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Olive Plants (*Olea europaea* L.) as a Bioindicator for Pollution

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Abstract: In the present work, olive plant (*Olea europaea* L.) was used as a biological indicator for pollution in which, molecular and physiological parameters were studied. Olive plants were collected from polluted and non-polluted areas in Jeddah - Saudi Arabia, traffic area as an air polluted area, sewage treatment station as water polluted area, industrial area as solid waste polluted, costal area as marine polluted area and an area without a direct source of pollution far away from the city center, which was used as control. These changes conducted with nucleic acid content, minerals content, pigments and some growth parameters. Results showed significant reductions in DNA and RNA contents under all polluted sites. Mineral contents were varied widely depending on the different pollutants and locations of olive plant. Generally, micro-elements varied (increase/decrease) significantly within collected samples and the source of pollution. All growth parameters were decreased significantly within the studied samples of all pollutant areas except the relative water content was increased. The content of chlorophyll a has decreased highly significantly in all polluted leaves. While the content of chlorophyll b has increased significantly in all polluted leaves especially in air polluted leaves. The total content of carotenoid pigments has decreased highly significantly in all polluted leaves. It was concluded that olive plant can be used as a biological indicator to the environmental pollutants.

Key words: *Olea europaea* L., pollutants, nucleic acid content, minerals content, pigments, growth parameters

INTRODUCTION

The weather changes caused by certain gases and solid pollutants can be discussed because these atmospheric changes leave fingerprints certainly clear on living organisms and symptoms that appear along with the analysis can give an idea of the source of emissions and health damage (Ahmad *et al*., 2007). Human activities in everywhere have increased the number of environmental constraints that cultivated plants have to deal with and atmospheric pollution is probably the main one.

Atmospheric pollution was released from many different anthropogenic sources such as industry, combustion of fossil fuels in vehicular traffic and energy production. Transportation is the most significant source of air pollution in urban areas when it comes to toxic trace elements like lead, so special attention must given to traffic pollutants. Intense road traffic creates gridlock every day and is also responsible for large missions of traffic-related air pollutants (Oliva and Espinosa, 2007).

Sawidis *et al*. (2011) reported that the gasses of motor vehicles, metalworking industries and other anthropogenic sources are the main sources of air pollution. Atmospheric pollutants are categorized into gaseous (mainly SO_2, NO_x and O_3) and dust, which often includes heavy metals. The increase in concentrations of these pollutants causes progressive reduction in growth and productivity of plants mainly, in correlation with reduction in photosynthetic ability of leaves and closure of leaf stomata of plants (Larcher, 1995).

Olive (*Olea europaea* L.) is a popular evergreen tree grown and cultivated in the Mediterranean region where several environmental constraints are limiting factors for its productivity. Probably the main restrictions for olive cultivation in the Mediterranean climate are water deficit and salinity stresses, though olive behaves as an intermediate drought and salt tolerant species when compared with other temperate fruit trees (Vitagliano and Sebastiani, 2000).

There are many studies conducted on olive plants, which focused on the effect of salinity, the mechanism of salt tolerance, growth biomass, physiological, biochemical

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and molecular changes occurring during development and ripening (Chartzoulakis, 2005; Tabatabaei, 2006; Conde *et al*., 2008; Melgar *et al*., 2008; Perica *et al*., 2008).

Generally, plant responses to air pollution are helpful in establishing the early presence of air-borne contaminants, determining the regional distribution of the pollutants and estimating the concentration of each pollutant. Also, providing a passive system for collecting pollutants for chemical analyses later and obtaining direct identification of different pollutants on the basis of plant species and variety affected (Sikora and Chappelka, 2004).

Olive tree leaves were used by Turan *et al*. (2011) as a bio-indicator for environmental pollution in the Province of Aydin (Turkey) and the possibility to use olive tree (*Olea europaea* L.) leaves for bio-monitoring and assessment of environmental pollution was shown to be possible in the Mediterranean region where they are indigenous and cultivated.

The present work is aimed to investigate the effect of different sources of pollution on molecular and physiological parameters of olive plants and the possibility to use olive tree leaves as a bio-indicator for environmental pollution.

