



Research Article

Heavy metals transfer in leaves, stem and roots of olive tree near roads in the region of Sfax (Tunisia) and their impact on physiological characteristics

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Abstract

In this study, we attempted to study the contamination of the leaves, stems and roots of olive trees collected from 24 sampling sites throughout three roads in the region of Sfax, Tunisia. To better study this contamination, we used two contamination indices, the accumulation and translocation factors. For Gremda road, Pb and Zn contamination did not exceed 50m. For the Tunis road, contamination of leaves, stems and roots by Pb and Cr extended up to 50 m in the eastern and western sides of the road. In contrast, Zn contamination reached 50m in the eastern and 25 m in the western side of the road, respectively. The sites of Manzel Chaker were less affected, and the reduction percentage of Chl did not exceed 11% compared to the control site, while the most affected were those of Tunis and Gremda. The olive leaves of Manzel Chaker and Tunis roads recorded higher sugar content than those of the Gremda road at a distance of 3m. The leaves of olive trees at the sites close to the Gremda and Tunis roads showed weakened antioxidant activity compared to that recorded at the control sites.

1. Introduction

Plants are continuously exposed to multitude stress from biotic and abiotic origin. The major pollutants coming from industrial and road activities are lead, zinc, copper and chromium [1]. Metal distribution between soil and plant is a key issue in the environmental impact of metals in the environment

[2]. In road activity, these pollutants have several sources such as combustion, fluid leakage and corrosion of metals. Lead, cadmium, copper and zinc are the major metal pollutants of the roadside and are released from fuel burning, wear out of tires, leakage of oils and corrosion of batteries [3]. Plants are able to

take up heavy metals from all media, depending on their concentrations. There is a strong relationship between heavy metals and food crops [4-5]. In general, the bioavailability of heavy metals depends on the amount of exchangeable metals in soil. Carbonate-bound and exchangeable metals are more bioavailable than other fractions [6]. The distribution of trace elements within the plant is closely related to the plant species. Some countries plan to list plant species to be grown preferentially on polluted soils [7-9], to assess the effect of a specific source of pollution [10], to differentiate between background (unpolluted) and polluted sites [9], and to monitor or assess the level of pollution in an area used 3 species *Calopogonium mucunoides*, *Axonopus compressus* and *Sida acuta* as accumulator species of metallic pollutants [11-14]. Prasad et al. identified Fabaceae, Poaceae, Euphorbiaceae and Flacourtiaceae as potential hyperaccumulators of heavy metals [15]. Similarly, Bada et al. observed that Malvaceae is absorbed heavy metal from contaminated soil [16]. The bioavailability of heavy metals in plant varies for different plant organs, and the absorption and bioaccumulation rate is highest for roots as compared to other parts [17]. Heavy metals are first absorbed by the apoplast of roots and transported further into other parts of the cells. They are translocated to different plant parts through various pathways and resulted into reduction in growth by altering the physiological, biochemical and metabolic activities of the plants [18]. The trace elements are distributed in the different organs and then can be remobilized, that is to say to change the organ later via the conductive vessels of elaborated sap, the phloem, in particular according to the stages of development of the plant [19]. Phenolic compounds constitute an important class of secondary metabolites found in plants. Polyphenols are formed in almost all plant cells. However, they perform various functions, one of which is the protection of plants against abiotic and biotic stress [20-21]. Heavy metals can lead to an increase in the synthesis of phenolic compounds as a response to metal stress to protect plants from oxidative stress [22]. Olive (*Olea europaea* L) is a popular evergreen tree grown and cultivated in the Mediterranean environment with several environmental factors in the growing climate [23]. 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. Several studies have used olive leaves as a bioindicator of air pollution [24-26]

Several studies are interested in the study of the impact of pollutants on the molecular characteristics of the olive tree [1], while others focus on the effect of pollution on the morpho-anatomical characteristics of the olive tree [27]. In the region of Sfax, many research investigated the relationship between metal contamination and the contents of these elements in plant organs [25-26]. However, little information on heavy metals in roadside soils and plants, especially vegetables, is available.

The objective of this study was determined the distribution of heavy metals between the leaves, stem and roots of Olive tree (Chemlali) by using different contamination index and the impact of this contamination on the physiological characteristics of olive tree.

2. Materials and methods

2.1. Study area

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; therefore, it has a diversity of infrastructure related to road, rail, sea and air. Away from the city center, just after the ring road of 4 kilometers, traffic experiences a significant decline. However, the transit (essentially consisting of buses) maintains the same size, which contributes to the increase in total traffic. The studies conducted 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). The environment of the city of Sfax, as for other large cities, has been deteriorating rapidly over the last couple of decades due to various types of pollutants emitted from various types of anthropogenic activities affecting soils and plants. In order to emphasize the traffic pollution in the region of Sfax and its impact on soils and plants, our study focused on three roads i.e.

Gremda (toward the northwest), Manzel Chaker (toward the southwest) and Tunis (toward the north) (Fig.1) [68]. The traffic activity on these roads was 21.757 Average Annual Daily Traffic (AADT), 10.775 AADT and 17.007 AADT for Gremda, Manzel Chaker and Tunis, respectively. Sampling sites were chosen according to the distance from the road border (Fig. 1).

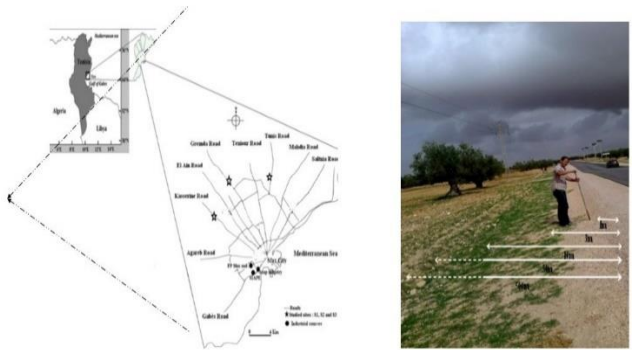


Figure 1. Study area [68].

Moreover, a study prepared by the municipality of Sfax in 2006 showed that the public transport away from the city center (mainly buses of the Regional Transport Company of the Sfax region (SORETRAS) was different between the three roads. The most important public transport activity was recorded for Manzel Chaker with 214 AADT (average annual daily traffic) followed by Gremda (192 AADT) and Tunis (164 AADT). Moreover, this study showed the contribution of light and two-wheeled vehicles in raising the level of traffic on Gremda, followed by Tunis and Manzel Chaker roads. The recorded levels were 17513 AADT, 13716 AADT and 8719 AADT for Gremda, Tunis and Manzel Chaker, respectively. Whereas for two-wheeled vehicles the traffic levels were 3629AADT, 1777 AADT and 919 AADT for Gremda, Tunis and Manzel Chaker, respectively. Thus, the studied roads were ranked as follows:
Gremda road: intense activity
Tunis Road: Average activity
Manzel Chaker road: Low Activity

The sampling points were labeled according to the road considered (MC for Manzel Chaker, G for Gremda and T for Tunis) followed by the distance from the road for every point and their positions were recorded (Table1).

Table 1. Soil physicochemical characteristic.

