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An Assessment Study of Usefulness of Using Olive (*Olea europaea* L.) Leaves in Biomonitoring the Air Pollution near Baniyas Oil Refinery, Syria: Estimating of Total Phenolic Compounds and Lead, Copper and Manganese in Olive Leaves

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Abstract

The aim of this study was to estimate the total amount of phenolic compounds of olive trees leaves (*Olea europaea* L.) as a biomarker for the assessment of heavy metals (HMs) air pollution (Pb, Cu and Mn) in Baniyas area. Olive trees were selected as the predominant species in the study area. Samples were collected from 6 locations at different distances from the vicinity of Baniyas refinery (0.1, 0.5, 2, 4, 6, 10) Km. The control was taken from an area about 20 km from the refinery to the north-east (Al-Qardaha rural). The concentration of total phenolic compounds (TPC) during the summer was (45.6 - 70.85) mg GAE/g dw, and during winter (35.6 - 52.9) mg GAE/g dw. The concentrations of the studied HMs (Pb, Cu and Mn) in unwashed leaves during summer were (0.879 - 2.170) ppm, (0.75 - 5.21) ppm and (54.38 - 8.78) ppm respectively, whilst during the winter concentrations were (0.479 - 1.023) ppm, (1.54 - 7.29) ppm and (53.79 - 7.58) ppm respectively. The results showed significant differences in the concentration of total phenolic compounds and HMs (Pb, Cu and Mn) between sites (ANOVA), significant differences in concentrations of both total phenols and HMs (Pb and Cu) between summer and winter at all sites (*t*-test, $p < 0.05$). TPC, Pb and Mn were higher in summer in all sites than in winter. Levels of Cu were significantly higher in winter than in summer at all sites (*t*-test, $p < 0.05$). The results showed a negative correlation (*t*-test, $p < 0.05$) between the concentration of

total phenolic compounds and both Pb and Mn with the distance from the refinery of Baniyas. Consequently, the result of this study enhanced the usefulness of using of TPC in olive leaves as biomarker of air pollution.

Keywords

Biomonitoring, Total Phenolic Compounds, Biomarker, Heavy Metals, Olive Leaves, Baniyas Oil Refinery

1. Introduction

Air pollution considered a worldwide issue due to its high health and environmental risk aspects [1] [2]. Previous studies and reviews elucidated the role of air pollution for 3.1% of disability-adjusted life [3]. A World Bank study estimated that annually 3500 deaths and 17,000 cases of bronchitis, respiratory disorders and cancer in Syria are the direct result of air pollution [4] [5]. Consequently, it is pivotal to assess and monitor the level of air pollutants in urban, rural and industrials areas.

Different outdoor air pollutants measurement methods were developed to maintain strict quality control on the emission of industrial and urban activities. Harvested rain water was used to determine trace metals on air particles [6]. This method was accomplished by employing either a filter or inertial separation to measure heavy metals (HMs) concentration on particles matter (PM) [7]. Risk assessment process of air pollution relied on two major points: local data of pollutants emissions and advanced air pollution monitor techniques. Due to the difficulty in access to previously mentioned points in Syria, it was in a critical importance to use alternative available methods in order to quantify the level of air pollutants and monitor the impact of these pollutants on ecological systems.

Biomonitoring of air pollution by plants is considered as a complementary sustainable affordable tool specifically in developing countries [5] [8]. Subsequently, biomonitoring can be defined as a rapid and economical method that has commonly been used for assessing environmental quality and potentially detrimental effects of pollutants to the biosphere [9] [10]. Biomonitoring of air pollution is carried out using biomarkers, bioindicators or/and bioaccumulators [11] [12] [13] [14]. Thus, biomarker can be defined as a xenobiotically-induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample [15].

Plants leaves can be used as effective bioaccumulators for HMs which emitted from various anthropogenic activities such as oil refineries, thermal power plants, transportations and other anthropogenic sources [16] [17] [18]. Air pollutants (as examples: HMs, SO_x, O_3 and NO_x) induced oxidative stress in plant, leading to cell damages through inciting the formation of ROS (reactive oxygen species); hence, as a defense mechanism, cells change their biochemical compositions [13] [19] [20]. ROSs are known to damage cell components, such as cel-

lular membranes, chlorophyll and DNA [21] [22]. Plant's cells have multiple defense mechanisms in order to maintain a higher capacity of antioxidant processes and detoxification mechanisms to control the production of ROS [23] [24].

