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*Research Article*

# Influence of the near roadsoil contamination on the germination and biochemical factors of two species: *Lycopersicum esculentum* and *Cucumis sativus*, in the region of Sfax (Tunisia)

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## Abstract

Plants are used as highly sensitive tools for the prediction and recognition of environmental stresses. In this work, we highlight the contamination of three soils in the Sfax region by heavy metals and polycyclic aromatic hydrocarbons and the impact of this contamination on the germination and biochemical factors of two selected species *Lycopersicum esculentum* and *Cucumis sativus*. Several contamination indices of soil (contamination factor and geoaccumulation factor) are used as well as some manipulations to control better contamination of studied species (speed germination and percentage of germination). Some antioxidant activities were used (catalase activity, peroxidase activity and ascorbate peroxidase). The use of the contamination index showed moderate soil contamination by Zn and Pb more remarkable in the vicinity of Gremda road than Tunis and Manzel Chaker roads. In contrast, no site was contaminated by Cu and Cr. On the other hand, the metal contamination of gremda soil and the hydrocarbon contamination of Manzel Chaker soil affected the cucumber germination less than tomato germination. We noted that the slight modification of chlorophyll, proline and soluble sugar contents expressed by cucumber in polluted soils are less than those expressed by the tomato. An increase in catalase and ascorbate peroxidase activity in tomato plants was observed in vicinity of Gremda road. In the case of cucumber, the levels of these antioxidant enzymes are higher than those recorded in tomato leaves, which leads us to the use of cucumber in the phytoremediation of polluted soils near roads in the Sfax region.

## 1. Introduction

Airborne particles are important metal carriers, especially in polluted areas such as urbanized and industrialized regions, which may negatively influence the vegetation health. Plants have long been used to remediate soils contaminated by heavy metals, which are pollutants of natural and anthropogenic origin [1-4]. The negative impact of these metals on the environment is due to their non-biodegradable nature, their long biological half-lives ranging from 10 to 3,000 years, and their potential for accumulation in different parts of the body [5]. Excessive exposure to these metals can cause various diseases, some of which can also have mutagenic and carcinogenic consequences [6-7].

Vegetables are reported to have the potential to accumulate heavy metals when grown on contaminated soils or when irrigated with wastewater [6, 8]. At higher levels, heavy metals cause inhibition of germination, growth and physio-biochemical disorders in plants [9]. Excessive concentrations of some of them (Cd, Cr, Cu, Ni, Zn) in the soil have caused disruption of ecosystems [10]. Plants growing in contaminated soils can absorb and accumulate metals in their edible tissues in large quantities, without any visible indication, thus becoming part of the human food chain [11]. It has been suggested that the factors related to the uptake of heavy metals by plants, resulting in the stress response, and thus the damage to plant physiological functioning, are essential for the long-term safety and conservation of agricultural resources and ecosystems [12]. Metal toxicity causes interference with the physiological functioning of plants, such as deleterious effects on chlorophyll biosynthesis [13-14], inhibition of growth of plants grown in soil contaminated with metals [15] and increased protein content due to greater synthesis of metal-binding proteins, plant metal chelators and antioxidant enzymes [15-16]. Likewise, proline accumulates under conditions of metal stress, due to increased synthesis or reduced degradation [17-18].

The metal response results in altered levels of plant protective enzymes, including peroxidase, catalase, and ascorbate peroxidase [19-20], and accumulation of free proline [19]. The enzymatic antioxidants, superoxydase dismutase (SOD) and catalase (CAT), along with ascorbate peroxydase (APX) and total peroxydase (POX) constitute the major defense system against reactive oxygen species produced by the electron transport chain located in chloroplasts [21]. *Lycopersicum esculentum* and *cucumis sativus*. Tomatos and cucumbers contained large amounts of phenols with anti-free radical properties, and certain antioxidant enzymes (such as superoxydase dismutase (SOD), catalase (CAT), ascorbate preoxydase (APX), total peroxydase (POX) and indole-3-acetic (IAA) were shown to regulate their activity in response to abiotic stress [22-27]. Tomato was selected as the planting material because reports on the effect of heavy metals on physiological and biochemical parameters of tomatos are rare.

Another type of pollutant (hydrocarbon contamination) generated by road activities, in the soil, is well known to decrease seed germination [28], negatively influence plant physiology and morphology [29], and decrease photosynthetic activity and overall plant growth [30-32]. Studies have reported that PAHs uptake into the plant induces morphological symptoms like growth reduction, chlorosis and necrosis, while physiologically they induce oxidative stress, DNA damage and cell death [33-34]. The degree of toxicity varies not only with the kind of PAHs but also with species of plants [35-37]. The mechanism by which they cause toxicity is not yet completely understood. When the amount of hydrocarbons in the environment is higher than the amount that can be metabolized, these become adverse effects on the soil biota [38], delay germination, decrease biomass and leaf area and can cause necrosis and death to the plants [39-40]. It has been shown that tomatos and cucumbers contain large amounts of phenols with anti-radical properties, and that certain antioxidant enzymes (such as SOD, CAT, POX, APX, IAA oxidase, polyphenol oxidase) regulate their activity in response to pollution by hydrocarbons [41-42].

In Tunisia, according to the Technical Agency for Land Transport "ATTT", the car fleet in Tunisia is estimated, at the end of 2014, at nearly 1,800,000 vehicles circulating on the roads of Tunisia, knowing that this figure may be revised downwards due to the fact that certain vehicles have been destroyed or scrapped without their owners reporting them. According to the same source, the car fleet is growing annually at the rate of 7000 to 8000 vehicles. In the region of Sfax, a proximity station, operational since June 1996, was set up at a roundabout where six heavily trafficked roads intersect. It continuously measures ozone and its precursors, sulfur dioxide, hydrogen sulfide and dust. However, research on particulate pollutants near roads in rural areas remains rare.

The literature on soil contamination near roads is poor. Similarly, several studies have been interested in industrial pollution on plants in the Sfax region (olive and almond trees) and other plants (rose bush, oleander, vine, pomegranate, etc.), while to date no study has addressed the impact of road pollutants on plant cover, specifically, the olive tree in rural areas


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[41-44]. In this study, we have tried to study the impact of double contamination of soils near three roads in the Sfax region on the germinative capacity and the expression of antioxidant enzymes of two species *Lycopersicum esculentum* and *Cucumis sativus.*

## 2. Materials and methods

*2.1. Study area*

The city of Sfax plays an important role in trade at the national and international level. Moreover, a study prepared by the municipality of Sfax in 2006 showed that the governorate's road network includes 4 national roads, 8 regional roads, 26 local roads and 816 km of paved agricultural roads.

