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Research Article

Metal accumulation and oil quality characteristics of Chemlali olive tree (*Olea europaea* L.) grown near road soil.

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Abstract

The metal contamination's study focused on soils and olive trees near three roads in the Sfax region (Gremda, Manzel chaker and Tunis road). This study has shown that Gremda soils are contaminated with Pb and Zn up to a distance of 50m, while for the Tunis and Manzel Chaker roads, the lead contamination does not exceed 25m. For Zn, there is contamination up to 50m from the Gremda and Tunis roads, and no more than 25m for the Manzel Chaker road. Likewise, we have noted that the absence of copper contamination. Only a few sites on Gremda road are contaminated with Cr. This soil contamination leads to the root accumulation of Pb and Zn in the roots of olive trees, especially near Gremda road. These metals are transferred from the roots to the leaves, disrupting physiological mechanisms such as soluble sugars. The study of the olive oil quality showed that the oil of Manzel Chaker trees is less affected than the oil of Gremda and Tunis trees. The results of Olive oil analyses revealed the reduction of oleic acid and the increase of the palmitic one in the case of Gremda and Tunis roads.

1. Introduction

Atmospheric pollution is a major problem in urban and industrial areas. The potentially toxic metals have preferentially accumulated in tissues from air or water [1]. They play an important role in plant growth, but at higher doses, these elements exert a toxic effect on plants [2]. The impact of atmospheric pollutants on plants will depend on the physiological and biochemical characteristics of the affected plant, and the properties of the pollutants. The damage caused by air pollution can manifest itself in several ways. They can appear on the foliage, in the form of necrotic lesions [3]. They can also lead to yellowing or chlorosis of the leaf. Sometimes plants die right away, but death usually occurs after repeated attacks. In addition to natural stress, plants are subject to attacks of anthropogenic origin, partly from atmospheric pollution. Indeed, the development of industrial activities in recent decades has led to the contamination of agro systems by atmospheric pollutants, particularly the potential toxic metals.

Studies on the stems and leaves contamination have shown an accumulation of lead in the root of beets harvested 15 m from roadways, this researcher also found that, in the vicinity of the road, Pb accumulate-ion in beet roots are in the order of 12 µgg-1 (8 m from the road); on the other hand, it is 1 µgg-1 at more than 500 m. Birch leaves show an accumulation four times greater when polluted (110 µgg-1) compared to control leaves (26 µgg-1) [4]. Regarding the accumulation of metals by plants from the soil, [5] the maximum Pb content of beets grown on lead-rich soil of the mining complex is 3.8 µgg-1. [6] found in some species that there are direct relationships between the metal contents of their tissues and the levels in the soil. For example, for Tansy vulgare (*Tanacetum vulgare*, family: Asteraceae), they found 15.8 µgg-1 and 12 µgg-1 for Cu and Zn respectively, compared to 9.5 µgg-1 and 44.5 µgg-1 in the soil. On the other hand, the soil is very rich in Pb (3440 µgg-1), the level of this metal in the aerial part of Viper (*Echium vulgare*, Boraginaceae family) is multiplied practically by 4 times between June (1.11 µgg-1) and September (4.07 µgg-1). This significant increase is explained by the transfer of Pb absorbed by the roots to the aerial part during flowering which takes place in June 1997 Some workers have reported a positive correlation between Cu, Zn and Pb and have interpreted it as a derivation of the same source of contaminants [7]. It was reported that the concentration values of Fe, Pb, Cu, Mn in plants on roadsides were approximately four times greater than values in plants at control sites [8]. Several authors used many indexes to assess the level of soil contamination, such as the geoacumulation index and enrichment factor [9-10]. Other works have used some index to evaluate the passage of soil metal pollution to different organs of plant, such as the accumulation factor (AF) and the translocation factor (TF) [11-13].

Several studies have used leaves as bioindicators of air pollution [14-17,19] and to monitor or assess the level of pollution in many sites [18-21]. Other works have chosen to study the impact of road and industrial emissions on the quality of olive oils [22-23].

In the region of Sfax for ten years, the environment of the city has been the subject of numerous research works concerning the impact of sulfur dioxide, fluorinated compounds, phosphorus and cadmium on plants [24-27]. The research showed the effect of gaseous pollutants on the morphological characteristics of fruit species (pomegranate, almond, etc.) [28]. Similarly, [25] chose to study the effect of metallic pollutants on the enzymatic activity of certain plant species. Similarly, plants located near phosphoric factories (olive, date palm, almond, pomegranate) showed a significant accumulation of Pb and other metals [26].

The highest concentrations, around 400 µ/g DM, were recorded in the unwashed leaves of the olive trees near the plant. So, after several years and several studies which have focused on industrial emissions, we choose another axis, by studying road pollution in the region of Sfax. In this study, we try to study the accumulation of metallic pollutants in the different organs of olive trees planted near the three roads (Gremda road, ManzelChaker road and Tunis road) in the region of Sfax and the role of meteorological factors in the spatial distribution of metal contamination. Likewise, we are trying to study the impact of road pollution on olive oil quality.

2. Materials and methods

2.1. Study area

Sfax, a southern city of Tunisia (latitude 34° 43′ N; longitude 10° 46′ E), borders the Mediterranean Sea. Its semiarid climate is, therefore, influenced by marine exposure generating a well ventilated area by low to moderate wind, rarely exceeding 5 m/s (DGAT, 1995). Urban expansion, linked to population growth and major industrial development in the region of Sfax is accompanied by intense road network development. All modes of transport are present in the region of Sfax, it has therefore a diversity of infrastructure related to road, rail, sea and air.

All modes of transport are present in the region of Sfax; it has therefore a diversity of infrastructures related to road, rail, sea and air. Away from the city center, just after the ring road of 4 km, traffic is experiencing a significant decline. However, the transit (consisting essentially by buses) almost keeps the same size, which contributes to the increase in the total traffic. The studies done by the Ministry of Equipment, Accommodation and Territorial Development (MEATD) between 2002 and 2007 showed that the traffic away from the city center recorded a growth rate of more than 9% annually (MEATD, 2016).

In order to emphasize the traffic pollution in the region of Sfax and their impact on soils and plants, our study focused on three roads (the road Gremda (toward the North West), the road ManzelChaker toward the South West) and road Tunis (toward the North) (Table1).

Four sites were chosen in the eastern side; 1mE. 2mE. 3mE and 4mE and four in the western side of ManzelChaker road 3mW. 25mW .50mW and 500mW distant 3m, 25m, 50m and 500m. A control side is selected with 1000m of distance. The same sampling was for the Tunis road.5 sites were chosen in the west south side of Gremda road distant 3mSW. 25mSW. 50mSW. 500mSW and a control side distance of 1000m (Figure1) respectively.

2.2. Sample collection

The studied soils and plants were collected near the three major roads in the region of Sfax, road Gremda (toward the northwest), road Manzel Chaker (toward the southwest) and road Tunis (toward the north) (Figure 1). Soil samples were collected at a depth of 0–20 cm using hand-driven steel augers. Sampling was distant from the coastal area approximately 30 km. A auperficial horizon up to a depth of 20 cm was considered.

2.3 Sample analysis

Two grams of the oven dried soil and plant samples were weighed and digested in aqua-regia using HNO_3 and HCI (1:1 HNO_3: HCI) and a ratio of soil sample (1:5). For each 1g of soil and plants 5mL of HNO_3 and 5mL of HCl were added and adjusted to 50 mL with distilled water [30]. Soil samples were analyzed for potentially toxic metals using Atomic Absorption Spectrometer (Pyeunicam, SPQ Philips) (laboratory of enzyme and bioconversion; National School of Engineers of Sfax: Tunisia) (The limits of detection of Cr, Zn, Cu and Pb were 0.078 ppm, 0.006 ppm, 0.025 ppm and 0.078 ppm respectively). Standard stock solutions for all the elements were prepared in the laboratory of olive institute (Sfax, Tunisia) following the procedures as described in APHA (1989). All samples were analyzed in triplicates.

2.4. Bioaccumulation factor

The bio-accumulation factor (BAF) is defined as the ratio of an element's concentration in plant's grain to that element's concentration in the corresponding soil. BAF was calculated for each plant sample to quantify the plant's bioaccumulation effect up-taking heavy metals from the soils [31]. The BAF was computed with the following formula (eq. 1)

$BAF = Cr/Cs$ eq. 1

Where Cr and Cs represented the heavy metals concentrations in the grain and soil extracts; re-spectively, on a dry weight basis [31-32].

