

Influence of the near roadsoil contamination on the germination and biochemical factors of two species in the region of Sfax (Tunisia)

Chaker Mbadra (✉ chakermarai@yahoo.com)

University of Sfax

kamel gargouri

University of Sfax

hadda ben mbarek

University of Sfax

lina trabelsi

University of Sfax

Bhekumtheto Ncube

University of KwaZulu-Natal

semia ellouz chaabouni

University of Sfax

Research Article

Keywords: Sfax, heavy metals, hydrocarbons, germination, chlorophyll, antioxydants enzymes

Posted Date: February 7th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3915060/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Influence of the near roadsoil contamination on the germination and biochemical factors of two species in the region of Sfax (Tunisia)

Chaker Mbadra^{1*}, Kamel Gargouri¹, Hadda Ben Mbarek^{1,2}, Lina Trabelsi^{1,3}, Bhekumtheto Ncube⁴, Semia Ellouz Chaabouni³.

* Corresponding author: chakermbadra@gmail.com

1 Olive Institute, University of Sfax, road aeroport km2, Sfax, Tunisia.

2 Faculty of science, University of Sfax, road soukra km4, Sfax, Tunisia.

3 National School of engineers of Sfax, University of Sfax, road Soukra km 4 Sfax, Tunisia.

4 School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Scottsville, South Africa.

Abstract

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 in the germination and biochemical factors of two selected species *Lycopersicum esculentum* and *Cucumis sativus*. The use of contamination index showed a moderate soil contamination by Zn and Pb up to 50m from gremda road, up to 10m from Manzel Chaker road and 3m from Tunis road. In contrast, no site was contaminated by Cu and Cr. The highest levels of hydrocarbon aromatic polycyclic are recorded in the vicinity of Manzel Chaker soil. On the other hand, the physico-chemical characteristic of studied soils affect the speciation of metals and hydrocarbon. We noted that the metal contamination of gremda soil and the hydrocarbon contamination of Manzel Chaker soil affected the cucumber germination less than tomato germination. The use of germination tests for the two selected species highlighted the capacity of cucumber to resist to both types of pollutants. This resistance is manifested by the slight modification of chlorophyll, proline and soluble sugars contents expressed by the 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 but for cucumber, the levels of these antioxidants 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.

Key words Sfax, heavy metals, hydrocarbons, germination, chlorophyll, antioxidants enzymes.

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

Acknowledgements This work was carried out in the Olive Tree Institute. The facilities and services of the Olive Institute are gratefully acknowledged. This study was achieved in partnership with the teams of Laboratory of Enzymes and Bioconversion and Laboratory of Sustainability of Olive and Arboriculture Production. It was supported by the Ministry of Agriculture and Water Resources and the Ministry of Higher Education and Scientific Research. Special acknowledgments are presented to Mr. Nabil Soua and Mrs. Mouna Khlif for their technical help and advices. The authors acknowledge Prof. Anne Lise Haenni for her help in improving the quality of this paper.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

Author Contributions.

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

Consent to participate

The Corresponding author and the order of authors are all correct at submission.

Consent to Publish

The Corresponding author and the order of authors are all correct to publish.

Introduction

Airborne particles are important metal carriers, especially in polluted areas such as in urbanized and industrialized regions which may negatively influence in vegetation health (Pariyar et al., 2013; Yan et al., 2014). Plants have long been used to remediate soils contaminated by heavy metals which are pollutants of natural and anthropogenic origin. (Govarthan et al., 2019; Javed et al., 2021; Ahmad et al., 2020; Alatawi et al., 2022). The negative impact of these metals on the environment 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 (Han 2007). Excessive exposure to these metals can cause various diseases, some of which can also have mutagenic and carcinogenic consequences (Gorsuch 1991; Bo et al., 2009).

Vegetables are reported to have the potential to accumulate heavy metals when grown on contaminated soils or when irrigated with wastewater (Gorsuch 1991; Bo et al., 2009). At higher levels, heavy metals cause inhibition of germination, growth and physio-biochemical disorders in plants (Claire et al., 1991). Excessive concentrations of some of them (Cd, Cr, Cu, Ni, Zn) in the soil have caused disruption of ecosystems (Meagher 2000). 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 (Monteiro et al., 2008). It has been suggested that the factors related to the uptake of heavy metals by plants, resulting to the stress response, and thus the damage to plant physiological functioning, is essential for the long-term safety and conservation of agricultural resources and ecosystems (Monteiro et al., 2009). Metal toxicity causes interference with the physiological functioning of plants, such as deleterious effects on chlorophyll biosynthesis (Qin 1994; Hattab et al., 2009), inhibition of growth of plants grown in soil contaminated with metals (Hattab et al., 2009), and increased protein content due to greater synthesis of metal-binding proteins, plant metal chelators and antioxidant enzymes (Gratao et al. 2006; Nayek et al. 2010). Likewise, proline accumulates under conditions of metal stress, due to increased synthesis or reduced degradation (Mehla et al., 2017; Dar et al., 2017).

