m altitude (Fig. 1). The levels of air pollutants were lowest at S1 site and hence this site was treated as control site for comparing the response pattern observed at sites S2, S3, S4 and S5 respectively. Plant samples were collected next to the cement factory for site S2; within a range of about one kilometer for site S3; 15 km for site S4; 10 km for site S5, and 30 km for site S1, site considered as pilot station.

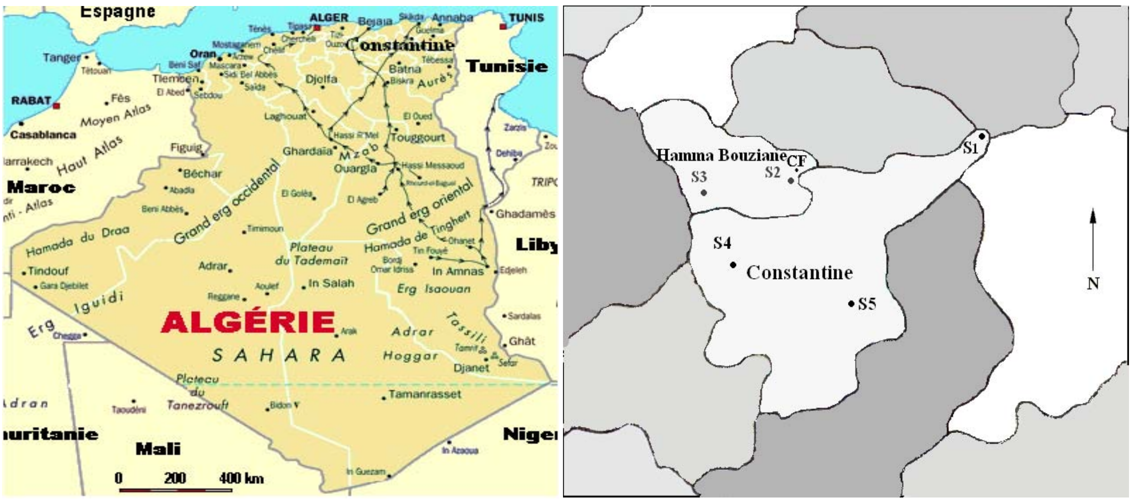

Figure 1: Map of the region of Constantine indicating the different sampling points: Site S1 – Pilot site, located at 30 km, 1325 m altitude, and with no apparent pollution source. Site S2 – Industrial area just near cement factory (CF), located in a depression at 525m altitude. Site S3 – Residential areas. Site 4 – University site and residential area. Site 5 – industrial area near a highway.

The speed of the strongest winds were recorded during April (13.32 km/h) and December (12.24 km/h) when the most frequent winds blow in NW and NNW directions during 25 days. The maximum wind speed was 25.2 km/h (NW) during 4 days although it reached 32 km/h on one occasion. 14.4 to 18 km/h is the maximum speed recorded in direction NNW during 7 days. During the remainder of the month of April**,** the wind's speed reached 25.2 km/h (NE) although rarely exceeding 18 km/h on average.

These weather conditions are unfavorable for pollutants dispersion in the atmosphere, because generally, the risks are more important when the winds are weak and the air stable. This weather configuration is most frequent in summer in Constantine where the atmosphere is hot, and sunny and slightly weathered fostering a pollution climate. Two sampling campaigns were carried out with a one year interval, the first series in June 2006, and the last one, second series, in July 2007.

The analyses on dust under air pollution effect are done 15 days after the last rains (Harrison et al., 1981).

2.2. Plant material

The study was conducted on *Cupressus sempervirens*, *Cupressus macrocarpa* and *Pinus halepensis* growing under natural conditions. Each conifer's sample consisted of five individual plants collected at selected sites. The reference samples were collected similarly from the control site, without visible sources of pollution and located at 30 km from the cement factory.

2.3. Needles washing technique

In order to measure the importance of the surface deposits, we carried out a washing of the needles, using the Stenbock-Farmer (1978) technique, which recommends solutions of 5 g/l Ethylene Diamine Tetra Acetic Acid (EDTA) pH 2 and 0,1N chlorydric acid pH 2. The number of treatments was often prolonged for up to 5 with duration of washing of 5 minutes. At the end, filters are charged even for the trees needles from the control site. By extending the duration of treatment to 10 minutes, the amount of extracted dust is greater.

2.4. Chlorophyll concentrations measurement

Leaf tissue samples were collected by four repetitions each sample (1g). Chl. contents were determined based on methods recommended by Witham et al. (1971) i.e. by extracting the Chl. using a 10 ml solution made up of 80% acetone and 20% ethanol. The samples were then placed for 48 hours at 30°C to enable extraction in the dark, and then analyzed using a Spectronic 20D spectrophotometer. The Chl. contents were calculated using optical density equations, given by the same authors, at λ=663 nm for Chl ***a***, λ=645 nm for Chl ***b*** and λ=652 nm for total Chl. Results are given in mg/g fresh matter (FM).

2.5. Proline concentrations measurement

To determine the Pro. content we used the Troll and Lindsley (1955) technique, modified by Dreier and Goring (1974).

100 mg of needles FM were taken, by four repetitions each sample and put in test tubes to which 2 ml of methanol 40% were added. The tubes were hermetically closed and heated in a bain-marie at 85°C for 1 hour. After cooling, 1ml from each one is added to 1 ml of acetic acid, 1 ml of reagent (prepared with a mixture of 120 ml distilled water, 300 ml acetic acid and 80 ml orthophosphoric acid), and 25 mg of ninhydrine; the whole is carried to boiling at 95°C for 30 minutes. After cooling, the toluene addition and agitation with the vortex make it possible to distinguish 2 phases: the higher containing Pro. and lower, aqueous, without it. After recovery of the higher phase, 5 mg of oxidized sodium sulphate were added to eliminate moisture. The reading of the

optical density was carried out at wavelength λ=528 nm using a Spectronic 20D spectrophotometer. Results were given in mg/g FM. Standard curves are obtained using L–proline.

2.6. Soluble sugars concentrations measurement

The assay technique of soluble sugars to the anthrone used was that of Shields and Burnet (1960). The Method of measurement of soluble Sgr by anthrone relates mainly to its concentration in reagent (2 g anthrone in 1 liter of sulphuric acid) and to the heating duration. 100 mg of needles fresh matter were taken by four repetitions each sample, put in test tubes in 3 ml ethanol at 80%, and kept at darkness and ambient temperature during 48 hours. In each sample, after evaporation of ethanol, 20 ml of distilled water were added. 2 ml from each sample were put in new tubes, to which we added 4 ml solution with anthrone at 0 °C, in ice bath. The tubes were hermetically closed, and left to mix content, heated in abain-marie for 8 minutes at 90 °C then left 30 minutes in darkness. The reading of optical densities is carried out at wavelength λ=585 nm using a Spectronic 20D spectrophotometer. We obtained Standard curves using D–glucose. Results are given in mg/g FM.