Physio-chem Parameters	Manzel Chaker																													
	Gremda						Tunis						W						N						S					
	SW																													
	3 m	25 m	50 m	50 m	500 m	T	3 m	25 m	50 m	50 m	500 m	3 m	25 m	50 m	50 m	500 m	T	3 m	25 m	50 m	50 m	500 m	3 m	25 m	50 m	50 m	500 m	T		
pH	5.1	5.5	6.2	7.2	7.2	7.9	5.7	6.4	7.11	7.11	7.06	6.2	7.3	6.3	7.4	7.4	7.6	7.50	7.3	7.7	7.7	7.19	8.3	7.0	7.9	7.9	7.69	7.74		
K+ (%)	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		
	1.2	0.5	1.4	1.27	0.41	2.2	0.9	0.8	1	1.3	1.9	1.7	1.8	1.8	0.8	0.5	0.6	0.6	2.2	1.4	0.1	1.3	0.41	0.6	0.8	1.6				
	0.93	0.83	0.54	0.41	0.41	0.3	0.9	0.8	0.51	0.64	0.81	0.73	0.61	0.61	0.7	0.91	0.72	0.52	0.42	0.5	±0	0.5	0.2	0.2	0.4	0.6				
Na+ (%)	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		
	0.4	0.37	0.21	0.11	0.11	0.08	0.41	0.12	0.3	0.2	0.4	0.2	0.2	0.5	0.12	0.5	0.01	0.01	0.2	0.2	0.2	0.2	0.12	0.1	0.2	0.2	0.2			
	0.6	0.93	0.73	0.8	0.8	0.7	0.7	0.63	0.8	0.9	0.72	0.75	0.75	0.73	0.68	0.73	0.43	0.69	0.74	0.58	0.36	0.94	0.83	0.88	0.63±					
Ca2+ (%)	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		
	0.3	0.1	0.2	0.2	0.2	0.3	0.14	0.17	0.2	0.14	0.2	0.1	0.07	0.1	0.1	0.2	0.1	0.1	0.25	0.33	0.14	0.31	0.22	0.4	0.4	0.4	0.4			
	0.9	0.7	1.4	0.88	0.7	0.7	0.9	1.7	0.8	0.7	0.51	0.75	0.9	0.8	0.8	0.23	1.34	1.53	1.61	1.51	1.66	1.52	1.4	1.55	0.65					
OM (%)	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		
	0.3	0.12	0.5	0.32	0.2	0.2	0.08	0.13	0.4	0.2	0.3	0.09	0.31	0.31	0.42	0.08	0.21	0.21	0.3	0.6	0.6	0.8	0.34	0.26	0.51	0.09	0.09			
	0.62	0.5	0.8	0.65	0.65	0.32	0.98	0.7	0.9	0.7	0.53	0.75	0.33	0.4	0.38	1.92	0.64	0.88	0.5	1.01	0.93	0.5	0.71	0.58	0.58					
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		
	0.2	0.2	0.1	0.1	0.1	0.2	0.4	0.24	0.3	0.32	0.4	0.2	0.1	0.1	0.1	0.2	0.4	0.2	0.09	0.1	0.2	0.1	0.1	0.4	0.2	0.2	0.2			

Sampling was carried out in rural areas, but these sites link the Sfax region to other important regions, such as Tunis, Kairouan, Sidi Bouzid and Kasserine.

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

2.2. Roads characteristic

2.2.1. Gremda road

Created in 1935, the total length was 103 km and several reconstructions have been made, but they vary along the road. In 1993, an asphalt mix was spread on the 99 km-100.5 km section at a rate of 150 kg/m². In 1999, a 7 cm thick asphalt pavement was spread over the sampling section. In 2001, bilateral widening and reinforcement by monolayer and bilayer coatings were realized. In 2008, bilayer coating of gravel from Hwareb was made up to the same section. In the same year, asphalt modernization was carried out only for the 91 and 92km section. In 2008, reinforcement made of bituminous concrete 6 cm thick and variable width was realized for the 91 and 97 km section.

2.2.2. Manzel Chaker road

Created in 1976. The width was between 6.5 m and 7.5 m; in the same year, pavement was realized for the 1-6 km section. In 1992, a waterproof bilayer coating of gravel (17 and 10 l/m²) for the sampling section. In 2004, reinforcement and asphalt pavement for the same section. In 2005, the strength reinforcement was 6-12 km.

2.2.3. Tunis road

The length is 274km. The width was between 6.5m and 7.5 m. Several reconstructions were performed. In 1972, a section 240-254 km (from Tunis to Sfax) in sandstone layer from El Hwareb, (14 l/m²). In 1983, a widening and a reinforcement of the sampling section in Tuf from Rejich, 35cm thick, also coated with 6cm thickness from Elfaiedh for the same section.

In 1993, asphalt, sand, gravel and bitumen were added to the sampling section, (244 and 252km), and in the same year, a bilateral expansion was made for this section. In 2010, a reinforcement of 238 to 256 km

stretch in heavy bitumen, thickness 10 to 12 cm and reinforcement accotes by sandstone up to 254 km.

2.3. Soil analysis

Samples were collected in June 2022. To make a quality soil sample, it is important to respect certain rules concerning the homogeneity and size of the plot, sampling depth, number of individual samples and equipment. The samples were collected along an axis perpendicular to the road using a manual auger. The samples were collected at a depth of 20 cm. After this operation, the samples were brought back to the laboratory, dried in the open air and sieved through a 2 mm mesh sieve [28].

Soil organic carbon was obtained using the dichromate oxidation method described by Walkley et al. [29]. Soil pH was measured in a soil–water mixture with a weight ratio of 1:2.5 (soil/water) using a glass electrode of pH meter (Neomet).

2.4. Extraction of soluble sugar in the leaves of olive tree

The extraction of sugar was carried out according to the method described by McCready et al. [30]. A 0.1 mg of dry vegetable matter was dissolved in 10 mL of ethanol 80%. After the water bath and centrifugation, anthrone was added to the extract. The readings were performed at a wavelength of 640 nm. A calibration curve was created from a mother glucose solution with a concentration of 1g/L.

2.5. Extraction of metal elements

The extraction of metal elements in the soil or plants was achieved by an acid attack using regular water; nitric and hydrochloric acid (V/V) [31] the determination of contents is carried out by the atomic absorption.

2.6. Extraction of chlorophyll, polyphenols and proline in the leaves of olive tree

Chlorophylls (a and b) were extracted from fresh leaves. Five disks of fresh material were extracted in acetone. Absorbance was measured spectrophotometrically and chlorophyll concentrations were calculated according to the following formulas [32-33]:

$$\text{- Chl a } (\mu\text{g/mL}) = 11.24 * (661.6) - 2.04 * A (644.8) \quad (1)$$

$$\text{- Chl b } (\mu\text{g/mL}) = 20.13 * A (644.8) - 4.19 * A (661.6) \quad (2)$$

$$\text{- Chl a + b} = 7.05 * A (661.6) + 18.09 * A (644.8) \quad (3)$$

n = number of disks = 5

The total polyphenol content was determined by spectrophotometry, according to the colorimetric method using the Folin-Ciocalteu reagent. This dosage was based on the quantification of the total concentration of hydroxyl groups in the extract.

The protocol used was based on that described by Singleton et al. [34], with some modifications. Briefly, in glass hemolysis tubes, a volume of 200 μ L of each extract was added, with a mixture of 1 mL of Folin-Ciocalteu reagent (diluted 10 times), and 800 μ L of a 7.5% sodium carbonate solution. The tubes were shaken and kept for 30 min. The absorbances were recorded at 765 nm.

The protocol for the proline extraction was based on that described as Bates et al. [35]. A sample of 300 mg fresh weight (FW) was homogenized in 10 mL of 3% aqueous sulfosalicylic acid solution and the homogenate was passed through a filter paper (Whatman No. 1). Then, 2 mL of the filtrate was reacted with 2 mL of 0.2% acid ninhydrin and 2 mL of concentrated glacial acetic acid in a test tube for 1 h at 100 °C. The reaction was stopped on ice for 15 min. The mixture was extracted with 4 mL of toluene for 20 s with a vortex. The absorbance of the upper phase was measured by spectrophotometry (Shimadzu UV-1800, Japan) at 520 nm. Proline concentration was calculated using a calibration curve and expressed as μ mol proline g⁻¹ FW.

2.7. Bioconcentration and translocation factors.

The calculation of the bioaccumulation factor (BF) and the translocation factor (TF) were calculated to assess whether plants could be categorized as accumulators, as follows:

Bioaccumulation factor (BF)

$$= \frac{\text{concentration in mangrove organ}}{\text{concentration in the soil}} \quad (4)$$

$$\text{Translocation factor (TF)} = \frac{\text{BCF of stem or leaf}}{\text{BCF of root}} \quad (5)$$

3. Results and discussion

3.1. Soil characteristic and contamination

The soil pH of Manzel Chaker varied between 7 and 8.7 (Table 1). A low pH value (pH = 7.3) was recorded at 50m from the right side of the road, and an alkaline pH (8.7) was recorded at a distance of 3 m from the south side of the road. For the two other sites, Tunis and Gremda, the pH values were in the range 5.1-7.2

and 5.7-7.6 mg/kg respectively. The acidity of the soil solution of these last two sites generally increased the solubility of the trace elements by modifying the distribution equilibrium of metals between the liquid phase (solubilized element) and solid (precipitate). Thus the pH of the medium influences speciation and their mobility. In fact, the percolation of lead in soil and its availability for plants depends mainly on soil pH. When the soil is more acidic, metals are more soluble and bioavailable [33]. Indeed, the availability of metals in the soil-plant system depends on soil pH, organic matter concentration and plant developmental stage [36-37].