MATERIALS AND METHODS

Plant material: The olive plant (*Olea europaea* L.) is commonly found in all five studied areas. Olive leaves of the same age, uniformly around the lower foliage and an initial quantity of about 100 g of each sample were collected. Trees of approximately the same age were chosen and care was taken to avoid selecting leaves with imperfections such as insect infestation, presence of honeydew, bird dropping, pesticide treatment, chlorosis or necrosisand coarse. The sampling was conducted simultaneously at each study area in early summer. Trees were selected randomly, which means no trees standing next to each other were chosen. Immediately after the collection the samples were placed in paper envelopes. The olive leaves were divided into three sets. The first one was taken in fresh state for estimation of pigments and growth parameters. The second set of leaves was air dried and used for estimation of total nucleic acids content. The third one was oven dried and used to estimate some minerals.

Quantitative estimation of nucleic acids: DNA and RNA were extracted following the method adopted by Schmidt and Thannhauser (1945) as described by Morse and Carter (1949). RNA was estimated colorimetrically by the orcinol reaction as described by Dische (1953) while DNA was estimated by diphenylamine (DPA) color reaction as described by Burton (1956).

Determination of certain minerals: The method of extraction adopted was that of Chapman and Pratt (1982). Sodium, potassium, calcium were determined photometrically by using flame emission photometer (B-7-E). Magnesium, chromium, cobalt, cadmium, lead, iron, manganese, zinc and copper were estimated by atomic absorption spectrophotometry (FMD3).

Quantitative estimation of pigments: The photosynthetic pigments chlorophyll a, chlorophyll b and carotenoids were determined in the leaves of plant. The spectrophotometric method recommended by Metzner *et al*. (1965) was used. Five gram fresh weight of leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged and the supernatant was made up to volume with 85% acetone. The extraction was measured against a blank of pure 85% aqueous acetone at three wave lengths of 452.5, 644 and 663 nm using Spekol Spectrocolourimeter VEB Carl Zeiss. Taking into consideration the dilutions made of the pigment fractions, chlorophyll a, chlorophyll b and carotenoids were determined as μg mL^{-1} using the following equations:

$$\text{Chlorophyll a} = 10.3\ E663 - 0.918\ E644 = \mu g\ mL^{-1}$$

$$\text{Chlorophyll b} = 19.7\ E644 - 3.87\ E663 = \mu g\ mL^{-1}$$

$$\text{Carotenoids} = 4.2\ E452.5 - (0.264\ \text{Chlorophyll a} + 0.426\ \text{Chlorophyll b}) = \mu g\ mL^{-1}$$

Then, the fractions were calculated as mg 100 g^{-1} fresh weight of leaves.

Determination of some growth parameters: Olive leaves were randomly collected from each area and used for the measurements of fresh weight (g), dry weight (g), relative water content (%) and leaf area (cm^2).

Determination of leaf relative water content: Leaf relative water content was measured using the method of Yamasaki and Dillenburg (1999).

Determination of leaf area: Leaf area calculated from the weight of leaves. A leaf disk with known surface area was cut with a calibrated cork borer and then weighed. The rest of the leaf was also weighed and leaf area was determined.

Statistical analysis: The statistical analysis of the obtained data was done using the Least Significance Difference test (LSD) at 1 and 5% levels of probability (Snedecor and Cochran, 1973).

RESULTS

Nucleic acid content: The changes in nucleic acid contents (DNA and RNA) in the *Olea europaea* L. leaves affected by pollutants were recorded in Table 1. DNA and RNA contents were varied according to pollutant's site. As shown in Table 1 that highly significant reductions in DNA and RNA contents under all polluted sites. In which the solid waste pollution gave the highest reduction in DNA and RNA content, while the air pollution gave the lowest effect in DNA content and marine pollution gave the lowest effect on RNA content.

Minerals content: Twelve minerals were determined in all samples of the different polluted sites. Mineral contents vary widely depending on the different pollutant and locations of olive plants (Table 2, 3). Four macro-elements were investigated (Na, K, Ca and Mg) in leaves samples collected from polluted sites. Among the macro nutrients, the concentration of Na recorded high significant increase (14.06, 21.42, 19.63 and 17.85 mg 100 g^{-1}) than the control value (13.09 mg 100 g^{-1}) in all samples studied. The content of K decreased significantly within all samples and the highest value for reduction was observed in leaves samples collected from marine pollution (Table 2).

Also, the content of Ca decreased significantly within all samples and the lowest value (11.00 mg 100 g^{-1}) was observed in leaves samples collected from solid waste pollution (Table 2).

The content of Mg was varied within the studied samples, its high significant increase observed in samples of air pollution (6.89 mg 100 g^{-1}), in water pollution (7.23 mg 100 g^{-1}) and in marine pollution (6.78 mg 100 g^{-1}). While, its high significant decreased (5.19 mg 100 g^{-1}) recorded in leaves samples collected from solid waste pollution (Table 2).

