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Research Paper

Impact of atmospheric pollution by the cement plant on the biochemical composition of the wild olive

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Abstract

The purpose of the present study was to determine the effect of air pollution resulting from the cement plant dust deposits on certain biochemical parameters of the *Oleaster (Olea europaea subsp. europaea var. silvestris)*. The results show that the levels of chlorophyll a, b, carotenoids and proteins were significantly reduced compared with the control. Carbohydrates show a very small increase in the polluted station; however the polyphenol content and flavonoids have increased. The increase of phenolic compounds leads to an antioxidant activity higher compared to the non-polluted area.

Keywords: Chlorophyll, Proteins, Polyphenol, Flavonoids, Antioxidant.

Introduction

The most common sources of air pollution are anthropogenic activities, particularly the cement industry[1].The emissions from cement plants which cause greatest concern are dust, carbon dioxide CO_2, nitrogen oxides (NOx) and sulphur dioxide (SO_2). Cement dust contains heavy metals like nickel, cobalt, lead, chromium, which are causing an adverse effect on plants[2]. In the field of environmental biotechnology, biomarkers are the first tools for measuring and evaluating the quality of the environment, their importance resides in their properties to be easily measurable using different biochemical and molecular approaches[3]. In stations subject to atmospheric pollution, especially cement dust, plants are directly exposed to this pollution by continuous deposits of this dust on the leaf surfaces which are considered as the main photosynthetic organs which can reduce their physiological activities, which makes it possible to use them as the best sources of biochemical biomarkers. Wild olive (*Olea europaea* subsp. *Europaea* var. *Sylvestris*) commonly called Oleaster, and also known in Algeria by Zebboudj. In addition to being considered one of the most representative trees of the specific species of the Mediterranean basin, it can also be an important source of wood biomass production[4]. It was domesticated 5850 years ago[5], and several molecular studies confirm that it is native to the Mediterranean basin[6, 7]. It grows in different environmental conditions even the most extreme like drought, low temperatures and salinity [6, 8, 9]. It's why we are interested in carrying out a comparative biochemical study between a wild olive tree that grows in the Zahana cement plant located in the mascara region and a wild olive tree that grows in the Tessala Mountains of the Sidi Bel

Abbes region characterized by a clean atmosphere and considered as a control site. This study makes it possible to evaluate the impact of atmospheric pollution on the various chlorophyllian pigments, soluble sugars, proteins, phenolic compounds (polyphenols and flavonoids) and the antioxidative activity of this species.

Materials and Methods

In the present study the main focus was on the comparative biochemical analyzes of the wild olive, species: *Olea europaea* subsp. europaea var. silvestris (L.) locally called Zebouj growing in two different areas: the Zahana cement plant which is known as a zone with high atmospheric pollution especially the cement dust and the second zone is the mountain of Tessala (control site) area known for its clean atmosphere. The distance between the two stations is 20 km, they undergo the same climatic and edaphic natural conditions, and the only difference between the two stations is the existence of the cement plant which is the origin of air pollution. The freshly collected leaves were kept in kraft paper in an ice box and then taken to the laboratory for analysis of various biochemical parameters. The species has been identified by Professor Djahed Benyounes, professor of environmental sciences at the Faculty of Science of Nature and Life, University DjillaliLiabes of Sidi Bel Abbes Algeria.

Water content

The fresh leaves of each sample were weighed and record as Wf, then dried at 50 °C for one week. The dry matter weighed was record as Wd. The leaf water content was calculated as the following: Water content (%) = (Wf−Wd) / Wf∗ 100

Where, Wf, fresh weight and Wd, dry weight.

Chlorophyll content

The contents of chlorophyll a, b and carotenoids were determined after their extraction from 1 g of fresh material, previously milled in 25 ml of 100% pure acetone. The samples were then centrifuged for 5 min at 500 g. After recovering the supernatant, the absorbance readings were made at 662 and 645 nm for chlorophyll a and b respectively and 470 nm for carotenoids which are the major absorption peaks.

The content was calculated using the formula of [10]:
Chlorophyll a: $C\,a$ (µg / ml) = $11.24\ A_{662} - 2.04\ A_{645}$
Chlorophyll b: $C\,b$ (µg / ml) = $20.13\ A_{645} - 4.19\ A_{662}$
Total carotenoids: C_{x+c} (µg / ml) = $(1000\ A_{470} - 1.90\ C\,a - 63.14\ C\,b) / 214$
(x + c = xanthophylls and carotenes).