Antioxidants defense mechanisms in plants are divided into two main groups: enzymatic and non-enzymatic antioxidants, this mechanism can slow down or even stop the oxidation of biomolecules and block the process of oxidative chain reactions [24]. Non-enzymatic scavengers are essential in the protection of cellular components against ROS [25]. Phenolic compounds are one of the most effective non-enzymatic scavengers (antioxidants) in view of their molecular structures [26] [27].

The reducing properties of polyphenols as hydrogen or electron-donating agents predict their potential for action as free-radical scavengers (antioxidants) [28]. Antioxidants properties of phenolic compounds manifested by their high tendency to chelate metals as a result of possess hydroxyl and carboxyl groups [27]. The induced accumulation of phenolic compounds as a result of heavy metals accumulation in plants leaves, supports the role of phenolic compounds as preventative antioxidants in terms of inhibiting transition metal-catalyzed free radical formation [13] [29] [30] [31] [32].

2. Materials and Methods

2.1. Characteristics and Meteorology of Study Area

Sampling was conducted in Baniyas city—Syria, which located on the western coast of the Mediterranean Sea, east of Syria (35˚13'19" N, 35˚57'55"). The area has a Mediterranean dry summer with mild winter [33] the average annual temperature is 19.3˚, precipitation ranged from 600 - 1200 mm with an average of 862 mm, over 80% occurring between December and March [34]. The prevailing wind in the study area is westerly-southwesterly [33].

2.2. Sources of Air Pollution in the Study Area

The city of Baniyas and the rural land around the city are mainly affected by two types of pollution sources: mobile sources, such as cars, buses, and trucks, and stationary sources, which are Baniyas oil refinery (located to the north of the city) and the thermal power plants (located to the west of the city). Emissions from both Baniyas oil refinery and the thermal power plant gained a growing concern, as a result of estimating an increasing number of cardiovascular and respiratory diseases among populations of the area [35].

2.3. Objectives

The present study was designed to elucidate the usefulness and effectiveness of olive leaves in biomonitoring of atmospheric pollution in Baniyas area. The study was designed to assess the HMs concentration and their distribution pattern in the ambient air of Baniyas city and rural lands around Baniyas oil refi-

nery (7 sites) (Figure 1) using olive trees leaves as a bioaccumulation of (Pb, Cu and Mn). Also, in our present study we estimated the concentration of total phenolic compounds (TPC) in olive leaves samples as a biomarker of air pollution through studying the correlation between both TPC and HMs (Pb, Cu and Mn) concentrations in all studied sites.

2.4. Sampling and Samples Preparation

The sampling was carried out two time, in March and September 2016, leaves of olive trees (*Olea europaea* L.) were collected from surrounding areas of Baniyas oil refinery (6 sites and a control (background) site in Al-Qardaha area). Samples locations are shown in (Table 1). Three samples were taken from each site. Healthy looking leaves were collecting from 3 trees per site. At the height of about two meters, fully expanded mature leaves (were collected from each plant in the polythene bags and transported to the laboratory. Samples pretreatment

Figure 1. Sampling location and Baniyas oil refinery, Syria. https://www.google.com/earth/.

Table 1. Summary of baseline characteristics and meteorology of study areas*.

Location name	Distance from refinery (Km)	Direction from refinery	Altitude	Latitude and Longitude
ST1	0.1	E**	30	35˚13'39"N 35˚57'54"E
ST2	0.5	E	56	35˚13'18"N 35˚58'53"E
ST3	2	E	145	35˚12'59"N 35˚59'53"E
ST4	4	E	127	35˚12'32"N 36˚00'51"E
ST5	6	E	406	35˚12'20"N 36˚02'24"
ST6	10	E	535	35˚11'42"N 36˚04'23"
ST7	20	NE***	235	35˚24'59"N 36˚03'14"

*Google earth; **East; ***North East.

and preparation were done using standard procedures. After mixing each sample, each sample was divided into two groups to determine both heavy metals (Pb, Cu and Mn) and TPC.