2.7. Statistical analysis

In ecotoxicology, the analysis of variance (Anova) is used to detect differences between groups (Landis and Yu, 1995). In a first instance, biochemical data were analyzed using Sample Power Sample Size (SPSS) 8.0, to calculate Fisher coefficient, classify homogeneous groups with Student Newman Keuils test (SNK) and multiple comparison in order to study the significance of site effect on variables. In second time, we used SYSTAT 5.0 which carries out Anova with multiple regressions (Kenneth et al., 1995). The alphabetical order (**a**, **b**, **c**, **d**, **e**) indicates the growth of the values. The same letter corresponds to different non significant averages. A Principal Component Analysis (PCA) was carried out in order to have correlations between variables and axis.

3. RESULTS

3.1. Results of dust quantification by washing technique

3.1.1. pH of washing solutions

Tables 1 and 2 show the experimental values obtained for pH measurements, and dust weight after washing of the needles. In the majority of treatments, the measured pH could reach values higher than that of the recommended solution (pH 2), suggesting that dust contains compounds playing the part of alkaline plug with values close to neutrality sometimes, thus for EDTA solution of *Pinus* needles at S5 which presents a high capacity of fixing/retention of particles compared to *Cupressus* sp. A pH 7.2 was measured in EDTA solution in 1st treatment of *Pinus* needles at S4 (Tab. 2). At the end of the 5th treatment, pH values generally reach low values. On the other hand, the highest pH values for HCl solution are those found at S2 for *Pinus* needles treatment (pH 2,5). The examination of the data gathered in tables highlights in some sites of the fluctuations between 6.2 at *C. macrocarpa* in S2 and 7.2 at *C. sempervirens* in S3, conversely with the values estimated at pilot site and which did not exceed 2,5 in no case for *C. macrocarpa* and 3.9 for *C. sempervirens* (Tab. 1).

Table 1: pH and dust quantities collected on *Cupressus* verticils in EDTA solution

Sites	*Cupressus sempervirens*			*Cupressus macrocarpa*		
	Washing Number and (mn / trmt)	**pH**	**dust quantities (µg/cm^2)**	**Washing Number and (mn / trmt)**	**pH**	**dust quantities (µg/cm^2)**
S1	3 10	3.97 3.96 3.99	4.20	5 5	2.47 2.25 2.25 2.31 2.23	4.40
S2	5 5	**5.80** 3.44 2.74 2.46 2.54	9.80	5 5	**6.23** 4.30 3.35 2.70 2.29	25.20
S3	5 5	**7.23** 5.30 4.35 3.70 3.29	16.80	5 5	**3.71** 2.47 2.40 2.35 2.38	5.20
S4	3 10	2.44 2.43 2.43	4.80	3 10	2.45 2.46 2.42	4.80
S5	5 5	2.97 2.34 2.27 2.27 2.31	2.54	5 5	2.97 2.34 2.27 2.27 2.31	3.00

mn / trmt : minute per treatment.

3.1.2. Dust quantification

Following these pH evaluations, the solutions were filtered. The quantities of dust, retained on Millipore filters, informed us that the species with small leaves, such as conifers, are equipped with the capacity of fixing-retention of air particles while contributing to its purification. The winds in Constantine area are not strong enough to allow an important dispersion of the pollutants. This phenomenon contributes to increasing the thickness of the deposits on the leaves.

a. Dust Quantities collected on *Cupressus* needles

On cypresses trees, there was a clear relation between the highest values of pH and the strong tendencies to accumulate important masses of dust, with 25.20 µg/cm^2 for *C. macrocarpa* and 16.80 µg/cm² for *C. sempervirens* (tab. 1), while the S4 site presents the weakest pH value with few variations between treatments.

The comparison of dust mass deposited on needles shows that in polluted sites, Cypresses have various behaviors. Indeed, under S2 medium conditions *C. macrocarpa* shows a higher Quantitie than the double of that measured at *C. sempervirens* which shows, on the other hand, at S3 a thicker dust mass. But at the other site, these quantities are close (Fig. 2a). Perhaps the exhaust fumes in S3 support the fixing of the deposits on *C. sempervirens* needles.

At S2 and S3 sites, cement factory dusts and roads sides as well as hydrocarbons from road traffic, especially at S5, are strongly deposited and adheres to the leaves, which makes them very difficult to eliminate during the use of the needles in experimentation.

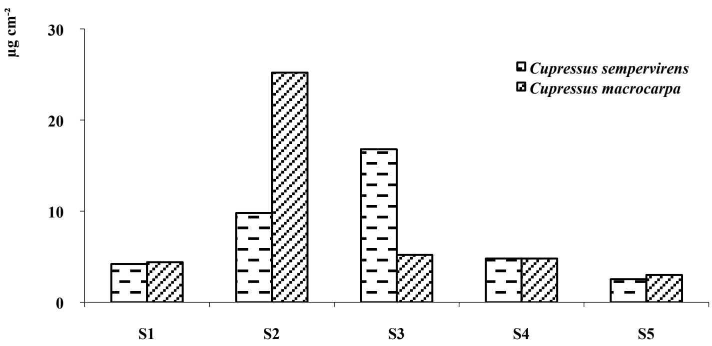

Figure 2a: Dust quantities comparison measured and extracted on *Cupressus sempervirens* and *Cupressus macrocrpa* in EDTA.

b. Dust Quantities extracted on *Pinus halepensis* needles

As illustrated in table 2, we found most important dust mass raised on the foliar deposits using EDTA (104.27 µg/cm²). We could note that the pine at S2 shows a very important capacity of fixing dusts. Compared to the quantity observed at S1, the rate of increase is 97.61%. Similar results were obtained with HCl (Fig. 2b).

Table 2: pH and dust quantities collected on *Pinus halepensis* needles.

Sites	Washing number and (mn / trmt)		pH		Dust quantities extracted by EDTA (µg/cm²)
	EDTA	HCl	EDTA	HCl	
S1	3 10	3 10	3.89 3.52 3.73	2.44 2.44 2.43	2.49
S2	5 5	3 10	2.40 2.27 3.20 2.50 2.37	2.43 2.45 2.37	104.27
S3	5 5	3 10	1.63 1.55 2.70 2.70 2.60	1.96 2.41 2.36	27.89
S4	3 10	—	**7.22** 4.23 4.05	—	2.68
S5	3 10	—	6.27 **6.86** 4.43	—	8.77

Thus, it arises from the whole of the results corresponding to the evaluation of dust that the deposits rejected by the cement factory have an important impact on the variation of Chl., Pro. and soluble Sgr. contents mainly at S2, S3 and S5.

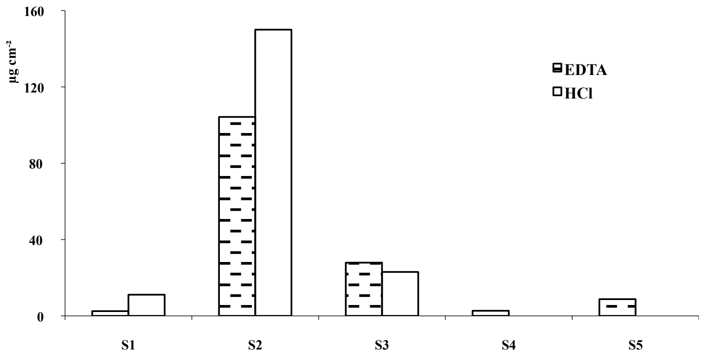

Figure 2b: Dust quantities measured and extracted on *Pinus halepensis* needles.