The organic content in soil also has a high affinity for metal cations due to the presence of ligands or functional groups that can form complexes with metals. Our results showed that the soil OM content of Manzel Chaker was close to 2% at the first site in north of the road and remained above 1% up to a distance of 10m. Only the first site in the south of the road had a content greater than 1%. In the immediate vicinity of the Gremda road (3m) the OM content (0.62%) was almost twice the value recorded at 500 m (0.3%). Similarly, there was a depletion in OM for the soil of Tunis where the contents did not exceed 1% for all sites.

Therefore, the low pH and low organic matter content increased the solubility at the Tunis and Gremda sites, whereas the increase of these two parameters favored the immobilization of metals at the Manzel Chaker sites.

In addition to pH and organic matter, the richness of major elements can contribute to the metal mobility. The soil of Tunis was poor in K⁺ (between 0.009% and 0.026%), Na⁺ (0.005% and 0.052%) and Ca²⁺ (not exceed 1%), likewise for the soil of Gremda where we observed low contents of K⁺, Na⁺ and Ca²⁺, Manzel Chaker soil, showed a richness of Ca²⁺ for the 3 mN (3.36%) and 25 mN (2.31%) sites. These major elements can participate in the reduction of metals by forming complexes with them.

The metallic contamination of soil is shown in Fig. 2. The highest Zn contamination (214 μ g/g) was recorded the 50 mSW site, which is 5 times higher than the Tunisian standard (39 μ g/g) [38]. Near Tunis road, Zn levels above the thresholds reached a distance of

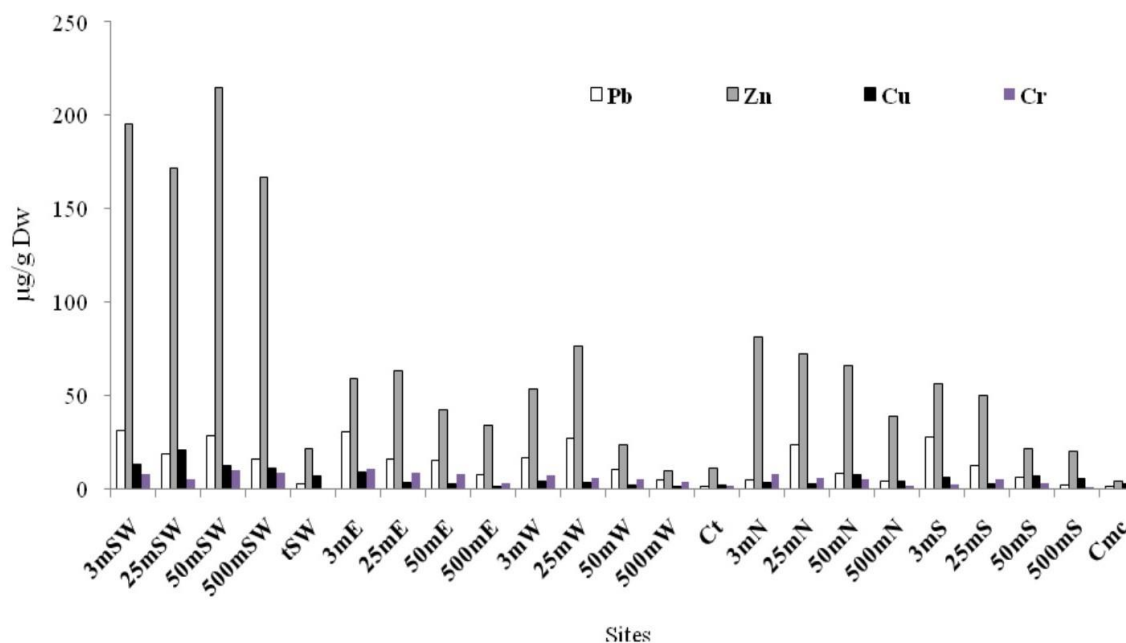


Figure 2. Heavy metals content in soil.

50m east and 25m west of the road. The highest grades were recorded at the 25mE and 25mW sites. They are 1.6 and 1.9 times higher than the standard, respectively. However, for Manzel Chaker road, Zn contamination was 2 and 1.4 times higher than the standard at the 3mN and 3mS sites. This contamination arrived at a distance of 25m north and south of the road.

For Pb, the levels recorded were 1.7, 1.68 and 1.5 times higher than the standard ($18\mu\text{g/g}$) at the 3mSW, 3mE and 3mS sites, respectively. For the Gremda road, the rate exceeding the limit reached a distance of 50m. Similarly, for Tunis road, lead contamination was observed at a distance of 25m east of the road. Only, the 25mW site exceeded the standard west of the road. For Manzel Chaker road, two sites exceeded the standard: 25mN and 3mS. Pb is one of the marker of road pollution and its elevation is related to road intensity [39].

Similar to other metals, Pb persists in the soil for many years, and is non-biodegradable. Tunisia implemented the use of Pb-free gasoline since 2002, but Pb in soil is likely to remain for a longer time. Like all other countries, Tunisia banned the use of lead in gasoline in 2002. In Algeria, until 2015, 103 million people were still exposed to lead from gasoline (PNU, 2015). In contrast many studies in countries that have

signaled the corruption of Pb in gasoline have shown an elevation of Pb in soil [40-42]. About 60% of informal trade with Algeria concerns petroleum products (Confederation of Corporate Citizens in Tunisia, 2017). The smuggling of petroleum products (gasoline and diesel) with Libya and Algeria is estimated to be 1 billion liters annually (IPEMED, 2017).

Approximately, one million cubic meters (m^3) per year, or a quarter of the national consumption of fuels, gasoline and, above all, diesel fuel, would come from smuggling from Algeria and Libya [43].

A study published by the World Bank in December 2013, entitled "Estimating Informal Trade across Tunisia's Land Borders", estimated the volume of smuggled fuels, from Algeria, to a quarter of national consumption. Algerian gas contained Pb till 2015. Similarly, the studies carried out by TISS, at the end of 2015 estimated the quantities of smuggled fuels, from Algeria and Libya, at 26% of national consumption, which is a first approximation of 900,000 tons in 2015. For the other two metals, no site exceeded the standard, and there was variation in levels between sites. The 3mSW and 25mSW sites recorded the highest Cr contents at 13.12 and $21.04\mu\text{g/g}$, respectively. For Cu, the contents were 3mE and 25mE. Generally, we observed a decrease in pollutant levels

when moving away from roads. The decrease in metals as a function of distance was demonstrated [44].

The use of dendrogram (Fig. 3) based on the variation of heavy metals showed for Gremda soils, two clades, one containing the control site, and the other containing two subgroups: 3mSW and 25mSW; and 50mSW and 500mSW.