2.5. Translocation factor

Concentrations of heavy metals in plants vary depending on total metal concentrations in their paddy soils [33]. Therefore, the transfer factor (TF) of the heavy metals was calculated by dividing the concentration of every metal in the plant by its total concentration in the soil.

Moreover, the translocation factor is calculated as the ratio of metal concentration in aerial parts of any plant over that metal's concentration in the plant root.

$TF = (C_{aerial} / C_{root})$, eq. 2

where, Caerial is the metal concentration in plant's aerial part, and Croot is that metal concentration in the plant's root [4, 32-34].

2.6. Sugars extraction

The extraction of sugars is carried out according to the method of [35]. We put 0.1 mg of dry vegetable matter in a solution with 10 ml of 80% Ethanol. After the water bath and centrifugation, the anthrone is added to the extract. The reading was taken at a wavelength of 640nm. A calibration curve was prepared from a glucose stock solution with a concentration of 1g/l.

2.7 Chlorophyll extraction

The Chlorophyll extraction (a and b) is carried out on fresh leaves. 5 discs of fresh material are extracted into acetone. Absorbance is measured spectrophotometrically and chlorophyll concentrations are calculated according to the formulas following [36-37] (eq. 3 and eq. 4)

Chl a (μg /ml)=11.24*A (661.6) -2.04 * A (644.8) (eq. 3)

Chl b (μg / ml) = 20.13 * A (644.8) - 4.19 * A (661.6) (eq. 4)

2.8 Oil quality indices, photosynthetic pigments contents and fatty acid composition

Free acidity (% oleic acid) and peroxide value (meq O_2/kg) were measured following the analytical method as described in the European Regulation.

Table 1. Roads characteristics

Roads	Gremda road	Tunis road	Manzel Chaker
Characteristics	*Created in 1935. *The total length was 103 km *Several reconstructions have been made, but they vary along the road. -In 1999, a 7-cm-thick asphalt pavement was spread. -In 2001, a bilateral widening and reinforcement by monolayer and two-layer coating was realized up. -In 2008, a two-layer coating of gravel from Hwareb (at 150 km far toward northwest) was made up to the same section.	*Created in 1971 *The length is 274 km with a width varying between 6.5 and 7.5 m. * Several reconstructions have been made: -In 1983, the road was widened and reinforced with soil from Rejich (100 km far on the coast) at 35 cm thick that was coated with 6 cm thick by soil from Elfaiedh (100 km far toward west). - In 1993, asphalt, sand, gravel and bitumen were added. -In the same year, a bilateral expansion was ma by sandstone	*Created in 1976 * The total length was 203 km and with width ranging between 6.5 m and 7.5 m. *Several reconstructions have been made -In 1992, a waterproof two-layer coating of gravel (17 l/m2 and 10 l/m2) was realized. -In 2004, reinforcement and asphalt pavement were done.

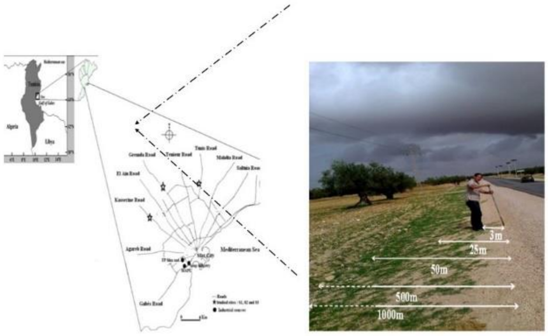

Figure 1. Study area

Extinction coefficients K232 and K270 were measured at 232 and 270 nm, respectively.

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm was evaluated from the absorption spectrum of each oil sample (7.5g) dissolved in cyclohexane as described by [38].
The fatty acid composition was determined based on the European Regulations EEC 2586/91 method as described by [28]

2.9. Statistical Analyses

Statistical analyses were performed using SPSS 10. Windows and treatment means were compared using the Least Significant Difference (LSD) test at $p < 0.05$. At least three replicates were used for each analysis.

3. Results and discussion

3.1 Soil contamination

The levels of metallic elements in soils near three roads are shown in Figure 2. The content of Zn exceeds the standard (39 μgg^{-1}) [39] in many sites near the three studied roads. For Gremda road, the highest values of Zn and Pb in the soil were recorded at 3m from the road. The values exceeding the thresholds (18 μgg^{-1}) [39] for Gremda soils reach a distance of 50m. For copper, only the two sites 3m and 25m have contents which exceed the threshold (13 μgg^{-1}). On the other hand, for Cr, no site exceeded the threshold (41 μgg^{-1}). While for Tunis road, the sites up to 25m in the East of Tunis road are more contaminated by Pb than the sites in the Western side of the road, the value of Zn recorded in 3m East of road (92.2 μgg^{-1}) is a twice of the one recorded in the same distance in the West. The contamination by Cu and Cr was not registered for all the sites of Tunis road.

For Manzel Chaker road, we noted contamination by Pb in the site 25mN and the absence of contamination in the site 3mN. In addition, we noted contamination by Pb only in the first site in the Southern side of the road (3m), while, for Zn, the contamination reaches 50m in the North and 25m in the South of the road. The higher Pb content is linked, on the one hand, to a persistent old accumulation of Pb, because Gremda road created in 1935 is older than the other two roads created in the following years. Nevertheless, the modest increase in Pb is quite reasonable, given the type of vehicles in circulation, the age of the roads and the absence of other sources such as industries. Pb accumulated for several years until 2002 when Tunisia banned Pb in gasoline. However, gasoline containing lead from Algeria until 2015 was used at a rate of 25%. The highest Pb and Zn contents are recorded at the Gremda sites and the sites East of Tunis road.

Monitoring of the exposure of the sites to prevailing winds showed that the Gremda sites and the sites East of Tunis road are the most exposed to road emissions (Figure 3).

In order to study the relationship between the sites, particularly, the degree of contamination, the use of a dendrogram (Figure 4) showed a link between the sites 3m and 25m of Gremda road and on the other hand, a correlation between the 3m and 25m sites in the Eastern and Western sides of the road, with the existence of 3mE, 25mE and 3m sites under the same clade. The comparison of our results with others in the world is illustrated in Table 2.