The results obtained are used to calculate the pigment contents on the basis of µg / g fresh weight. The indicator of plant greenness is also calculated using the following equation *(a+b)/(x+c),* which represents the weight ratio of *chlorophyll* a and *b* to total carotenoids[10].

Total soluble carbohydrates content:

The amount of total soluble sugars was estimated by phenol sulphuric acid reagent method[11].Leaf samples (500 mg) were homogenized with 10 ml of 80% ethanol and placed in water bath at 80°C for 30 min. after centrifugation at 2000 rpm for 10 minutes, the supernatant was used for estimation of total soluble sugars (sucrose, fructose and glucose and their methyl derivatives). Two milliliters of alcoholic extract is pipetted into a colorimetric tube, and 1 ml of 5 % phenol is added. Then 5 ml of concentrated sulfuric acid is added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing, the tubes were allowed to stand 10 minutes, then they were shaken and placed for 10 to 20 minutes in a water bath at 25°to 30° C before readings are taken. The absorbance of the characteristic yellow- orange color is measured at 485 nm. Standard curve was prepared by using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight.

Total soluble protein content:

One g of fresh leaf was grinded in 10 ml of 0.06 M phosphate pH: 7 at 4°C. The homogenate was centrifuged at 3200 rpm for 30 minutes and the supernatant was recovered.

Protein content of extracts was determined by adding 0.8 ml of Bradford's reagent to 0.2 ml of the supernatant followed by vortexing. Absorbance of the mixture was recorded at 595 nm. Bovine Serum Albumin (BSA) was used as the standard for calibration[12].

Phenolic content and antioxidative activity:

Extraction of phenolic compounds:

The leaves were washed with distilled water, dried at room temperature, and then ground to a fine powder.

10 gm of powder were exhaustively three times extracted with methanol 80% at a ratio of 1:10 (w/v) using the maceration method for 12 h at ambient temperature. Both supernatants were mixed and concentrated using a rotary vacuum evaporator at 40°C then the solid crude extract was kept in the refrigerator (4°C) for further analysis.

Total polyphenol content:

The total phenolic contents were determined by the Folin-Ciocalteu method[13]. 20 µl of each extract (1 mg/ml) were mixed with 1.58 ml water, and then add 100 µL of the Folin-Ciocalteu (1/10) and 300 µL of Na_2CO_3 (7.5%). After 90 min at room temperature, the absorbance was measured at 765 nm. The total polyphenol content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (10 to 100 mg/ml). The estimation of the phenolic compounds was carried out in triplicate. The total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per g of dried extract.

Total flavonoid content:

The total flavonoids content of each plant extract was estimated by the method that uses aluminum chloride [14]. One ml of each sample containing 1mg/ml of dry extract was mixed with 5 mL of distilled water and subsequently with 0.3 ml of a NaNO2 solution (5%) (Merck). After 6 min, 0.3 ml of 10% AlCl3.6H2O (Merck) was added and allowed to stand for 6 min, then 2 ml of 1mol/L NaOH was added to the mixture. Immediately, water was added to bring the final volume to 10 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was then determined at 510 nm. Flavonoid contents were calculated using a standard calibration curve developed by using the known concentrations of catechin (10-100 mg/ml). The results were expressed as mg per g equivalent to catechin.

Antioxidant activity:

The antioxidant activity of the samples and standards was determined by way of the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). At 0.1 ml of the samples at different concentrations (0.05–10 mg/ml) were each added to 3.9 ml of DPPH methanolic solution (60 µmol/L). The blank sample consisted of 0.1 ml of methanol added to 3.9 ml of DPPH. After a 30 min incubation period at room temperature in the dark, the absorbance was measured at 517 nm [15].

% scavenging activity = $[(A_0-A_1)/A_0]$ X100. Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. IC_{50} value was calculated from the graph. Ascorbic acid was used for comparison.

Statistical analysis

All determinations were performed in triplicate, in three separate experiments and the values were averaged and reported along with the standard deviation (± Standard Deviation). Statistical analysis was carried out by using Microsoft Excel 2010 software.

Results and Discussion

Water content:

The highest water content was observed for Oleaster from the control site (clean) with a content of 63%, while the water content was observed for Oleaster from the polluted site with a rate of 47%. It has been reported that water content is associated with protoplasmic permeability in cells, and under

stress conditions of air pollution the transpiration rates are usually high, causes loss of water and dissolved nutrients, resulting in early senescence of leaves[16, 17, 18].

