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Leaf biochemical responses and fruit oil quality parameters in olive plants subjected to airborne metal pollution

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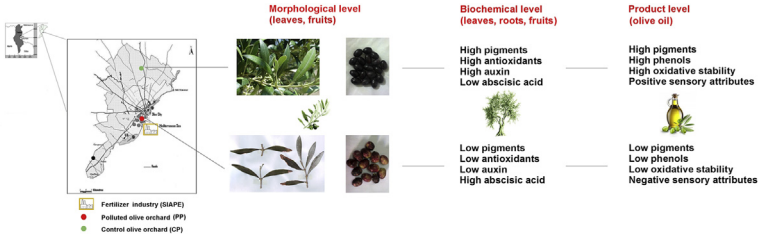
HIGHLIGHTS

- Olive trees and fruits exposed to airborne metal pollution were studied for two years.
- High levels of Cd, Cu, Fe, Mn, Ni and Pb were found on the fruits but not in the oil.
- A depression of the antioxidant system and changes in hormone levels occurred.
- Atmospheric metals negatively affected olive oil chemical and sensory quality.
- Airborne metal pollution could indirectly have negative effects on oil consumers.

GRAPHICAL ABSTRACT



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ABSTRACT

This study was carried out in two olive orchards (*Olea europaea* L., cv. Chemlali) located in a polluted area near a fertilizers factory and in a control unpolluted site, managed with similar cultivation techniques. The aim was to investigate the physiological and biochemical responses of polluted plants (PP), exposed to atmospheric metal contamination (Cd, Cu, Fe, Mn, Ni and Pb) as compared to control plants (CP). Leaves, roots and fruits of PP showed a depression of their non-enzymatic and enzymatic antioxidant defences and a disruption of their hormonal homeostasis. The anomalous physiological status of PP was also demonstrated by the lower values of pigments in leaves and fruits, as compared to CP. Atmospheric metals negatively affected olive oil chemical and sensory quality. However, despite metal deposition on fruit surfaces, the accumulation of potentially toxic metals in olive oil was negligible. Considering that olive oil is an important food product worldwide and that many productive olive orchards are exposed to several sources of pollution, this work could contribute to clarify the effects of atmospheric metal pollution on olive oil quality and its potential toxicity for humans.

1. Introduction

Environmental abiotic stresses, such as extreme temperature, salinity, drought, flooding, metal exposure and air pollutants,

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greatly affect plant metabolism and productivity. The plant surfaces are a major sink for aerosol and airborne particles, which may negatively influence vegetation health (Pariyar et al., 2013; Yan et al., 2014). Airborne particles are important metal carriers, especially in polluted areas such as in urbanized and industrialized regions. Particularly, fertilizers production is one the major sources of anthropogenic atmospheric particles and metal pollution (Wang et al., 2011).

Nowadays, in Tunisia, due to the intensive increase of the industrial productions, the olive cultivation, which is one of the main agricultural activities in the country, is facing the combined effects of arid climate and air pollution. In Sfax city, the main industrial area in Tunisia, it has been recently observed that the landscape around the factory called 'Société Industrielle d'Acide Phosphorique et d'Engrais' (SIAPE) ('Industrial Society of Phosphoric Acid and Fertilizers') is characterized by the progressive degradation of surrounding vegetation. Metals (particularly Cd, Cu, Fe, Mn, Ni, Pb and Zn) are among the most phytotoxic air pollutants emitted from this industry (Ben Abdallah and Boukhris, 1990; Azri et al., 2002; Mezghani et al., 2005).

Regarding their relation with plants, metals can be classified into two categories: essential metals, which are necessary in many physiological processes and whose deficiency or excess may cause harmful effects in plant cells (e.g., Cu, Fe, Mn, Ni), and non-essential metals, which can be toxic even at relatively low concentrations (e.g., Cd, Pb) (Appenroth, 2010; Krzeslowska, 2011). Usually, metals are loosely bound to the surface of dust particles, thus they might be highly mobile and potentially bioavailable (Marx et al., 2008). Their residence time in the atmosphere varies according to the size of metal-bearing particles. Coarse particles settle rapidly and near the pollution source, whereas fine particles settle very slowly and are usually dispersed to higher distances by the wind (Candeias et al., 2014). Thus, deposition is a significant pathway for the transfer of metals from the atmosphere to plants and soils. Metals can be absorbed by plants via root uptake and/or via direct foliar contact, and exert detrimental effects on plant physiology.

A great deal of research has been performed to evaluate the effects of metal pollution on plant productivity and/or to determine metal accumulation in the different plant parts. However, few works have focussed on the changes in plant biochemical traits induced by plant exposure to metals. Moreover, the effects of metal pollution on plants have been mainly studied in annual or forest species, while information on the response of tree crops is scarce. Indeed, to our knowledge, no study has addressed the effect of airborne metal pollution on both plant biochemical responses and oil quality of olive trees. Olive tree (*Olea europaea* L.) is one of the most important commercially valuable crops in Tunisia, particularly in Sfax region, not only for its socio-economic importance, but also for the benefits of olive fruits in limiting several cardiovascular diseases, certain types of cancers and artherosclerosis (Romero et al., 2002; Somova et al., 2003). Recently, it has been shown that olive leaves possess interesting antimicrobial and antioxidant activities and can be used as a natural and functional ingredient in food technology (Lee and Lee, 2010; Taamalli et al., 2012). Moreover, local populations used decoction of olive leaves in folk medicine for its beneficial effects against several diseases, as described by Japon-Lujan and Luque De Castro (2007). For this reason, the consumption of olive oil and table olives has steadily increased in recent years, even in countries that do not have a tradition (International Olive Council, 2015). To the best of our knowledge, most papers dealing with responses of olive trees grown around industrial activity have focused only on gaseous pollution and its effects on some morphological and biochemical effects on leaves (Mezghani et al., 2005; Nanos and Ilias, 2007; Dilek et al., 2011).

In this study, the biochemical responses of olive plants exposed to atmospheric metal pollution in an area near a fertilizer factory have been investigated. The results were compared with those of olive trees growing in a relatively clean area, not directly exposed to metal pollution. For this purpose, the following determinations were carried out: (a) accumulation of metals on olive fruits and into the oils extracted from the same fruits; (b) levels of plant growth regulators, pigments, and enzymatic and non-enzymatic antioxidants in olive organs exposed to metal-bearing aerosol; and (c) chemical and sensory quality indices of the extracted oils.

2. Materials and methods

2.1. Plant material, experimental design and oil extraction

The region of Sfax is located in the south east of Tunisia on the Mediterranean Sea (34°43'N; 10°46'E, area of 7086 km^2). It is characterized by an arid Mediterranean climate largely influenced by its mild and gentle topography, and its maritime exposure. The region is well ventilated with low to moderate wind velocities rarely exceeding 5 m s^{-1} (Bahloul et al., 2015). Over a 52 year-period, the average total year rainfall is 210 mm, the average minimum air temperature of the coldest month (January) is 6.5 °C and the average maximum air temperature of the hottest month (August) is 31 °C, with a yearly mean of 23 °C. Most of the total annual rainfall is mostly occurring from October to December; the dry period is during June–September (data provided by the Meteorological Station of Sfax city).

The studied polluted region is a low plain along the Mediterranean sea-side lined up with series of 100-m-high hills and situated about 20 km along the coast. It is submitted both to continental dry winds and to highly humid sea coastal winds. The prevailing winds from the southeastern sector have a frequency of 25.5%, and those of the southwestern sector appear with a frequency of about 16.3%. However, northwest and north-east winds occur with intermediate frequencies. The studied olive trees (*Olea europaea* L., cv. Chemlali) were located in a land plot located at 0.7 km from the "Société Industrielle d'Acide Phosphorique et d'Engrais" (SIAPE) factory, close to Sfax city. They consist of 35-year-old trees planted on a loamy sand soil and are referred to as polluted plants (PP).

The SIAPE is the main pollution source in Sfax city. It is a phosphate fertilizer producing factory, located in the southern suburb of Sfax, that converts crude phosphate with a high fluoroapatite [$Ca_5(PO_4)_3F$] content into a granule phosphate fertilizer easily assimilated by plants. During the phosphate attack by sulphuric and phosphoric acids, metals (particularly Cd, Cu, Fe, Mn, Ni, Pb and Zn) are released from the industry chimney in the form of inorganic particulate (dust emission at source = 160 kg h^{-1}). The factory emissions of metal in the particulate were found at concentrations of 2.0 (Cd), 3.2 (Cu), 7.4 (Fe), 2.4 (Mn), 3.1 (Ni), 0.3 (Pb), and 9.4 (Zn) g t^{-1} of raw material (Ben Abdallah and Boukhris, 1990; Azri et al., 2002; Mezghani et al., 2005). Generally, wet deposition dominates during winter (in coincidence with high precipitation). It may occur as washout from smoke below clouds or rainout of particulates taken up by clouds. Whereas, dry deposition would dominate in summer period (in association with low precipitation). Therefore, vegetation developed around the SIAPE Society is continuously exposed to air metal pollution. On the other side, the control plot (with control plants, CP) was located in El Hencha region, 40 km north of SIAPE, in an inland rural area, without any industries, where winds occur with scarce frequencies. In the control plot, the same atmospheric metals examined for PP were found at concentrations below the instrument detection limits.