But, weak precipitations accentuated the phenomenon of pollution and did not contribute to the "rain scrubbing" which is a natural phenomenon of partial elimination of dust, increases the pollutants adsorption on vegetable aerial surfaces and then direct absorption.

3.2. Results of the biochemical analyses

3.2.1. Chlorophyll contents (Chl)

The Chl. contents present great variations between sites and sampling campaigns (Fig. 3A, B,C). All results shows that the pilot site S1 reveals the highest contents, whereas the S2 site, near the cement factory, expresses the lowest contents. Deployed groups by SNK test confirms the results advanced on figures. At S2 and S3 sites, the results of total Chl. contents measurement showed that these contents fall considerably for all species. We notes 5.42±0.46 mg/g FM at *C. semperverens*, which accounts for the 1/5th of S1 content; and 5.52±0.03 mg/g FM for *C. macrocarpa*. It is also valid for *Pinus* (6.23±0.06), which accounts for the 1/10th.

a. *Cupressus sempervirens* and *Cupressus macrocarpa*

At S2, S3 and S5 sites we observed that reduction of total Chl. content is due to an increased matter deposition following permanent exposure to dust effluents. These Chl. contents decrease slightly in S4 at *C. sempervirens* (Fig. 3a); whereas they decrease of more than half at *C. macrocarpa* in S4 (Fig. 3b).

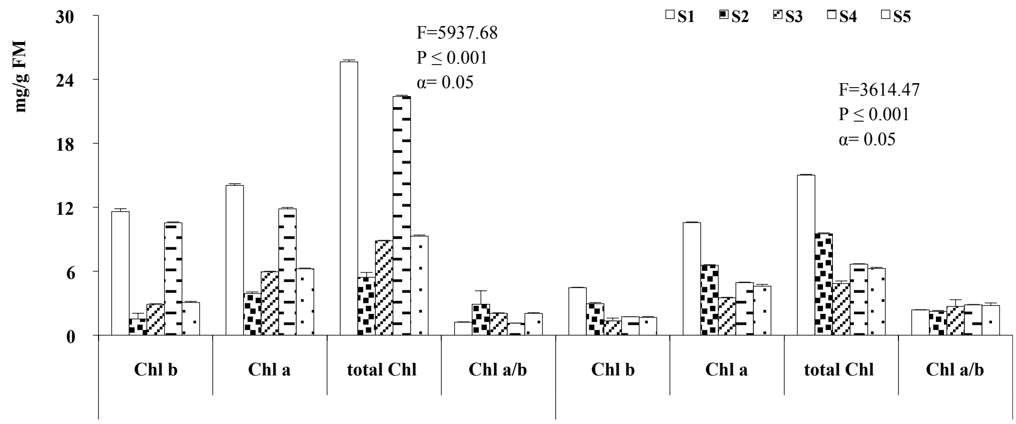

Figure 3a: Chlorophyll contents at *Cupressus sempervirens*. At second sampling series, compared to S1, the reduction of total Chl is estimated at 36.68 % for S2, 67.72 % for S3, 55.62 % for S4, and 58.34 % for S5.

We note also, that *C. macrocarpa* and *C. Sempervirens* do not answer in the same way to an environmental variation at the other sites. Indeed, Chl. ***a*** content at *C. Sempervirens* is about 11.84±0.08 mg/g FM, on the other hand, it approximately lowered half at *C. macrocarpa* at S4 site. At the pilot site (S1), the contents of Chl. ***b*** present an important variation between these two species: 11.59±0.27 mg/g FM for *C. sempervirens* and 6.66±0.24 for *C. macrocarpa*. However, at S2 and S5 sites, Chl. ***b*** contents do not differ as slightly as well at *Cupressus* that at *Pinus*.

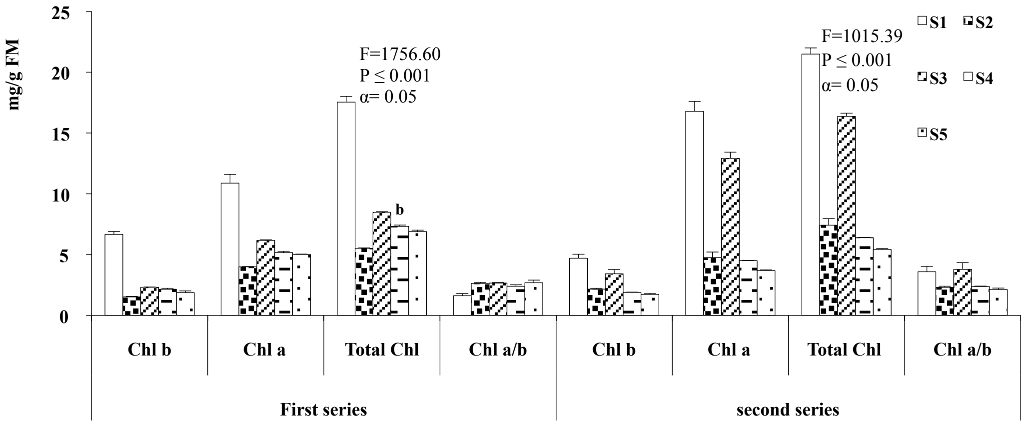

Figure 3b: Chlorophyll contents at *Cupressus macrocarpa*.

Anova for total Chl. at *Cupressus* revealed highly significant differences for the first series and for the second one, whatever considered site, testifying to inter-sites variability; the assumption of equality of the sites averages is rejected**.** Consequently, *C. sempervirens* can be regarded as more resistant to pollution than the other coniferous. While for the second series, SNK test reveal the release of **5** distinct groups indicating the existence of inter-sites differences with a maximum raised at S1 site while being allotted to the group **e** and a minimum recorded at S3, affected to group **a** at α=0.05.

b. *Pinus halepensis*

Chl content for *Pinus* during the 1st series testify to great diversity between sites and an important intra-specific variability. Total Chl content presents some variations when the species are subjected to dust pollution. Indeed, we observed a strong concentration at the pilot site (68.03±0.30), value appreciably close to that recorded in S4 (51.45±0.68) (Fig. 3C). On the other hand, we noticed that the behavior of the Pine seems very different with the fluctuations of medium; it is in S2 that we notes a very important fall of the Chl. content illustrated by the lowest content (6.23±0.06), the rate of fall is estimated at 90.84 %. Between these two limits are the values, considered as rather weak, observed in S3 and S5 where the rate of fall is between 13.08 and 13.58% respectively; resulting in rather close values about 8.90±0.02 and 9.24±0.07 mg/g FM.

With regards to the 2nd series, the Pine shows a similar behavior to that noticed in 1st series for this parameter by showing the highest content at S1, of the intermediate values at S4 and S5 then at S3 and a minimum of accumulation at S2. For this parameter, the Anova revealed highly significant differences (F=2207.23).