*2.2. Soil collection and analysis*

The samples were taken along an axis perpendicular to the axis of the road using a manual auger. Four sites were chosen on the east side and four on the west side of Manzel Chaker and Tunis roads at a distance of 3 m, 10 m, 50 m and 100 m from the road. A control site was selected at a distance of 1000 m. The sites were chosen on the southwest side of the Gremda road at 3 m, 10 m, 50 m and 100 m and a distant witness at 1000m (Fig. 1) [93].

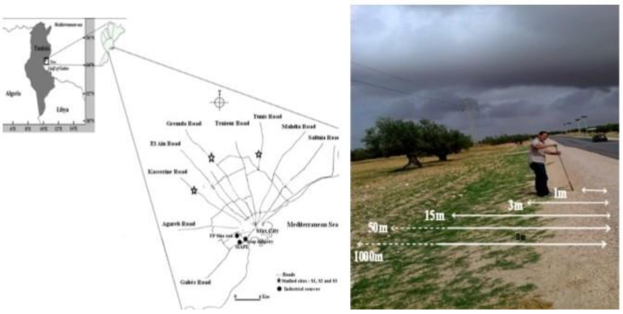


**Figure 1.** Study area [93]

After this operation, the samples are brought back to the laboratory, dried in the open air and then sieved through a 2 mm sieve [45]. Samples were collected from a depth of 0 to 20 cm using hand-operated steel augers. Soil pH was measured by a Neomet brand pH meter equipped with a glass electrode and a reference electrode [46]. Soil organic carbon was obtained using the soil pH method or dichromate oxidation of [47]. For hydrocarbon analysis, pre-dried soil samples were heated between 40 and 60°C to remove all traces of moisture before extraction. The dried soil samples were placed in the Soxhlet and extraction is carried out with acetone and dichloromethane (ratio 1:1) [48-51]. The filtrate was concentrated to a volume of 1 mL using a vacuum evaporator. The prepared samples are stored in the refrigerator at a temperature below 4°C before analysis. The analytical separation is carried out on a column (Lichosphere 100 RP – 18.5µm). The elution is detected at 278 nm. For heavy metal analysis, soil samples were digested in aqua regia (1:1 v/v $HNO_3$/HCl) [52]. Metals in the final solutions were determined using Pye Unicam, SPQ Philips, Atomic Absorption Spectrometer (AAS). Three replicates were analyzed for each test.

*2.2.1. Geoaccumulation index*

The geoaccumulation index (geo I) is based on the use of the following formula [53]:

$$Geo\ I = \log_2(Cn/1.5Bn)$$

Where n is the particle size fraction of soils; Bn is the average (crustal) concentration of element n, and 1.5 is the correction factor. The following interpretation for the geoaccumulation index: Geo I <0 = unpolluted; 0 < Geo I <1 = unpolluted to moderately polluted; 1 < Geo I <2 = moderately polluted, 2 < Geo I <3 = moderately to heavily polluted, 3 < Geo I Cn is the measured total concentration of the element <4 = heavily polluted, 4 < Geo I <5 = heavily to extremely polluted; and Geo I > 5 = extremely polluted [54].

*2.2.2. Contamination factor*

The contamination factor (Cf) was suggested and defined as follows [55]:

$$Cf = Cm/Cr$$

Cm is the measured concentration of the examined metal in the sample and Cr is the geochemical background concentration or reference value of the metal or the background value of heavy metals in the uncontaminated soil [55-56] suggested four classes of Cf to assess soil contamination levels: low (Cf <1), moderate (1 ≤ Cf <3), considerable (3 ≤ Cf <6), and very high levels of contamination (Cf ≥6).

*2.3. Plant analysis*

*2.3.1. Physiological analysis*

For the chlorophyll analysis, we added 5 mL of 100% acetone for 5 olive leaf discs, then put them into a mortar. After a filtration and adjust with acetone up to 20 mL, the sample is stored in tubes covered with aluminum foil to prevent degradation of the pigments.


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Finally, we measure the chlorophyll with a spectrophotometer using 3 different wavelengths 661.6 and 644.8 nm. The soluble sugars extraction is carried out according to the method of [57].

The formula used for the calculation is:

[Chl a] (μg/ mL) = (11.24 * A 661.6) – (2.04 * A644.8)

[Chl b] (μg/ mL) = (20.13 * A644.8) – (4.19 * A661.6)

A: Absorbance

*2.3.2. Germination test*

The germination capacity of the seeds is assessed using a germination test based on current international standards (International Seed Testing Association (ISTA), 1999). This involves evaluating the inhibition of germination of seeds placed in contact with polluted soils to be tested. During each test, 25 seeds of each species are germinated on 200 g of each type of soil. The number of germinated seeds was recorded every six hours for the duration of the experiment. The duration of the test is 10 days. The data measured are germination capacity and germination speed. The percentage of germination corresponds to the total number of seeds having germinated during the duration of the test reduced to the number of seeds germinated. The germination speed corresponds to the time necessary for the germination of 50% of the seeds (T50). This last parameter is expressed in hours [56].

To follow the elongation of the plant height, we transferred the plants to the same type of soil. After sieving (ø = 2 mm), 800 g of each soil are placed in waterproof bags, then in pots. On each of these pots a seedling from the germination phase is transplanted. As part of this study, six repetitions are carried out. the length of the aerial parts of each plant is determined in 45 days.

*2.4. Biochemical analysis*

The catalase activity was determined as the rate of decomposition of $H_2O_2$ (240 nm/min) against 0.036% $H_2O_2$used as the blank and expressed as μmol of $H_2O_2$ decomposed min/g fresh weight. The activity was calculated using the molar extinction coefficient of $H_2O_2$ at 240 nm. Peroxidase activity was measured as the increase in absorbance at 420 nm/min due to oxidation of pyrogallol [59]. A reaction mixture without the addition of crude enzyme extract was used as the blank.

Peroxidase activity was calculated using the molar extinction coefficient of pyrogallol at 420 nm (19 L/mol/cm) and expressed as μmol of $H_2O_2$ decomposed/min/g fresh weight of leaves. Ascorbate peroxidase assay was done by recording the decrease in absorbance at 290 nm due to the oxidation of ascorbate to dehydroascorbate by $H_2O_2$ [60]. Ascorbate peroxidase activity was calculated using the molar extinction coefficient of sodium ascorbate at 290 nm (2800 L/mol/cm) and expressed in μmol of ascorbate oxidized/min/g fresh weight of leaves.

*2.5 Statistical analysis*

Data were subjected to analysis of variance (ANOVA) using SPSS IMB SPSS version 26, Chicago, USA).