The olive plants of the two sites (PP and CP) were similar in age,

plant density and training system. For each experimental site, ten trees from two adjacent rows (total 20 trees per treatment) were selected to be similar in potential yield and canopy architecture. For both PP and CP, four composite leaf samples (each from five selected trees) were collected, at 06:00–07:00 h, in selected days of February, May, August and November 2013 and 2014. Fully expanded leaves with similar light exposition and position in the canopy were sampled from each plant along the median segment of new-growth shoots (young leaves) or one-year-old shoots (mature leaves) (the average life of an olive leaf is 30 months, according to Sofo et al., 2004). Well-developed fruits were chosen inside the canopy using the same method adopted for leaves. Active roots (at least 10–15 g fresh weight, with a diameter < 5 mm) were sampled (composite samples), at 06:00–07:00 h, in November 2013, and May and August 2014 by excavating the part of the soil around the plant. All the samples were immediately frozen in liquid nitrogen and then stored at −80 °C. The morphology of olive leaves was analysed in both PP and CP.

Oil extraction was carried out using an Abencor system within 24 h after fruit sampling. Olives (5 kg) were crushed with a hammer mill and were slowly mixed for 30 min at 25 °C. Next, the obtained paste was centrifuged at 3500 × *g* over 3 min. The oil was then separated by decanting, transferred into dark glass bottles and stored in the dark at 4 °C.

2.2. Determination of metals

Metal extraction from fruit surface was carried out by shaking, at 100 rpm for 4 h, 50 g of fruits with 80 ml of 5% HNO_3 (TraceSELECT®; Sigma Aldrich, St. Louis, MO, USA). The liquid phase was then sampled, filtered with Whatman® 42 filter paper and stored at 4 °C until analysis. For oil analysis, an aliquot of 0.5 g of oil was added with 7 mL of 69% HNO_3 and 1 mL of 30% H_2O_2 (TraceSELECT®; Sigma Aldrich) and pre-digested overnight in PTFE-TFM liners. Oil samples were then mineralized using a microwave oven Multiwave 3000 (Anton Paar, Graz, Austria), according to the following digestion programme: 8 min to reach a power of 800 W (held for 8 min); 8 min from 800 W to 1000 W (held for 7 min); 6 min from 1000 W to 1200 W (held for 7 min); final cooling for 25 min. Digested oil samples were diluted to 25 mL with deionised water produced by a Milli-Q® system (Merck Millipore, Billerica, MA, USA), then filtered and stored at 4 °C, analogously to surface extracts. A certified standard reference oil sample (Certipur® Multi-element standard II dissolved in oil; Merck KGaA, Darmstadt, Germany) and blank sample (7 mL HNO_3 and 1 mL H_2O_2) were digested and prepared following the same procedures used for oil samples. The deviation between obtained concentrations and expected values for reference oil sample was less than 7% for all the elements analysed, so confirming the validity of the method.

Total concentrations of Cd, Cu, Fe, Mn, Ni, Pb and Zn were determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES; Thermo iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, MA, USA). The emission wavelengths selected for element quantification were: Cd 228.8 nm; Cu 324.7 nm; Fe 259.9 nm; Mn 257.6 nm; Ni 231.6 nm; Pb 220.35 nm; Zn 206.2 nm. The curve calibration was built on two points, using the blank solution as zero point and a multi-element calibration standard (Certipur® ICP Multi-element standard solution IV; Merck Millipore) at a concentration of 2 mg L^{-1}. The latter was prepared by dilution of a certified 1 g L^{-1} calibration standard in the blank acidic solution. Instrument detection limits were calculated for each metal as three times the standard deviation of ten replicates of the blank, and corresponded to 0.018 mg kg^{-1} for Cd, 0.053 mg kg^{-1} for Cu, 0.060 mg kg^{-1} for Fe, 0.015 mg kg^{-1} for Mn, 0.061 mg kg^{-1} for Ni, 0.103 mg kg^{-1} for Pb, and 0.052 mg kg^{-1} for Zn. The deviation between obtained concentrations and expected values for reference oil sample was less than 7% for all the elements analysed, so confirming the validity of the analytical method.

2.3. Chlorophyll and carotenoids determination

The extraction was carried out at 4 °C in dark conditions. Samples of leaves (25 mg) and fruits (250 mg) were homogenized in a mortar and pestle with 1.5 mL of 80% acetone (v/v). The extracts were centrifuged in sealed tubes at 15,000 × *g* for 5 min.

The supernatant was collected and the absorbance was read at 663 and 647 nm for chlorophyll *a* and chlorophyll *b*, respectively, and at 470 nm for carotenoid content. The concentrations for total chlorophyll (chlorophyll *a* + chlorophyll *b*), and the total carotenoids (xanthophylls and carotenes) were calculated according to the equations of Lichtenthaler and Buschmann (2001).

2.4. Anthocyanins and phenols determination

Aliquots of leaves and fruits (2.5 g) were extracted in 15 mL of acidified methanol (1% HCl) for 2 h at room temperature in the dark, and then centrifuged at 1000 × *g* for 15 min.

Anthocyanin levels in the methanolic extracts were calculated with the formula [A530 − (0.24 × A653)] (Gould et al., 2000), where A530 and A653 are the absorbances determined at 530 nm and 653 nm, respectively, using a Jasco V-530 UV–vis spectrophotometer (Jasco Corp., Tokyo, Japan). Total anthocyanin content was determined as mg cyaniding 3-glucoside equivalents (CGE) per 100 g of fresh weight, using an extinction coefficient of 26.900 L mol^{-1} cm^{-1} at 535 nm and a molar mass (MW) of 449.2 g mol^{-1}.

Total phenolic content was determined by the Folin-Ciocalteu colorimetric method using chlorogenic acid as a standard. Each reaction mixture contained 20 μL of 1:1 sample extract:distilled water and 100 μl of freshly prepared 4% sodium carbonate (anhydrous). The solution was vortexed and let stand for 5 min. Successively, 20 μL of Folin-Ciocalteu reagent were added. After 30 min, absorbance was measured at 750 nm. Total phenolic content was expressed as mg chlorogenic equivalents (CAE) per gram of fresh weight of tissue.

2.5. Activities of antioxidant enzymes

For the assays of the antioxidant enzymes, 500 mg (fresh weight) of tissue (leaves, roots and fruits) were homogenized with a pestle in an ice-cold mortar in 3 mL of an ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 1% (w/v) polyvinylpyrrolidone. The homogenates were filtered through four layers of cheesecloth, then centrifuged at 4 °C for 20 min at 15,000 × *g*. The supernatant was collected and used for the assays of enzymatic activities.

Total superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed in terms of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Sofo et al. (2004) with some modifications. The reaction mixture (final volume of 165 μL) contained 150 μL of 100 mM K/P buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM NBT, 2 μM riboflavin, and 15 μL of the diluted (1:10) extract. Samples were incubated for 10 min under fluorescent lamp (Osram R80, Milan, Italy) at 150 W and absorbance at 560 nm was read against unilluminated samples. One unit of SOD activity was defined as the amount of the enzyme required to cause 50% inhibition of the rate of NBT reduction.

The activity of catalase (CAT; EC 1.11.1.6) was measured in terms of H_2O_2 decomposition rate, according to the method of Sofo et al. (2004) with some modifications. The reaction mixture (final

volume of 165 μl) contained 150 μL of 50 mM K/P buffer (pH 7.0) + 8.8 mM 30% H_2O_2, and 15 μL of extract. The decline in absorbance at 240 nm was followed for 1 min and the amount of decomposed H_2O_2 was measured using an extinction coefficient of 39.4 $mM^{-1}cm^{-1}$. Samples without H_2O_2 were used as a blank. One unit of CAT activity was defined as the amount of the enzyme required to decompose 1 μmol of H_2O_2 in 1 min.