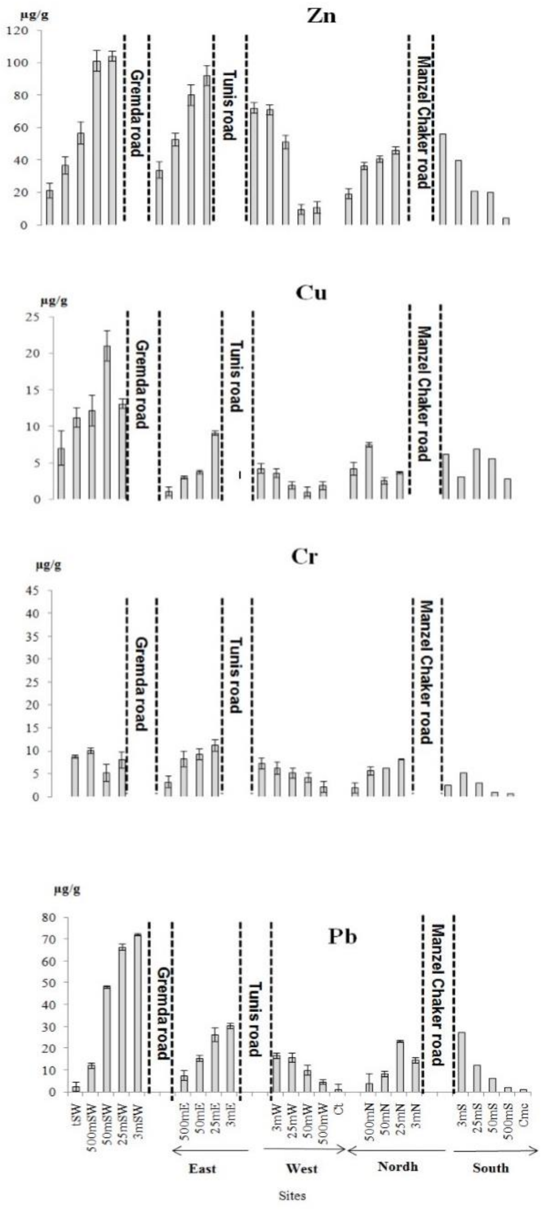

Figure 2. Heavy metals content in soil

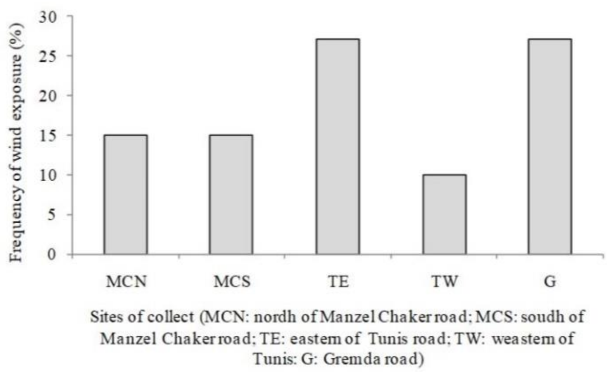

Figure 3. Frequency of exposure of studied sites to road emissions

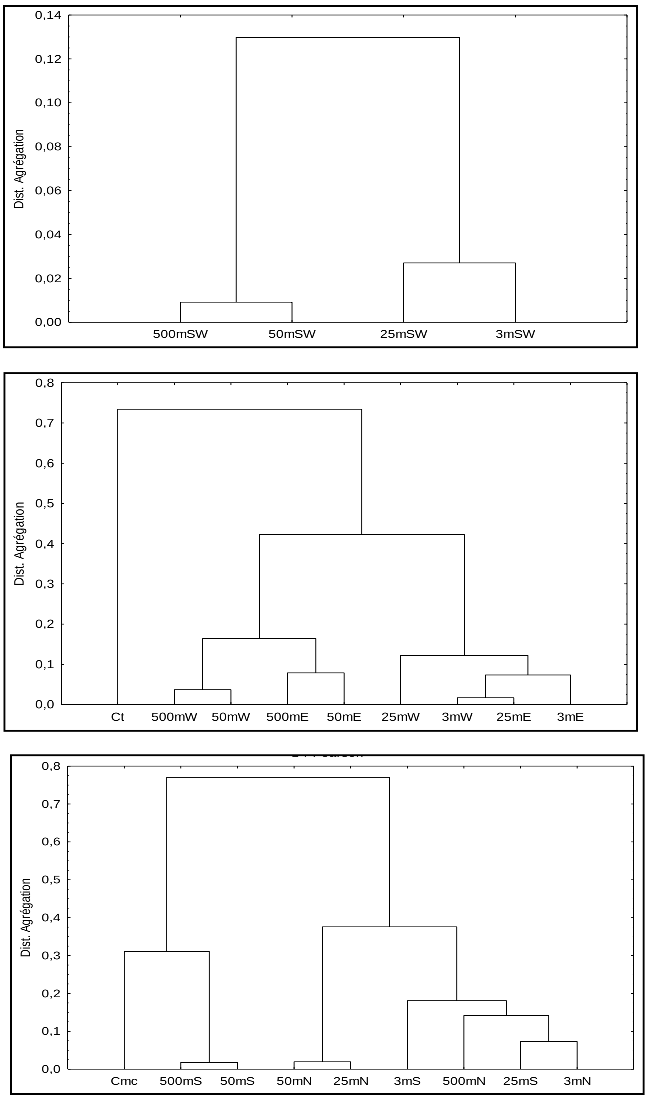

Figure 4. Dendrogram of studied sites according to soil contamination

3.2 Distribution of heavy metals in olive tree

For Gremda road, the Pb contents varied between 1.32 and 12.2 µgg^{-1} Ms in the leaves, between 0.19 and 2.58

Table 2. The levels of, Pb, Zn, Cu and Cr (µg g-1) in roadside soil compared with other studies worldwide**.**

Study areas	Pb	Zn	Cu	Cr	Authors
Sfax, Tunisia	1.2 - 72.2	4-14.2	1-21	0.5-11.2	Currently study
Dubai, United Arab Emirates	259.66–2784.45	1.34–166.43	5.51–65.90	.	[40]
Belgrade, Serbia	13–616	29–1199	16–96	21–89	[41]
Jiangsu provinc, eastern China	19.6	64.0	24.1	52.9	[42]
Kavala's region. Greece	359.4	137.8	42.7	193.2	[43]
Amman, jordan	156-308	241-426	37-131	-	[44]
Lancaster, Engalnd		300-530	19-199	-	[45]
Kaduna, Nigeria	4-32	27-185	1.6-5	-	[46]

μgg^{-1} in the stems and between 1.45 and 74.2 μgg^{-1} in the roots. While for Zn, the contents varied between 8.2 and 88.7 mg kg^{-1}, between 0.91 and 18.3 $\mu gg^{-1}$and between 4.3 and 114.2 $\mu gg^{-1}$for leaves, stems and roots respectively. For copper, the values are between 1.8 and 9 mg kg^{-1}, between 0.5 and 10.4 μgg^{-1} and between 1.95 and 8.5 μgg^{-1} for leaves, stems and roots respectively. While for chromium, the contents are between 0.35 and 5.3 μgg^{-1}; between 0.4 and 6.5 μgg^{-1} and between 0.6 and 9.5 µgg-1 for leaves, stems and roots respectively (Table 3)

For Tunis road (Table 4), the variation of the metal contamination showed a spatial variation on both sides of the roads. In the leaves of the Eastern side, the contents varied between 4.32 µg g-1 and 3.66 µg g-1; between 9.2 µg g-1 and 98.16 µg g-1; between 0.7 µg g-1 and 5.44 µg g^{-1} and between 1.2 µg g^{-1} and 3.09 µg g^{-1} for Pb, Zn, Cu and Cr respectively. While, in the Western side, the levels are between 0.16μgg^{-1} and 7.8μgg^{-1}; between 4.2 μgg^{-1} and 71.6 μgg^{-1}; between 0.2 μgg^{-1} and 3. μgg^{-1} and between 0.27 μgg^{-1} and 6.5 μgg^{-1} for Pb, Zn, Cu and Cr respectively. We notice an increase in the contents in the roots. The values were between 3.66 μgg^{-1} and 33.2 μgg^{-1}; between 14.2 μgg^{-1} and 171.6 μgg^{-1}; between 0.72 μgg^{-1} and 7.37 μgg^{-1} and between 1.75 μgg^{-1} and 10.16 μgg^{-1} for Pb, Zn, Cu and Cr respectively in the Eastern side of the road. While in the Western side, except the Cr, we noticed a decrease in the contents of Pb, Zn and Cu. For leaves, the highest levels are recorded for Zn (108.4 µgg-1) (3mW).

In the stems, the highest levels are recorded for Zn in the 25mW site (57.4 μgg^{-1}), followed by Pb (8.2 μgg^{-1} in the 3mW site), Cr (9.9 μgg^{-1} in 3mW) and Cu (6.22 μgg^{-1} in 3mW). We notice an increase in the contents of heavy metals in the roots. The values were between 0.37 μgg^{-1} and 11.96 μgg^{-1} between 7.2 μgg^{-1} and 108.4 μgg^{-1}; between 1.22 μgg^{-1} and 6.6 μgg^{-1} and between 0.3 μgg^{-1} and 13.98 μgg^{-1} for Pb, Zn, Cu and Cr respectively.

For Manzel Chaker road (Table 5), elevated levels of metallic elements were reported for the three plant organs on the Northern side than the Southern one. The leaf contents of Pb, Zn, Cu and Cr in the Northern side are between 1.1 μgg^{-1} and 4.3 μgg^{-1}; between 25.5 μgg^{-1} and 62 μgg^{-1}; between 3.6 μgg^{-1} and 7.2 μgg^{-1} and between 1.2 μgg^{-1} and 3.2 μgg^{-1} respectively. While, in the Southern side, the levels are between 1.2 μgg^{-1} and 3.7 μgg^{-1}; between 23.1 mg kg^{-1} and 56.3 μgg^{-1}; between 1.4 μgg^{-1} and 6.8 $\mu gg^{-1}$and between 0.4 μgg^{-1} and 1.9 mg kg^{-1} μgg^{-1}respectively. The root contents are higher for the four elements studied. On the Northern side of the road, they varied between 2.2 mg kg^{-1} and 8.6 mg kg^{-1}, between 28.3 μgg^{-1} and 72.2 μgg^{-1}; between 2.2 μgg^{-1} and 10.3 μgg^{-1} and between 2.02 μgg^{-1} and 12.2 μgg^{-1} for the same order of metals. Likewise, for the south side, the root contents are higher than those on the leaves.