2.4.1. Heavy Metals Determination

Portion of each samples were used to estimate HMs (Pb, Cu and Mn). Samples kept unwashed in order to study the atmospheric deposition of HMs which are carried by PM that are emitted from the refinery, and transformed distances before deposited on the surface of plant leaves leading to accumulate some HMs like Pb, Cu and Mn [36] [37] [38]. Samples were dried in an oven at 105˚C to a consistent weighed, dried leaves samples were grounding using a stainless steel grinder, then samples were passing through a sieve with a mesh size of 0.25 mm.

1) Nitric acid digestion

The approach used in this study was modified from that mention by [39] which partly was modified from [40]. A weight of 0.2 g of dried grounded sample was placed in a 50 ml Erlenmeyer flask and 5 ml of concentrated HNO_3 was added. The sample was heated for 45 min at 90˚C, and then the temperature was increased to 200˚C at which the sample was boiled until a clear solution was obtained. Concentrated HNO_3 was added to the sample (2 ml was added at least one times) until the volume was reduced to about 1 ml. The interior walls of the flask were washed down with a little distilled water and the tube was swirled throughout the digestion to keep the wall clean and prevent the loss of the sample. After cooling, the solution was filtered with filter paper, then it was transferred to a 25 ml polyethylene bottles by adding distilled water.

2) Instrumental analysis

The Cu and Mn concentrations of digestion solutions were determined using an atomic Absorpotion Spectrophotometer (AAS) (Varian 220). Pb concentration was determined using a graphite furnace AAS technique, Cu and Mn were measured using Flame AAS. The samples solutions were aspirated into the instrument and the absorbance obtained was used to determine the concentrations of the metals in the different samples from calibration curves.

2.4.2. Total Phenolic Compound Content Determination

1) Preparation of plant extracts

Samples were air-dried in darkness at room temperature. Dried plant parts were cut up and directly been extracted. Plant extracts were prepared according to a standard protocol mentioned in [41] with some modifications. Dried plant material (20 g) was transferred to dark-colored flasks and mixed with 250 ml of methanol (methanol was used as one of the best solvent to extract total phenolic compounds [42]) and stored at room temperature. After 48 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated, and again re-extracted the samples for another 48 h. Combined supernatants were evaporated to dryness under vacuum at 32˚C using a rotary evaporator (**Figure 2**).

The obtained extracts were kept in glass sample bottle and stored in a refrigerator.

2) Determination of TPC content in samples extracts (Folin-Ciocalteu regent method)

The concentration of TPC in plant extracts was determined using spectrophotometric method [43] with some modified by [44] [41]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, after two minutes 2.5 ml of 7.5% $NaHCO_3$ was added (Folin-Ciocalteu's reagent was added before $NaHCO_3$ to avoid the oxidation of TPC with air). Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were left for another two minutes in dark place (Folin-Ciocalteu's reagent is sensitive to light), thereafter incubated in a thermostat at 40˚C for an hour (Figure 3).

The absorbance was determined using a spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolic compounds was read (mg/ml) from the calibration line; then the content of TPC in extracts was expressed in terms of Gallic acid equivalent in dried weight (mg of GA/g of dw).

Figure 2. Extraction process of TPC from olive leaves.

Figure 3. Folin Ciocalteu reagent method.

2.5. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS statistics 20 software. Significance of differences between mean values was tested using Fisher's Least Significant Difference at $\alpha = 0.05$. Values presented on bar charts are means ± SD, n = 3, correlations coefficient (r) were determined by linear regression analysis employing Microsoft excel software. T test ($p < 0.05$) was used to find the difference in the mean values for two groups (summer and winter) for each experiment.

3. Results and Discussion

3.1. Leaf Metals Concentrations

Concentrations of HMs (Pb, Cu and Mn) in summer and winter for each site, with means values expressed as ppm (mg/kg dry weight ± standard deviations) in olive leaves samples are shown in Table 2 and Table 3 respectively. Mean

Table 2. Mean (±SD) concentrations ppm ($mg \cdot kg^{-1}$) for Pb, Cu and Mn in olive leaves (*Olea europaea* L.) in summer (*t*-test $p < 0.05$).