The metal response results in altered levels of plant protective enzymes, including peroxidase, catalase, and ascorbate peroxidase (Cheng 2003; Israr et al., 2011), and accumulation of free proline (Cheng 2003). The enzymatic antioxidants SOD and CAT, along with APX and POX, constitute the major defense system against reactive oxygen species produced by the electron transport chain located in chloroplast (Sofo et al., 2005). Tomato and cucumbers contained large amounts of phenols with anti-free radical properties, and certain antioxidant enzymes (such as SOD, CAT, POX, APX, IAA oxidase, polyphenol oxidase) were shown to regulate their activity in response to abiotic stress (Iseri et al., 2011; Saeed et al., 2013; Pati et al., 2014; Nasibi et al., 2020; Aazami et al., 2021; Yusuf et al., 2021). Tomato was selected as the planting material because reports on the effect of heavy metals on physiological and biochemical parameters of tomato are rare.

Another type of pollutants generated by road activities, hydrocarbon contamination in the soil is well known to decrease seed germination (Oguntimehinet al., 2010), negatively influences plant physiology and morphology (Haider et al., 2021), and decreases photosynthetic activity and overall plant growth (Masakorala et al., 2013; Gawryluk et al., 2022). So, by altering the fundamental components of habitat, crude oil has an impact on the ecosystem (Ilyas et al., 2021). Several studies have reported that PAHs uptake into the plant induce morphological symptoms like growth reduction, chlorosis and necrosis; while physiologically they induce oxidative stress, DNA damage, cell death, alter antioxidant enzymes and inhibit photosynthesis (Alkio et al., 2005; Liu et al., 2009; Oguntimehin et al., 2010). The degree of toxicity varies not only with the kind of PAHs but also with species of plants (Maliszewska-Kordybach and Smreczak, 2000; Sverdrup et al., 2003). The mechanism by which they cause toxicity is not yet completely understood.

Due to its natural origin, hydrocarbons can be reincorporated into the soil to be degraded in the biogeochemical cycles by microorganisms (Youssef et al., 2009). When the amount of hydrocarbons in the environment is higher than the amount that can be metabolized, these become with adverse effects in the soil biota (Pardo-Castro et al., 2004), and delay germination, decrease biomass and leaf area, and can cause necrosis and death to the plants (Adam and Duncan 2002; Ramana-Rao et al., 2012).

It has been shown that tomato and cucumber 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 (Xishan et al., 2021; Xishan et al., 2022)

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 while 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. On this point, pollution near three roads in Sfax region were studied during this period with emphasis on the impact of pollution on the surrounding soil as well as on olive trees, the endemic tree in the region of Sfax. Several studies are interested in the olive tree by studying its response to different abiotic stresses (ben abdallah et al., 1990, Mezghanni et al., 2001; elloumi et al., 2003)

In this study we try 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*

Materials and Methods

Study area

The city of Sfax (34°44'24 North, 10°46'36 East) located in the eastern center of the Tunisian Republic, on the Mediterranean coast, 270 km south of the capital Tunis. Sfax plays an important role in trade at the national and international level. The governorate's road network includes: 4 national roads, 8 regional roads (368km in 2014), 26 local roads and 816 km of paved agricultural tracks. The asphalted agricultural tracks did not exceed 402 km throughout the governorate in 2009, almost all of which (98%) in the hinterland of Sfax.

To carry out a quality soil sample, it is important to respect certain rules concerning the homogeneity and size of the plot, the sampling depth, the number of individual samples and the equipment. The samples were taken along an axis perpendicular to the road axis using a manual auger. Four sites were chosen on the east side and four on the west side of the 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 1000m. The sites were chosen on the southwest side of the Gremda road 1m, 5m, 15m, 50m and a distant witness 1000m. Samples are taken at a depth of 20cm

After this operation, the samples are brought back to the laboratory, dried in the open air then sieved through a 2mm mesh sieve (Elloumi et al., 2003)