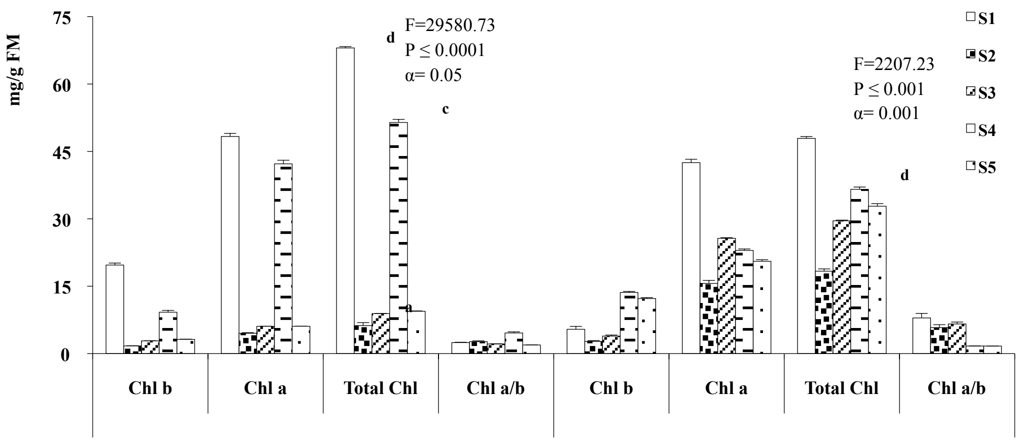

Figure 3c: Chlorophyll contents at *Pinus halepensis*.

The specimens of pine used in experimentation showing that the lowest Chl. contents are located in S2, S3, S5 and present chlorosis and necrosis (Fig. 4 c,f). These observations are on line with those of Wulff et al. (1996) which subjected a species of Pine (*Pinus sylvestris*) with chronic ozone amounts with the aim of estimating the degrees of necrosis and chlorosis observed on the needles. Moreover, these results reveal the significant difference between the sites to have a better photosynthesis and organic matter development.

The layer of "epicuticlar" wax is a characteristic which makes it possible for conifers to survive in adverse conditions, such as temporary dryness, air pollution, wind, high temperatures and strong radiations (Kinnunen et al., 1999). This leads to suppose that this physiomorphologic characteristic is responsible for their apparent resistance to the pollution.

This leads us to deduce that S2 is the most subjected site to pollution by dust deposits and which would require a strong rainfall to eliminate these deposits and to support a regeneration of Chl. biosynthesis to ensure the release of a greater leaf area and a better photosynthesis. It should be noted that Chl. ***a*** content constitute in the majority of cases the double of that in Chl. ***b*** (Lehninger, 1989). This could be explained by the resistance of the species which showed a resilience under pollution in keeping contents minima for the photosynthetic assimilation. The Anova showed that the Site factor explains to 100 % of the variation of the total Chl. content. Chlorophyll contents at S3 site, located at 1 km from the cement factory, displayed slight increases compared to S1, which may demonstrate the direct effect of dust deposits on the inhibition of Chl. biosynthesis. Ali (1993) found that total Chl. content of potted plants transferred to three different sites in Egypt showed a negative correlation with pollution at sites. Clover and mauve developing in increasingly polluted sites had a reduction of total Chl. content up to 29%.

Cypresses are particularly sensitive to the pollution generated by cement dusts (Fig. 4). The low Chl. contents calculated at affected sites illustrate such important reduction. In other terms, the dust quantities emitted and travelling at large distances cause a strong disturbance of Chl. synthesis and result in a metabolic deceleration of the biological processes.

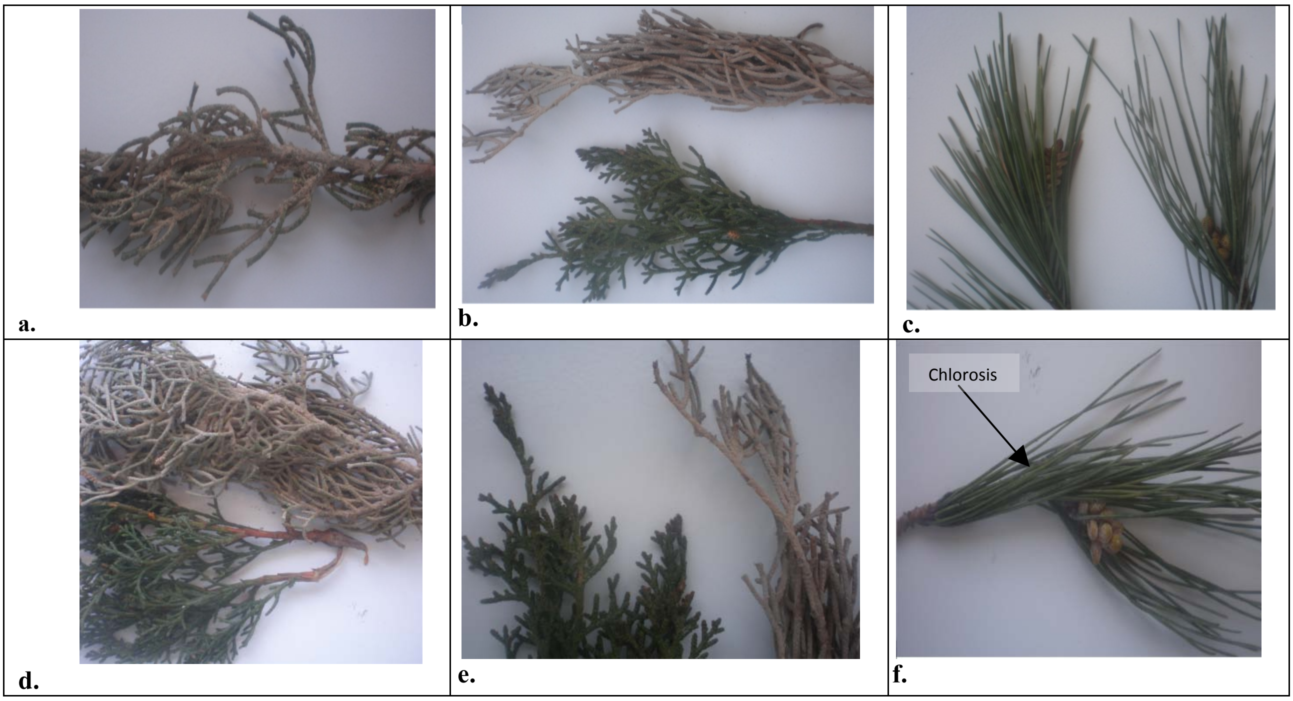

Figure 4: Aspects and comparison between branches of cypress and pine needles coming from the sites near Cement Factory and the pilot site. **a.** *Cupressus* verticils collected at S2 site. **b.** *C. sempervirens* verticils collected at S2 (up) and at S1 (down). c. *Pinus halepensis* needles collected at S1 (left) and S2 (right). d. *Cupressus* verticils collected at S3 (up) and at S1 (down). e. Apical area of cypress branches collected at S1 (left) and at S2 (right). f. Chlorosis on *Pinus halepensis* needles collected at S2

3.2.2. Proline Contents

a. Proline accumulation in *Cupressus sempervirens* and *Cupressus macrocarpa*

For the first series, Pro. contents only slightly vary for *C. macrocarpa* with 0.42±0.01 mg/g FM at S3 and 0.47±0.05 at S1; and are not so different between S2 and S4 (fig. 5). The two cypresses considered behave in the roughly the same manner and present tendencies to have rather close Pro. contents. Whereas for *C. sempervirens*, a strong content was found at site S5 with 0.69±0.01 mg/g FM, which preserves its tolerance character to the stress and where the species are subjected to exhaust fumes and hydrocarbons which seem to play an important part in the activation of Pro. biosynthesis. On sites S2 and S3, contents were above the moderate ones observed varying between 0.36±0.06 at S2 and 0.21±0.03 at S3.