## 3. Results and discussion

*3.1. Soil contamination*

The contents of metallic elements found in the sites studied are illustrated in Table 1. We notice a decrease in the levels of these pollutants as we move away from the roads. Referring to the standards reported by Hentati et al. [66], Pb (18 mg/kg), Zn (39 mg/kg), Cr (41 mg/kg), Cu (13 mg/kg), we noted that the Zn contents exceeding the threshold have reached a distance of 50m near Gremda road (195.42 mg/kg), 10 m near Tunis (48.89 mg/kg) and Manzel Chaker roads (37.55 mg/kg). For Pb, the contamination has reached the same distance as Zn for Gremda (28.55 mg/kg and 44.83 mg/kg respectively) and Tunis roads while for Manzel Chaker road, the contamination did not exceed three meters in the vicinity of the road (36.16 mg/kg). For Cu, only the first site near Gremda road exceeded the threshold. On the other hand, no site has been contaminated by Cr.

Many previous studies have concluded that heavy metal content in roadside soils has an exponential distribution with an increasing distance from the road [61-65]. However, other research results showed that the distribution profiles of heavy metals in roadside soils were not always significantly correlated with road distance [65]. To better study the metal contamination of the soils, two contamination indices were used: Igeo and FC.

The use of the contamination index showed that the contamination of the gremda sites by Zn is a moderate contamination, up to 50 m from the road. Moderate contamination, up to 10 m, from Manzel Chaker road



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**Table 1.** The metals and hydrocarbons content in the studied soil

| Stations | Pb (mg/kg) | Igeo (Pb) | Cu (mg/kg) | Igeo (Cu) | Zn (mg/kg) | Igeo (Zn) | Cr (mg/kg) | Igeo (Cr) | CF (Pb) | CF (Zn) | CF (Cu) | CF (Cr) | PAH (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1G** | 32.04±3.2$^{a}$ | 0.25 | 13.6±0.54$^{a}$ | -1.53 | 214.67±5.89$^{a}$ | 1.74 | 8±1.77$^{a}$ | -2.94 | 1.70 | 5.01 | 0.51 | 0.20 | 623.2±34.2$^{a}$ |
| **2G** | 29.33±1.4$^{a}$ | 0.12 | 6.73±1.77$^{c}$ | -0.51 | 171.83±4.77$^{a}$ | 1.55 | 5.21±0.77$^{ab}$ | -3.56 | 1.63 | 4.40 | 0.45 | 0.13 | 536.32±64.8$^{ab}$ |
| **3G** | 28.55±3.6$^{a}$ | 0.08 | 3.99±0.88$^{d}$ | -2.29 | 195.42± 6.6$^{a}$ | 1.88 | 8.94±1.87$^{a}$ | -2.78 | 1.47 | 5.50 | 0.30 | 0.22 | 478.3±54.1$^{ab}$ |
| **4G** | 7.81±1.5$^{c}$ | -1.79 | 3.5±0.58$^{d}$ | -2.48 | 21.33±3.3$^{ac}$ | -1.46 | nd | nd | 0.43 | 0.54 | 0.26 | nd | 271.12±24.6$^{c}$ |
| **TG** | 2.53±0.54$^{c}$ | -3.41 | 2.38±0.54$^{d}$ | -3.03 | 26.89±1.33$^{ac}$ | -1.12 | nd | nd | 0.14 | 0.68 | 0.18 | nd | 112.2±14.2$^{cd}$ |
| **1MC** | 36.16±3.2$^{A}$ | 0.42 | 5.87±0.48$^{A}$ | -1.73 | 73.58±3.2$^{A}$ | 0.33 | 7.28±0.77$^{A}$ | -1.42 | 2.01 | 0.96 | 0.45 | 0.01 | 1650±84.9$^{A}$ |
| **2MC** | 14.64±2.4$^{C}$ | -0.88 | 2.63±0.18$^{AB}$ | -2.89 | 37.55±2.9$^{A}$ | -0.81 | 13.32±0.67$^{AB}$ | -0.55 | 0.81 | 1.88 | 0.20 | 0.03 | 1100±64.2$^{AB}$ |
| **3MC** | 7.85±1.34$^{CD}$ | -1.,78 | 5.98±1.28$^{A}$ | -1.71 | 23.02±1.4$^{AB}$ | -1.35 | 7.85±0.87$^{A}$ | -1.31 | 0.43 | 0.59 | 0.46 | 0.07 | 490±14.2$^{C}$ |
| **4MC** | 2.79±0.54$^{D}$ | -3.27 | 5±0.48$^{A}$ | -1.96 | 20.24±1.12$^{AB}$ | -1.53 | 2.79±1.77$^{C}$ | -2.80 | 0.16 | 0.51 | 0.38 | 0.00 | 129±13.2$^{CD}$ |
| **TMC** | 1.32±0.33$^{D}$ | -4.35 | 2.28±0.88$^{A}$ | -3.09 | 9.79±0.43$^{C}$ | -2.58 | 1.32±0.27$^{C}$ | -3.88 | 0.07 | 0.25 | 0.17 | 0.00 | 101±14.1$^{CD}$ |
| **T1** | 59.6±1.3* | 0.23 | 10.52±1.48* | -2.26 | 57.61±2.22* | 0.51 | 2.86± 1.11* | -4.22 | 0.812 | 2.13 | 0.104 | 0.08 | 467±21.2* |
| **T2** | 44.83±2.45** | -0.52 | 4.46±0.98** | -3.84 | 48.89±1.78* | -0.07 | 0.14±0.07** | -8.84 | 0.48 | 1.42 | 0.034 | 0.003 | 348±17.2** |
| **T3** | 13.2±1.54*** | -1.85 | 6.10±1.11** | -2.93 | 8.25±0.44** | -3.15 | 0.19±0.05** | -8.02 | 0.19 | 0.16 | 0.065 | 0.005 | 289±20.9** |
| **T4** | 4.98±0.54*** | -2.53 | 1.58±0.67*** | -4.17 | 8.72±0.54** | -2.62 | 0.04±0.01** | -9.26 | 0.11 | 0.24 | 0.027 | 0.002 | 166.2±34.2*** |
| **TT** | 2.4±0.75*** | -3.84 | 2.21±0.87*** | -4.44 | 8.02±1.34** | -2.41 | 0.13±0.01** | -8.60 | 0.031 | 0.28 | 0.049 | 0.004 | 101.4±24.1*** |

G: Gremda road; MC: Manzel Chaker road; T: Tunis road)

and 3 m from Tunis road. For Pb, Gremda soil is moderately polluted up to 50 m from the road, while only the first site of Manzel Chaker and Tunis roads are moderately contaminated by Pb. According to this index, no site was contaminated by Cu and Cr.