Total peroxidase (POX; EC 1.11.1.17) activity was determined measuring the oxidation of guaiacol to tetraguaiacol, which results in an increase in absorbance at 470 nm, according to the method of Chance and Maehly (1955) with some modifications. The reaction mixture (final volume of 200 μL) contained 150 μL of 50 mM K/P buffer (pH 6.1) + 50 mM guaiacol, and 50 μL of the diluted (1:8) extract. The reaction was started with the addition of 1 μL of 30% H_2O_2. The mixture was incubated at 25 °C for 15 min before reading the absorbance. The amount of tetraguaiacol was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1}, after subtracting the background absorbance due to the buffer solution and to the assay reagents. One unit of POX activity was defined as the amount of the enzyme required to produce 1 μmol of tetraguaiacol in 1 min.

The ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed according to the method of Francini et al. (2006) with some modifications, by measuring the oxidation of ascorbate at 290 nm at 25 °C for 1 min. The reaction mixture (final volume of 160 μL) contained 150 μL of 50 mM potassium phosphate (pH 6.6) + 1 mM ascorbate + 0.4 mM Na_2EDTA, and 15 μL of enzymatic extract. The reaction was started with the addition of 2 μL of 30% H_2O_2 (2 μL of hydroxylamine as inhibitor). One unit of APX activity was defined as the amount of enzyme required to oxidize 1 μmol of AsA in 1 min. All the enzymatic activities were expressed as units on mg of proteins. Protein content was measured according to Bradford (1976).

2.6. Indole-3-acetic and abscisic acids

For each tissue (leaves, roots and fruits), an aliquot of 250 mg was ground with a mortar and pestle and put in a tube. To each tube, 2.5 mL extraction solvent (2-propanol/H_2O/HCl 37%; 2:1:0.002, v/v/v) was added. The tubes were shaken at a speed of 100 rpm for 30 min at 4 °C. To each tube, 2.5 mL of dichloromethane was added and then the samples were shaken for 30 min at 4 °C and centrifuged at 13,000 × *g* for 5 min. After centrifugation, two phases were formed. An aliquot (1.0 mL) of the solvent from the lower phase was transferred using a Pasteur pipette into a screw-cap vial, and the solvent mixture was concentrated using an evaporator with nitrogen flow. Finally, the samples were re-dissolved in 0.1 mL methanol and stored at −20 °C.

The quantitative determinations of IAA and ABA were carried out by a competitive enzyme-linked immunosorbent assay (ELISA) using the following kits, respectively: Phytodetek® *t*-ZR Test Kit, Phytodetek® DHZR Test Kit, and Phytodetek® IAA Test Kit (Agdia Biofords, Evry, France). The means of the optical densities and binding percentage of duplicate standards or samples (100 μL) were calculated and plotted in a semi-logarithmical scale, in order to linearize the equation.

2.7. Oil chemical and sensory analysis

Oil total phenols, chlorophyll and carotenoids were analysed by spectrophotometric analyses. In particular, the total phenol content was determined by Folin-Ciocalteu method, applied on the phenolic extract obtained following the procedure of Favati et al. (1994). The phenolic content was expressed as mg gallic acid equivalents (GAE) kg^{-1} oil. Chlorophyll content was determined according to Pokorny et al. (1995) and expressed as mg pheophytine kg^{-1} oil. Carotenoids were analysed according to the method proposed by Minguez-Mosquera et al. (1991) and expressed as mg lutein kg^{-1} oil. Oxidative stability was measured with the Oxitest apparatus (Velp Scientifica, Usmate, MB, Italy), using 10 g of oil sample under high temperature (90 °C) and oxygen pressure (6 bar). The result was obtained graphically by using the two-tangent method and expressed as induction period (IP).

The method used for the panel test was developed by the International Olive Council (2015), according to European Commission Regulation 2568/91. A group of experts panelists identified oil specific characteristics and flavors by tastings (Morales et al., 1995; Angerosa, 2000; Angerosa et al., 2004). Panelists also monitored the eventual presence of any defects that might declass oil from the best-quality designation of 'extra-virgin' to other merchandise classes having lower quality.

2.8. Statistical analysis

The number of replicates (*n*) for each measured parameter is specified in the table and figure captions. In the case of composite samples obtained from fruits harvested or oil extracted in September, October, November and December of the same year, the same amounts of fruits or oil were used for each month. Statistical analysis of data was carried out using the software Sigmastat 3.1 SPSS Inc. software (SPSS Inc., IL, USA). Analysis of variance (ANOVA) of all the analysed parameters was performed. Means were statistically analysed by Fisher's LSD test at $P \leq 0.05$.

3. Results and discussion

3.1. Metal accumulation on fruit surface and in oil

Generally, air pollution caused a significantly higher accumulation of metals on PP fruit surface, compared to CP (Table 1). In 2013, Cu, Mn, Pb and Zn concentrations in PP surface extracts were significantly higher than the control (CP) as well as, in 2014, Fe and Pb concentrations in PP samples appeared statistically higher compared to CP (Table 1).

Metal concentrations in oil samples were in almost all cases below the instrument detection limits (see Supplementary Table 1 for the complete results on oil metals). Among the metals quantified in this study, only Pb is considered a contaminant by the Codex Alimentarius Commission (2011) which set Pb maximum permissible concentration in virgin olive oil at 0.1 mg kg^{-1}. Both in PP and CP oil samples, Pb concentration was below the detection limit and, consequently, it complied with the legislation limit. The International Olive Council (2015) has recently established some quality criteria for the international trade of olive oil. One of these standards is the maximum admissible content of two trace metals, *i.e.* Fe (3.0 mg kg^{-1}) and Cu (0.1 mg kg^{-1}). In this study, only in one case the Fe content exceeded this limit. Indeed, PP oil of December 2013 contained 5.6 mg kg^{-1} Fe, against 1.0 mg kg^{-1} Fe found in the corresponding CP oil. With regard to Cu concentration, it was slightly higher than the quality limit only in two samples, CP oil of December 2013 (0.16 mg kg^{-1}) and PP oil of December 2014 (0.12 mg kg^{-1}), thus revealing no correlation between air pollution and metal accumulation in oil. In the other samples, Cu level was always below the detection limit (0.05 mg kg^{-1}).

Although air pollution was associated with a higher metal accumulation on fruit epicarp, no correlation was found between air pollution and metal accumulation in the oil. Results of this study revealed that the repartition of metals to oil is negligible, but major risks might exist for olive mill wastewaters due to the atmospheric deposition of metals on olive surfaces and the higher solubility of these elements in water rather than in oil. Indeed, Llorent-Martínez

Table 1
Metals extracted from olive fruit surface of control plants (CP) and polluted plants (PP). The values represent means ($n = 4$) ± SD of composite samples obtained from fruits harvested in September, October, November and December of the same year. Values followed by different letters are statistically different ($P < 0.05$) within columns. LOD = limit of detection.

Sampling time	Treatment	Cd	Cu	Fe	Mn	Ni	Pb	Zn
		($\mu g\ mL^{-1}$)						
2013	CP	<LOD	0.0630 ± 0.0020 b	0.4498 ± 0.0927 a	0.0510 ± 0.0018 b	<LOD	<LOD	4.1315 ± 0.9388 b
	PP	<LOD	0.1498 ± 0.0092 a	0.5278 ± 0.0421 a	0.1213 ± 0.0089 a	0.0053 ± 0.0008 b	0.0393 ± 0.0047 a	6.1418 ± 0.5321 a
2014	CP	<LOD	0.0775 ± 0.0045 b	0.2145 ± 0.0177 b	0.0580 ± 0.0053 b	0.0665 ± 0.0063 a	<LOD	3.9693 ± 0.1566 b
	PP	<LOD	0.0585 ± 0.0014 b	0.4715 ± 0.0093 a	0.0550 ± 0.0069 b	0.0840 ± 0.0040 a	0.0245 ± 0.0039 a	3.0845 ± 0.2780 b

et al. (2014) found that less than 10% metals passed in the olive oil during oil extraction.

3.2. Leaf morphology and pigment analysis

In the polluted area, olive leaves exhibited marginal and apical necrosis of brick-red color, while fruits were caracterized by necrotic spots on their surface (Supplementary Fig. 1). In addition, the observation of PP leaves established the existence of a film of whitish dust, difficult to remove even after washing, probably due to air pollutants (Supplementary Fig. 1). Likely, metals are absorbed through leaf stomata and moved by transpiration into the principal sites of accumulation at the leaf tip and margins, where they caused the observed structural damages and necrosis. Indeed, no evident necrotic areas were found in CP leaves and fruits.