The lowest content observed was in S4 for the 1st series of sampling (Fig. 5). An inter-specific variability was observed with respect to the Pro. accumulation for S5 where we could note that *C. sempervirens* accumulate approximately the double of the content accumulated by *C. macrocarpa*.

For the 2nd series, *C. macrocarpa* revealed little variability and showed a Prl contents brought closer enough (Fig**.** 5). We note however, the highest content at S4 (0.40±0.11) compared with those reported at S1 (0.34±0.04). No significant difference was statistically observed with Anova or SNK test. All values were superimposed in a same homogeny group.

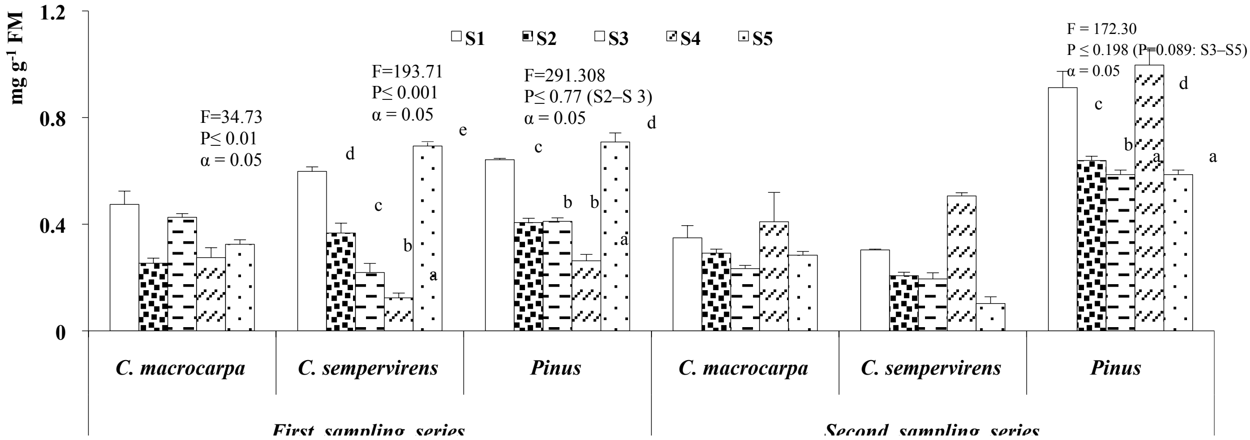

Figure 5: Proline contents.

b. Proline accumulation in *Pinus*

For the 2st series, Pro. content measured at S5 is most important with 0.70±0.03 mg/g FM, value rather close to that observed in S1 (0.64±0.005), against S4 where we could observe the lowest content (0.26±0.02). It is at S2 and S3 that we notes a very low rate of variability for Pro., considering the needles of the pine at these two sites are subjected to comparable deposits of dust, generating the same response of plants to this abiotic stress. This fact was noticed also when taken measurements one year later, during the 2nd series, where we noted that the pine roughly behaves in a similar manner at the sites considered the most polluted, namely: S2, S3 and S5. The Anova reveals highly significant differences (F=291.308, P≤0.001). The separation of homogeneous groups at α=0.001 distinguish 4 groups. The test of the multiple comparisons of the SPSS at α=0.001 confirms the very high significance of SNK test, except for the Prl contents between S3 and S5 sites which are not significant even at threshold α=0.05 (P=0.089) (Fig. 5).

3.2.3. Soluble sugars contents

a. Soluble sugar contents at *Cupressus*

For the 1st series, we note at *C. sempervirens* that the soluble Sgr. contents are appreciably close and do not present pronounced variations between pilot site S1 and that considered the most subjected to dusts (S2) with respectively 298.97±7.43 mg/g FM and 268.15±2,70 (Fig. 6). A maximum of accumulation was in S5 (453.27±12.60).

A fairly low content is recorded at S4, while the lowest was raised in S3. The strong concentrations recorded out in S5 indicates an activation of the degradation of the glucidic reserves that suggests that a plant adaptation to the constraints of pollution with the risk especially causing an exhaustion of reserves. A rate of fall of the Chl content was noticed, which resulted in a weak development of the organic matter, mainly the glucids.

b. Soluble sugars contents at *Pinus halepensis*

Pinus does not behave in the same manner under the various constraints of sites. However, it had tendencies to have the Sgr. contents most raised at site in the vicinity of the cement factory (S2) for the 1st series with 214.36±2.08 mg/g FM (Fig**.** 5). Contrary, at the 2nd series, this same site testified to the weakest average (142.90±1.99) (Fig**.** 6), whereas the maximum value was calculated at S1 (526.33±10.63). Contrary to the weakest Sgr concentrations at S3, appreciably equalizes with that raised in S5, for the 1st series. These sites, crossings both of primary automobile distributors, which suggest that sugars degradation could be activated by the emission of exhaust fumes.

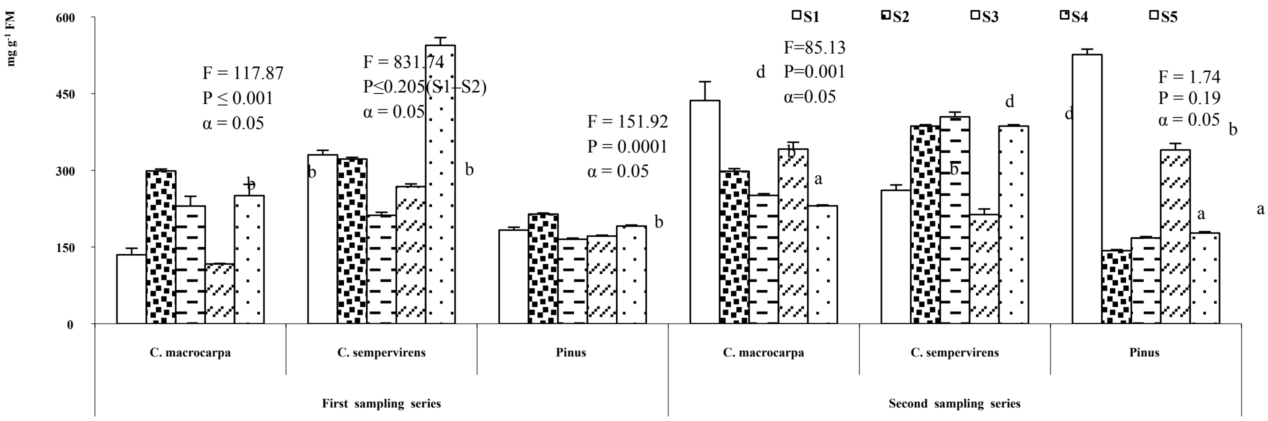

Figure 6: Soluble sugars contents.