So, to summarize, the metal contamination in the three selected sites is in the order of Zn>Pb> Cr> Cu. To compare the metallic distribution between the three organs, we find the following order roots> leaf> stems. Several studies have indicated that metals preferentially accumulate in the root part of plants [25-26]. On the other hand, the comparison between the sites gave the following order: Gremda> Tunis EST>Tunis Ouest>ManzelChaker Nord>Manzel ChakerSud. This is linked, as we have already mentioned, to the frequency of exposure to road emissions (Figure3).

Table 3. Variation of heavy metal accumulation on olive near Gremda road (μg g^{-1})

Elements	Samples	3mSW	25mSW	50mSW	500mSW	Control site
	Leaves	12.2±3.1^{a}	3.6±1.6^{c}	3.7±0.38^{c}	1.32±0.26^{c}	2.4±0.12^{c}
Pb	Stem	2.58±0.23^{A}	1.18±0.2^{B}	0.37±0.12^{B}	0.19±0.08^{B}	
	Root	74.2±6.1*	20.7±2.13**	8.5±0.9**	5.3±0.62***	1.45±0.2***
	Leaves	88.7±12^{a}	78.8±2.3^{a}	66±5.4^{a}	44.2±1.9^{b}	8.2±1.2^{c}
Zn	Stem	18.3^{A}	8.6^{B}	5.52^{C}	3.53^{C}	0.9±0.22^{C}
	Root	114.2±8.1*	82.2±2.32*	82.2±2.5*	51.2±1.3**	4.3±1.1***
	Leaves	9±1.9^{a}	6±1^{a}	4.2±1.3^{a}	2.4±1.53^{b}	1.75±0.44^{c}
Cu	Stem	10.4^{A}	5.66^{B}	4.02^{B}	2.6^{B}	0.5±0.1^{C}
	Root	42.2±2.9*	8.88±1.8***	5.2±2.4***	3.1±0.8***	1.92±0.33***
	Leaves	5.3±0.5^{a}	6.2±0.5^{a}	2.3±0.38^{b}	1.25±0.25^{b}	0.35±0.09^{b}
	Stem	6.5±1.2^{A}	5.6±1.3^{A}	3.34±0.69^{B}	0.5±0.14^{C}	0.4±0.1^{C}
Cr	Root	3.15±0.66*	8.8±0.45**	9.5±2.6**	2±0.3*	0.65±0.2**

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b, c, A, B and C) and (*, ** and ***) indicate significant differences ($p < 0.05$) between treatments.

Table 4. Variation of heavy metal accumulation on olive near Tunis road (μg g^{-1}).

Elements	**Samples**	**E**				**W**				**Control site**
		3m	**25m**	**50m**	**500m**	**3m**	**25m**	**50m**	**500m**	
	Leaves	3.66±1.3^{a}	4.32±1.1^{a}	4.3±1.42^{a}	3.2±0.56^{a}	7.81±0.41^{a}	7.75±0.2^{a}	6.5±0.52^{a}	2.98±1.56^{bc}	0.16±0.14^{bc}
Pb	Stem	12.6±11^{A}	6.5±2.2^{B}	1.55±0.68^{B}	3.5±1.14^{B}	8.2±2.4^{A}	5.2±0.74^{A}	6.45±1.63^{A}	2.93±0.54^{C}	0.32±0.084^{C}
	Root	33.2±1.32*	12.2±1.4**	14.6±2.1**	3.66±0.62***	11.96±0.8*	11.2±1.6*	10.04±0.85*	3.5±1.2**	0.37±0.17**
	Leaves	98.16±2.4^{a}	87.3±4.2^{a}	68.9±3.22^{b}	9.2±1.65^{c}	71.6±4.2^{a}	66±4.2^{a}	44.9±1.85^{ab}	14.2±2.2^{bc}	4.2±1.2^{c}
Zn	Stem	65.6±6.6^{A}	45.1±4.8^{A}	35.5±3.85^{AB}	6.2±5.25^{C}	42.1±6.54^{A}	57.4±7.5^{A}	16.8±5.4^{C}	3±0.66^{C}	0.48±0.22^{C}
	Root	171.6±1.66*	87.4±6.6**	40.4±4.4**	14.2±4.2***	108.4±7.6*	70±3.74**	26.2±6.2**	8.2±1.5**	7.2±3.1**
	Leaves	5.44±2.24^{a}	0.76±0.1^{c}	0.71±0.12^{c}	0.72±0.12^{c}	3.6±1.23^{a}	0.45±0.15^{bc}	0.2±1.02^{bc}	0.87±0.18^{bc}	0.66±0.28^{bc}
Cu	Stem	5.18±1.18^{A}	4.52±1.52^{A}	1.98±0.11^{C}	0.51±0.11^{C}	6.22±2.2^{A}	1.02±0.41^{C}	2.65±0.74^{C}	0.85±0.22^{C}	0.62±0.18^{C}
	Root	7.37±2.31*	5.86±1.86*	2.29±1.28**	0.72±0.16**	6.6±0.85*	1.22±0.28**	5±0.7**	1.4±0.4**	2.41±1.4**
	Leaves	3.09±1.09^{a}	1.97±0.87^{b}	2.9±0.19^{b}	1.2±0.2^{ab}	6.5±1.5^{a}	3.85±0.87^{ab}	0.5±0.11^{bc}	1.66±0.52^{bc}	0.27±0.13^{c}
Cr	Stem	5.65±0.78^{A}	8.27±1.27^{AB}	3.36±1.36^{A}	1.340.3^{C}	9.9±0.9^{A}	8.1±0.7^{A}	2.23±0.2^{BC}	0.37±0.1^{C}	0.22±0.09^{C}
	Root	7.58±1.58*	10.16±2.85*	3.5±1.35**	1.75±0.11**	13.98±1.98*	10.3±1.52*	2.68±0.9**	1.8±0.1**	0.3±0.2**

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b,c,A,B and C) and (*, ** and ***) indicate significant differences ($p < 0.05$) between treatments.

Table 5. Variation of heavy metal accumulation on olive near Manzel Chaker road (μg g^{-1}).

Elements	**Samples**	**N**				**S**				**Control site**
		1m	**3m**	**10m**	**50m**	**1m**	**3m**	**10m**	**50m**	
	Leaves	4.3±2.1^{a}	2.8±0.41^{ab}	0.9±0.2^{c}	1.1±0.2^{c}	3.6±1.1^{a}	3.7±1.3^{a}	1.2±0.5^{b}	1.2±0.1^{b}	0.9±0.14^{b}
Pb	Stem	3.8±1.5^{A}	3.2±1.2^{A}	3.1±1.1^{A}	2.1±0.2^{AB}	3.1±1.01^{A}	3.7±1.4^{A}	1.2±0.41^{AB}	0.4±0.1^{C}	0.3±0.02^{C}
	Root	4.5±2.3*	8.6±1.1**	4.6±1.3*	2.2±0.52*	3.9±1.3*	6.4±1.02**	1.4±0.11**	1.8±0.6**	1.1±0.2**
	Leaves	62±6.3^{a}	45.9±5.1^{b}	29.5±1.3^{bc}	25.5±1.2^{bc}	55.5±6.11^{a}	56.3±3.2^{a}	32.1±2.1^{bc}	23.1±1.1^{c}	16.2±2.4^{c}
Zn	Stem	42.2±1.6^{A}	44.7±1.2^{A}	24.8±1.3^{AB}	17.2±3.2^{C}	52.8±3.12^{A}	45.6±1.3^{AB}	28.5±3.41^{B}	11.2±2.1^{B}	1.8±0.52^{C}
	Root	72.2±0.9*	52.2±2.2**	132.5±3.61**	28.3±1.2**	59.6±6.1*	49.6±2.2*	38.3±2.1**	31.8±3.2**	26.9±2.1**
	Leaves	7.2±2.3^{a}	5.6±1.3^{ab}	3.8±1.1^{b}	3.6±1.1^{b}	6.8±3.14^{a}	4.1±1.1^{ab}	2.2±1.4^{ab}	1.4±0.4^{c}	0.5±1.2^{c}
Cu	Stem	2.7±1.2^{A}	0.4±0.1^{AB}	1.9±0.85^{A}	1.3±0.41^{AB}	1.4±0.32^{A}	2.6±0.6^{AB}	1.8±0.11^{A}	0.9±0.2^{A}	0.4±0.15^{A}
	Root	10.3±1.1*	3.6±1.1**	4.7±1.3**	2.22±0.6**	3.9±1.14*	4.2±1.2*	4.7±1.1*	1.8±0.2**	0.6±0.1**
	Leaves	3.2±0.85^{a}	2±0.52^{ab}	1.7±0.3^{ab}	1.2±0.22^{ab}	1.9±0.84^{a}	0.4±0.2^{ab}	0.9±0.1^{ab}	0.9±0.1^{ab}	0.4±0.15^{c}
Cr	Stem	6.2±2.3^{A}	3.03±1.1^{AB}	0.9±0.11^{C}	1.12±0.11^{C}	18.2±2.6^{A}	0.41±0.12^{C}	0.85±0.2^{C}	0.7±0.1^{C}	0.25±0.08^{C}
	Root	12.2±1.3*	3.5±0.6**	1.8±0.4**	2.02±0.4**	19.2±1.7*	2.9±0.8**	1.8±0.6**	0.7±0.2**	0.51±0.21**