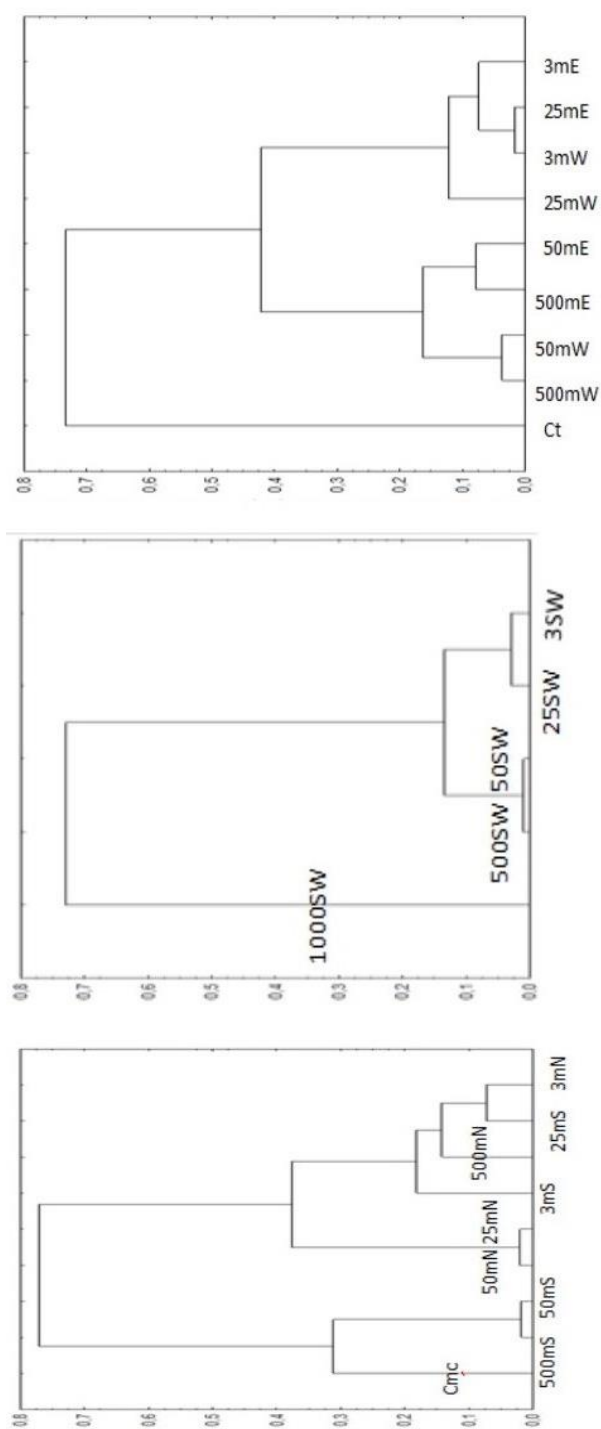


Figure 3. Correlation between sites.

For Tunis road, 2 clades were observed, one is formed of 2 subgroups: 3mE and 25mE; and 3mW and 25mW with the other represented by the control site. For the Manzel Chaker sites, the distribution of the sites did not show definite groups. The impact of road traffic sometimes reached, a hundred meters on both sides of the road, and was particularly sensitive in the first 80 to 100 meters [45].

In addition to the intense road activity on the Gremda and Tunis roads, meteorological factors can play a role in the contamination of soil and plants in the vicinity of these roads. Gremda sites are influenced by N, NNE, NNW, SE, NE, ENE, E and ESE winds. The sites in the eastern side of Tunis road are subject to winds from the NW, WNW, W and WSW sectors, whereas those on the western side are subject to winds from the NNE, NE, ENE, E and ESE sectors. For sites south of Manzel Chaker road, the wind direction was ESE, E, NE, NNE and N, however, sites to the north were subject to wind direction S, SSE, SWS, SW, W and WNW. Thus, we noticed that the percentages of winds that brought emissions to the Gremda sites were between 58% and 84% (for August, September, October November and December, 2021) and between 51% and 75% (January, February, March, April and May, 2022). The frequencies of winds which bring emissions to the eastern side of Tunis road were between 9% and 24% (in 2021) and between 12% and 32% (in 2022). For the western side of Tunis, the frequencies of winds were between 27% and 46% (in 2021) and between 43% and 66% (in 2022). On the other hand, the wind frequencies bring emissions to the northern side of Manzel Chaker road are between 7% and 32% (in 2021) and between 16% and 39% (in 2022).

3.2. Leaves, stem and roots contamination

The contents of metallic elements (Pb, Zn, Cu and Cr) are presented in Tables 2, 3 and 4. For Gremda road, Pb levels varied between 0.32 and 6.2 $\mu\text{g/g}$ in the leaves, between 0.19 and 2.5 $\mu\text{g/g}$ in the stems and between 0.31 and 39.4 $\mu\text{g/g}$ in the roots. For Zn, the highest levels were recorded in the roots (between 20.2 and 198.62 $\mu\text{g/g}$), followed by the leaves (between 18.6 and 98.7 $\mu\text{g/g}$) and the stems (between 0.19 and 2.58 $\mu\text{g/g}$). For copper, the values were 1.8-9, 0.5-10.4 and 1.95-8.5 $\mu\text{g/g}$ for leaves, stems and roots, respectively. Whereas for chromium, the contents

Table 2. Variation of heavy metal accumulation on olive near Gremda road.

Elements	Sample	3m SW	25m SW	50m SW	500m SW	Control
Pb	Leaves	6.2 ± 3.1	6.6 ± 1.6	0.6 ± 0.08	0.32 ± 0.26	0.5 ± 0.15
	Stem	2.58 ± 0.44	1.18 ± 0.17	0.37 ± 0.21	0.19 ± 0.09	0.13 ± 0.05
	Root	39.4 ± 6.1	22.2 ± 2.13	0.8 ± 0.09	0.3 ± 0.62	0.31 ± 0.13
Zn	Leaves	98.7 ± 12	78.8 ± 2.3	36 ± 5.4	34.2 ± 1.9	18.6 ± 2.3
	Stem	18.3 ± 3.4	8.6 ± 2.2	5.52 ± 1.45	3.53 ± 1.65	0.91 ± 0.32
	Root	198.62 ± 8.1	60.32 ± 2.32	47.2 ± 2.5	35 ± 1.3	20.2 ± 1.6
Cu	Leaves	5.5 ± 1.9	6 ± 1.4	4.2 ± 1.3	2.4 ± 1.53	1.75 ± 0.8
	Stem	6.2 ± 1.89	5.66 ± 1.87	4.02 ± 0.87	2.6 ± 0.43	0.5 ± 0.21
	Root	8.6 ± 2.9	8.88 ± 1.8	5.2 ± 2.4	3.1 ± 0.8	1.95 ± 0.3
Cr	Leaves	5.3 ± 0.5	6.2 ± 0.5	2.3 ± 0.38	1.3 ± 0.25	0.4 ± 0.09
	Stem	6.5 ± 1.43	3.53 ± 1.55	3.34 ± 0.67	0.5 ± 0.12	0.4 ± 0.11
	Root	3.15 ± 0.66	5.96 ± 0.45	9.5 ± 2.6	1.2 ± 0.3	0.65 ± 0.2

Table 3. Variation of heavy metal accumulation on olive near Tunis road.

Elements	Samples	E				W				Control
		3m	25m	50m	500m	3m	25m	50m	500m	
Pb	Leaves	13.66	4.32	4.3	0.62	1.81	1.75	1.5	0.98	0.159
		±1.3	±1.1	±1.42	±0.56	±0.41	±0.2	±0.52	±1.56	±0.14
	Stem	2.6	6.5	1.55	0.5	1.2	2.8	0.45	0.93	0.32
		±11	±2.2	±0.68	±1.14	±2.4	±0.74	±1.63	±0.54	±0.084
	Root	35.6	17.2	16.2	0.66	1.96	2.58	1.84	0.5	0.37
Zn	Leaves	104.2 ± 2.4	87.3	68.9	29.08	94.6	94.2	44.9	16.2	11.2
			±4.2	±3.22	±1.65	±4.2	±4.2	±1.85	±2.2	±1.2
	Stem	65.6	45.1	35.5	28.58	42.1	57.4	16.8	3	0.48
		±6.6	±4.8	±3.85	±5.25	±6.54	±7.5	±5.4	±0.66	±0.22
	Root	101.6	79.36	40.4	34.5	108.4	73	46.2	19.2	13.33
Cu	Leaves	5.44	0.76	0.71	0.72	3.6	0.45	0.2	0.87	0.66
		±2.24	±0.13	±0.12	±0.12	±1.23	±0.15	±1.02	±0.18	±0.28
	Stem	5.18	4.52	1.98	0.51	6.22	1.02	2.65	0.85	0.62
		±1.18	±1.52	±0.62	±0.11	±2.2	±0.41	±0.74	±0.22	±0.18
	Root	7.37	5.86	2.29	0.72	6.6	1.22	5	1.4	2.41
Cr	Leaves	3.09	1.97	2.9	1.2	6.5	3.85	0.5	1.66	0.27
		±1.09	±0.87	±0.19	±0.2	±1.5	±0.87	±0.11	±0.523	±0.13
	Stem	5.65	8.27	3.36	1.34	9.9	8.1	2.23	0.37	0.22
		±0.78	±1.27	±1.36	0.3	±0.9	±0.75	±0.23	±0.11	±0.09
	Root	7.58	10.16	3.5	1.75	13.98	10.3	2.68	1.08	0.3
		±1.58	±2.85	±1.35	±0.11	±1.98	±1.52	±0.95	±0.12	±0.19

were: 0.4-5.3, 3.3-6.5, and 0.6-9.5 µg/g for leaves, stems and roots, respectively. With reference to the normal values for metals in plants according to [46] (Pb: 1 µg/g; Zn: 50 µg/g; Cu: 10 µg/g; Cr: 1.5 µg/g), the contamination by Cu, does not exceed 3m, while it

reaches 25m for Pb and Zn, and 50m for Cr.