The use of the contamination factor confirmed the results of the geoaccumulation index. This index showed that contamination is moderate for Pb up to a distance of 50 m from Gremda road, 3 m from Manzel Chaker road and was low for all sites of Tunis road. Indeed, studies by [67] indicated that Pb emissions can reach a distance of 50 m. For Zn, the use of CF showed considerable contamination up to a distance of 50 m, moderate at a distance of 3m from Manzelchaker road and up to 50 m from Tunis road. For Cu and Cr, the sites studied are slightly contaminated by these two elements. So, the order of contamination is as follows: Zn> Pb> Cu > Cr. For lead, despite the fact that Tunisia has implemented the use of unleaded gasoline since 2001, it seems difficult to explain the contamination of soils by this element. This may be due to the old persistent contamination by Pb. Indeed, the majority of informal exchanges with Algeria concern petroleum products. Likewise, the smuggling of petroleum products (petrol and diesel) with Libya and Algeria is estimated at 1 billion liters per year (TISS 2015). Around a quarter of national fuel consumption, especially diesel, comes from smuggling with Algeria and Libya. For hydrocarbon contamination, we noted that the highest levels (1650 mg/kg) are recorded 1 m from Manzel Chaker road, then the 5 m site, we recorded a reduction of 33.3 % in hydrocarbon content. In the distance (3 m), in the vicinity of Tunis and Gremda roads, the contents recorded represent 41% and 38%, respetively of the contents recorded in the soils of Manzel Chaker. In the same region, the analyzes of hydrocarbons in the soil of Sfax showed values ranging from zero to 134 ppm [68]. On the other hand, a content reported between zero to 11.63 mg/g in the soil of the Sfax region [69].

### *3.2. Soil characteristics*

The physicochemical characteristics of the studied soils are illustrated in Table 2. The pH of Gremda road sites ranges between 5.9 and 7.9. The soil is acidic up to 50 m from the road. On the other hand, the pH is alkaline for Tunis and Manzel Chaker road soils in all sites. The acidity of the soil on the sites generally increases the solubility of trace metal elements by modifying the metal distribution between the liquid (solubilized element) and solid (precipitated) phase. Indeed, the percolation of lead in the soil and its availability to plants essentially depend on the pH of the soil [70]. The increase of soil acidity led to the elevation of the bioavailability of metals [71]. Organic matter levels exceed 1 % only near Manzel Chaker road. While for the sites of Tunis and Gremda roads the contents do not exceed 7 % and 8 % respectively. Indeed, the availability of metals in the soil-plant system depends on soil pH and organic matter concentration [71-72]. The organic part of the soil also has a great affinity for metal cations through the



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**Table 2.** Localization, physical and chemical characteristic of the studied soils

| Stations | Latitude | $Ca^{2+}$ (%) | k+ (%) | $Na^{+}$ (%) | Ph | OM (%) | Silt (%) | Sand (%) | Clay (%) |
|---|---|---|---|---|---|---|---|---|---|
| **1MC** | 34°52′40.73 | 1.49±0.01 | 0.06±0.035 | 0.09±0.02 | 7.64±0.25 | 1.12±0.04 | 50.81±0.8 | 40.13±0.78 | 8.9±0.1 |
| **2MC** | 34°52′40.68 | 1.42±00.8 | 0.08±0.009 | 0.07±0.009 | 8.24±0.25 | 1.05±0.2 | 48.29±6.3 | 45.56±3.21 | 5.53±3.17 |
| **3MC** | 34°52′40.51 | 1.18±0.15 | 0.14±0.019 | 0.08±0.012 | 8.57±0.4 | 0.6±0.2 | 52.81±0.8 | 38.13±0.78 | 7.9±0.1 |
| **4MC** | 34°52′40.10 | 0.83±0.14 | 0.16±0.002 | 0.066±0.01 | 8.09±0.26 | 0.65±0.2 | 50.19±6.3 | 43.66±3.21 | 5.83±3.17 |
| **TMC** | 34°52′37.60 | 0.67±1.017 | 0.08±0.036 | 0.071±0.13 | 8.15±0.6 | 0.55±0.14 | 49.81±0.8 | 42.13±0.78 | 7.9±0.1 |
| **1T** | 34°59′59.45 | 0.64±0.08 | 0.06±0.01 | 0.082±0.006 | 7.77±0.1 | 0.66±0.04 | 45.19±6 | 53.96±6.33 | 0.94±0.22 |
| **2T** | 34°59′59.47 | 0.85±0.18 | 0.06±0.013 | 0.08±0.005 | 7.02±0.15 | 0.65±0.2 | 53.95±5.2 | 44.49±5.02 | 0. 97±0.19 |
| **3T** | 34°59′59.44 | 0.83±0.05 | 0.13±0.03 | 0.06±0.006 | 7.26±0.5 | 0.56±0.1 | 42.19±6 | 55.96±6.33 | 1.04±0.22 |
| **4T** | 34°59′59.28 | 0.78±0.06 | 0.17±0.02 | 0.077±0.017 | 7.95±0.15 | 0.57±0.24 | 51.95±5.2 | 46.49±5.02 | 0.97±0.19 |
| **TT** | 34°59′59.50 | 0.85±0.042 | 0.15±0,03 | 0.081±0.012 | 7.72±0.12 | 0.66±0.015 | 44.19±6 | 54.96±6.33 | 0.94±0.22 |
| **1G** | 34°56′08.92 | 0.63±0.24 | 0.64±0.06 | 0.29±0.04 | 5.9±0.6 | 0.72±0.04 | 52.95±5.2 | 45.49±5.02 | 1. 07±0.19 |
| **2G** | 34°56′08. 87 | 0.55±0.18 | 0.65±0.05 | 0.28±0.015 | 6.3±0.8 | 0.65±0.2 | 59.16±2 | 40.3±1.8 | 0.46±0.34 |
| **3G** | 34°56′08.74 | 0.78±0.02 | 0.72±0.06 | 0.3±0.015 | 6,7±0.3 | 0.7±0.2 | 60.29±13.5 | 39.44±13.3 | 0.26±0.1 |
| **4G** | 34°56′08.69 | 0.71±0.05 | 0.54±0.02 | 0.22±0.04 | 7.32±0.35 | 0.65±0.2 | 55.19±6 | 43.96±6.33 | 0.94±0.22 |
| **TG** | 34°56′00.73 | 0.66±0.028 | 0.63±0.07 | 0.18±0.015 | 7.9±0.55 | 0.65±0.14 | 51.95±5.2 | 47.49±5.02 | 0.97±0.19 |

presence of ligands or functional groups, which can form complexes with metals [72].