Sample	Pb ppm	Mean ± SD	Cu ppm	Mean ± SD	Mn ppm	Mean ± SD
ST1	2.318 1.82375 2.37	$2.170^{a} \pm 0.301$	1.5 0.25 0.5	$0.75^{a} \pm 0.66$	60.38 52 50.75	$54.38^{a} \pm 5.24$
ST2	1.9375 1.34875 1.32875	$1.538^{b} \pm 0.346$	3.25 2.5 2.75	$2.83^{b} \pm 0.382$	53.38 55.25 50.63	$53.09^{a} \pm 2.3$
ST3	1.694 1.289 1.3	$1.428^{b} \pm 0.23$	2.25 1.375 1.75	$1.79^{ab} \pm 0.44$	53.38 44.25 49.75	$49.13^{ab} \pm 4.6$
ST4	1.4025 1.4265 1.4065	$1.41^{b} \pm 0.013$	0.75 1.25 2.875	$1.625^{ab} \pm 1.11$	46.63 43.25 42.25	$44.04^{b} \pm 2.3$
ST5	1.16125 0.9175 1.10125	$1.06^{c} \pm 0.13$	5.25 2.375 2.125	$3.25^{bc} \pm 1.74$	37.75 33.88 36.13	$35.92^{c} \pm 1.94$
ST6	0.9175 0.83375 0.8875	$0.88^{c} \pm 0.042$	3.25 1.125 2.875	$2.42^{ab} \pm 1.134$	34.21 33.8 31.5	$33.17^{c} \pm 1.46$
ST7	0.0073 0.0029 0.0052	$0.0051^{d} \pm 0.002$	3.5 5.125 7	$5.21^{c} \pm 1.75$	8.6 7.4 10.33	$8.78^{d} \pm 1.48$
LSD ($p < 0.05$)		0.455		1.34		7.03
Correlation coefficient r ($p < 0.05$)		0.941		0.754		0.922

Table 3. Mean (±SD) concentrations ppm ($mg \cdot kg^{-1}$) for Pb, Cu and Mn in olive leaves (*Olea europaea* L.) in winter, (*t*-test $p < 0.05$).

Sample	Pb ppm	Mean ± SD	Cu ppm	Mean ± SD	Mn ppm	Mean ± SD
ST1	0.88125 0.8155 1.375	$1.024^{a} \pm 0.306$	0.375 1.5 2.75	$1.542^{a} \pm 1.19$	58 54.88 48.5	$53.79^{a} \pm 4.84$
ST2	1.005 1 0.9075	$0.971^{ab} \pm 0.055$	2.625 3.125 3.375	$3.04^{a} \pm 0.389$	51.25 52.75 47.63	$50.54^{ab} \pm 2.63$
ST3	0.92875 0.875 1.00625	$0.94^{ab} \pm 0.066$	3.5 3.375 4.125	$3.67^{a} \pm 0.41$	40.13 52.38 47	$46.50^{bc} \pm 6.14$
ST4	0.8775 0.6205 0.66375	$0.721^{bc} \pm 0.14$	3.125 3.375 2.875	$3.13^{a} \pm 0.25$	44.25 40.5 42.5	$42.42^{c} \pm 1.88$
ST5	0.755 0.525 0.6295	$0.637^{bc} \pm 0.115$	2 1.375 11.75	$5.042^{a} \pm 5.82$	33.38 34.88 33.13	$33.79^{d} \pm 0.95$
ST6	0.53375 0.3275 0.57625	$0.48^{c} \pm 0.133$	4.75 4.25 6.625	$5.21^{a} \pm 1.25$	32.75 31.75 32.25	$32.25^{d} \pm 0.40$
ST7	0.00075 0.0005 0.00125	$0.00083^{d} \pm 0.00038$	5.125 13.25 3.5	$7.29^{b} \pm 5.22$	7.81 7.12 7.82	$7.58^{e} \pm 0.40$
LSD ($p < 0.05$)		0.333		2.623		7.314
Correlation coefficient r ($p < 0.05$)		0.935		0.945		0.925

values of heavy metals were placed in the following descending order: Mn > Cu > Pb. Concentrations of both Pb and Mn negatively correlated with distance ($p < 0.05$) in summer and winter ($r = -0.94$, $r = -0.922$ in summer and $r = -0.935$, $r = -0.925$ in winter, respectively) as shown in **Figure 4**. Cu concentrations were positively correlated with distance from Baniyas oil refinery in summer and winter ($r = 0.754$, $r = 0.945$ respectively) as shown in **Figure 4**. Concentrations of studied metals (Pb, Cu and Mn) in leaves of Olive trees did not indicate HMs air pollution in the studied area; this result could be attributed to the fact that from mid-2015, oil production stood at approximately 3% of pre-war levels [45].