Chlorophyll content:
The determination of the chlorophyll content a, b and carotenoids of Oleaster shows a significant difference between the two stations (Table 1)

Table 1: Concentration of chlorophylls a, b, carotenoids (µg/g) and the pigment ratio in leaves of, the wild olive (*Olea europaea*) collected from Zahana station (polluted) and Tessala station (control sites)

Stations	Chlorophyll a	Chlorophyll b	Total carotenoids	Pigment ratio (a+b)/(x+c)
Zahana (polluted)	201 ± 1.2	100 ± 1.9	93 ± 1	3.22 ± 0.6
Tessala (control site)	388 ± 2.4	324 ± 3.1	101 ± 0.8	7.04 ± 0.7

The concentration of Chlorophyll a in the leaves at polluted sites was recorded as 201 µg/g which was 388 µg/g at the control site. Thus a reduction of 48.2 % in Chlorophyll a content was recorded in the samples from the polluted sites in comparison to control. The concentration of Chlorophyll b was 100 µg/g in the leaf samples collected from polluted sites while it was 324 µg/g in the samples from control site. The polluted sites sample thus had 69 % less Chlorophyll b content. The concentration of total carotenoids in the leaf samples from polluted and control site was recorded as 93 µ/g and 101 µg/g respectively with a reduction of 7.9 % in leaf samples from polluted sites. It is noted that the content of chlorophyll a and b are too low in the Zahana station (station subject to atmospheric pollution), while there is not a big difference in the amount of carotenoids. According to[19, 20]a reduction in chlorophyll content might be due the replacement of magnesium ions by two hydrogen atoms and degradation of chlorophyll molecules to phaeophytin. On another side, the photosystems are often damaged due to contamination by dust cement [21]. The alkaline nature of cement dust reduces the absorption of mineral substances from the soil, in particular Mg, Fe, Mn, which has a negative influence on the biosynthesis of chlorophyll[22]. The pigment ratio between chlorophyll and carotenoids is about 3.22 in the polluted station and is considered very low. According to [10], lower values for the ratio (a + b)/(x + c) are indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus, which is expressed by a faster breakdown of chlorophyll than carotenoids.

Total soluble carbohydrates content:
Despite the degradation of the Oleaster's chlorohyllof the station polluted by the cement plant, the soluble sugars show a very slight increase (16.44 mg/g) compared to the control station (15.46 mg/g) with a rate increase of 5.34%. The increase in soluble sugar content has also been observed by several authors due to metal stress[23, 24, 25], this is probably the result of perturbations in the hydrolysis of starch that can trigger leaf senescence[26].

Total soluble protein content:
Soluble protein content was reduced from 6.75 to 3.5 mg/g in the polluted area compared to the control zone with a reduction rate of 48.15%.The reduction of the total soluble protein content in areas subject to atmospheric pollution may be due to the degradation of chlorophyll with damage or closure of the stomata of polluted leaves which greatly reduces the photosynthetic activity, the increase of the activity of proteases and their decompositions into amino acids and reduction of the amount of newly synthesized proteins[27, 28, 29].

Phenolic content and antioxidative activity:
Total polyphenolic content significantly increased in the polluted station. For control station, the content of phenolics was 9.14, while at the cement plant it was 15.9 mg/g GAE DW, (Table 2). Flavonoids also increased in comparison to the control from 6.08 to 8.31 mg/g CE DW, (Table 2). Accumulation of polyphenols and flavonoids in leaves under air pollution have also been observed in other studies[30, 31]. There is an inverse relation between IC_{50} values and antioxidant activities; a lower IC_{50} values indicate a higher antioxidant activity. The methanolic extract of the leaves of the Zahana station (polluted) showed a greater DPPH radical scavenging capacity compared to the methanolic extract of the leaves of the Tessala station (control site), with an IC_{50} of 0.5 mg/ml against 0.6 mg/ml respectively (Table 2). During stress, phenolic compounds and especially flavonoids can be metal

chelators, they also act as scavengers of free radicals to protect plants from damage caused by oxidative stress, and their antioxidant action is mainly due in their chemical structure[32].

Table 2: Contents of polyphenols, flavonoids and IC 50 of the antioxidant activity of Oleaster extracts, from the Tessala station and the Zahana station

Stations	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg EC/g)	IC_{50} (DPPH) (mg/ml)
Zahana (polluted)	15.9 ± 0.1	8.31 ± 0.9	0.5
Tessala (control site)	9.14 ± 0.4	6.08 ± 0.3	0.6

Conclusion

Although the Oleaster of the zahana station polluted by the cement factory shows a degradation of the chlorophyll composition, it resists these stressful conditions by an accumulation of phenolic compounds; in particular the flavonoids that help fight against oxidations. These molecules can serve as biomarkers of atmospheric pollution for *Olea europaea* subsp**.** *europaea*.

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