Eight micro-elements were determined in the present study; Mn, Cr, Co, Fe, Zn, Cd, Pb and Cu. In generally, Mn has decreased in all studied samples except in marine pollution samples were highly increased. Cr and Cu have increased in all studied samples except in marine pollution samples. Co and Zn have decreased in all studied samples, Fe and Pb have increased in all studied samples, Cd has decreased in water and marine pollution samples and increased in air and solid waste pollution samples. (Table 2, 3).

Growth parameters: Growth parameters of the studied samples were recorded such as fresh weight, dry weight and leaf area. All these parameters were decreased significantly within the studied samples of all pollutant areas (Table 4). The fresh weight has decreased highly significantly from 2.8 g at the control to the values of 2.4, 1.7, 2.1 and 2.3 g in air polluted, water polluted, solid waste polluted and marine polluted leaves respectively. The dry weight has decreased highly significantly from 1.78 g at the control to the value of 1.2, 0.8, 1.0 and 1.4 g in air polluted, water polluted, solid waste polluted and marine polluted leaves, respectively. Relative water content was increased highly significantly from 36.43% at the control to the values of 50.00, 52.94, 52.38 and 39.13% within the studied samples of all pollutant areas, respectively. Leaf area scored high significant decreased in all samples of polluted areas.

Table 1: Changes in DNA and RNA contents of *Olea europeaa* L. leaves, values listed are expressed as µg nucleic acid 100 g^{-1} air dried weight, each value is a mean of three determinations

Site of pollution	DNA content	RNA content
Control	10.52	91.40
Air pollution	8.21-HS	58.70-HS
Water pollution	3.83-HS	53.00-HS
Solid waste pollution	3.69 -HS	45.70-HS
Marine pollution	3.88-HS	77.10-HS
LSD at 5%	0.45	2.67
LSD at 1%	0.65	3.84

HS: Highly significant change

Table 2: Changes in minerals of *Olea europaea* L. leaves (mg 100 g^{-1}) oven dried weight

	Macro elements			
Site of pollution	Na	K	Ca	Mg
Control	13.09	70.74	81.92	6.32
Air pollution	14.06+HS	68.04-HS	24.42-HS	6.89+HS
Water pollution	21.42+HS	54.97-HS	21.32-HS	7.23+HS
Solid waste pollution	19.63+HS	44.12-HS	11.00-HS	5.19-HS
Marine pollution	17.85+HS	34.58-HS	24.42-HS	6.78+HS
LSD at 5%	0.51	2.20	4.02	0.11
LSD at 1%	0.73	3.17	5.78	0.16

HS: Highly significant change

Pigment content: Differences in pigment contents in the *Olea europaea* L. leaves affected by pollutants are recorded in Table 5. The content in chlorophyll a has decreased highly significantly in all polluted leaves. The content in chlorophyll b has increased significantly from 29.668 mg g^{-1} at the control to 42.496, 39.504, 38.392 and 41.520 mg g^{-1} fresh within the studied samples of all pollutant areas, respectively (Table 5). The total content of carotenoid pigments has decreased highly significantly from 3.700 mg g^{-1} fresh matter at the control to the values of 1.552, 3.332, 2.828 and 1.984 mg g^{-1} fresh matter, respectively.

Table 3: Changes in minerals of *Olea europaea* L. leaves (mg 100 g^{-1}) oven dried weight

	Micro elements							
Site of pollution	Co	Fe	Zn	Cd	Pb	Cu	Mn	Cr
Control	1.01	2.48	0.34	1.99	4.16	0.60	0.29	1.63
Air pollution	1.01 NS	3.03+HS	0.27-HS	2.45+HS	6.12+HS	0.98+HS	0.29 NS	1.85+HS
Water pollution	0.92-HS	5.24+HS	0.32-HS	1.62-HS	5.16+HS	0.87+HS	0.23-HS	1.88+HS
Solid waste pollution	0.64-HS	6.21+HS	0.30-HS	2.33+HS	6.32+HS	0.66+HS	0.21-HS	1.79+HS
Marine pollution	1.01 NS	2.89+HS	0.32-HS	1.85-HS	4.57+HS	0.59 NS	0.36+HS	1.57-HS
LSD at 5%	0.02	0.24	0.004	0.05	0.13	0.03	0.008	0.02
LSD at 1%	0.03	0.34	0.005	0.07	0.19	0.04	0.012	0.03