Normal concentration of Pb in plants tissues should not exceed 6 or 10 ppm [46] [47] and according to [48] normal level of Pb in plant is less than 3 ppm. In comparison with local study [49] a higher level of Pb in leaves of *Pinus bruita* and *Cupressus sempervirens* around Baniyas refinery was obtained, 30 and 31 ppm respectively. Variations of HMs levels in plants are attributed to two

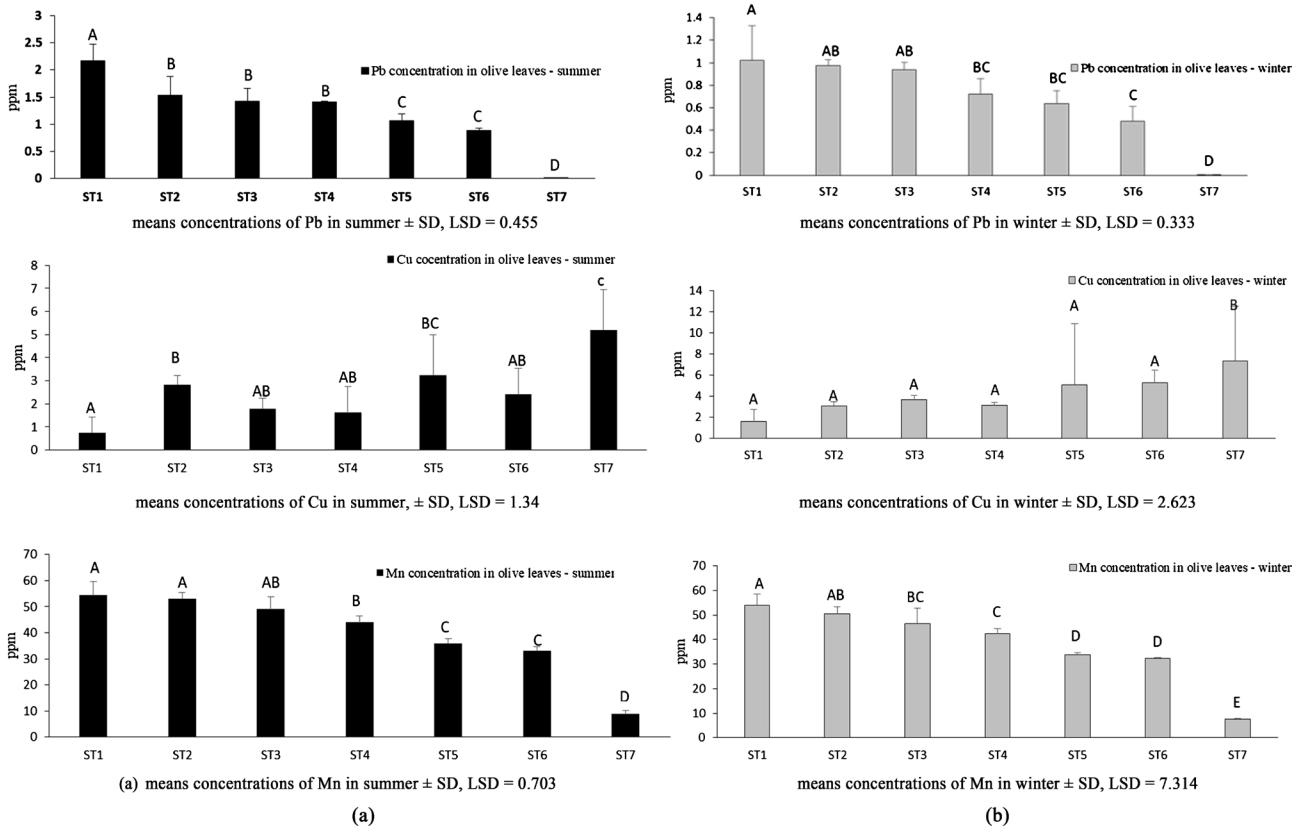

Figure 4. Heavy metals (Pb, Cu and Mn) concentration ppm (mg·kg^{-1}) in olive leaves (*Olea europaea* L.) in summer (a) and winter (b) (mean values ± SD, LSD $p < 0.05$). Different letters above the column indicate significant differences ($p < 0.05$).

factors: the species of plant and the location of samples [50] [51].

Our findings indicate a significant difference (*t*-test, $p < 0.05$) in Pb concentrations between sites in summer and winter. Cu concentrations were less than the normal level; Cu deficiency could happen in concentration of 2 - 5 ppm [47].

Cu level in winter was significantly higher than in summer in all sites (*t*-test $p < 0.05$). These results are compatible with previous few studies. [52] confirmed significant seasonal variations in the level of Cu, with a higher concentration in winter in leaves of *Taraxacum officinal*, *Plantago major* and *Plantago lanceolata*. [53] suggested the same seasonal variation in leaves of *Typha latifolia* and *Phragmites australis*. This pattern of seasonal variations for copper in plants can be explained that some of Cu is actively removed from the leaves (remobilization) once their initial period of rapid growth is finished (which occurred in winter and spring) [54]. In contrast with Pb and Mn, highest level of Cu is observed in ST7 (background site).

Natural Manganese contents in plants range from 30 to 300 mg/kg of dry matter [47]. Our results showed a seasonal variation in concentrations of Mn in olive leaves samples. Mn levels negatively correlated with distance from the refinery in summer ($r = -0.922$) and winter ($r = -0.925$). The seasonal variation of Mn concentrations in our study agreed with [52], which indicated a higher level of Mn in summer samples in comparison to winter samples. The same pattern of