The Anova of 1st series results, revealed highly significant differences for soluble sugars and total Chl. accumulation at P≤0.001. Strong soluble Sgr contents in S5 at *C. sempervirens* (Fig. 6) let suppose that pollution would act in two different ways: maybe that it inhibits the activity of the degradation of glucidic reserves or that *C. sempervirens* is not able any more to work out reserves allowing him to adapt to the pollution climate, which supports the assumption of conifers deterioration in the area undergo.

In the pilot site, Sgr. accumulation at *C. macrocarpa* and *P. halepensis* is correlated with Chl. contents and it is inhibited at all other sites where the Chl. is negatively influenced by the deposits of dust. This suggests that the species use the synthesized sugars by photosynthesis in the event of stress. According to Heineke et al. (1992), the soluble Sgr concentrations raised out are proportional to the Pro. cellular contents. In our case, this assertion is not verified since the Pro. and soluble Sgr. contents are very independent in the test of the PCA (Tab. 3). This could be confirmed by the work results of Hellmann et al. (2000) which advances that the biosynthesis of the carboxylate enzyme pyrroline-5 synthétase (P5CS) is induced by the high percentages of Sgr. but which it is inhibited by the proline itself.

Table 3: Correlation matrix between variables and axis in PCA test.

Variables	Chl	Prl	Scr	Axis 1 Correlation	Axis 2 Correlation
Chl	1.000			**0.8558**	0.3086
Prl	**0.605*****	1.000		**0.8970**	0.0629
Scr	0.051	0.202	1.000	0.3435	**-0.9329*****

*** : highly significant

Chl. and Pro. variables are significantly and positively correlated between them and with axis 1 (Tab. 3) and express the tolerance which constitute the first principal component, while there is no significant correlation between Pro. and Sgr. or between Chl. and Sgr. (Tab. 3). In the same way, the soluble Sgr. variable is significantly correlated with the axis 2 whose significance is deterioration and which constitute the second principal component (Fig.7).
Individuals located in the positive part of axis 1 are best adapted to the environmental medium. But, there was no individual from the S2 site (individuals: Cm4, Ph5, Cs6) (Tab. 4) assigned to the groups of people situated on the positive part of axis 1 and 2 (Fig. 7). This may implies that trees from S2 do not tolerate particle deposition and have a poor adaptation. In the main plan (Axis 1 and 2), it seems clear that there are two groups of trees on axis 1 and two groups on axis 2 (Fig. 7).

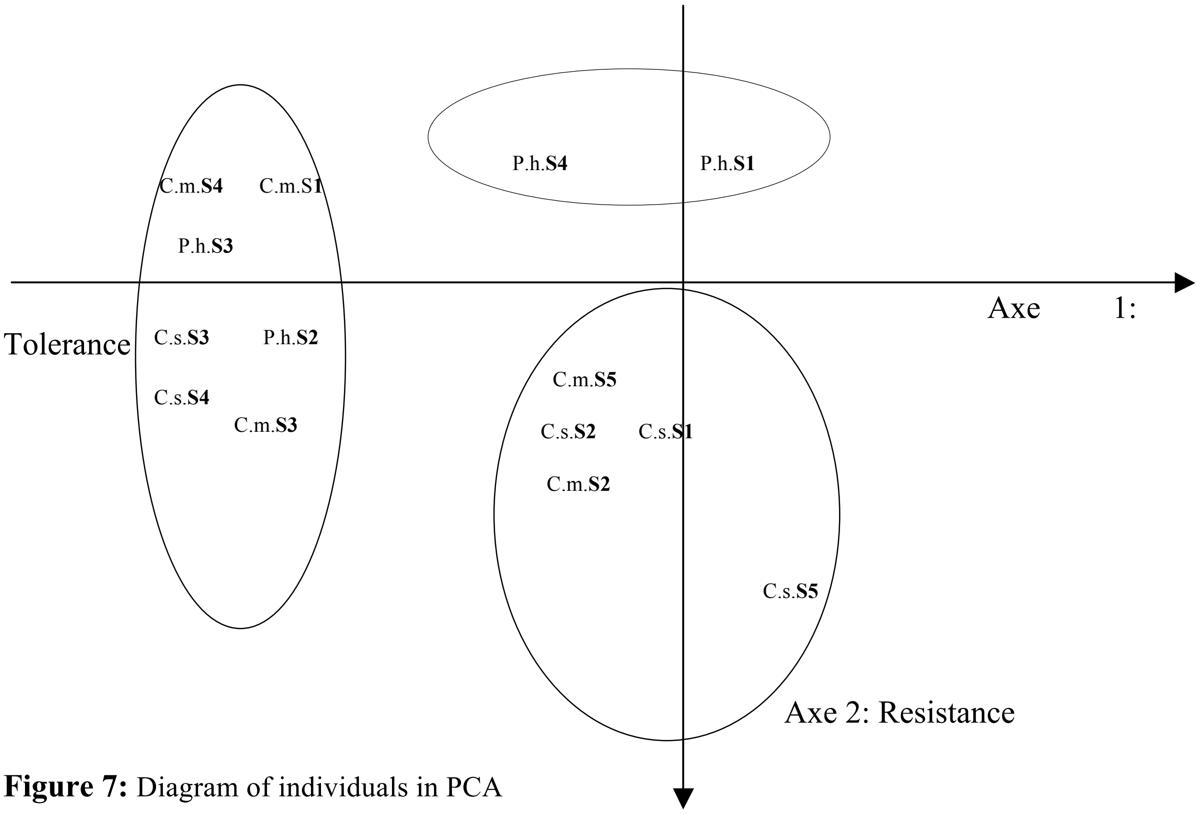

Figure 7: Diagram of individuals in PCA

As axis 1 is mainly formed by the tolerance variables, it would be permitted to say that these groups are positioned relatively to their Chl. and Pro. accumulation; contrary to the groups which are positioned on axis 2 and relatively to their accumulation of soluble sugars.

Table 4: Coding of the individuals in PCA diagram.

	S1	S2	S3	S4	S5
Cupressus macrocarpa	C.m.S1	C.m.S2	C.m.S3	C.m.S4	C.m.S5
Pinus halepensis	P.h.S1	P.h. S2	P.h.S3	P.h.S4	P.h.S5
Cupressus sempervirens	C.s.S1	C.s. S2	C.s.S3	C.s.S4	C.s.S5

4. DISCUSSION

Differentiation of changes in the leaf or needle physiology, through ageing, senescence, accelerated cell senescence, programmed cell death and oxidative stress, provides additional clues raising diagnosis efficiency, especially in combination with information about the target of the stress agent at the tree, leaf/needle, tissue, cell and ultrastructural level (Günthardt-Goerg and Vollenweider, 2007).