Therefore, the low content of organic matter increases the solubility of heavy metals in Tunis and Gremda sites, while the increase in this parameter promotes the immobilization of metallic elements for the Manzel Chaker sites. In addition, the sites close to Manzel Chaker road are characterized by an enrichment in $Ca^{2+}$ with values approaching 1.5% (1MC). Increased $Ca^{2+}$ concentrations could reduce heavy metal solubility [73-74]. The $K^{+}$ contents are similar between the different studied, the values are between 0.09 (%) and 0.14 (%). The same for Na+, the values are similar between sites and do not exceed 0.08 $k^{+}$. The variation of clay contents between the three sites can affect the availability of the metals, which may be more available near Gremda and Tunis roads. The clay soil retains a high amount of metals [75]. The bioavailable fraction of anthropogenic heavy metals, such as Cu, Pb and Zn, decreased with decreasing soil particle size, such as clay particles [76].

*3.3. Effect of contamination on seed germination*

The percentage and speed of germination of the seeds of the two studied species are illustrated in Fig. 2. For Gremda road, we noted 15 % at 3 m, 16% at 10m while it reaches 62% in the control site. So, a reduction of 76% in the 3m site compared to the control site. For the same distance of Tunis road, we noted 15 % at 3 m, 23 % at 10 m while it reaches 42 % in the control site. So, a reduction of 65 % in the 3 m site compared to the control site. Finally, for the Manzel Chaker road, we noted 63 % at 3 m, 70 % at 10 m while it reaches 90 % in the control site, so a reduction of 30 % in the 3 m site compared to the control site. While no difference was noted in the percentage of germination from a distance of 50 m from the three roads.

For cucumber, the results obtained showed that, in 3m away from Gremda road, the germination was 50.7%, while it is in order of 64 % and 70.7 % at the same distance from Tunis and Manzel Chaker roads respectively. The germination percentage reductions of cucumber germinated on 3m from Gremda, Tunis and Manzel Chaker roads compared to the control soil were 25 %, 7% and 16 % respectively. On the other hand, this percentage reduction is negligible from 50 m of three roads, when compared with the control sites.

For the germination speed, contamination of soils 1 and 10 m from the gremda inhibited the germination of half of the seeds sown. For the same distances from the Tunis road; germination of 50% of the seeds requires 200 and 140 hours for 1m and 10m respectively. While on the soils of Manzel Chaker, germination of 50% of tomato seeds requires 245 and 240 hours for distances of 1 and 10 m respectively. For the same distances, contamination of sites near the gremda and Manzel chaker roads does not have the same effect as that of tomato; hence it was noted that


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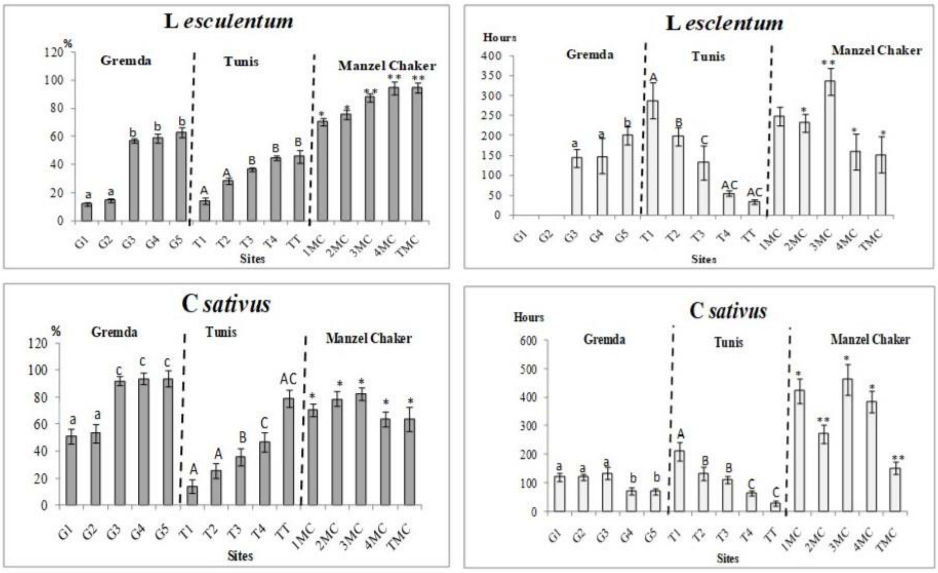


**Figure 2.** Capacity and speed of germination of the studied species on contaminated and control soils for the three studied areas

germination of 50 % of cucumber seeds on soils 1m and 10m from the gremda road requires 110h. While it requires 230 and 140 hours on the soils of Tunis respectively and requires 410 and 275 hours on the soils of Manzel Chaker respectively.

The use of principal component analysis (Fig. 3) showed the intervention of heavy metals, especially Pb and Zn in the percentage and speed of tomato germination.

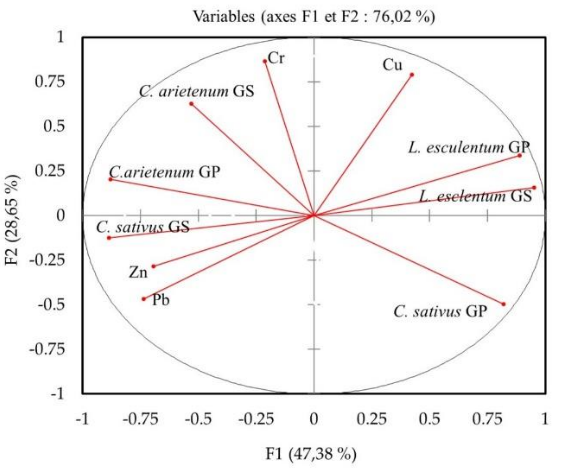


**Figure 3.** 2D projection of variables according to factors 1 (explaining 47, 38 % of variability) and 2 (explaining 28.65% of variability) where: GS germination speed, GP germination percentage

On the other hand, this analysis showed no effect of Cu and Cr on the germination of this species.

From these results, we conclude the effect of metal contamination of Gremda and Tunis soils and the organic contamination of Manzel Chaker soil on the germination percentage and germination speed of tomato. Likewise, the metal contamination of the gremda soil and the hydrocarbon contamination of Manzel Chaker soil affected the cucumber germination less than tomato germination. The effect of soil contamination on plant germination is due to the ability of metals to bind to the sulfur group of certain amino acids, leading to inhibition of the activity of essential enzymes [58]. While, for organic pollutants [77-78], it was proved that petroleum hydrocarbons can form a film on the seed, preventing the entry of oxygen and water.

Studies by [79] on cucumber germination showed that only mercury affects the germination of this species and neglected the effect of other metals. However, the Gremda study area has never been exposed to sources of pollution other than road traffic. This source of pollution is not reported as a source of mercury. Thus, the impact observed on seed germination would probably be due to the pollution detected by Pb and Zn. Tesar et al. [80] showed that the effect of Zn on the germination of several plant species from 100 mg/kg. Besides, Zn with 500 µg/g can reduce the germination percentage of tomato [81].