seasonal variations was also observed in *Nuphar lutea* [55]. Different levels of Mn in olive trees leaves were noticed in previous study in Turkey [56]. Mn concentrations ranged from 0.35 to 4.49 ppm. These variations with our findings demonstrate the effect of air pollution near Baniyas oil refinery. Compatible with our results, which indicate a higher level of Mn near Baniyas oil refinery, study by [57] observed a higher content of Mn in leaves of various trees near Bahrain oil refinery compared with the control site. The level of Mn in trees leaves also varied between species [58].

3.2. TPC Concentrations in Olive Leaves Samples

Total phenolic contents in samples extracts are expressed in terms of Gallic acid equivalent. Concentrations of total phenolic compounds (TPC) in olive leaves samples in summer and winter are shown in **Table 4**. Findings showed significant

Table 4. Mean (±SD) concentrations of TPC (mg GAE/g dw) in olive leaves (*Olea europaea* L.) in summer and winter (*t*-test $p < 0.05$).

Sample	TPC (mg GAE/g dw) summer	Mean ± SD	TPC (mg GAE/g dw) winter	Mean ± SD
ST1	70.85 69.94 71.76	70.85 ± 0.91	59.66 50.19 48.98	52.94 ± 5.84
ST2	70.62 72.06 71.46	71.38 ± 0.722	60.41 61.77 52.77	58.32 ± 4.85
ST3	71.3 70.85 69.94	70.7 ± 0.69	56.25 59.12 61.77	59.05 ± 2.76
ST4	64.72 54.89 61.54	60.38 ± 5.02	49.36 51.40 52.99	51.25 ± 1.82
ST5	49.82 51.1 49.97	50.29 ± 0.70	37.03 42.55 44.22	41.27 ± 3.76
ST6	42.48 44.29 50.04	45.60 ± 3.95	31.73 38.32 36.95	35.67 ± 3.47
ST7	38.77 42.48 40.06	40.43 ± 1.88	28.71 21.14 27.57	25.81 ± 4.08
LSD ($p < 0.05$)		5.85		9.05
Correlation coefficient r ($p < 0.05$)		0.96		0.894

differences (*t*-test, $p < 0.05$) between summer and winter in all sites, with descending order of total phenolic compounds concentrations as ST1 > ST2 > ST3 > ST4 > ST5 > ST6 > ST7. TPC concentrations negatively correlated with distance ($p < 0.05$) from Baniyas oil refinery in summer ($r = -0.96$) and winter ($r = -0.935$) (Figure 5).