HS: Highly significant change

Table 4: Changes in some growth parameters of *Olea europaea* L. leaves

Site of pollution	Fresh weight (g)	Dry weight (g)	Relative water content (%)	Leafarea (cm^2)
Control	2.8	1.78	36.43	6.75
Air pollution	2.4-HS	1.2-HS	50.00+HS	4.75-HS
Water pollution	1.7-HS	0.8-HS	52.94+HS	5.55-HS
Solid waste pollution	2.1-HS	1.0-HS	52.38+HS	3.75-HS
Marine pollution	2.3-HS	1.4-HS	39.13+HS	5.23-HS
LSD at 5%	0.06	0.05	1.12	0.16
LSD at 1%	0.08	0.08	1.60	0.23

HS: Highly significant change

Table 5: Changes in pigments of *Olea europaea* L. leaves (mg 100 g^{-1}) fresh weight of leaves

Site of pollution	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a/b	Carotenoids
Control	43.560	29.668	73.228	1.468	3.700
Air pollution	35.216-HS	42.496+HS	77.712+HS	0.829-HS	1.552-HS
Water pollution	37.276-HS	39.504+HS	76.78+HS	0.944-HS	3.332-HS
Solid waste pollution	36.112-HS	38.392+HS	74.504+HS	0.941-HS	2.828-HS
Marine pollution	37.876-HS	41.520+HS	79.396+HS	0.912-HS	1.984-HS
LSD at 5%	0.47	0.73	0.35	0.04	0.13
LSD at 1%	0.67	1.05	0.51	0.05	0.19

HS: Highly significant change

DISCUSSION

Turan *et al*. (2011) suggested that the usage of olive tree leaves or other trees as bio-indicators for environmental pollution is very common and useful instead of physical indicators.

Now a day, plants can be accepted and effectively used as biomonitors of environmental pollution. Micro and Macro element analysis of plant samples has for many years been an alternative, easy and effective way of conducting ecological research in urban areas (Aksoy *et al*., 2000). The use of vegetation as passive sampler in biomonitoring bears the advantage of high spatial and temporal resolution due to the excellent availability of plants and low sampling costs. Many plant groups, including the evergreen trees, have been used for monitoring air pollution and their (dis) advantages for this purpose have been pointed out (Laaksovirta and Olkkonen, 1977; Murin, 1995).

This study has shown significant changes in the contents of nucleic acids and mineral elements, in all growth parameters and also in pigments content according to different type of pollutants.

Atmospheric pollution is a direct and undesirable effect of human activities. Air pollutants are those gases which should not be present at all in the clean atmosphere or which are normally present in it, but reach quantities that impair crop production. Visible symptoms of air pollutant injury on plants are not easily observable and can be monitored only in particularly experimental conditions (for example; sensitive cultivars and controlled fumigation). Vitagliano and Sebastiani (2000) reported that the effects of air pollutants (such as O_3 and CO_2) on plants may be very subtle and the effects on crop productivity may be very slow and may escape control. Mansfield (1998) suggested that CO_2 pollution may disrupt the control of water relations in some species because their stomata do not close sufficiently in CO_2-enriched air.

In this study a highly reduction in chlorophyll a under pollution was recorded, these result concomitant with Nanos and Ilias (2007). They demonstrated that reduction of light quantity and/or quality reaching chloroplasts possibly caused the reduction of photosynthetic rate and quantum yield. They reported that photo system damage may have occurred due to heavy metal toxicity, often associated with cement dust contamination. In addition, the presence of dust between the peltate on the lower leaf surface must have caused-among other possible effects-a decrease in leaf conductance to water vapor and CO_2 movement, although without significantly affecting transpiration.

Al-Absi *et al.* (2009) and Madejon *et al.* (2006) reported that the content of micro elements in olive leaves are lower than in olive fruits and the concentration of trace elements in the leaves and fruits decreased with time, in consequence, the toxicity risk to the food web diminished.

It is clearly that, tree leaves absorb dusts of elemental pollutant both from the air and from the soil through roots. Therefore, chemical composition of leaves varies with the seasons, especially of heavy metals, which is generally higher at the end of summer. Therefore, this season seems to be appropriate period of sampling. There many trees and shrubs have been particularly used for establishing selected elements even in trace and ultra-trace levels (Ahmad *et al.*, 2007).

Results showed that marine pollution in olive trees decreased potassium concentrations, this may be due to a direct effect of sodium, displacing potassium and/or causing loss of potassium from the root tissue (Kchaou *et al.*, 2010). A positive correlation between the leaf sodium concentration and total leaf area per plant was recorded in the present work and decreases in total leaf area per plant were correlated with increases of leaf sodium concentration.