The values of pH close to neutrality raised at S2 and S5 could be explained by the existence of limestone and carbonates in the thick foliar deposits of fixed dust. These observations corroborate with those obtained by Kaminski and Landsberger (2000), which observed a pH 7,4 during analyses out of thirteen samples of grounds and aerosols of the area of St Louis where factories are abundant. None of the thirteen samples had a pH<6,5 or >8,0. Results surprising to consider seen that the industry of the area emitted SO_2 which could acidify the layers grounds, the most plausible explanation was that the existence of carbonates in the ground is playing the part of buffer solution.

The winds in Constantine area are not enough strong to allow an important dispersion of the pollutants. This phenomenon contributes to amplify the thickness of the deposits on the needles. These same deposits have an effect on photosynthesis and breathing by clogging the stomata (Laitat, 1990).

Strong mineral acid HCl pH 2 shows an undeniable effectiveness in the dust extraction. This effectiveness varies considerably from species. The present approach, elaborate with an aim of determining the capacity of binding of dust by the plants, highlighted that conifers are species most equipped with this capacity by the important quantities of dust extracted. This phenomenon contributes enormously to the air-cleaning by the foliar system of the trees which absorbs, in addition to the quantities of suspended particles in the air, poisons such as: HF, SO_2, NO_X (Stenbock-fermor, 1978). The EDTA, efficient chealate, is a detergent whose technique of use is regarded as most effective for the dust extraction (Taylor, 1956 cited by Stenbock-Farmer, 1978).

At *Cupressus* and *Pinus*, recognizable damages to a red-brown or yellow-brown coloring of needles were noticed, starting at the end and being able to extend until the base in form of chlorosis which facilitates the detachment of needles (Fig.2). This phenomenon was described by Mulgrew and Williams (2000) under air pollution conditions and can probably induce early needles senescence.

Changes in Chl contents are an indicator of stress (Naumann et al., 2008). Foliar concentrations of pigments, most notably chlorophylls and carotenoids, are affected by a variety of stress factors (Carter, 1998). In stressed vegetation, leaf Chl content decreases, thereby changing the proportion of light-absorbing pigments and leading to less overall absorption (Zarco-Tejada et al., 2000). The photosynthetic apparatus, especially photosystem II, may be temporarily affected by environmental stresses before irreversible morphological damage is observed (Naumann et al., 2008). Cypress who seems indifferent to pollution by their always green aspect, present morphological apparent damage, following their exposure to a permanent environmental pollution by dust and exhaust fumes. Their contamination is revealed by an apparent change of the foliage color and poor growth, especially when this species are characterized by a fast growth under normal conditions. It was also noticed that the apical buds present a strong inclination and tend to break. This damage is similar to those described following an exposure of conifers to atmospheric pollutants by many authors (Laitat, 1990; Nilsson and Sallinäs, 1999; Mulgrew and William, 2000). The Chl content is weak in the one year old needles of a fir tree presenting of the damage in comparison with a healthy specimen according to a study carried out in three different sites in Germany without correlation with a specific pollutant (Godbold et al., 1993).

The cement factory contributes significantly in the rise of air pollution levels. Great quantities of pulverized materials are handled. Consequently, the major problem of the air pollution lies in the emissions of dust which remains the problem dominating of the environment throughout the world by the movements of the winds constituting a harmful effect with the environment bordering the factory (Debell, 1997).

In response to the varied abiotic stress, several plants accumulate amino acids or their derivatives (Bagni, 1994). For Schwacke et al. (1999), the Prl represents the free amino acid most abundant in the pollen of *Petunia* under conditions of stress. Corroborated the role of Prl in the protection of plants against abiotic stress, as the tolerant line plants, which had higher Prl content, were less damaged, and the sensitive-line plants, with lower Prl levels, suffered greater injury than the tolerant-line plants (Giannakoula et al., 2008).

Sometimes, we note a strong analogy between the total Chl contents and the Prl contents, which appears contradictory, since these two metabolites are in competition for their common precursor, the glutamate (Roosens et al., 1999). Although the dryness was rather marked for the periods of sampling, Prl accumulation is lower than in pilot content, this leads to believe that this metabolite can constitute an indicator of hydrous stress only when the medium does not undergo constraints of the air particulate pollution.

Prl accumulation may be an injury result in plant, rather than an adaptive metabolic response (Qian et al, 2001). These very low Prl contents can inform us that this metabolite could serve, while being degraded, as available and fast source of nitrogen, carbon or reducing equivalents during a stress (Hellmann et al., 2000). Prl accumulates heavily in several plants under stress, providing plants protection against damage by reactive oxygen species and plays important role in osmoregulation (Ahmad and Hellebust, 1988; Roosens et al., 1999), protection of enzymes (Paleg et al., 1984), enhance antioxidant defense systems in plant responses to oxidative stresses **(**Banu et al, 2009**),** and scavenging of free radicals (Smirnoff and Cumbes, 1989).

Under environmental stress conditions, many plants accumulate several kinds of compatible solutes such as Prl, glycinebetaine, Sgr and polyols (Choudhary et al., 2007).

We could conclude that the activity of the enzyme Pyroline-5-carboxylate synthetase (P5CS) is inhibited during the reaction of the precursor of Prl then supporting the formation of the gluthamine. Extreme sensitivity of the metabolic processes of Prl synthesis and degradation themselves may be of benefit by regulating metabolic processes adversely affected by stress (Hare and Cress, 1997). Although all the species are exposed to a polluted climate, especially in S2, S3 and S5 sites, there is not an important accumulation of free Prl in the needles. Nevertheless, among the studied perennial species, it *Pinus* represent the most accumulating specie of Prl at polluted sites.

Sugar degradation affects the plant metabolism and can lead to retarded plant growth (Sharma, 2009). Contrary to the low Chl contents which thus imply an inhibition of the degradation of Sgr, so a poor adaptation to polluted climate with conservation of plant reserves, and corelation between Chl and Sgr (Fig. 4, 7) and (Fig. 5, 7). For *C. sempervirens*, a simple comparison between Figures, let suppose that this species accumulates more Prl (0.69±0.01) and sugars (453.27±12.60) at S5, which shows that these two metabolites could be influenced by the rejections of exhaust.

Hellmann et al. (2000) showed that a specific increase in Prl is induced by a metabolisable sugars contribution. The vegetable cells can adapt their metabolism to the conditions of pollution by degrading Sgr. A concentration raised out of sugars suggests a good metabolism as the low contents can indicate an imminent lack (Loreti et al., 2001).

An explanation often advanced to justify the increase in pollution by the particulate matters calls upon the increased road transport. Conifers can be selected like better vegetable species to introduce in town or in industrial parks for the air-cleaning.

Plants provide an enormous leaf area for impingement, absorption, and accumulation of air pollutants to reduce the pollutant level in the air environment (Shannigrahi et al., 2004). Schulz et al. (1999) reported that the pine bark surface is very porous and the absence of metabolic processes makes it almost inert for inorganic and organic substances.

5. CONCLUSION

The study related to the foliar widespread forest species system in the urban perimeter of the town of Constantine. Following a continuous exposure to polluted environment, conifers testified to the high significant variations in Chl. contents. The two *Cupresses* who seems indifferent to pollution by their always green aspect and their right port present some morphological apparent damages.