The trends of total chlorophyll and total carotenoids in leaves and fruits of PP and CP were similar (Table 2 and Fig. 1a,b). Particularly, mature leaves of both treatments showed significantly higher values of these two classes of compounds compared to young leaves Fig. 1(a,b). The values of leaf and fruit chlorophyll and carotenoids in CP were generally higher than those found in PP, and this difference was more marked for carotenoids (Table 2 and Fig. 1b). Interestingly, the peaks of leaf chlorophyll content corresponded to May of both years, in correspondence with plant vegetative recovery, while the highest carotenoid content was revealed in August, when the need of photo-protectant pigments against adverse arid climatic conditions is higher (Fig. 1a). Fruit chlorophyll reached the lowest values in December, while fruit carotenoids were quite constant during the year (Table 2).

Anthocyanins are pigment compounds responsible for a dark blue coloration. They are located in the vacuoles of the hypodermal cells of fruit skins and leaf epidermis (Sofo et al., 2012a). In this experiment, significant differences of anthocyanin levels were found between PP and CP, but a clear differentiation between young and mature leaves was not clearly detected (Fig. 1c). Regarding the differences in anthocyanin levels in fruits, CP had significantly higher values than PP (Table 2). The maximum values of anthocyanins were observed in May and August for leaves (Fig. 1c), and in November for fruits (Table 2).

The observed plant biochemical response suggests that PP did not manage to face the metal stress, with a consequent decline of photosynthetic (chlorophyll), photo-protectant and free-radical scavenging pigments (carotenoids and anthocyanins), as pigments synthesis in PP did not keep pace with their own degradation.

3.3. Enzymatic and non-enzymatic antioxidants

It was demonstrated that olive tissues have high amounts of phenols with free radical-scavenging properties, and that some antioxidant enzymes (such as SOD, CAT, POX, APX, IAA oxidase, polyphenol oxidase) regulate their activity in response to abiotic stresses (Sofo et al., 2004; Bacelar et al., 2007; Lopez-Huertas and del Río, 2014). Here, total phenol content in PP leaves resulted to be significantly higher in August and November 2013, and in February, August and November 2014 (Table 2 and Fig. 1d). Within the same treatment (PP or CP), young leaves contained increased levels of phenols (almost two times more in some dates), compared to mature leaves (Fig. 1d). In fruits, total phenols were significantly higher in CP, expecting October and November 2014, where significant differences were not detected between the two treatments (Table 2). Fruits of CP contained more phenols than PP, with significantly differences in all the considered dates (Table 2).

The antioxidant enzymes APX and POX are involved in the detoxification of H_2O_2 both within the cell and in the apoplast. While POX are less specific and can use a broad range of substrates as electron donors, preferably some phenolic compounds, APX is more specific and use ascorbate as electron donor (Sofo et al., 2005; Lopez-Huertas and del Río, 2014). The trends of APX and POX activity in leaves had a parallel trend, with CP leaves showing

Table 2
Total chlorophyll, total carotenoids, total anthocyanins and total phenols in fruits of control plants (CP) and polluted plants (PP). The values represent means ($n = 4$) ± SD. Values followed by different letters are statistically different ($P < 0.05$) within columns. CGE = cyaniding 3-glucoside equivalents; FW = fresh weight; GAE = gallic acid equivalents.

Sampling time	Treatment	Total chlorophyll (mg g^{-1} FW)	Total carotenoids (mg g^{-1} FW)	Total anthocyanins (mg CGE g^{-1} FW)	Total phenols (mg GAE g^{-1} FW)
November 2013	CP	48.140 ± 4.380 ab	2.704 ± 0.376 a	0.660 ± 0.087 a	0.279 ± 0.039 ab
	PP	37.860 ± 2.644 c	1.278 ± 0.172 b	0.538 ± 0.065 ab	0.186 ± 0.032 c
September 2014	CP	47.777 ± 1.975 ab	2.780 ± 0.119 a	0.463 ± 0.084 b	0.233 ± 0.017 b
	PP	41.220 ± 3.109 c	1.043 ± 0.222 b	0.332 ± 0.025 c	0.150 ± 0.010 c
October 2014	CP	46.754 ± 3.060 b	2.814 ± 0.619 a	0.429 ± 0.039 b	0.331 ± 0.011 a
	PP	40.266 ± 3.594 c	0.956 ± 0.182 b	0.331 ± 0.051 bc	0.284 ± 0.029 ab
November 2014	CP	54.223 ± 3.097 a	2.431 ± 0.304 a	0.629 ± 0.040 a	0.189 ± 0.026 c
	PP	46.192 ± 3.693 b	1.028 ± 0.223 b	0.484 ± 0.045 b	0.166 ± 0.013 c
December 2014	CP	49.913 ± 4.706 a	2.285 ± 0.110 a	0.573 ± 0.082 a	0.278 ± 0021 a
	PP	30.267 ± 0.976 c	1.027 ± 0.22 b	0.518 ± 0.065 b	0.198 ± 0.021 bc

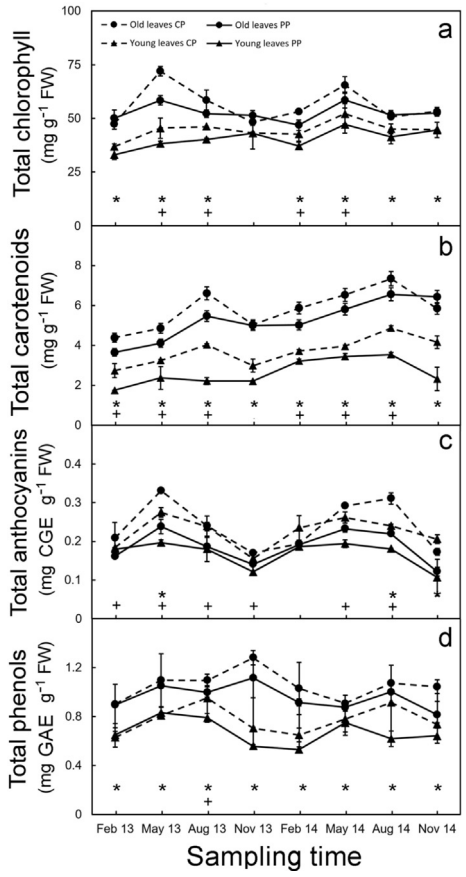

Fig. 1. (a) Total chlorophyll, (b) total carotenoids, (c) total anthocyanins, and (d) total phenols in leaves of polluted plants (PP; continuous line) and control plants (CP; dashed line). The values represent means ($n = 4$) ± SD. Values in the same sampling time with a cross (+) are statistically different ($P < 0.05$) between CP and PP; values in the same sampling time with an asterisk (*) are statistically different ($P < 0.05$) between mature (circles) and young (triangles) leaves. CGE = cyaniding 3-glucoside equivalents; FW = fresh weight; GAE = gallic acid equivalents.

significantly higher values of APX and POX activities compared to PP (Fig. 2a,c). Within each treatment, mature leaves generally had values of APX and POX activities significantly higher, compared to young leaves (Fig. 2a,c). Only for POX, two peaks were observed for all the treatments in August 2013 and May 2014 (Fig. 2c). In roots, the activities of both enzymes were generally significantly higher in CP (Table 3), while the differences in fruits were marked for APX (higher in CP) but not for POX (no statistical differences between the treatments, excepting for November 2014) (Fig. 2b,d). The values of POX activity in roots and fruits were generally lower than those found in leaves, while these differences were not observed for APX (Table 3 and Fig. 2a–d).

The enzymatic antioxidants SOD and CAT, together with APX and POX, constitute the major defence system against the reactive oxygen species produced by the electron transport chain located in chloroplast (Sofo et al., 2005). In this study, SOD activity showed a clear distinction between CP (higher values) and PP, independently by leaf age (Fig. 2e). The significantly higher values of SOD activity in CP leaves were also observed in roots, excepting in November 2013 (Table 3), whereas the differences between CP and PP in fruits were not evident in all the dates (Fig. 2f). Regarding CAT activity, no statistically differences between CP and PP nor between mature and young leaves were found (Fig. 2g). As for SOD, CAT activities in CP roots were generally higher (excepting November 2013) (Table 3), while the activities measured in fruits did not show a clear difference between the two treatments, excepting for three dates (September and December 2013; November 2014) (Fig. 2h).

On the basis of the results obtained (Fig. 2a–h), olive trees of the CP treatment showed a better antioxidative capacity, both enzymatic and non-enzymatic. Sofo et al. (2005) observed an increase in some antioxidant enzymes in plants experiencing drought, with a kind of compensatory response that allowed plants to recover their physiological status after rewatering. In this case, the multi-metal stress, probably because continuous and chronic, resulted to be detrimental for plant and the antioxidant capacity of olive tissue was depressed.