For Tunis road, contamination of leaves, stems and roots by Pb and Cr (with the exception of 50mW stems and leaves sites with Pb and Cr contents below standard) has arrived up to 50m east and west of the

Table 4. Variation of heavy metal accumulation on olive near Manzel Chaker road.

Elements	Samples	N				S				Control
		3m	25m	50m	500m	3m	25m	50m	500m	
Pb	Leaves	4.9	0.8	0.9	0.1	3.6	0.7	0.2	0.2	0.1
		±2.1	±0.41	±0.2	±0.2	±1.1	±0.3	±0.15	±0.1	±0.14
	Stem	3.8	0.5	0.1	0.1	3.1	0.7	0.2	0.4	0.3
		±1.5	±0.2	±0.01	±0.22	±1.01	±1.4	±0.41	±0.1	±0.02
Zn	Root	5.3	0.9	0.6	0.2	3.9	1.4	0.4	0.8	0.1
		±2.3	±0.14	±0.32	±0.52	±1.3	±1.02	±0.11	±0.6	±0.21
	Leaves	62	45.9	29.5	25.5	55.5	36.3	17.6	23.1	16.2
		±6.3	±5.1	±1.3	±1.2	±6.11	±3.2	±2.1	±1.1	±2.4
Cu	Stem	52.2	44.7	24.8	17.2	52.8	45.6	16.2	11.2	1.8
		±1.6	±1.2	±1.3	±3.2	±3.12	±1.3	±3.41	±2.1	±0.52
	Root	83.2	49.2	42.5	28.3	59.6	49.6	19.8	31.8	26.9
		±0.9	±2.2	±3.61	±1.2	±6.1	±2.2	±2.11	±3.2	±2.1
Cr	Leaves	3.2	0.6	3.8	0.45	1.8	2.9	2.2	1.4	0.5
		±2.3	±1.3	±1.1	±1.1	±3.14	±1.1	±1.4	±0.4	±1.2
	Stem	2.7	0.4	1.9	0.32	1.4	2.6	1.8	0.9	0.4
		±1.2	±0.1	±0.85	±0.41	±0.32	±0.6	±0.11	±0.2	±0.15
Pb	Root	8.3	1.6	4.7	0.85	2.5	4.2	4.7	1.8	0.6
		±1.1	±1.1	±1.3	±0.6	±1.14	±1.2	±1.1	±0.2	±0.1
	Leaves	3.2	2	1.7	0.62	1.9	0.4	0.66	0.8	0.4
		±0.85	±0.52	±0.3	±0.22	±0.84	±0.2	±0.1	±0.1	±0.15
Cr	Stem	2.2	3.03	0.9	0.32	1.82	0.41	0.45	0.47	0.25
		±2.3	±1.1	±0.11	±0.11	±0.6	±0.14	±0.2	±0.1	±0.08
	Root	3.9	3.5	1.8	0.71	1.92	0.9	0.8	0.7	0.51
		±1.3	±0.62	±0.4	±0.01	±1.7	±0.08	±0.03	±0.2	±0.21

road. Zn contamination reached 50m east and 25m west of the road. On the other hand, Cu content did not exceed the norm. Generally, root levels were higher than leaf and stem levels.

For Manzel Chaker road, Pb levels above the norm did not exceed the first site north of the road, while only for the roots, that arrived at 25m in the southern side. For Zn, the distribution of contamination was similar to that of Pb and did not exceed the 3m. For Cr, leaf contamination was observed at a distance of 50 m, north of the road. On the other hand, in the south of the road, the contamination by Cr affected the three organs but did not exceed the 3m. Similar to the Grmeda and Tunis road sites, the plants on the Manzel Chaker road were not contaminated by Cu.

So, to summarize, the metal contamination in the three chosen sites were in the order: Zn> Pb> Cr> Cu. These metals were accumulated mainly in the sites close to the roads, confirming the intervention of road emissions in this contamination. Some workers have

reported a positive correlation between Cu, Zn and Pb in plants grown near roads and have shown that they are derived from the same source of contaminants [47-48]. Similarly, studies of Deruelle et al. [49] on birch leaves showed an accumulation four times greater when they were close to roads (110 µg / g against 26 µg / g in the control leaves).

Comparing the metal distribution between the three organs, we found the following order: Roots> Leaves> Stems. Several studies have indicated that metals preferentially accumulate in the root parts of the plants [25-26]. Similarly, in another study on *Calopogonium mucunoides*, *Axonopus compressus* and *Sida acuta* showed that leaves had higher heavy metal content, followed by stems and roots [44]. This can inform us of the relationship between soil and olive tree contamination near roads and the importance of soil-plant passage as the air-plant passage. Contrary to these results, the work of Capnesi et al. [50] indicated that Pb and Zn are more likely to be found

Table 5. Accumulation and translocation factor of heavy metals on the olive tree near Gremda road (AF: accumulation factor; TF: translocation factor).

Elements	Samples	3mSW		25mSW		50mSW		500mSW		Control	
		AF	TF	AF	TF	AF	TF	AF	TF	AF	TF
Pb	Leaves	0.2	0.15	0.347	0.29	0.02	0.43	0.011	0.24	0.19	0.7
	Stem	0.08	0.06	0.062	0.05	0.013	0.04	0.0086	0.03	Nd	-
	Root	1.27	-	1.16	-	0.02	-	0.0135	-	0.12	-
Zn	Leaves	0.5	0.5	0.45	0.41	0.16	0.81	0.205	0.86	0.87	0.92
	Stem	0.09	0.09	0.05	0.04	0.02	0.06	0.02	0.06	0.04	0.04
	Root	1.016	-	1.095	-	0.21	-	0.209	-	0.94	-
Cu	Leaves	0.419	0.63	0.29	0.7	0.34	0.71	0.214	0.56	0.24	0.89
	Stem	0.472	0.72	0.26	0.63	0.32	0.32	0.23	0.21	0.07	0.25
	Root	0.65	-	0.42	-	0.42	-	0.278	-	0.28	-
Cr	Leaves	0.65	1.66	1.15	1.006	0.23	0.24	0.14	0.62	-	0.53
	Stem	0.81	2.05	0.67	0.59	0.33	0.34	0.05	0.25	-	0.6
	Root	0.39	-	1.14	-	0.95	-	0.22	-	-	-

in leafy vegetables than in roots.