3.3. Relationship between TPC and Heavy Metals Concentrations in Olive Leaves

Our findings suggested a significant positive correlation ($p < 0.05$) between TPC and HMs (Pb and Mn) concentrations in summer and winter (Figure 6). This result is identical with previous studies, which showed a remarkable increasing in the TPC content significantly enhanced by high level of Mn treatment [59]. However, the concentration of Cu, as shown in (Figure 6), negatively correlated with TPC contents.

3.4. Discussion

Plants require a constant nutritional supply to preserve and enhance metabolic and growth processes. Hence, deficient or excess level of HMs has various impacts on plant growth. Excessive levels of HMs can cause cells damaged, enzymes inhibition and may lead to mortality if occurring in toxic level [60] [61]. Pb is not considered as a necessary metal for plant growth; however high levels of Pb were accumulated in tissues of different plants in some industrials or urban areas in comparison to rural clean areas, indicating the rule of anthropogenic activities in Pb air pollution [17] [57]. Accumulation of Pb in plants leaves is mainly attributed to airborne particles (particles matters) (PM) deposition, which originated from the emission of industrials activities (Baniyas refinery and transportation in

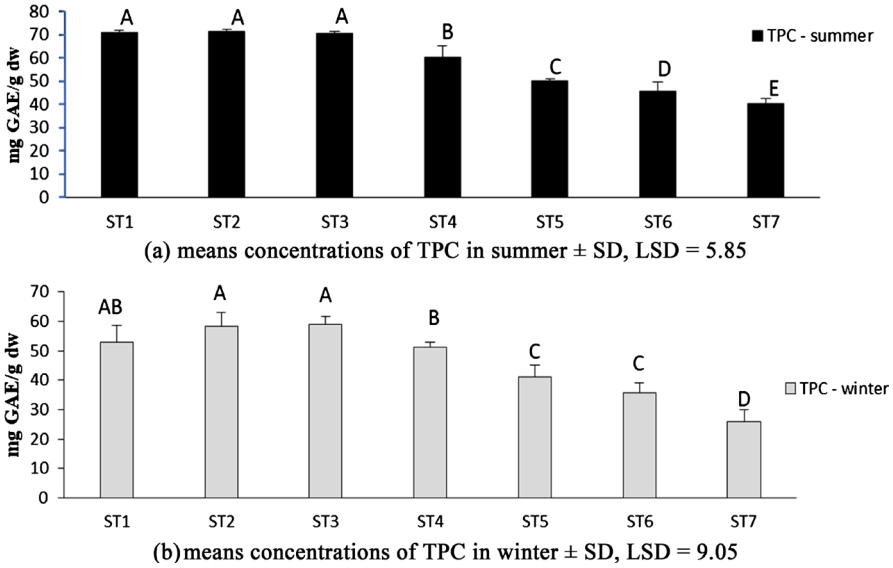

Figure 5. Total phenolic compounds (TPC) concentrations (mg GAE/g dw) in olive leaves (*Olea europaea* L.) in summer (a) and winter (b) (means concentrations ± SD, LSD $p < 0.05$) different letters above column indicate significant differences ($p < 0.05$).