3.4. Hormonal balance

Different approaches have been used in plants to investigate the effects of metals at genomic, transcriptomic and post-transcriptomic levels (Semane et al., 2010). It is known that the activation of responses induced by abiotic stresses, including metals, is mediated through the synthesis of molecules with signal function, such as phytohormones. Particularly, indole-3-acetic acid (IAA), the most abundant auxin in plants, stimulates cell division, expansion and differentiation in a majority of plants, including tree species (Sofo et al., 2012b). In this experiment, the value of foliar levels of IAA were significantly higher in CP-2013 in all the sampling dates, whereas the differences between the two treatments in 2014 were less marked (significantly different only in August and November) (Fig. 2i). In roots, the levels of IAA were always significantly higher in CP for all the sampling dates considered (Table 3). Regarding fruits, differences in IAA content, significantly higher in CP, were detected in all the months of 2013 and in November 2014 (Fig. 2j). These results could be explained by the increased activity of IAA oxidase (IAAox, an IAA catabolic enzyme) in leaves and roots of olive plants experiencing abiotic stresses, as in the case of drought. Even if less specific than IAAox, POX can use IAA as electron donor, acting also as IAAox (Shinshi and Noguchi, 1975). Moreover, IAA seems to induce an increase of POX activity (Sofo et al., 2004, 2005), as observed in this trial, where POX activities were generally higher in CP plants (Table 3 and Fig. 2), that in turn had higher IAA contents than PP (Table 3 and Fig. 2).

Generally, abscisic acid (ABA) acts as a general inhibitor of growth and metabolism, and negatively affects the synthesis of proteins and nucleic acids (Kobashi et al., 2001; Srivastava, 2002). Its synthesis is enhanced in plants experiencing various types of abiotic stresses and it can be accumulated in leaves, where it is able to reduce stomatal conductance (Davies et al., 2000). A metal-induced inhibitory action has been reported to be concomitant with an increased endogenous ABA content, indicating that this hormone takes part in the metal-imposed phytotoxicity (Sharma and Kumar, 2002). The trends of ABA concentrations in olive organs were very different from those found for IAA (Table 3 and Fig. 2i–l). In leaves, ABA was statistically higher (excepting May 2013) in PP from February 2013 to May 2014, while no statistical differences were detected in the last two sampling dates of 2014 (Fig. 2k). In roots, the ABA levels of PP were higher of those of CP in all the dates studied (Table 3). Finally, PP fruits showed the highest ABA values in May 2013, February 2014 and August 2014, when they were statistically different from CP values (Fig. 2l).

In most of the plants, IAA and ABA, together with other plant growth regulators, affect almost every event of plant growth and development, particularly stem and root growth, through cell elongation, and increased expression of several genes (Hedden and Thomas, 2006; Peleg and Blumwald, 2011). Furthermore, IAA and ABA appear to play a key but opposite role in the changes of growth processes mediated by metals (Maciejewska and Kopcewicz, 2002; Maksymiec, 2011). In this case, the generally higher values of IAA in

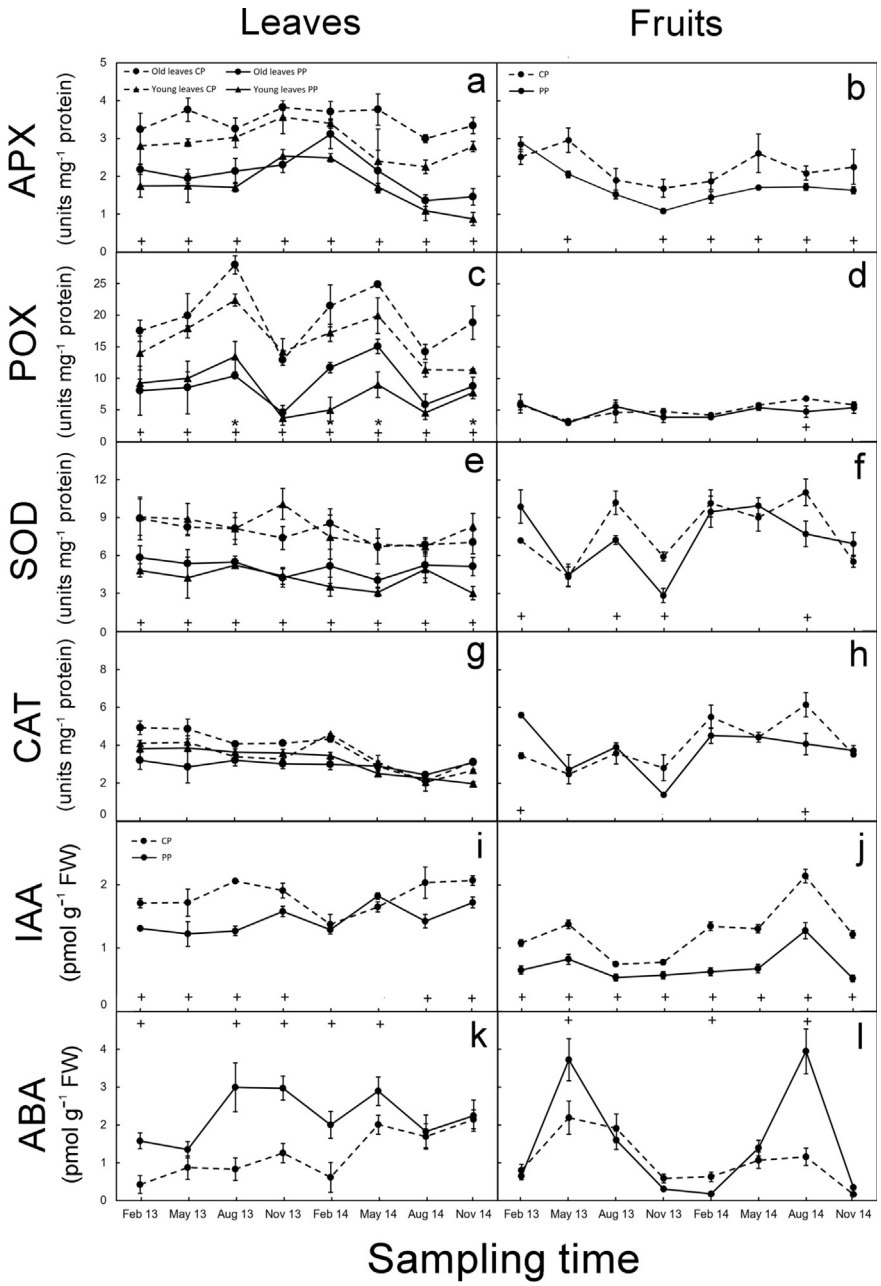

Fig. 2. Activities of (a, b) ascorbate peroxidase (APX), (c, d) total peroxidase (POX), (e, f) superoxide dismutase (SOD) and (g, h) catalase (CAT), and concentrations of (i, j) indole-3-acetic acid (IAA) and (k, l) absicic acid (ABA) in leaves and fruits of polluted plants (PP; continuous line) and control plants (CP; dashed line). The values represent means ($n = 4$) ± SD. Values in the same sampling time with a cross (+) are statistically different ($P < 0.05$) between CP and PP; values in the same sampling time with an asterisk (*) are statistically different ($P < 0.05$) between mature (circles) and young (triangles) leaves. FW = fresh weight.

Table 3
Activities of ascorbate peroxidase (APX), total peroxidase (POX), superoxide dismutase (SOD) and catalase (CTA), and concentrations of indole-3-acetic acid (IAA) and absicic acid (ABA) in roots of control plants (CP) and polluted plants (PP). The values represent means ($n = 4$) ± SD. Values followed by different letters are statistically different ($P < 0.05$) within columns. FW = fresh weight.

Sampling time	Treatment	APX	POX	SOD	CAT	IAA	ABA
		(units mg^{-1} protein)				(pmol g^{-1} FW)	
November 2013	CP	2.116 ± 0.468 c	12.348 ± 0.543 a	5.494 ± 0.380 b	2.255 ± 0.160 c	1.514 ± 0.061 ab	16.599 ± 2.217 a
	PP	3.217 ± 0.681 cb	3.033 ± 0.295 c	6.193 ± 2.179 b	2.424 ± 0.117 c	1.226 ± 0.191 c	3.435 ± 0.776 c
May 2014	CP	5.653 ± 0.163 a	10.619 ± 0.420 a	12.934 ± 1.632 a	5.276 ± 0.390 a	1.835 ± 0.110 a	10.358 ± 1.100 a
	PP	2.397 ± 0.237 c	7.866 ± 1.638 b	7.500 ± 2.185 b	3.572 ± 0.109 b	1.361 ± 0.199 b	7.439 ± 2.338 b
August 2014	CP	3.635 ± 0.745 b	10.535 ± 0.916 a	13.148 ± 2.177 a	5.332 ± 0.242 a	1.489 ± 0.250 b	9.082 ± 0.928 ab
	PP	2.142 ± 0.267 c	3.514 ± 0.765 c	4.461 ± 1.960 bc	2.016 ± 0.106 c	1.276 ± 0.197 c	5.997 ± 1.069 c

Table 4
Chemical parameters of olive oils from control plants (CP) and polluted plants (PP). The values represent means ($n = 4$) ± SD of composite samples obtained from oils extracted in September, October, November and December of the same year. Values followed by different letters are statistically different ($P < 0.05$) within columns. GAE = gallic acid equivalents; IP = induction period.