The variation in contamination between the three study sites revealed the intervention of several factors, such as road intensity, leaf age, and leaf surface area [39]. In addition, we cannot ignore the role of meteorological factors. The sample sites were agricultural fields plowed 4 to 6 times per year to a depth ranging between 5 to 25 cm and planted with olive trees at a planting density ranging between 17 and 204 trees/ha. The wind observations recorded the weather station of Sfax (Tina Airport) often indicate bi-directional dominance: "East" and "West". Indeed, the direction of the monthly winds established over a period of 10 years (between 2002 and 2011) reveals a clear predominance of the winds coming from the directions ESE, SE, E, ENE, NNW and SW, as well as that the average wind speed is moderate, hardly exceeding 5 m/s [51]. The Gremda road sites were influenced by winds from the N, NNE, NNW, SE, NE, ENE, E and ESE. So, the five directions brought to the emissions from road to sampling area. Tunis road faces NNE, NE, ENE, E and ESE winds. Thus, the three wind directions were able to bring road traffic emissions to the sampling sites. Manzel Chaker road was under the dominance of ESE, E, ENE, NE, NNE, N and NNW winds and 4 of them enhanced spreading of pollutants toward sampling sites. The extent of metal contamination up to 50m in the sites of Tunis and the stop at a distance of 25m for the sites of Gremda and 3m for the sites of Manzel Chaker was

linked to the intense traffic activity for Gremda and Tunis roads, compared to Manzel Chaker, however, in the case of Gremda sites, the intensive olive cultivation regime behaved as an obstacle and prevented the dispersion of pollutants over long distances. This is why the highest grades for Pb (39.4µg/g) and Zn (128.6µg/g) were recorded at 3 m from the Gremda road compared to those of other sites at the same distance.

The study by Goldbach et al. [54] on a rich-soil by lead (3440 ppm) showed that the contents of this metal in the aerial part of Viperine (*Echium vulgare*, family Boraginaceae) were multiplied practically by 4 times in passing from June (1.11 ppm) to September (4.07 ppm). This important increase was explained by the transfer of lead absorbed by the roots to the aerial parts during blooming in June.

3.3. Index of contamination

To better study the accumulation of metals by the different organs of the olive tree, we used two factors of contamination: The accumulation factor (Af) and translocation factor (Tf). The calculation of these factors are shown in Tables 5, 6 and 7.

For the Gremda road, the accumulation factor showed an accumulation of Pb and Zn in the roots, up to a distance of 25m. For Cr, accumulation was only noted in olive roots at the site 25mSW. However, no accumulation of Cu was observed. The translocation factor showed only the passage of Cu from the roots and leaves of the olive trees, while the others

Table 6. Accumulation and bioconcentration factor of heavy metals on the olive tree near Manzel Chaker road (AF: accumulation factor; TF: translocation factor).

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	1.05	0.92	0.03	0.8	0.11	0.75	0.02	0.5	0.13	0.9	0.16	0.5	0.03	0.55	0.36	0.25	0.21	1
	Stem	0.81	0.71	0.02	0.5	0.01	0.08	0.02	0.5	0.66	0.79	0.06	0.5	0.03	0.55	0.14	0.5	Nd	3
	Root	0.19	-	0.04	-	0.07	-	0.05	-	0.83	-	0.11	-	0.06	-	1.09	-	0.76	-
Zn	Leaves	0.76	0.74	0.64	0.4	0.45	0.69	0.65	0.9	0.99	0.93	0.73	0.83	0.88	0.71	0.72	0.49	0.6	0.6
	Stem	0.64	0.62	0.62	0.9	0.37	0.58	0.44	0.6	0.65	0.88	0.91	0.91	0.76	0.81	0.35	0.35	0.043	0.06
	Root	1.02	-	0.68	-	0.64	-	0.72	-	0.73	-	0.99	-	0.98	-	0.98	-	0.6	-
Cu	Leaves	0.87	0.38	0.01	0.3	0.51	0.83	0.11	0.52	0.29	0.72	0.84	0.69	0.52	0.46	0.75	0.77	0.04	0.83
	Stem	0.7	0.32	0.15	0.2	0.26	0.4	0.08	0.4	0.38	0.7	0.94	0.6	0.42	0.38	0.36	0.5	0.14	0.66
	Root	0.9	-	0.62	-	0.63	-	0.2	-	0.9	-	0.9	-	0.5	-	1.1	-	0.24	-
Cr	Leaves	0.39	0.81	0.32	0.56	0.3	0.94	0.33	0.8	0.74	0.98	0.4	0.13	0.82	0.1	0.67	0.78	0.78	0.78
	Stem	0.27	0.56	0.49	0.86	0.16	0.5	0.8	0.45	0.22	0.94	0.45	0.13	0.56	0.07	0.45	0.45	0.49	0.49
	Root	0.95	-	0.56	-	0.32	-	0.5	-	0.24	-	-	-	0.34	-	0.38	-	0.92	-

Table 7. Accumulation and bio-concentration factor of heavy metals on the olive tree near Tunis road (AF: accumulation factor; TF: translocation factor).

Elements	Samples	3mE		25mE		50mE		500mE		3mW		25mW		50mW		500mW		Control	
		AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF	AF	TF
Pb	Leaves	0.45	0.38	0.26	0.25	0.28	0.26	0.08	0.93	0.107	0.9	0.06	0.6	0.14	0.81	0.2	1.9	0.12	0.4
	Stem	0.08	0.073	0.4	0.37	0.11	0.09	0.06	0.75	0.07	0.61	0.1	1.08	0.04	0.24	0.2	1.86	0.26	0.82
	Root	1.17	-	1.05	-	1.04	-	0.086	-	0.1	-	0.09	-	0.18	-	0.1	-	0.3	-
Zn	Leaves	0.65	1.02	1.38	1.09	1.6	1.7	0.85	0.84	1.77	0.8	1.23	1.3	1.89	0.97	0.2	-	1.02	-
	Stem	0.412	0.6	0.71	0.51	0.83	0.87	0.84	0.82	0.78	0.38	0.75	0.78	0.71	0.36	0.3	-	0.04	-
	Root	0.63	-	1.26	-	0.95	-	1.01	-	0.86	-	0.95	-	1.95	-	2	-	-	-
Cu	Leaves	0.59	0.7	0.2	0.12	0.23	0.31	0.63	1	0.84	0.5	0.12	0.37	0.1	0.04	0.6	0.34	0.27	0.27
	Stem	0.57	0.7	1.2	0.77	0.65	0.86	0.44	0.71	1.45	0.93	0.28	0.83	1.38	0.53	0.78	0.6	0.32	0.25
	Root	0.81	-	1.55	-	0.76	0.63	0.63	0.63	1.17	0.33	0.33	0.33	2.6	1.3	1.27	1.27	1.27	1.27
Cr	Leaves	0.2	0.4	0.21	0.2	0.35	0.82	0.3	0.68	0.9	0.46	0.62	0.37	0.1	0.18	0.9	0.12	0.9	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	0.43	0.83	0.09	0.21	0.1	0.73
	Root	0.67	-	1.1	-	0.42	-	0.54	-	0.37	-	1.66	-	0.52	-	0.09	-	0.14	-

remained in the roots.

For the sites of Manzel Chaker road, we recorded an accumulation of Pb in the leaves of 3mN and Zn in the roots of the same site. However, for the other two other metals, we did not record any accumulation at any of the sites. For the translocation factor, all sites had TF below 1 for all metals.

For the Tunis road, the accumulation of Zn in the leaves reached a distance of 50 m in both directions, whereas for the roots, accumulation stopped at 25m. Similarly, copper and Cr accumulation was within 25m east of the road and no accumulation was observed in the west of the road. Similarly, the use of the translocation factor has shown that for Zn, this factor is greater than 1 at the east of the road up to a distance of 50 m. Whereas for Pb, Tf was less than 1 for all sites, which is the same as the difficulty passage of this metal from roots to stems and leaves. For Cu and Cr, the accumulation of these metals in the roots or leaves of sites not exceeding 25 m was observed. No Pb accumulation was recorded at the Manzel Chaker sites. The translocation factor exceeded 1 for Zn only in the 25mN site sheets. For Cu, TF exceeded 1 in the 25mN, 500mN and 3mS sites. Cr contamination was indicated by the accumulation in the leaves at the 3 mN site. Similarly, the roots and stems of the 3mS site were examined.

The concentration of lead in the driving force is increased by the driving distance (99% of Pb) from the vehicle is distributed within 50m from the road [52-53].

Comparing the three sites studied, the largest accumulation was recorded for the sites of the Tunis road, which arrived at 50m for the Zn, followed by the sites of the Gremda road (up to a distance of 25m) and finally the Manzel Chaker road sites that did not exceed 3m. Despite the intense road traffic on the Gremda road, the intensive regime of planting olive trees has prevented the distribution of pollutants at great distances, while the ordinary regime of the plantation for the olive trees on the Tunis road has facilitated the distribution of these metals up to a distance of 50 m. The low distribution of metals on Manzel Chaker roads is linked to low road activity and the intervention of meteorological factors.