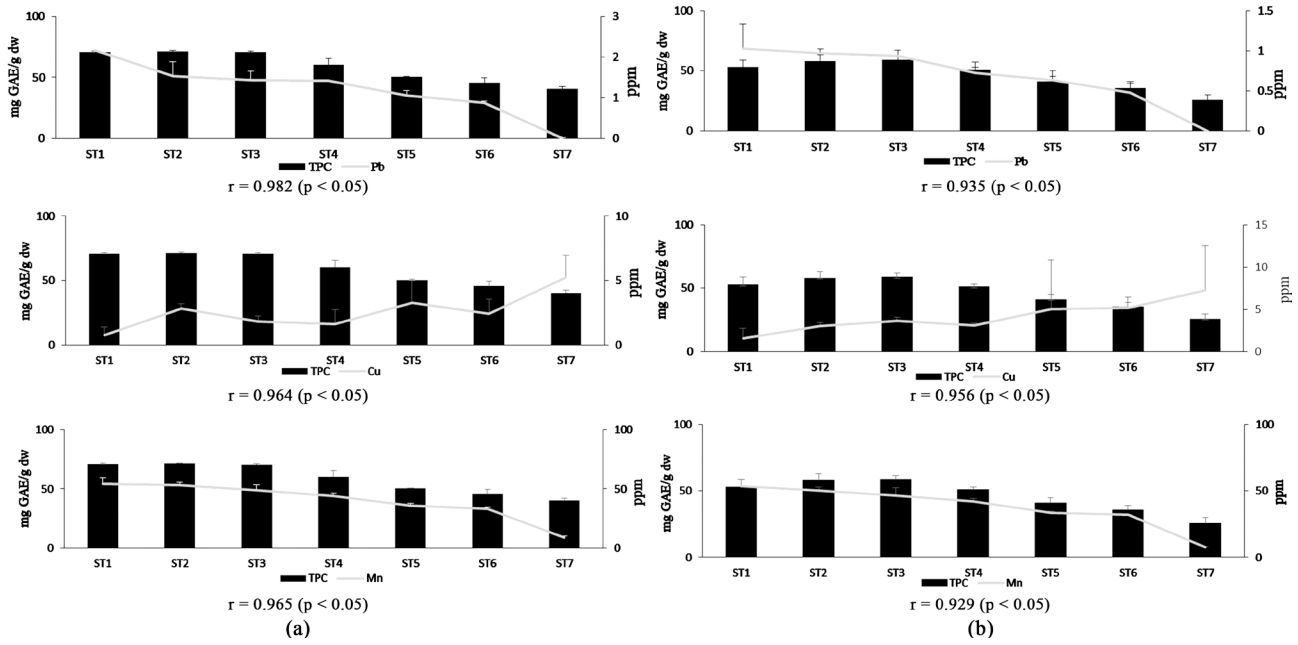

Figure 6. Pearson's correlation coefficient (r) ($p < 0.05$) (bar graph) between (TPC) and HMs (Pb, Cu and Mn) concentrations in summer (a) and winter (b) (means concentrations).

the cause of the present study) [18] [62] [37]. High level of Pb and Mn in plants tissues induces oxidative stress through increasing the production of reactive oxygen species (ROS) which damage cell components [27] [63]. However, plants responds to the induced ROS stress though some defense mechanisms that help plants in surviving under stress conditions [64] [65]. Phenolic compounds play a vital role in plant growth and as a non-enzymatic antioxidant against various induced stress factors like HMs, UV, microbes, cell injuries, drought and other factors [19] [66] [67] [68] [69]. The structure of phenolic compounds illustrates the antioxidant activity against ROS; phenolic compounds consist of aromatic ring with at least one hydroxyl group [66]. Antioxidants activity of TPC is attributed due to its ability of scavenging free radicles like ROS through donating electrons or hydrogen atoms from the hydroxyl groups, or through chelating heavy metals [26] [70]. Despite our findings which didn't indicate any exceed in permissible limits for (Pb and Mn), TPC positively correlated with (Pb and Mn) in all sites, this result highlights the variation in permissible limits of HMs between species. Furthermore, different pollutants emitted from Baniyas oil refinery, hence, different stress factors effect olive leaves around the refinery. Regard to Cu levels, results showed a negative correlation between Cu and TPC. Copper has antioxidants properties which can limit the formation of ROS; our result agreed with the results observed by [71] which indicate a negative correlation between Cu and TPC in leaves of olive trees; leaves were affected by exceed level of Cu. Copper cooperates with TPC as a defense mechanism, through limiting the production of ROS; therefore higher level of TPC might decree the level of Cu. The use of plants in air pollution risk assessment in developing countries [72] is considered as one of the most abundant and effective methods, where less

environmental policies and awareness are threatening ecological systems in both urban and rural areas. Overall, biomonitoring studies can contribute in urban planning to achieve higher standard in regard to air quality and healthy environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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