Monitoring of aerial part height of tomato and cucumbers for 43 days is illustrated in Fig. 4. The tomato growth on Gremda road soils shows the


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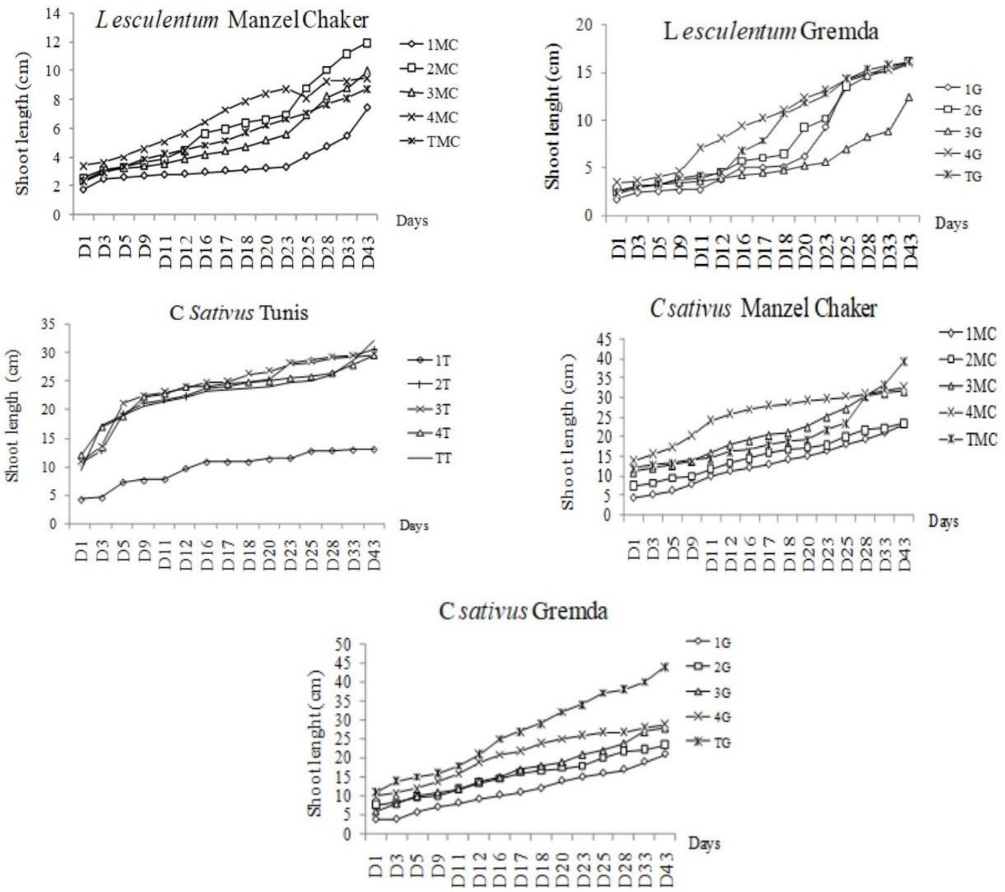


**Figure 4.** Elongation of aerial part of the studied species

absence of metal contamination effect on tomato height. We noted that, at the end of the experiment, the height is 15.5 cm for the tomatoes germinated on the polluted soils and the control site. On the other hand, on the soils of Manzel Chaker road, we notice a reduction of 51% in the tomatoes height 1 m away from the road compared to the control soil (1000 m). For the cucumber, we notice that on the three road soils, aerial growth did not affect the distance from the studied roads. We noted that the height of the cucumber near roads and in the control sites are similar. We record 31 cm, 32 cm and 33 cm in the sites of Tunis, Gemda and Manzel Chaker roads respectively. So unlike the tomato, the contamination of the soils of Manzel Chaker by hydrocarbons has no impact on the aerial growth of cucumber, which shows resistance to metal contamination of gremda soils and organic contamination of Manzel Chaker soils. The high hydrocarbon content recorded at 1 m from Manzel Chaker road (1650 mg/kg) affected the tomatoes height. The hydrocarbons can participate in the inhibition of cell division of the leaf [82-83] noted a 96% reduction in ryegrass biomass after 30 days of growth on soil contaminated with 25 g/kg of petroleum hydrocarbons.

*3.4. Physiological response*

In addition to the disruption of germination, we report a variation in the physiological characteristics of the species studied in response to metallic and organic pollution. The variation in the contents of chlorophylls, soluble sugar and proline profiles is illustrated in Fig. 5.

On the away of 3 m from Gremda road soil, the Chla and Chlb contents of tomato recorded a reduction of 81 % and 53 % compared to those recorded in the control site. The percentages decrease away from the road. At the same distance from Manzel Chaker road; we recorded a reduction in chlorophyll contents with percentages of 40% and 39% compared to the control site. While the reduction in chlorophyll A and B contents are in the order of 76% and 44% respectively, in the soils of Tunis road.

For cucumber, on 3 m away from Gremda road, metal contamination contributes to the reduction in Chla