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b,c,A,B and C) and (*, ** and ***) indicate significant differences ($p < 0.05$) between treatments.

In addition, this is linked to the variation in road intensity between the three roads. The study carried out by the municipality of Sfax in 2004 showed the increase in road activity in the Gremda road (21757 TJMA) compared to that of the Tunis road (17007TJMA) and the Manzel Chaker road (10775TJMA).

3.3 Contamination index

To better study the accumulation of metals by the different organs of the olive tree, we used two indexes; accumulation factor (Af) and translocation factor (Tf). The calculation of these factors is illustrated in tables 6, 7 and 8. For Gremda road, we record a root accumulation of Pb up to a distance of 25m and an accumulation of Zn up to a distance of 50m. For Cu, we recorded root accumulation up to a distance of 50m. While the foliar accumulation of Cu appears at a distance of 50m. For Cr, a root and leaf accumulation of this metal appears at a distance of 25m. The use of the translocation factor showed that for all sites, Pb did not transfer from the roots to the aerial part. Likewise, for Zn and Cu, the translocation of Cr was recorded only for the first site. For ManzelChaker road, we did not record any root or foliar accumulation of Pb for the olive trees at all the sites. For Zn, we recorded a root accumulation up to 50m in Northern and Southern sides of the road, the foliar accumulation in the sites 3m and 50m in North of the road and an accumulation up to 50m in the Southern side of the road. For Cu, our results showed a root accumulation that reaches 25m in north of the road and 3m in the Southern side. While, leaf accumulation did not exceed 3m in the Northern and Southern side of the road. Finally, for Cr, root accumulation was recorded only for 3m in the North of the road and no leaf accumulation was recorded for all sites North and South of the road. As with Gremda road, our results for Manzel Chaker road showed the absence of Pb translocation from roots to leaves for all sites. For Zn, this translocation factor exceeded 1 only in the 25mS site leaves. For Cu, TF exceeded 1 in the leaves from the 25mN and 3mS sites. Finally, for Cr, only for the 3mS site, this metal was transferred from the roots to the aerial part. For Tunis road, root accumulation of Pb was recorded only at the site 3m in the East of the road. Likewise, for Zn, there is root accumulation up to a distance of 25m in the East of the road and leaf accumulation up to a distance of 50m in the Eastern side of the road. While in the Western side, no accumulation was recorded, neither root nor aerial. For Cu, root accumulation is shown at the 25mE, 3mW, and 50mW sites. However, there was no foliar accumulation of Cu. Finally, for Cr, the root accumulation of this metal is recorded at 25m East and West of the road. On the other hand, no foliar accumulation was recorded for this metal. Table 7 showed that only Zn was transferred from the roots to the aerial part only in the 50m E site.

Table 6. Accumulation and translocation factor of heavy metals on the olive tree near Gremda road

Elements	Samples	3mSW		25mSW		50mSW		500mSW		Control site	
		AF	TF	AF	TF	AF	TF	AF	TF	AF	TF
	Leaves	0.16	0.15	0.14	0.17	0.12	0.43	0.04	0.24	0.9	1.6
Pb	Stem	0.08	0.07	0.04	0.05	0.01	0.04	0.008	0.03	Nd	
	Root	1.02		1.09		0.3		0.24		0.57	
	Leaves	0.45	0.8	0.77	0.95	0.31	0.81	0.26	086	0.38	1.89
Zn	Stem	0.09	0.17	0.05	0.10	0.02	0.06	0.02	0.06	0.04	0.21
	Root	1.09		1.02		1.4		0.3		0.2	
	Leaves	0.68	0.21	0.29	0.7	1.36	0.71	0.5	0.56	0.24	0.89
Cu	Stem	0.79	0.24	0.62	0.63	0.63	0.32	0.6	0.21	0.07	0.25
	Root	2.21		0.42		1.9		0.81		0.27	
	Leaves	0.65	1.66	1.15	0.88	0.26	0.24	0.14	0.62	-	0.53
Cr	Stem	0.81	2.05	0.67	0.51	0.36	0.34	0.05	0.25	-	0.6
	Root	0.39		1.3		0.95		0.22		-	

3.4 Chlorophyll and sugar content

The soluble sugars and chlorophyll contents of the leaves of olive trees planted in the vicinity of three roads are illustrated in figure 5. Our results showed a significant reduction in Chla contents in the site 3m from Gremda road in the order of 55 %, followed by the site 25m with a reduction of 50%. Likewise, for chl b and chla+b, the sites 3m and 25m recorded the greatest reduction in chlorophyll pigments. On the other hand, we noted a fluctuation in the percentages of reduction of chlorophyll pigments for Manzel Chaker sites. The sites closest to the road (3mN, 25mN, 3mS and 25mS) did not record a significant reduction in chlorophyll pigments. For Tunis road, there is a significant reduction in Chla content for the 3mE and 25mE sites with percentages of 53% and 48% respectively. Likewise, for Chl b with percentages of

Table 7. Accumulation and bio-concentration factor of heavy metals on the olive tree near Manzel Chaker road

Elements	Samples	3mN		25mN		50mN		500mN		3mS		25mS		50mS		500mS		Control	
		AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF
Pb	Leaves	0.92	0.96	0.12	0.32	0.11	0.19	0.27	0.9	0.13	0.9	0.156	0.85	0.18	0.85	0.36	0.66	0.21	0.81
	Stem	0.81	0.84	0.14	0.37	0.37	0.67	0.51	0.79	0.66	0.79	0.09	0.85	0.18	0.85	0.14	0.22	nd	0.27
	Root	0.19	-	0.37	-	0.55	-	0.54	-	0.83	-	0.11	-	0.21	-	1.09	-	0.76	-
Zn	Leaves	2.41	0.85	0.64	0.34	1.32	0.22	0.65	0.9	4.2	0.93	1.38	1.03	1.51	0.83	0.71	0.72	0.49	0.6
	Stem	1.64	0.54	0.62	0.33	1.11	0.18	0.44	0.88	2.06	0.88	1.58	0.84	1.34	0.74	0.35	0.35	0.043	0.06
	Root	3.09	-	1.84		2	-	0.72	-	2.32		1.88		1.9		0.98		0.6	-
Cu	Leaves	1.95	0.69	0.25	1.55	0.51	0.83	0.85	0.7	1.1	1.74	0.84	0.97	0.52	0.46	0.75	0.77	0.04	0.83
	Stem	0.73	0.26	0.15	0.10	0.26	0.41	0.31	0.35	0.38	0.35	1.33	0.61	0.42	0.38	0.36	0.5	0.14	0.66
	Root	1.23	-	1.39		0.63	-	0.52	-	1.51	-	0.84	-	0.55	-	0.66	-	0.24	-
Cr	Leaves	0.39	0.25	0.32	0.56	0.3	0.92	0.63	0.09	0.74	0.09		0.14	0.13	0.5	0.1	0.9		0.78
	Stem	0.77	0.91	0.49	0.86	0.16	0.5	0.19	0.5	2.25	0.94		0.14	0.13	0.47	0.07	0.8	0.45	0.49
	Root	2.97	-	0.56		0.32	-	0.38	-	2.37	-		-	0.34	-	0.38	-	0.92	-