Sampling time	Treatment	Oxidative stability (IP) (h:min)	Chlorophyll (mg pheophytine equivalents kg^{-1} oil)	Carotenoids (mg lutein equivalents kg^{-1} oil)	Total phenols (mg GAE kg^{-1} oil)
2013	CP	16:37 ± 1:43 c	20.75 ± 2.01 a	9.21 ± 0.28 a	266.43 ± 20.97 a
	PP	2:36 ± 0.22 d	7.07 ± 0.45 d	3.82 ± 0.16 b	127.61 ± 10.82 d
2014	CP	27:15 ± 1.10 a	15.79 ± 1.29 b	8.07 ± 0.85 a	171.43 ± 18.06 b
	PP	24:27 ± 0.67 b	12.49 ± 0.58 c	4.68 ± 0.23 b	159.97 ± 10.90 c

Table 5
Sensory attributes of olive oils from control plants (CP) and polluted plants (PP). The values represent means ($n = 4$) ± SD of composite samples obtained from oils extracted in September, October, November and December of the same year. Oil classification was evaluated according to the EC Regulation 1989/03. Score ranges from 0 to 5.

Sampling time	Treatment	Positive sensory attributes			Negative sensory attributes			Oil classification
		Fruitness	Bitterness	Pungency	Rancid	Musty	Others	
2013	CP	2.3 ± 0.4	2.5 ± 0.6	2.8 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Extra-virgin
	PP	0.5 ± 0.0	0.8 ± 0.4	1.8 ± 0.4	3.0 ± 0.4	2.7 ± 0.4	1.3 ± 0.3	Lampante
2014	CP	2.8 ± 0.8	2.3 ± 0.3	2.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Extra-virgin
	PP	1.0 ± 0.8	1.0 ± 0.0	0.5 ± 0.5	1.0 ± 0.3	1.0 ± 0.0	0.5 ± 0.2	Virgin

CP and of ABA in PP (Table 3 and Fig. 2i–l), indicated a better plant physiological and biochemical status of the olive plants not exposed to atmospheric metal depositions.

3.5. Oil chemical and sensory analysis

Differences in bioactive and antioxidant compounds between oils from the PP and CP, as well as in oxidative stability, were recorded. Moreover, differences were noted comparing the analytical data of oils of the same field in the two consecutive years. In particular, chlorophyll, carotenoid and phenol contents detected in PP-2013 were significantly lower than those found in CP (Table 4). A very low oxidative stability was observed for the sample PP-2013 (2:51 h:min), whereas in all the other samples, the oxidative stability was higher than that normally reported for extra virgin olive oil (see Comandini et al., 2009) (Table 4). The difference in carotenoid content observed in 2014 oil samples was similar to 2013 samples (Table 4). Indeed, the sample PP-2014 showed a carotenoid content corresponding to 61.5% of that of CP-2014 (Table 4). Chlorophyll, total phenols and oxidative stability were significantly lower in PP-2014 than in CP-2014, but the differences were less marked than those found in the corresponding samples of 2013 (Table 4).

In the analysed oils, chlorophyll content was in the range reported by Condelli et al. (2015) for extra virgin olive oils from Southern Italy, whereas carotenoid content was lower. The concentration reported in literature for phenolic compounds is rather variable (200–1500 mg kg^{-1}) and influenced by variety, climatic conditions, fruit ripeness, oil extraction process and oil storage (Hrncirik and Fritsche, 2005). The investigated oils showed a low total phenol content. However, excepting PP-2013 sample, the oils had a good oxidative stability. This could be due not only to the total antioxidant compounds, but also to the concentration of specific compounds.

Table 5 shows three positive oil descriptors (fruitiness, bitterness and pungency) varied between PP and CP. The CP oils were classified as “extra-virgin” olive oils, according to the sensorial parameters listed in the European Commission Regulation 1989/03. The sensory analysis of the CP oil showed an intense fruity flavour (apple and almond), and well balanced bitter notes and pungent tastes, able to give to oil the feeling of freshness and pleasantness, with the two main negative attributes (particularly, musty and rancid) equal to zero. On the other side, the sensory profile of PP oil was completely different from CP oil (Table 5). In fact, lower values of positive attributes, together with the presence of negative attributes (defects), were detected in PP oils, that were classified as “lampante” in 2013 (defects > 0 and ≤ 2.5) and “virgin” (defects > 2.5) in 2014 (Table 5). Actually, it became clear that sensory quality was the result of the synergistic effect of the various components of the oil, whose composition was influenced by plant physiological status and fruit quality (Issaoui et al., 2011). Detailed results on olive sensory test for each month (September, October, November and December) of 2014 are reported as radar diagrams (Supplementary Fig. 2).

4. Conclusions

This study demonstrated that the atmospheric pollution is responsible for the deposition of metals on the epicarp of olive fruits but without evidence of oil contamination. The plants exposed to metals (PP) showed reduced antioxidant defences, and IAA and ABA homeostasis was disrupted. The physiological status of metal-exposed plants worsened, as demonstrated by the lower values of chlorophylls, carotenoids and anthocyanins. Furthermore, aerial deposition of metals, even if does not seem to pose a risk for oil safety, influenced the chemical quality of olive oils, negatively affecting their content in phenols, carotenoids and chlorophyll. In addition, oil sensory quality was strongly worsened. Considering that olive oil is an important food product worldwide and that many productive olive orchards are exposed to several sources of pollution, this work could contribute to clarify the processes and factors regarding the effects of atmospheric metal pollution on olive oil quality and its potential toxicity for humans.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.11.041.

References

Angerosa, F., 2000. Sensory quality of olive oil. In: Harwood, J., Aparicio, R. (Eds.), Handbook of Olive Oil: Analysis and Properties. Springer, New York, USA, pp. 355–392.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., Montedoro, G., 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J. Chromatog. A 1054, 17–31.

Appenroth, K.-J., 2010. What are "heavy metals" in plant sciences? Acta Physiol. Plant 32, 615–619.

Azri, C., Tili, A., Serbaji, M., Methioub, K., 2002. Étude des résidus de combustion des fuels liquide et solide et de traitement chimique du phosphate brut dans la ville de Sfax (Tunisie). Pollut. Atmos. 174, 297–307.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Lopes, J.I., Gonçalves, B.C., Ferreira, T.C., Correia, C.M., 2007. Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. Plant Soil 292, 1–12.

Bahloul, M., Chabbi, I., Sdiri, A., Amdouni, R., Medhioub, K., Azri, C., 2015. Spatio-temporal variation of particulate fallout instances in Sfax city, Southern Tunisia: influence of sources and meteorology. Adv. Meteor. http://dx.doi.org/10.1155/2015/471396. Article ID 471396, 11 pages.

Ben Abdallah, F., Boukhris, M., 1990. Action des polluants atmosphériques sur la végétation de la région de Sfax (Tunisie). Pollut. Atmos. 127, 292–297.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Candeias, C., Melo, R., Avila, P.F., da Silva, E.F., Salgueiro, A.R., Teixeira, J.P., 2014. Heavy metal pollution in mine-soil-plant system in S. Francisco de Assis - Panasqueira mine (Portugal). Appl. Geochem 44, 12–26.

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. Meth. Enzym 2, 764–775.

Codex Alimentarius Commission, 2011. Working Document for Information and Use in Discussions Related to Contaminants and Toxins in the GSCTFF. Doc. CF/5 INF/1.

Comandini, P., Verardo, V., Maiocchi, P., Caboni, M.F., 2009. Accelerated oxidation: comparative study of a new reactor with oxidation stability instrument. Eur. J. Lipid Sci. Tech. 111, 933–940.

Condelli, N., Caruso, M.C., Galgano, F., Russo, D., Milella, L., Favati, F., 2015. Prediction of the antioxidant activity of extra virgin olive oils produced in the Mediterranean area. Food Chem. 177, 233–239.