The needles washing technique with acids revealed the existence of thick deposits which make it difficult and sometimes impossible for the plant to conduct natural exchanges with its medium. Chlorophyll Contents observed at *Pinus halepensis* are highly significant due to the existence of an important intra-specific variability and an inter-sites variation resulting in needles necrosis and chlorosis. Chl ***a*** contents remain relatively higher than those of Chl ***b*** at the polluted sites, and the ratio Chl **a/b** is always higher than 0; this may be results in the weak adaptation of the plant to the stress into spite of its strong total Chl. content (Pääkkönen et al., 1999). Based on these results, the chlorophyll parameter constitutes a good indicator of the particulate atmospheric pollution. *Cupressus* is very sensitive under the same conditions particularly by presenting moderate values. While for Pro., the answers of each plant are very slightly variable. The statistical tests showed very little significance between variations. We attend an inhibition of the synthesis of the amino acid being able to be related to the thickness of dust deposit. Within sight of the fluctuations observed in the Pro. accumulation and the very low contents calculated, this biochemical parameter does not constitute a good factor of tolerance or resistance of the trees to the constraints caused by particulate pollution.

On the other hand, the results of soluble Sgr. analysis presents varied accumulation enough between the various sites. The soluble Sgr. contents are weaker at the particularly exposed sites. Phenomenon probably due to their degradation in the form of energy and under the influence of the polluted medium like resistance and adaptation forms. Soluble Sgr parameter can constitute an indicator of the pollution by the use of Sgr by the plant in maintaining metabolic balance. The statistical analysis proved to be effective to put forward the site effect, plus we move away from the cement factory, conifers accumulate metabolites being used to them as forms of adaptation.

This study showed clearly the repercussions of cement industry localized near the town of Constantine. The trees are very often used for their property as indicators of polluting compounds presence in the ecosphere and, especially for the solid particles, their concentration gradients. Concretely, the air pollution in the periphery of Constantine is alarming and the consequences can be the modification in the climate and the aspect of deterioration of the vegetable cover of the area. The constraints of pollution by the particles have a determining influence on the presented situation of the trees and the strong harmful effects for the population.

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TABLE SOURCES

Table Source **Figure 2a**

Site	***C. sempervirens***	***C. macrocarpa***
S1	4,2	4,4
S2	9,8	25,2
S3	16,8	5,2
S4	4,8	4,8
S5	2,54	3

Table Source **Figure 2b**

Site	EDTA	HCl
S1	2,49	11,1
S2	104,27	150,01
S3	27,89	23,016
S4	2,68	
S5	8,77	

Table Source **Figure 3b**

Cuptressus macrocarpa								
	First sampling series				**Second sampling series**			
	Chl b	**Chl a**	**total Chl**	**Chl a/b**	**Chl b**	**Chl a**	**total Chl**	**Chl a/b**
S1	6,6612	10,8771	17,5383	1,619	4,704075	16,784375	21,48845	3,591275
S2	1,5227	3,9977	5,5205	2,627	2,164225	4,7568	7,421025	2,315525
S3	2,3171	6,1674	8,4845	2,6619	3,4242	12,918025	16,367225	3,789625
S4	2,1549	5,1658	7,3207	2,4007	1,894975	4,503825	6,3988	2,377025
S5	1,8758	5,0163	6,8922	2,6866	1,73465	3,69035	5,425	2,131425
σ 1	0,24	0,72	0,47	0,18	0,329278	0,815312	0,505478	0,447747
σ 2	0,043	0,024	0,03	0,086	0,070942	0,458086	0,539465	0,087850
σ 3	0,02	0,05	0,047	0,047	0,349198	0,506524	0,262804	0,549389
σ 4	0,08	0,11	0,11	0,12	0,024094	0,015639	0,018762	0,036271
σ 5	0,14	0,028	0,12	0,22	0,081581	0,050796	0,072905	0,117678

Table Source **Figure 3c**

	First sampling series				**second sampling series**			
	Chl b	**Chl a**	**Total Chl**	**Chl a/b**	**Chl b**	**Chl a**	**Total Chl**	**Chl a/b**
S1	19,7094	48,3285	68,0379	2,4535	5,4158	42,4972	47,913	7,9372
S2	1,7048	4,5291	6,234	2,6619	2,714	15,6283	18,3424	5,7913
S3	2,8337	6,0733	8,907	2,144	3,9024	25,6615	29,564	6,595
S4	9,2127	42,241	51,4537	4,5935	13,6064	22,9654	36,5719	1,6879
S5	3,1597	6,0805	9,4203	1,9249	12,2537	20,5525	32,8062	1,6772
σ 1	0,44	0,69	0,3	0,08	0,65	0,76	0,4	1
σ 2	0,06	0,13	0,66	0,18	0,18	0,67	0,51	0,64
σ 3	0,05	0,03	0,02	0,057	0,23	0,14	0,15	0,43
σ 4	0,41	0,8	0,68	0,26	0,23	0,32	0,51	0,02
σ 5	0,06	0,03	0,07	0,039	0,21	0,35	0,57	0

Table Source **Figure 5**

	First sampling series			**Second sampling series**		
	C. macrocarpa	***C. sempervirens***	***Pinus***	***C. macrocarpa***	***C. sempervirens***	***Pinus***
S1	0,4743	0,5981	0,6419	0,3489	0,3036	0,9123
S2	0,2537	0,367	0,4063	0,2915	0,2069	0,6389
S3	0,4259	0,219	0,4108	0,2336	0,1948	0,5861
S4	0,2749	0,1238	0,2628	0,4093	0,506	0,9969
S5	0,3247	0,6933	0,7084	0,2839	0,1027	0,5861
σ 1	0,05	0,017	0,005	0,046	0,003	0,061
σ 2	0,019	0,037	0,016	0,015	0,013	0,016
σ 3	0,014	0,034	0,013	0,012	0,023	0,017
σ 4	0,037	0,018	0,024	0,11	0,012	0,059
σ 5	0,017	0,016	0,034	0,014	0,025	0,017

Table Source **Figure 6**

	First sampling series			**Second sampling series**		
	C. macrocarpa	*C. sempervirens*	*Pinus*	*C. macrocarpa*	*C. sempervirens*	*Pinus*
S1	134,831	330,26222	183,10095	436,486292	260,934802	526,33548
S2	298,788	322,07478	214,36208	298,256775	386,511005	142,90805
S3	230,178	212,02281	165,23744	250,939745	404,587172	167,78937
S4	116,859	268,16526	171,40460	341,533247	213,511442	339,83196
S5	250,54	544,41165	190,96939	230,524307	386,511005	177,35910
σ 1	12,81	8,931753	5,658534	36,890161	10,541913	10,633037
σ 2	3,65	3,246128	2,083643	5,024978	2,675922	1,994936
σ 3	19,01	5,949435	2,237153	3,330931	9,289973	2,540085
σ 4	1,04	5,260938	1,770753	13,445365	10,915667	12,773815
σ 5	22,04	15,137309	2,024937	1,837601	2,675922	2,55193