Plants grown under NaCl salinity showed an increase in sodium and chloride concentrations and a decrease in potassium and calcium (Bartolini *et al.*, 1991; Tattini *et al.*, 1995; Chartzoulakis *et al.*, 2002; Vigo *et al.*, 2002). Salinity can directly affect nutrient uptake, such as sodium reducing potassium uptake or by chloride reducing nitrate uptake (Grattan and Grieve, 1998).

In olive cultivars, salt tolerance is associated with effective mechanisms of ion exclusion and retention of sodium and chloride in the roots (Massimiliano, 1994; Tattini *et al.*, 1995; Chartzoulakis *et al.*, 2002), limiting the accumulation of these ions into actively growing shoots. Massimiliano (1994) reported that the resistance mechanism of salt-tolerant olive cultivar is probably related to sodium exclusion by roots and the ability to maintain an appropriate K/Na ratio in actively growing tissues. Ion exclusion and compartmentation at the root level regulates ion concentration in the xylem sap preventing accumulation of potentially toxic ions in the aerial part (Flowers and Yeo, 1989; Drew *et al.*, 1990). The effectiveness of exclusion mechamism depends on the salinity level.

Also all growth parameters of the studied samples were recorded such as fresh weight, dry weight and leaf area were decreased significantly within the studied samples of all pollutant areas except the relative water content was increased.

Salinity stress caused deleterious effects on many growth parameters in trees and shrubs like olive trees (Therios and Misopolinos, 1988; Tattini *et al.*, 1992, 1995; Chartzoulakis *et al.*, 2002; Vigo *et al.*, 2005). An important mechanism to avoid the deleterious effects of salinity in olive trees is the ability to limit uptake and/or transport of saline ions (sodium and chloride) from the root zone to aerial parts (Chartzoulakis, 2005).

Total leaf area per plant was significantly reduced in all cultivars by salt treatment, which was due to both a decrease of leaf growth and a leaf abscission phenomenon during the salt treatments (Kchaou *et al.*, 2010).

Giorgelli *et al.* (1994) reported that treated plants by gradient of sulphur dioxide concentrations in the range 0-100 pp have not induced visible injury symptoms, but treatment induced significant depression in net CO_2 assimilation, stomatal conductance and transpiration and an increase in vapor pressure deficit. Transpirating surface was dramatically reduced in plants by the treatment and also total leaf thickness was also reduced.

Minnocci *et al.* (1999) reported that long-term exposure to low O_3 concentrations produces significant effects on olive leaves physiology (gas exchange and stomatal aperture) and morphology (individual leaf area and stomatal density). They observed that after 100 days of treatment, leaf drop and development of necrotic spots were observed in O_3-fumigated plants, significant reductions in photosynthetic activity (57%) and stomatal conductance (69%) were detected in O_3-fumigatedplants compared with control plants, a significant reduction in stomatal conductance (40%) was observed, leaves that developed after exposure to O_3 showed decreased stomatal aperture and also the large O_3-induced reduction in transpiring stomatal surface could have significant effects on olive productivity. The influence of ambient ozone pollution on olive leaf gas exchange irrigated with saline water was also studied by Abusafieh and Nanos (2011).

Mezghani *et al.* (2005) in a study on fluoride accumulation by vegetation in the vicinity of a phosphate fertilizer plants in Tunisia have reported that absorption and accumulation of fluoride are more important in trees with evergreen foliage (olive tree) than in with deciduous leaves. They also observed that there was a highly significant correlation between foliar fluoride concentration and atmospheric fluoride concentration up to a distance of 16 km from the pollution source.

Air and soil pollution with trace metals specially heavy metals is a matter of great interest, especially in urban areas. Biomonitoring of air quality using plants has been widely applied to detect and to monitor the effects of pollution (Sawidis *et al.*, 1995a-c; Bargagli, 1998; Mingorance and Oliva, 2006; Gajic *et al.*, 2009). Although

biomonitoring of air quality using plants has been practised for many years, in many European countries, it has still not been applied at a satisfactory level, due to different and even opposing results, depending first of all on the type of soil and bedrock. Trees are very efficient at trapping atmospheric particles and they play a special role in reducing the level of fine, "high risk" respirable particulates, which have the potential to cause serious human health problems (IPHRS, 2004).

In conclusion, the use of olive tree leaves as a biological indicator to environmental pollutants is a useful technique for the detection of the seriousness of those pollutants on the environment and human health, directly/indirectly, especially with regard to crops and plants of economic importance which are used as food for humans, either directly or through the manufacture of those plants.

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