Davies, W.J., Bacon, M.A., Thompson, D.S., Sobeih, W., Rodríguez, L.G., 2000. Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signalling system and hydraulic architecture to increase the efficiency of water use in agriculture. J. Exp. Bot. 51, 1617–1626.

Dilek, T., Cemre, K., Pınar, K., Handan, G., Levent, A., Cafer, T., Sait, C.S., 2011. The use of olive tree (*Olea europaea* L.) leaves as a bioindicator for environmental pollution in the Province of Aydın, Turkey. Environ. Sci. Pollut. Res. 18, 355–364.

Favati, F., Caporale, G., Bertuccioli, M., 1994. Rapid determination of phenol content in extra virgin olive oil. Grasas Aceitas 45, 68–70.

Francini, A., Galleschi, L., Saviozzi, F., Pinzino, C., Izzo, R., Sgherri, C., Navari-Izzo, F., 2006. Enzymatic and non-enzymatic protective mechanisms in recalcitrant seed of *Araucaria bidwillii* subjected to desiccation. Plant Physiol. Biochem. 44, 556–563.

Gould, K.S., Markham, K.R., Smith, R.H., Goris, J.J., 2000. Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Cunn. J. Exp. Bot. 51, 1107–1115.

Hedden, P., Thomas, S.G., 2006. Plant Hormone Signaling. Blackwell Publishing, Oxford, UK, pp. 37–66.

Hrncirik, K., Fritsche, S., 2005. Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. J. Agric. Food Chem. 53, 2103–2110.

International Olive Council, 2015. Sensory Analysis of Olive Oil. Method for the Organoleptic Assessment of Virgin Olive Oil. COI/T.20/Doc. No 15.

Issaoui, M., Dabbou, S., Mechri, B., Nakbi, A., Chehab, H., Hammami, M., 2011. Fatty acid profile, sugar composition, and antioxidant compounds of table olives as affected by different treatments. Eur. Food Res. Technol. 232, 867–876.

Japon-Lujan, R., Luque De Castro, M.D., 2007. Small branches of olive tree: a source of biophenols complementary to olive leaves. J. Agric. Food Chem. 55, 4584–4588.

Kobashi, K., Sugaya, S., Gemma, H., Iwahori, S., 2001. Effect of abscisic acid (ABA) on sugar accumulation in the flesh tissue of peach fruit at the start of the maturation stage. Plant Growth Regul. 35, 215–223.

Krzeslowska, M., 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol. Plant 33, 35–51.

Lee, O.H., Lee, B.Y., 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. Bioresour. Technol. 101, 3751–3754.

Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In: Current Protocols in Food Analytical Chemistry. John Wiley and Sons, Inc., New York, USA. Unit F4.3. Online ISBN: 9780471142911.

Lopez-Huertas, E., del Río, L.A., 2014. Characterization of antioxidant enzymes and peroxisomes of olive (*Olea europaea* L.) fruits. J. Plant Physiol. 171, 1463–1471.

Llorent-Martínez, E.J., Fernández-de Córdova, M.L., Ortega-Barrales, P., Ruiz-Medina, A., 2014. Quantitation of metals during the extraction of virgin olive oil from olives using ICP-MS after microwave-assisted acid digestion. J. Am. Oil Chem. Soc. 91, 1823–1830.

Maciejewska, B., Kopcewicz, J., 2002. Inhibitory effect of methyl jasmonate on flowering and elongation growth on *Pharbitis nil*. J. Plant Growth Regul. 21, 216–223.

Maksymiec, W., 2011. Effect of jasmonate and some other signaling factors on bean and onion growth during the initial phase of cadmium action. Biol. Plant 55, 112–118.

Marx, S.K., Kamber, B.S., McGowan, H.A., 2008. Scavenging of atmospheric trace metal pollutants by mineral dusts: inter-regional transport of Australian trace metal pollution to New Zealand. Atmos. Environ. 42, 2460–2478.

Mezghani, I., Elloumi, N., Ben Abdallah, F., Chaieb, M., Boukhris, M., 2005. Fluoride accumulation by vegetation in the vicinity of a phosphate fertiliser plant. Fluoride 38, 69–75.

Minguez-Mosquera, M.I., Rejano-Navarro, L., Gandul-Rojas, B., Sanchez-Gomez, A.H., Garrido Fernàndez, J., 1991. Color-pigment correlation in virgin olive oil. J. Am. Chem. Soc. 68, 332–336.

Morales, M.T., Alonso, M.V., Rios, J.J., Aparicio, R., 1995. Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. J. Agric. Food Chem. 43, 2925–2931.

Nanos, G.D., Ilias, I.F., 2007. Effects of inert dust on olive (*Olea europaea* L.) leaf physiological parameters. Env. Sci. Pollut. Res. 14, 212–214.

Pariyar, S., Eichert, T., Goldbach, H.E., Hunsche, M., Burkhardt, J., 2013. The exclusion of ambient aerosols changes the water relations of sunflower (*Helianthus annuus*) and bean (*Vicia faba*) plants. Env. Exp. Bot. 88, 43–52.

Peleg, Z., Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. Curr. Opin. Plant Biol. 14, 290–295.

Pokorny, J., Kalinova, L., Dysseleri, P., 1995. Determination of chlorophyll pigments in crude vegetables oils. Pure Appl. Chem. 67, 877–892.

Romero, C., García, P., Brenes, M., García, A., Garrido, A., 2002. Phenolic compounds in natural black Spanish olive varieties. Eur. Food Res. Technol. 215, 489–496.

Semane, B., Dupae, J., Cuypers, A., Noben, J.P., Tuomainen, M., Tervahauta, A., Kärenlampi, S., Van Belleghem, F., Smeets, K., Vangronsveld, J., 2010. Leaf proteome responses of *Arabidopsis thaliana* exposed to mild cadmium stress. J. Plant Physiol. 167, 247–254.

Sharma, S.S., Kumar, V., 2002. Responses of wild type and abscisic acid mutants of *Arabidopsis thaliana* to cadmium. J. Plant Physiol. 159, 1323–1327.

Shinshi, H., Noguchi, M., 1975. Relationship between peroxidase, IAA oxidase and polyphenol oxidase. Phytochemistry 14, 1255–1258.

Sofo, A., Nuzzo, V., Tataranni, G., Manfra, M., De Nisco, M., Scopa, A., 2012a. Berry morphology and composition in irrigated and non-irrigated grapevine (*Vitis vinifera* L.). J. Plant Physiol. 169, 1023–1031.

Sofo, A., Tataranni, G., Xiloyannis, C., Dichio, B., Scopa, A., 2012b. Direct effects of *Trichoderma harzianum* strain T-22 on micropropagated GiSeLa6® (Prunus cerasus x Prunus canescens) rootstocks. Env. Exp. Bot. 76, 33–38.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2005. Antioxidant defences in olive tree during drought stress: changes in activity of some antioxidant enzymes. Funct. Plant Biol. 32, 45–53.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2004. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Sci. 166, 293–302.

Somova, L.I., Shode, F.O., Ramnanana, P., Nadar, A., 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. J. Ethnopharmacol. 84, 299–305.

Srivastava, L.M., 2002. Plant, Growth and Development - Hormones and Environment. Elsevier Academic Press, San Diego, CA, USA, pp. 172–188.

Taamalli, A., Arráez-Román, D., Barrajón-Catalán, E., Ruiz-Torres, V., Pérez-Sánchez, A., Herrero, M., Ibañez, E., Micol, V., Zarrouk, M., Segura-Carretero, A., FernándezGutiérrez, A., 2012. Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: phenolic composition and cytotoxicity against human breast cancer cells. Food Chem. Toxicol. 50, 1817–1825.

Wang, Y., Hopke, P.K., Chalupa, D.C., Utell, M.J., 2011. Effect of the shutdown of a coal-fired power plant on urban ultrafine particles and other pollutants. Aerosol Sci. Tech. 45, 1245–1249.

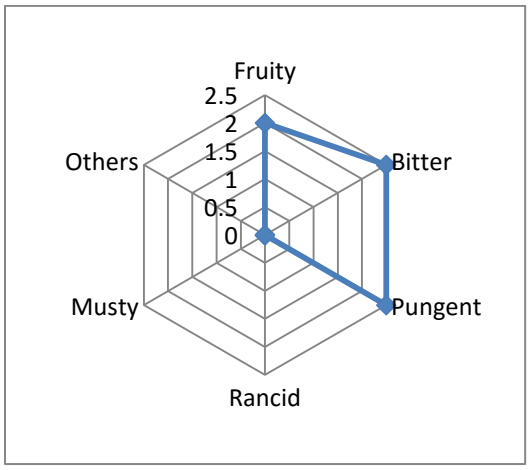
Yan, X., Shi, W.Z., Zhao, W.J., Luo, N.N., 2014. Impact of aerosols and atmospheric particles on plant leaf proteins. Atmos. Environ. 88, 115–122.