Table 8. Accumulation and bio-concentration factor of heavy metals on the olive tree near Tunis road

Elements	Semples	3mE		25mE		50mE		500mE		3mW		25mW		50mW		500mW		Control site	
		AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF
Pb	Leaves	0.12	0.11	0.16	0.35	0.92	0.98	0.41	0.87	0.04	0.06	0.28	0.69	0.64	0.64	0.18	0.8	0.12	0.4
	Stem	0.41	0.37	0.24	0.53	0.11	0.1	0.45	0.96	0.48	0.6	0.77	0.46	0.64	0.64	0.62	0.8	0.26	0.82
	Root	1.09		0.46		0.94		0.47		0.6		0.91		0.99		0.76		0.3	
Zn	Leaves	1.06	0.57	1.08	0.99	1.3	1.7	0.27	0.64	0.9	0.6	0.92	0.94	0.71	0.21	0.17		0.38	
	Stem	0.7	0.38	0.56	0.51	0.6	0.87	0.18	0.34	0.58	0.4	0.8	0. 8	0.49	0. 64	0.31		0.04	
	Root	1.86		1.09		0.76		0.41		0.36		0.98		0.17		0.86			
Cu	Leaves	0.5	0.7	0.2	0.12	0.23	0.31	0.63	0.8	0.84	0.5	0.12	0.45	0.1	0.04		0.6	0.34	0.62
	Stem	0.5	0.7	1.2	0.77	0.65	0.86	0.44	0.71	1.45	0.9	0.43	0.57	1.38	0.53	0.78	0.6	0.32	0.6
	Root	0.8		1.55		0.76		0.63		1.17		0.27		2.6		0.9		0.6	
Cr	Leaves	0.2	0.4	0.21	0.2	0.35	0.82	0.37	0.68	0.9	0.5	0.62	0.34	0.1	0.18		0.9	0.12	0.9
	Stem	0.5	0.74	0.9	0.81	0.4	0.96	0.41	0.76	1.37	0.7	1.3	0.78	0.34	0.83	0.09	0.2	0.1	0.73
	Root	0.67		1.1		0.42		0.54		0.37		1.66		0.52		0.09		0.14	

47% and 46% and Chla + b with percentages of reduction of 43% and 41% for the same sites respectively. The Pb and Zn contamination at the Gremda sites affected the Chlorophyll content. Similarly, the work of [47]. on a species of the Fabaceae family (*Vigna mungo* L) demonstrated the negative effect of Pb on chla and chlb.

Likewise, the synergism between the content of Pb (3.66 µgg-1) and Zn (98.16 µgg-1) recorded in the 3mE site affected the content of chlorophyll pigment. We noticed that the Chlb content is the least affected. Literature showed that road pollution affected Chla levels and has no effect on chlorophyll b in the olive leaves [48].

The dust can physically block stomata of plants and chemical characteristics of dust may affect either soil or plants [49]. Dust cover on leaf surfaces may affect yield in a variety of ways, with the yield reduction depending upon the thickness of cover and to an extent, the type of plant [50]. The study [51-52] demonstrated that photosystem of chloroplast damage may have occurred to due to heavy metals. The effect of metal pollution on chlorophyll content has been demonstrated by several studies [29, 53-55, 58-61, 63]. Similarly, [56-57], it shown a strong correlation between heavy metals and photosynthesis in trees and herbaceous plants.

The variation in soluble sugars contents is illustrated by the same figure (Figure 5). We notice a decrease in sugar content in the sites close to the polluting source. Taking the control site as a reference site, we reported a reduction of 36% and 28% for the 3m and 25m sites of Gremda road respectively. In addition, we noted a reduction in sugar content in the order of 26% and 22 % for the 3m and 25m in the Eastern side of Tunis road. While in the Western side of Tunis road, we registered a reduction in the order of 22% and 21% for the sites 3m and 25m respectively. In the same line, Pb

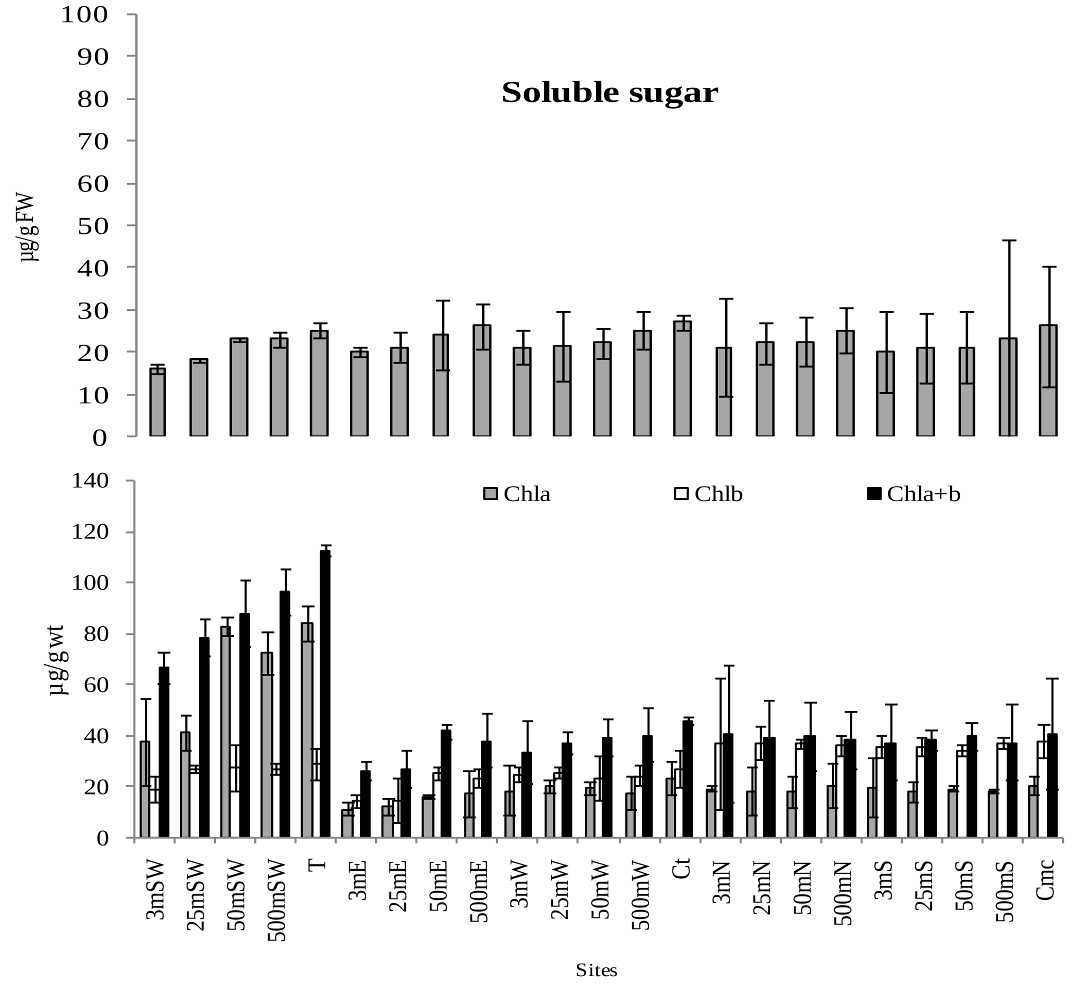

Figure 5 Determination of chlorophylls and soluble sugars content in the leaves of olive trees near three roads in the region of Sfax.

significantly affected the content of soluble sugars in the leaves of *Triticum aestivum* L and *Avena sativa* L [58].

For Manzel Chaker road sites, the reduction percentages are 19% and 15% for the sites 3m and 25m in the North of the road respectively; while, in the South of road, the percentage of a reduction in soluble sugar contents were 23% and 19% for the sites 3m and 25m sites respectively. The increase in soluble sugar content in olive tree leaves when moving away from roads have demonstrated the effect of metal pollution on the synthesis of these sugars. On the other hand, we notice that at 3m and 25m from the road. The olive trees of Manzel Chaker and the leaves of Tunis have higher sugar contents than those recorded in the leaves of Gremda. This is explained on the one hand, by the difference in the road intensity between the roads also, it should be mentioned that the irrigation system affects the synthesis of these metabolites, the olive trees of Gremda are irrigated with intensive irrigation. While, for the olive trees of Tunis and ManzelChaker, the irrigation is irregular, sometimes scarce, which generates an increase in water stress in these trees and consequently, an increase in soluble sugar content.