3.4. Chlorophyll, sugar, proline and polyphenol content

The olive leaves were collected from the sites 3 m and

25 m on the Tunis and Gremda roads presented brick-red necrosis (Fig. 1). It is likely that the metals were absorbed through leaf stomata and moved by transpiration into the main body accumulation sites at the leaf tips and margins, where they caused structural damage and necrosis. Indeed, no obvious necrotic areas were found in the leaves from the control sites.

The chlorophyll, proline and polyphenol contents are shown in Fig. 4. Levels of chlorophyll pigments were significantly reduced in the olive leaves at the sites close to the roads. For Chla, the most important reduction percentages of 60, 56 and 31% compared to control leaves were recorded at the site 3mSW, 25mSW and 50mE, respectively. The sites of Manzel Chaker were less affected and the reduction percentage of Chl did not exceed 11%, whereas, those of Tunis and Gremda were the most affected. Chlb has experienced a different evolution and the largest reduction has affected only 3 sites: 1mSW, 1mE and 50mE.

Chla + b showed a reduction of 41% in 3mSW leaves, 30% in 25 mSW and 21% in 50 mSW. Whereas for the Tunis road, the 3mE and 50mE sites showed a reduction in Chla + b with 27% and 19%, respectively. Therefore, we observed that the Chlb content was the least affected. [1] showed that road pollution negatively affects Chla levels and has no effect on chlorophyll b. Therefore, contamination with Pb (31 µg/g) and Zn (195 µg/g) affected the chlorophyll content at the Gremda sites. Similarly, the synergism between the content of Pb (30.4 µg/g) and Zn (195.25 µg/g is false) determined at the 3mE site affected the Chl content.

The effect of metal pollution on chlorophyll levels has been demonstrated in several studies [54-59]. For soluble sugars, the olive leaves of the Manzel Chaker and Tunis roads recorded higher sugar contents than those of the Gremda road at a distance of 3m. They were 37, 38 and 40.3 µg/g for the 3, 3, and 3 mS sites, respectively. Almost half of these content was recorded at the same distance from Gremda road (19.5 µg/g). Compared with the control sites, the 3mSW and 3mE sites near Gremda and Tunis roads recorded increases of 37% and 58%, respectively, whereas while the two sites close to the 3mS and 3mN roads had small increases of 10% and 14%, respectively,

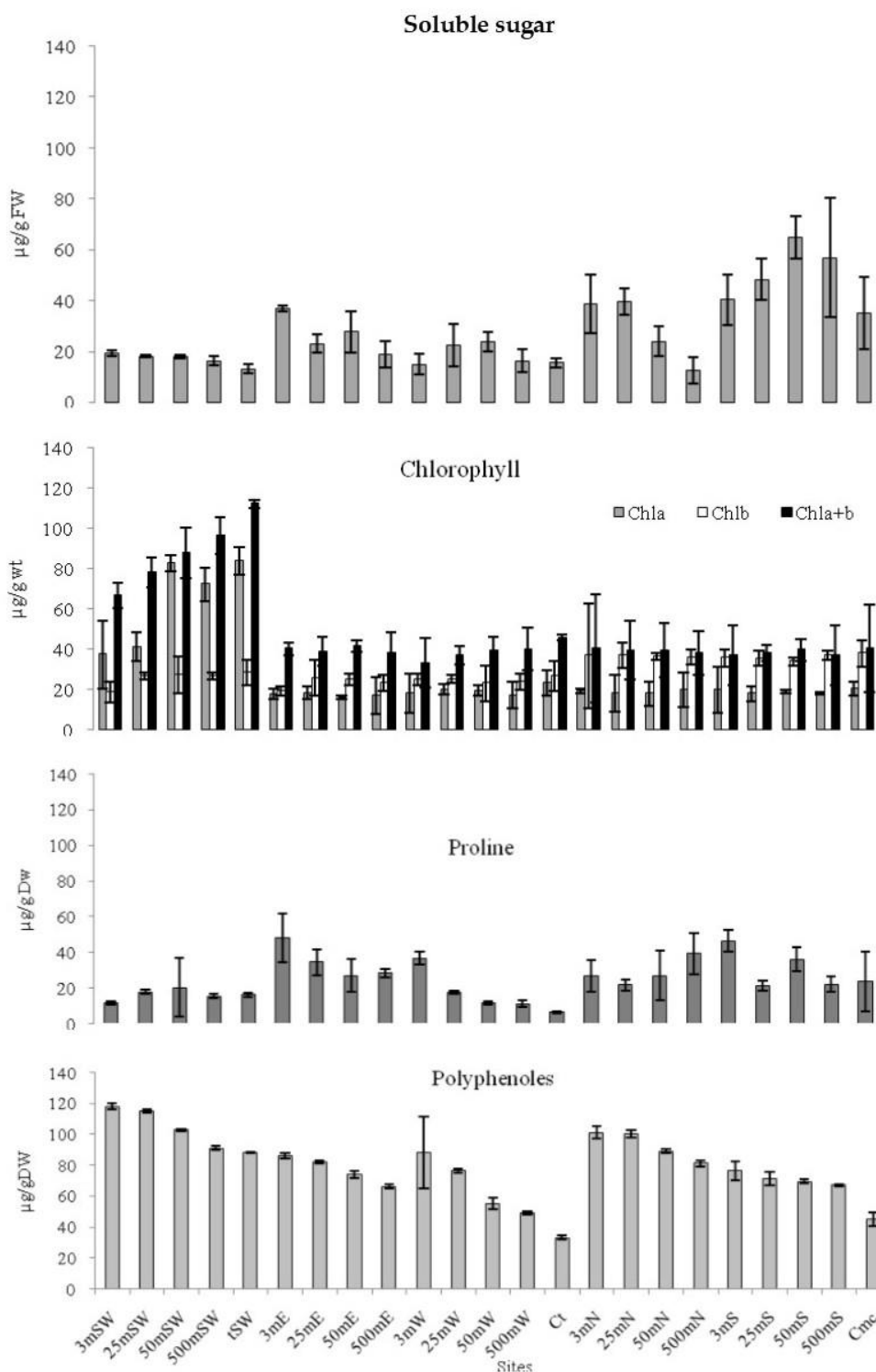


Figure 4. Variation of biochemical factors in the olive leaves.

compared to the control site. The levels of sugars in the Gremda sites were lower than those of Tunis and Manzel Chaker. This is related to the use of an irrigation regime on the farm near Gremda road, which can cause a dilution of these sugars. This is

confirmed by the low levels of proline recorded at the Gremda sites (11.7- 20.3 $\mu\text{g/g}$) compared with those of Manzel Chaker (21.8- 46.6 $\mu\text{g/g}$) and Tunis (6.7- 48.3 $\mu\text{g/g}$). For the pigments of polyphenols and referring to the standard levels in olive leaves (23-28 mg/g) [60-

[61], we noticed higher levels which exceeded the standards at the 3mSW and 25 mSW sites. These sites are characterized by significant Pb and Zn pollution. The evaluation of polyphenol content in the northern side of Manzel Chaker road seems to be linked to organic pollution (PAH) [62]. The increase in polyphenol content is a mechanism of plant resistance to metal stress. The study of the impact of road pollution on the polyphenol contents of olive trees is poor. Furlan et al. [63] showed the impact of metal pollution from industry on the polyphenol contents of *Tibouchina pulchra* (Melastomataceae). Pandey et al. [64] studied the impact of metal pollution (especially Pb and Cd) on the species *Albizia procera* (Roxb.) (Fabaceae) increase from 2% in the control site to 4.2% in the polluted site.

The use of PCA (Fig. 5) showed the existence of three groups, the most important being a group formed by Pb and Zn content in soil and olive root; the second group consisted of Chla, Chlb and Chl a + b, and these two groups were negatively correlated. The third group included the foliar Zn and Pb contents and the stem Pb content. The proline and soluble sugar contents were found to be in the same group. The existence of polyphenols in the same group as the foliar Pb and Zn contents indicates the absence of leaf contamination in the polyphenol contents.