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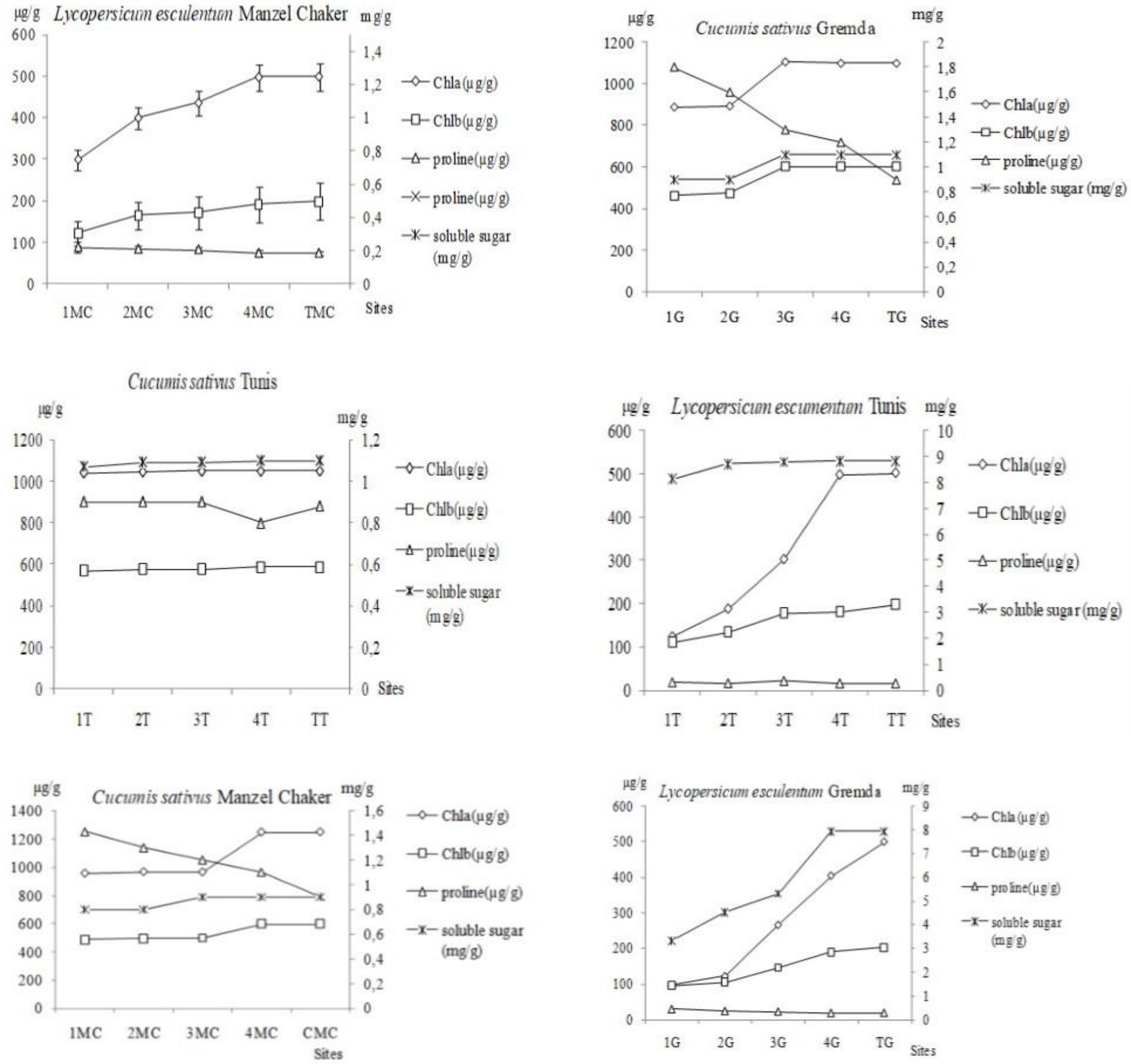


**Figure 5.** Variation of the physiological parameters in the study species

content with 20 % compared to the control site while the Chlb content was reduced by 24 %. Over the distance from the Tunis road, a small reduction in Chla content, which does not exceed 1% compared to the control site, while there is a reduction of 3 % in chlb. On the other hand, on the soil 3 m away from the Manzel Chaker road, the chla and chlb contents of cucumber were reduced by 24 % and 9 % respectively. The reduction in Chla and Chlb contents for tomatoes continues on the Gremda road up a distance of 10 m while on the Manzel Chaker road, the reduction in chlorophyll assimilation affects only the first two sites. The reduction of chlorophyll in tomato leaves sprouted on gremda soils, especially the soils up to a distance of 50m influenced the soluble sugar contents. The reduction percentages decrease as you move away from the road, it is 59 % at 3 m, 43 % at 10m and 33 % at 50 m from the road; then the content becomes similar to that recorded in the control site. The stability of chlorophyll content recorded in the tomato leaves sprouted on the ground of the Tunis road is reflected in the soluble sugar contents in these leaves, only the tomato leaves distant 3 m from the road which recorded a small reduction in soluble sugars of 8% compared to the control site, while for the other sites the contents were close to the control level. Contamination by hydrocarbons of sites of Manzelchaker road disrupts the synthesis of soluble sugars in tomato leaves. We note a reduction of 28 % and 26 % in the sugar contents synthesized in the


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leaves when the tomatoes are germinated on soil 3 and 10 m away respectively from the control site (1000 m) then the contents approach those recorded in the site witness.

Metallic contamination of Gremda soil and organic contamination of Manzel Chaker soil slightly affected the synthesis of soluble sugars with percentages not exceeding 19% in sites 1G and 2G and 12% in sites 1MC and 2MC. So, from these results, we concluded that cucumber showed resistance to metallic and organic pollutants.

Proline accumulation is another indicator of metallic stress [91]. The synthesis of proline in tomato leaves has experienced the same evolution as soluble sugars and chlorophyll where we notice an increase in the synthesis of this metabolite in the first three sites of Gremda road. The value recorded in the fourth site (0.287 µg/g Ms) is similar to that recorded in the control site (0.288 µg/g Ms). Likewise, on the soils of Manzel Chaker, we recorded an increase in proline contents for sites 1MC, 2MC and 3MC compared to the control site. The content recorded in the 4MC site is similar to that recorded in the TMC site (0.186 µg/g Ms). On the other hand, the synthesis of proline by cucumber leaves experienced a slight increase compared to the site content only in the 1G, 2G, 1MC and 2MC sites, which confirms that the effect of metallic and organic pollution of the soil on the expression of proline in cucumber leaves does not exceed the first two sites in the vicinity of Gremda and Tunis roads. In addition, the levels recorded in cucumber leaves are lower than those recorded in tomato leaves, which confirms the resistance of cucumber to metallic and organic pollutants from road activities.

So, we see that metal contamination of gremda soil affects the chlorophyll uptake of tomato. The reduction occurred in the chlorophyll of tomato which is mainly related to its biosynthesis [84]. The negative effect of metals on tomatoes is also demonstrated [85]. Recently, an inhibiting effect of metals has been observed in other species like cucumber [86]. The effect of metals on plastid pigments depends on leaf age and plant development [87]. Chlorophyll concentrations could be lowered by activating enzymatic degradation in metal-stressed plants [88]. Studies by [89] on the effect of hydrocarbons on the chlorophyll assimilation of *Cyperus brevifolius* showed that the accumulation of these pollutants on the cuticle of the cross section of leaves and shoots forms a thick dark layer and exerts harmful effects on the morphology, anatomy and chlorophyll content of plants grown in this particular environment.

Soluble sugars in tomato leaves grown under the stress of $Cu^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ were reduced by 57.5% [90]. A similar decrease in soluble sugars was observed in tomato, pea, and spinach plants irrigated with metals-contaminated (Cr, Cd, Pb, Zn, Cu) and waste water [16]. The decline of sugar content was related to the loss of photosynthetic pigments and the high energy needs of plants due to oxidative stress and toxicity response [16, 91].

The use of PCA analysis (Fig. 6) highlighted the effect of heavy metals and hydrocarbons on the chlorophyll assimilation of tomato and the absence of effect on cucumber. Likewise, we note the effect of organic and metallic pollutants on proline expression. It is also regarded as an abiotic stress marker against heavy metals [83]. On the other hand, [16] reported another source for proline, other than road pollution, which is wastewater.