The results presented above were refined by a factorial analysis of correspondences (Figure 6). The following parameters are used;
* Pb, Zn, Cu and Cr contents in the soils; Pbs, Zns, Cus and Crs respectively.
* Pb, Zn, Cu and Cr contents in the leaves of olive trees; PbL, ZnL, CuL and CrL respectively.
* Pb, Zn, Cu and Cr contents in the roots of Pbr, Znr, Cur and Crr olive trees respectively.
*The chlorophyll contents;Chla, Chlb, Chla + b.
* The polyphenol contents.

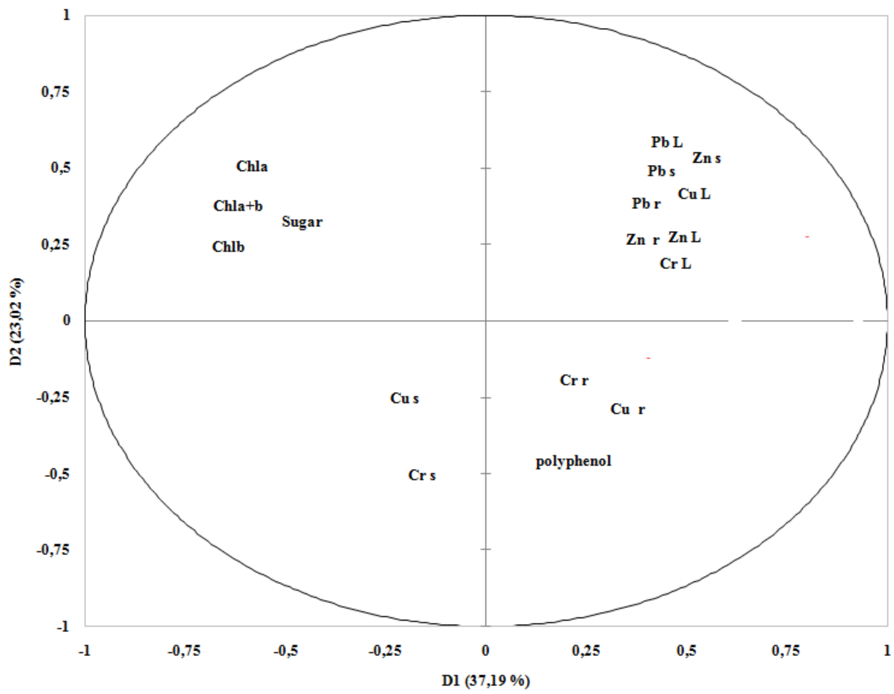

Figure 6. Projection of variables (analyzed elements) in the 1×2 factorial plane

The projection over the 1×2 factorial plane (presenting the maximum inertia) of all selected parameters showed two principals groups;

* **Group 1**; contains the root contamination by Pb and Zn (Pbr and Znr), soil contamination by Pb and Zn (Pbs and Zns), leaf contamination by Pb and Zn (PbL and ZnL) and the leaves contamination by Cu and Cr (CuL and CrL).

* **Group 2**; contains the contents of chlorophylls and soluble sugars in the leaves (Chla, Chlb, Chla + b and Sugar).

The rate of Cu and Cr in the soils and the roots and the polyphenole contents in the leaves are dispersed and out of any influence of other parameters. The use of PCA has shown, the influence of contamination by Pb and Zn for soils, roots and leaves on the chlorophyllic assimilation and the synthesis of soluble sugars. On the other hand, we do not record any influence of this contamination on the polyphenol contents of the leaves.

3.5 Olive oil characteristics

The increase in free acidity is linked to the increase in enzymatic activity responsible for damaged tissues [59]. In other words, the increase in the peroxide index in the oil in the area close to the roads, especially the Gremda road (Table 9), could be explained by the increase in the activity of lipoxygenase [28, 60]. On the other hand, the values recorded in the most polluted sites; 3m from the road to Gremda and 3m from the east side of the road to Tunis are higher than those recorded by [28] under fluoride pollution. The comparison of the spectrophotometric absorption characteristics in the UV region at 232 and 270 nm as a function of the distance from the roads do not show any significant differences. Taking into account the values of free acidity, peroxide value and K232 and K270, the oil samples obtained from Manzel Chaker met the European Union requirements for the extra virgin olive oil category. While olive oil near Gremda and Tunis roads has peroxide contents that exceed the limits, this appears to be related to the contamination of these two areas by different types of pollutants from the road source. Total chlorophyll and carotenoids contents in the oils for the olive of 3m and 25m near Tunis and Gremda roads, were affected by the air pollution level around these roads. In contrast, we noted any modification for the oil of plants near Manzelchaker road when compared with the control site. Indeed, the content of chlorophyll at 3m and 25m from Gremda road was reduced by 50% when compared the sites with the control site. For Tunis road, we noted a reduction of chlorophyll pigment in

Table 9. Free acidity, peroxide values, extinction coefficients, total chlorophyll (chl) and carotenoids (Car) contents of oils and olive yield of olive trees (Cv. Chemlali) grown near three roads in the regions of Sfax

Roads of Sfax	Distance	Free acidity (%)	Peroxide value (meq O2/kg)	K232	K270	Chl (mg/kg)	Car (mg/Kg)
Manzel Chaker	3m	2.25 ±0.23a	11.8±0.5a	1.215±0.1a	0. 55±0.14a	12.67±1.15a	6.86±1.46a
	25m	2.47±0.37b	11.02±0.71a	1.18±0.2a	0.2795±0.15a	12.9±4.9a	6.67±0.59a
	50m	1.36±0.15c	11.55±1.05a	1.5±0.07a	0.52±0.22b	12.92±1.62a	7.09±0.65a
	500m	0.86±0.13bc	11.97±0.4a	1.56±0.12a	0.54±0.014b	13.07±1.25a	6.17±1.1a
	Control site	0.82±0.11bc	11.52±0.4a	1.51±0.11a	0.51±0.011b	13.17±0.25a	5.87±0.8a
Tunis	3m	2.58±0.05A	21.91±0.61A	1.1±0.05A	0.592±0.05A	6.27±1.1A	9.85±0.06A
	25m	2.31±0.07A	14.67±1.01C	1.17±0.05A	0.52±0.14A	6.94±0.46A	7.97±0.35A
	50m	2.28±0.15C	12.51±1.13C	1.32±0..06A	0.55±0.01A	16.62±0.33C	17.1±0.72C
	500m	1.55±0.03BC	12.69±0.48C	0.95±0.07A	0.58±0.011A	15.367±0.47C	15.1±0.04C
	Control site	1.2±0.05BC	12.35±0.18C	0.85±0.04A	0.54±0.011A	15.12±0.47C	14.9±0.03C
Gremda	3m	0.6±0.015*	20.596±0.52*	1.91±0.049*	0.56±0.042*	4.05±1.96a	4.6±2.01*
	25m	0.57±0.14*	12.54±1.16*	0.81±0.06*	0.0.58±0.1*	4.176±1.09a	5.8±1.15*
	50m	0.53±0.15**	10.71±0.44**	1.76±0.014*	0.595±02*	9.053±1.96ab	12.6±2.3***
	500m	0.43±0.01**	10.73±0.42**	1.7±0.014*	0.627±0.08*	8.22±2.69ab	13.8±1.2***
	Control site	0.41±0.01**	10.51±0.22**	1.5±0.012*	0.547±0.08*	8.11±1.29ab	13.2±1.7***

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b,c,A,B and C) and (*, ** and ***) indicate significant differences ($p < 0.05$) between treatments.

3m and 25m with 61% and 54% compared with the control site respectively. Whereas, for the carotene pigment and in the same conditions, the value of reduction was 65% and 56% for the sites 3m and 25m from Gremda respectively, compare to the control site.

While for Tunis road and the same distances, there is a reduction of 34% and 47% respectively compared to the control site. This reduction could be attributed to the pigments degradation by pollutants, particularly the metal pollutant. Furthermore, the decrease of these pigments contents confirmed the oxidative stress induced by the pollutants and could be considered a toxicity sign caused by environmental stress.