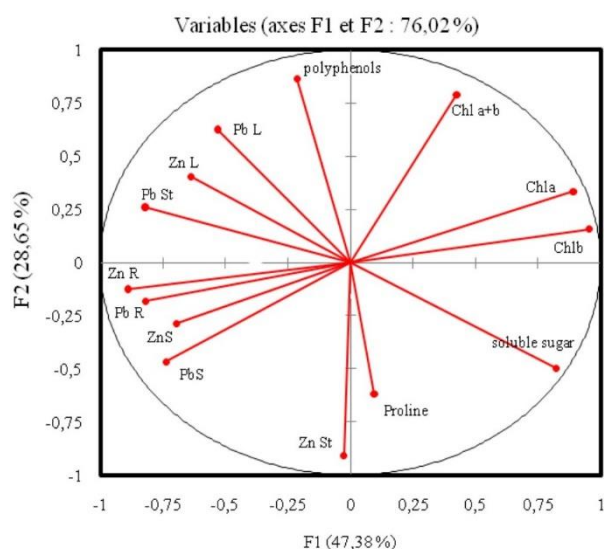


Figure 5. 2D projection of variables according to factors 1 (explaining 76, 02 % of variability) and 2 (explaining 28.65% of variability) (where: S: soil, St: Stem, L: leaves).

3.5. Antioxidants enzymes

The enzymatic antioxidants catalase, together with

ascorbate peroxidase and total peroxidase, constitute the major defense system against the reactive oxygen species produced by the electron transport chain located in chloroplast [65]. The results obtained showed (Fig. 6) that the peroxidase contents in the first two sites of the Gremda road represented 42% and 44% of the contents recorded in the control site (Tsw).

Likewise, a 50% reduction in peroxide content was recorded at these sites compared to the Manzel chaker sites at the same distances. Similar results were obtained for catalase and ascorbate peroxidase.

For the Tunis sites, in the eastern side, the polluted sites (3 m and 25 m) showed a 40% reduction in total peroxidase compared to the control site. The same results were obtained for the western sites of the road. For catalase and ascorbate peroxidase, the percentage of reduction was between 30% and 40 % for the eastern and western sites of the road compared to the control site. On the other hand, comparing the results with the sites close to the Tunis road (3m and 25m) with Manzel Chaker sites, only the total peroxidase content showed a reduction between 30% and 45%. According to studies by Fourati et al. [66], olive trees in the control site showed better antioxidant capacity, both enzymatic and non-enzymatic. Likewise, Pandey,et al. [64] observed an increase in certain antioxidant enzymes in plants facing drought, with a compensatory response that allowed the plants to recover their physiological state after rewatering. In this case, the multimetal stress, probably because it was continuous and chronic, was detrimental to the plant and the antioxidant capacity of the tissue of the olive tree was depressed. Moreover, under high Pb and Cd stress, superoxide dismutase (SOD) and ascorbate peroxidase were also inhibited to some extent, and excessive ROS resulted in a significantly higher degree of oxidative damage [67].

4. Conclusions

The study of metal transfer between soils and plants near the three roads in the Sfax region (Tunisia) highlighted the impact of variations in soil characteristics on metal speciation. This variation led to a variation in the metal contamination of olive roots, stems and leaves. This contamination influences olive leaves, which show brick-red necrosis. Chlorophyll

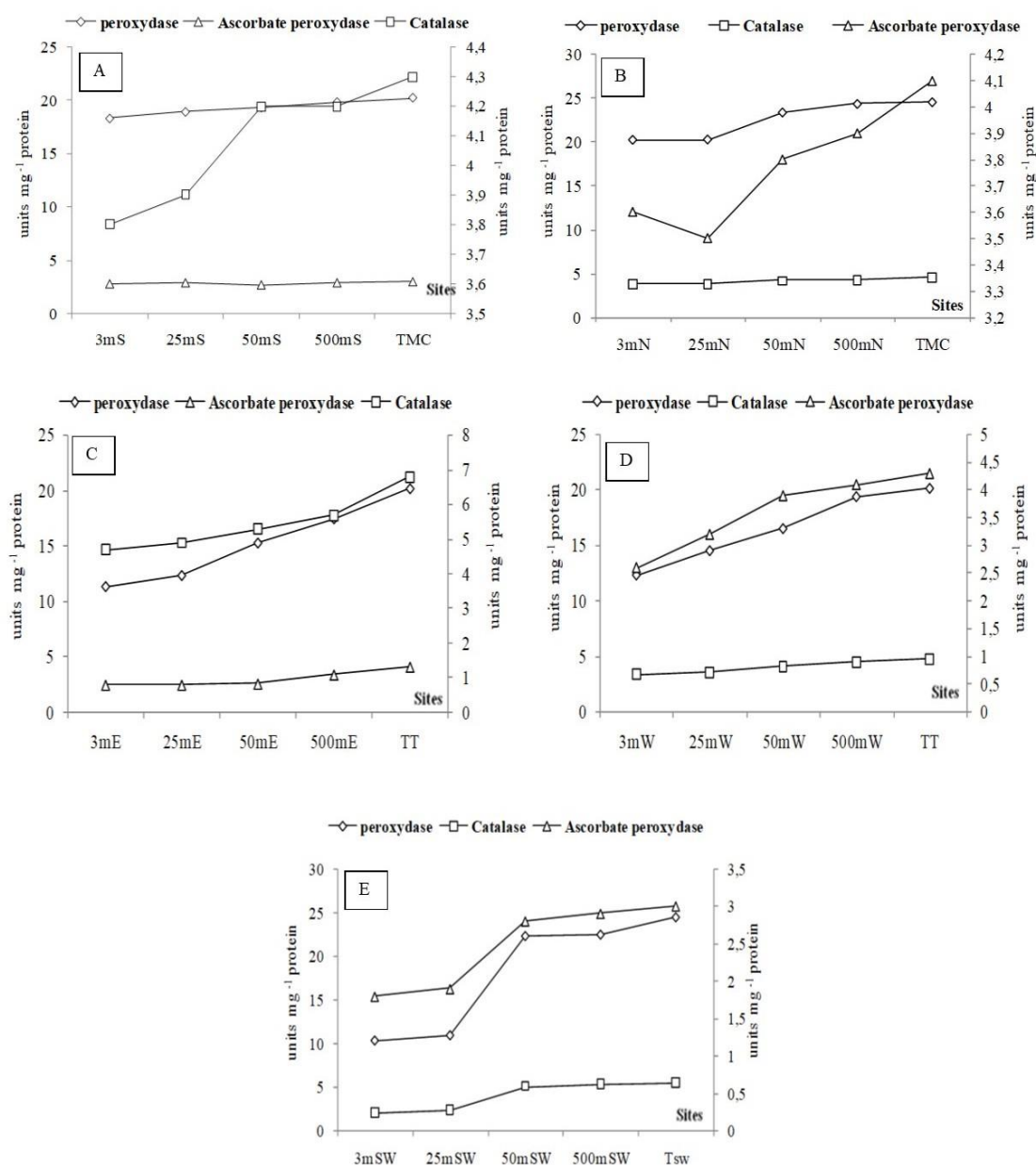


Figure 6. Activities of ascorbate peroxidase, peroxidase and catalase in leaves of polluted and control plants (A: Southern side of Manzel Chaker road; B: Northern side of Manzel Chaker road; C: Eastern side of Tunis road; D: Western side of Tunis road; E: sites of Gremda road).

pigment content was significantly reduced in olive leaves from sites close to roads. In this context, farmers are advised to avoid planting olive trees at a distance of 50m near the Gremda, Tunis and Gremda roads. We are trying to find a biological solution for this contamination, which will be the subject of a subsequent publication.

Authors' contributions

Drafted the manuscript, C.M.; Contributed to the

study conception and design, H.B.M., L.T., R.C., F.D.; Material preparation, data collection and analyses, K.G., S.E.C., B.N.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

All authors declare that there is no conflict of interest in this work.

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