Supplementary Table 1. Metals from oils of control plants (CP) and polluted plants (PP). The values represent means (n = 4). LOD = limit of detection.

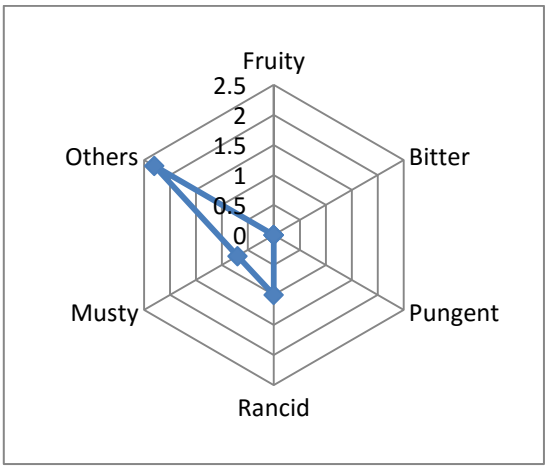
Sample	Description	Cd	Cu	Fe	Mn	Ni	Pb	Zn
					(µg g^{-1})			
Oil 1	CP SEPTEMBER 2013	<LOD	<LOD	**0.346**	<LOD	**0.075**	<LOD	<LOD
Oil 2	PP SEPTEMBER 2013	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 3	CP OCTOBER 2013	<LOD	<LOD	<LOD	<LOD	**0.223**	<LOD	<LOD
Oil 4	PP OCTOBER 2013	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.423**
Oil 5	CP NOVEMBER 2013	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 6	PP NOVEMBER 2013	<LOD	<LOD	<LOD	<LOD	**0.257**	<LOD	**2.616**
Oil 7	CP DECEMBER 2013	<LOD	**0.156**	**1.007**	<LOD	<LOD	<LOD	<LOD
Oil 8	PP DECEMBER 2013	<LOD	<LOD	**5.575**	**0.102**	<LOD	<LOD	<LOD
Oil 9	CP SEPTEMBER 2014	<LOD	<LOD	**1.479**	<LOD	<LOD	<LOD	<LOD
Oil 10	PP SEPTEMBER 2014	**0.026**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 11	CP OCTOBER 2014	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.060**
Oil 12	PP OCTOBER 2014	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 13	CP NOVEMBER 2014	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 14	PP NOVEMBER 2014	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 15	CP DECEMBER 2014	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oil 16	PP DECEMBER 2014	<LOD	**0.123**	<LOD	<LOD	<LOD	<LOD	<LOD

Supplementary Figure 1. Necrotic spots in leaves and fruits of polluted plants.

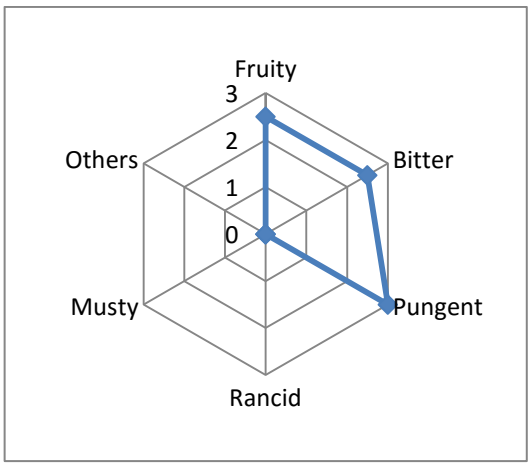
Supplementary Figure 2. Sensory radar diagrams of olive oils extracted in 2014 from control plants (CP) and polluted plants (PP). The values represent means (n = 4).



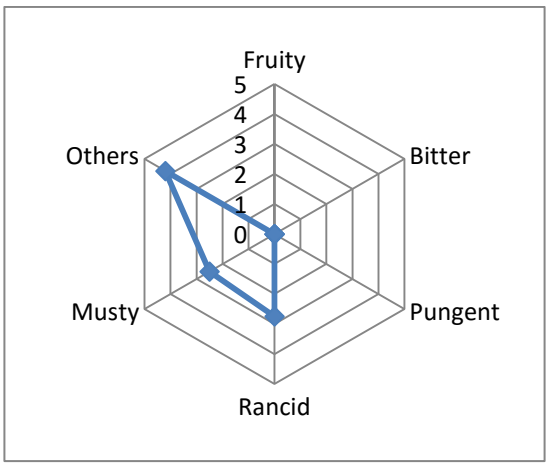
CP September 2014



PP September 2014



CP October 2014



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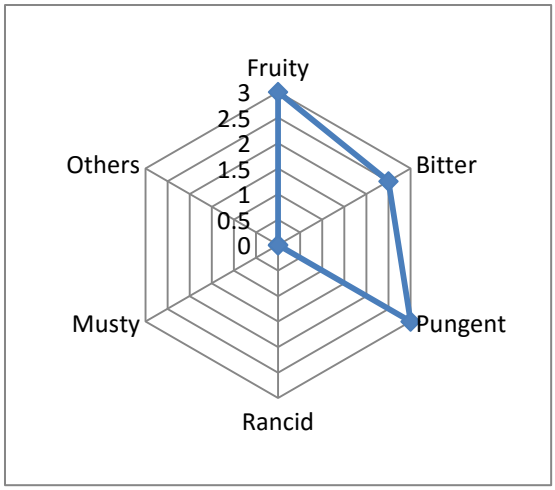
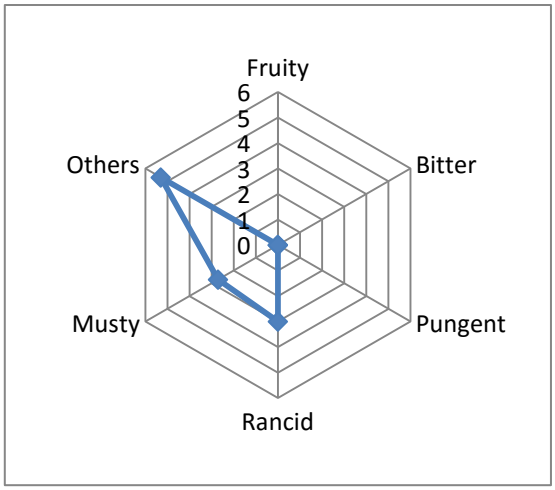


CP November 2014



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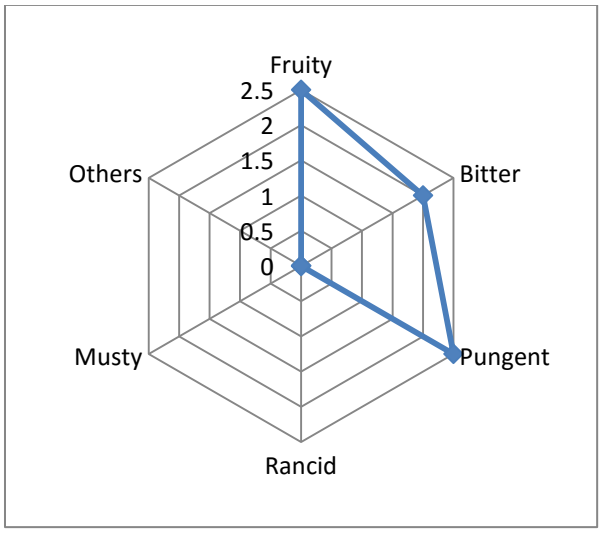
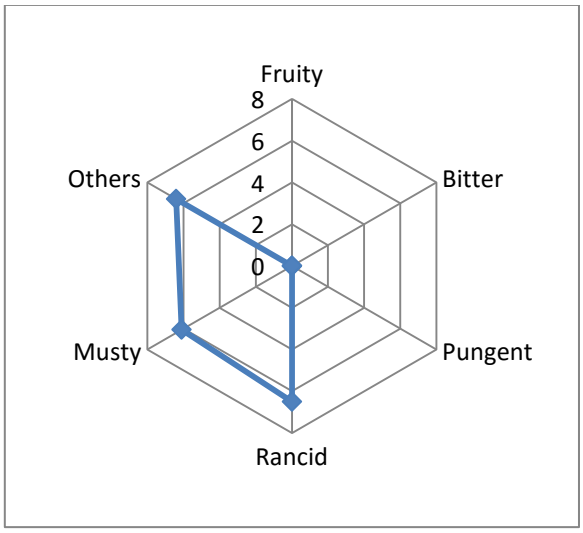


CP December 2014



PP December 2014