3.6 Fatty acid composition of olive oil

For both oil samples, the most abundant acid was the oleic one with values between 54.2µg g^{-1} and 56.82µg g^{-1}, 52.07µg/g- µg g^{-1} and 59.7 µg g^{-1}, 46.7 2µg g^{-1} and 61.18µg g^{-1} for ManzelChaker, Tunis and Gremda roads respectively (Table 10). Except the sample for ManzelChaker, oil samples near Gremda and Tunis (3m) roads were significantly lower than those in oil of control ones (1000m). However, the oil obtained from control plants would be nutritionally better than that obtained in the case of polluted ones.

On the other hand, the level of palmitic acid was 17.52 2 µg g^{-1}, 20.62 µg g^{-1} and 21.6 µg g^{-1} at a distance of 3m near Manzel Chaker, Tunis and Gremda roads respectively. These grades were higher than those recorded at a distance of 1000m, especially for Tunis and Gremda roads. We noticed an increase of 21% and 11% for the 3m and 25m sites of Tunis road respectively compared to the control site. While for Gremda road, and in the same distances, an increase of 31% and 17% was noted compared to the control site. In the oil olive trees located in the polluted area, the decrease in oleic acid in favor of palmitic acid could be due to disturbances in the biosynthesis of triacylglycerols [61-62]. The variation in the compound of olive oil (chemlali) is affected by atmospheric pollution linked to road traffic [28]. In addition to this environmental stress, the variation in the fatty acid composition of olive oil appeared to be affected by climatic factors [63], the time of harvest and the olive variety [59, 64-65].

4. Conclusions

This study focused on the distance from contamination of olive trees in the vicinity of three roads in the region of Sfax (Tunisia). Based on the frequencies of exposure of olive trees to road emissions, we notice that the olive trees of Gremda and Tunis road are more contaminated than the olive trees of Manzel Chaker, this is confirmed by the use of the factors of contamination. This contamination disrupted the chemical composition of the oil in the sites most exposed to road emissions and closest to Gremda and Tunis roads. In a later study, we try to evaluate the atmospheric particulate fallout near three main roads in the region of Sfax and we will study the role of some meteorological factors in the particles fallout variation.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mr. Kamelgargouri, Dr. Semiachaabouni and Dr. Bhekumtheto Ncube. The first draft of the manuscript was written by Dr. Chakermbadra and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 10. Fatty acid composition (%) of olive oils from olive trees (Cv.Chemlali) grown at different distance near three roads in the region of Sfax

Fatty acids	Manzel Chaker					Tunis					Gremda				
	3m	25m	50m	500m	Control	3m	25m	50m	500m	Control	3m	25m	50m	500m	Control
Palmitic acid (C16)	17.52± 0.03^{a}	17.5 ±0.13^{a}	17.8 ±0.07^{a}	17.3 ±0.26^{a}	16.9 ±0.16^{a}	20.6 ±0.8^{a}	18.1 ±0.7^{ac}	17.2 ±0.8^{bc}	17.28 ±0.9^{bc}	16.18 ±0.9^{bc}	21.6 ±3.2^{a}	17.41 ±0.2^{b}	15.6 ± 0.45^{c}	16.05 ±0.8^{bc}	15.09 ±0.7^{bc}
Palmitoleic acid (C16′)	1.15±0 .07^{a}	1.5 ±0.64^{a}	1.23 ±0.07^{a}	1.34 ±0.44^{a}	1.21 ±0.34^{a}	3.21 ±0.8^{a}	2 ±0.13^{ab}	1.8 ±0.14^{ab}	1.67 ±0.33^{ab}	1.44 ±0.13^{ab}	2.17 ±0.9^{a}	1.67 ±0.15^{a}	15.64 ±0.45^{a}	16.05 ±0.9^{a}	15.85 ±0.8^{a}
Heptadecanoic acid (C17)	0.055± 0.02^{a}	0.057 ±0.02^{a}	0.068 ±0.1^{b}	0.08 ±0.02^{b}	0.05 ±0.01^{b}	0.05 ±0.01^{a}	0.04 ±0.02^{a}	0.07 ±0.02^{b}	0.093 ±0.03^{bc}	0.082 ±0.02^{bc}	0.06 ±0.05^{ab}	0.06 ±0.03^{a}	0.07 ±0.01^{ab}	0.08 ±0.02^{c}	0.07 ±0.03^{c}
Heptadecenoic acid (C17′)	0.087 ±0.01^{a}	0.408 ±0.2^{a}	0.068±0. 01^{b}	0.06 ±0.02^{b}	0.04 ±0.01^{b}	0.07 ±0.03^{a}	0.04 ±0.01^{b}	0.048 ±0.02^{b}	0.046 ±0.012^{b}	0.034 ±0.011^{b}	0.067 ±0.03^{a}	0.06 ±0.01^{a}	0.04 ±0.025^{a}	0.03 ±0.01^{a}	0.03 ±0.01^{a}
0.Stearic acid (C18)	2.8 ±0.4^{ab}	2.2 ±0.2^{ab}	2.12± 0.45^{ab}	1.46 ±0.4^{a}	1.36 ±0.4^{a}	3.09 ±0.7^{a}	2.84 ±0.7^{a}	2.18 ±0.12^{a}	2.17 ±0.9^{a}	2.16 ±0.8^{a}	4.11 ±1.2^{a}	3.48 ±0.2^{ab}	2.67 ±0.65^{ab}	1.85 ±0.45^{c}	1.66 ±0.35^{c}
Oleic acid (C18′)	55.9 ±0.6^{a}	55.62 ±0.01^{a}	56.82 ±0.7^{a}	55.2 ±3.75^{a}	54.2 ±2.75^{a}	52.3 ±1.9^{ab}	59.3 ±0.9^{bc}	52.2 ±0.12^{bc}	59.07 ±0.13^{bc}	52.07 ±0.12^{bc}	46.7 ±3.25^{a}	49.25 ±0.5^{ab}	47.5 ±5.65^{ab}	61.18 ±2.18^{c}	59.08 ±2.08^{c}
Linoleic acid (C18′′)	12.9 ±4.26^{a}	14.89 ±1.31^{b}	12.57 ±0.1^{a}	12.8 ±0.6^{a}	11.6 ±0.5^{a}	18.3 ±0.7^{ab}	15.5 ±0.5^{ab}	15.16 ±0.7^{ab}	15.7 ±0.4^{bc}	14.6 ±0.3^{bc}	18.97 ±0.12a	14.4 ±0.2^{a}	14.07 ±0.13^{a}	13.5 ±0.12^{a}	11.4 ±0.02^{a}
Linolenic-eicosanoic acid (C18′′′)	0.816 ±0.15^{a}	0.77 ±0.18^{a}	0.615 ±0.12^{b}	0.715 ±0.16^{a}	0.605 ±0.13^{a}	0.7 ±0.04^{a}	0.61 ±0.2^{b}	0.61 ±0.2^{b}	0.58 ±0.17^{b}	0.47 ±0.17^{b}	0.26 ±0.16^{a}	0.35 ±0.02^{b}	0.69 ±0.015^{c}	0.41 ±0.2^{c}	0.31 ±0.12^{c}
Eicosanoic acid (C20)	0.537 ±0.17^{a}	0.52 ±0.15^{a}	0.48 ±0.15^{a}	0.49 ±0.14^{a}	0.38 ±0.12^{a}	0.56 ±0.01	0.42 ±0.02^{a}	0.43 ±0.02^{ab}	0.38 ±0.05^{ab}	0.32 ±0.07^{ab}	0.48 ±0.17^{c}	0.41 ±0.15^{a}	0.41 ±0.09^{a}	0.42 ±0.26^{a}	0.32 ±0.16^{a}
Eicosenoic acid (C20′)	0.227 ±0.11^{a}	0.26 ±0.13^{a}	0.23± 0.026^{a}	0.28 ±0.02^{a}	0.22 ±0.02^{a}	0.22 ±0.02^{a}	0.12 ±0.08^{b}	0.12 ±0.09^{b}	0.118 ±0.07^{b}	0.106 ±0.06^{b}	0.57 ±0.18^{a}	0.48 ±0.08^{a}	0.17 ±0.074^{a}	0.15 ±0.11^{a}	0.14 ±0.11^{a}

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b) indicate significant differences ($p < 0.05$) between treatments.

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