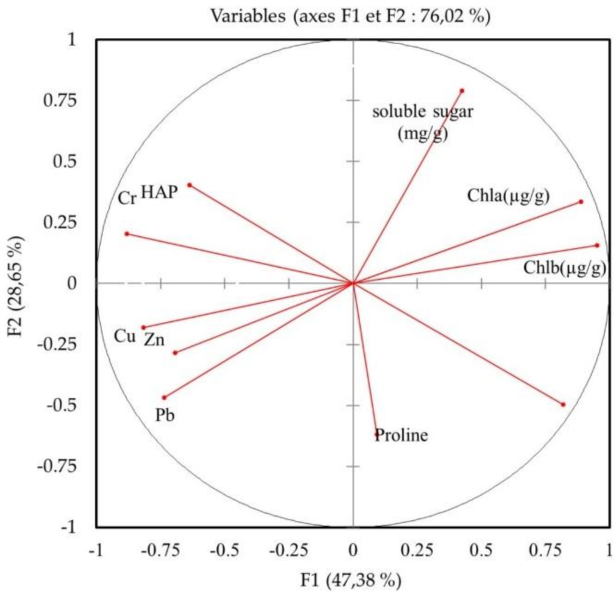


**Figure 6.** 2D projection of variables according to factors 1 (explaining 47, 38 % of variability) and 2 (explaining 28.65% of variability) where: GS germination speed, GP germination percentage


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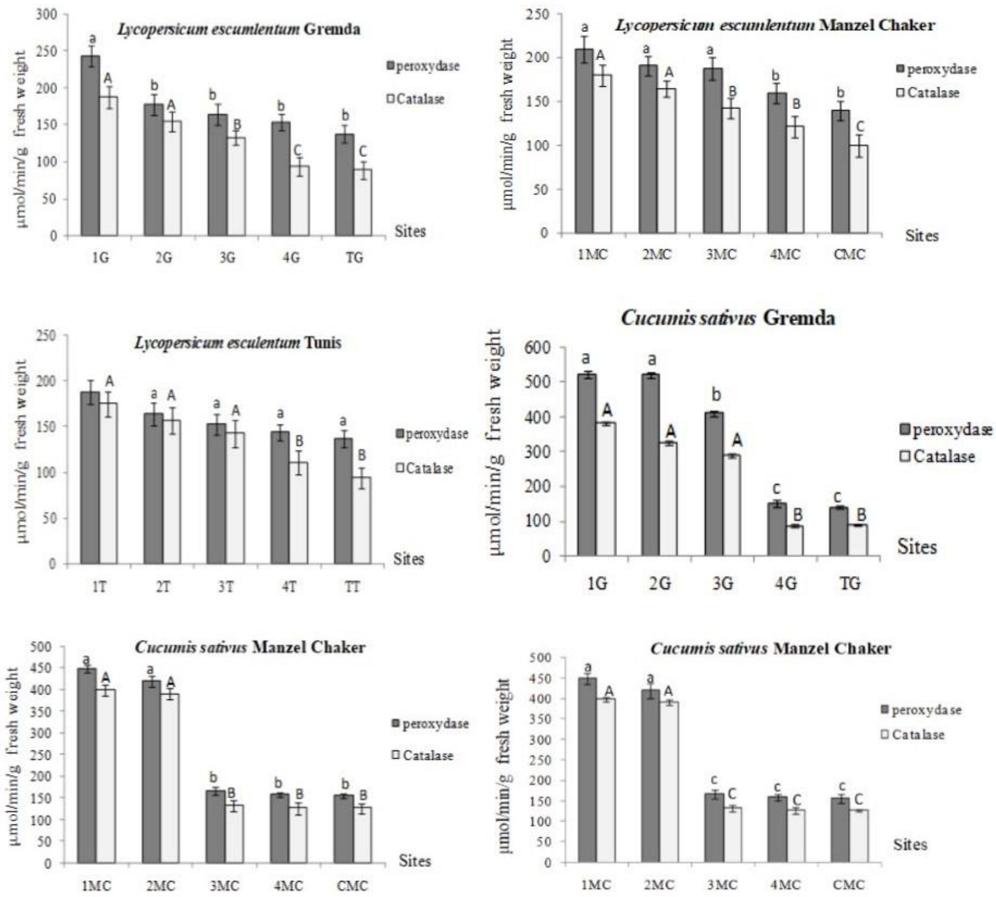


**Figure 7.** Catalase and peroxydase activity in the study species

*3.5. Biochemical response*

Tomato leaves grown at sites near Gremda road 1G, 2G and 3G showed an increase in preoxidase activities compared to the control site (138 µmol/min/g fresh weight of tomato leaves). The contents are 244,178,164 for 1G, 2G and 3G respectively. Likewise, for catalase and ascorbate peroxidase, we note the elevation in the expression of these enzymes up to a distance of 50m then the contents become similar to that recorded in the control site. The elevation in the expression of these enzymes respected the same distances from Manzel Chaker road. The highest contents are recorded in sites 1MC, 2MC and 3MC (in µmol/min/g of fresh weight of tomato leaves), 210, 191 and 188 respectively for peroxidase; 180, 165 and 143 respectively, for Catalase and 1.65, 1.24 and 1.15 for ascorbate peroxidase (Fig. 7). These results confirmed the contents found by [90]. An increase in catalase activity in tomato plants was observed when $Cd^{2+}$ was enriched in the soil [90]. Similarly, ascorbate peroxidase activity was induced due to $Cd^{2+}$ stress in *Bechmeria nivea*. The production of protective enzymes in plants has been considered a defense mechanism to reduce oxidative stress [89]. Oxidative stress, induced by the accumulation of $Cd^{2+}$ in plant cells, is manifested by increased accumulation of $H_2O_2$ [20, 86]. For cucumber, the levels of peroxidase, catalase and ascorbate peroxidase recorded are higher than those recorded in tomato leaves. The peroxide contents recorded in stations 1G, 2G, 3G, 1MC and 2MC are twice the values recorded in tomato leaves at the same stations. On the other hand, in the soils of the Tunis road, the peroxide levels in cucumber leaves are similar to those in tomato leaves. The high levels of these antioxidant enzymes explain the mechanisms of cucumber resistance to metal and organic stress [91], showed that cucumber can resist the pollution of 500 µM $HgCl_2$. Likewise, studies by [92] showed that cucumber can withstand contents in the order of 100 and 400 µmol $L^{-1}$ with the expression of high levels of antioxidant enzymes.


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## 4. Conclusions

Metallic and organic contamination of soils taken near three roads in the Sfax region revealed contamination that does not exceed a few meters near Manzel Chaker and Gremda roads. This contamination influenced the germination, the growth and contributed to the modification of the biochemical and physiological factors of two species in the Sfax region. After this study, we conclude an aspect of the remarkable resistance of cucumber to metallic and organic pollutants that can be exploited soon.

## 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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