Soil analysis

Samples were collected at a depth of 0–20 cm using hand-driven steel augers. The pH of the soil is measured by pH meter fitted with a glass electrode and a reference electrode. The soil pH is determined by a Neomet brand pH meter on a soil/water suspension with a 1/2.5 ratio (Pauweles et al., 1992). Organic matter plays a significant role in improving soil structure by binding soil particles and fertility by releasing nutrients into the soil solution. The content of organic matter was obtained from the determination of one of its constituents. Soil organic carbon was obtained using the dichromate oxidation method of Walkley and Black (1934). For the hydrocarbon analysis, pre-dried soil samples were heated to 40–60 °C to remove all traces of moisture before extraction. The dried soil samples were placed in the Soxhlet and the extraction is carried out by acetone and Dichloromethane (ratio 1:1) as solvents with 3 cycles per h for 8 h (Kumar et al., 2011). Soxhlet extraction is commonly practiced for the extraction of particulate PAHs (Kavouras et al., 1999; Dachs and Eisenreich 2000; Fernandez et al., 2002). The extract was cooled and filtered through Whatman filter paper. The filtrate was concentrated to 1 ml volume using a vacuum evaporator. The prepared samples are stored in the refrigerator below 4 °C before analysis. The qualitative analysis of PAHs is determined by HPLC (HP. 1100) coupled to a UV detector. The analytical separation is carried out on a column (Lichosphere 100 RP – 18.5µm). The elution is detected at 278 nm. For heavy metals analysis, soil samples were digested in aqua regia (1:1 v/v HNO₃/HCl) (McGrath and Cunliffe 1985). Metals in the final solutions were determined using Pye Unicam, SPQ Philips, Atomic Absorption Spectrometer (AAS). Three replicates were analyzed for each test. About 50 g of pre-dried soil samples was heated at 40–60 °C to remove any trace of moisture before extraction.

Geoaccumulation Index

To evaluate the degree of soil contamination by selected metals (Pb, Zn, Cu and Cr), the contamination criterion was addressed; this is the geoaccumulation index (geo I) which is based on the use of the following formula (Müller 1969):

$$\text{Geo I} = \log_2 \left(\frac{C_n}{1.5 B_n} \right)$$

where n in the particle size fraction of soils; B_n is the average (crustal) concentration of element n, and 1.5 is the correction factor. Lu et al. (2009) gave the following interpretation for the geoaccumulation index: I_{geo} < 0 = unpolluted; 0 < I_{geo} < 1 = unpolluted to moderately polluted; 1 < I_{geo} < 2 = moderately polluted, 2 < I_{geo} < 3 = moderately to heavily polluted, 3 < I_{geo} C_n is the measured total concentration of the element < 4 = heavily polluted, 4 < I_{geo} < 5 = heavily to extremely polluted; and I_{geo} > 5 = extremely polluted.

Contamination factor

The contamination factor (C_f) was suggested by Håkanson (1980) and defined as follows:

$$FC = C_m / C_r$$

C_m is the measured concentration of the examined metal in the sample and C_r is the geochemical background concentration or reference value of the metal or the background value of heavy metals in the uncontaminated soil (Khairy et al., 2011). Håkanson (1980) suggested four classes of C_f to assess soil contamination levels (Loska et al., 2004): low (C_f < 1), moderate (1 ≤ C_f < 3), considerable (3 ≤ C_f < 6), and very high levels of contamination (C_f ≥ 6).

Plant analysis

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 20ml, the sample is stored in tubes covered with aluminum foil to prevent degradation of the pigments. Finally, we measure the chlorophyll with a spectrophotometer using 3 different wavelengths 661.6; 644.8 and 470 nm. The soluble sugars extraction is carried out according to the method of McCready et al (1950).

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 germinative capacity 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 (Vernay et al. 2009).

To follow the elongation of the aerial parts, we transferred the plants to the same type of soil. After sieving ($\phi = 2$ 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. Calculations on the length of the aerial parts of each plant are determined in 45days.

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 (Aebi, 1984). The activity was calculated using molar extinction co-efficient 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 (Chance 1955). Reaction mixture without the addition of crude enzyme extract was used as the blank.

Peroxidase activity was calculated using molar extinction co-efficient 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 (Nakano and Asada, 1981). Ascorbate peroxidase activity was calculated using molar extinction co-efficient of sodium ascorbate at 290 nm (2800 L/mol/cm) and expressed in μmol of ascorbate oxidized/min/g fresh weight of leaves.

Statistical analysis

The ANOVA analysis of variance and the determination of significant differences by the Tukey test were carried out by SPSS 13.0 software for Windows.

Results and discussion

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 elements as we move away from the roads. Many previous studies have concluded that heavy metal content in roadside soils has an exponential distribution with an increasing distance from the road (Akbar et al. 2006, Nabulo et al. 2006, Ndiokwere et al. 1984, Pagotto et al. 2001, Zhang et al. 2012). However, other research results showed that the distribution profiles of heavy metals in roadside soils were not always significantly correlated with road distance (Zhang et al., 2012). Referring to the standards reported by Serbaji (2000) 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, 10m near Tunis and Manzel Chaker roads. For Pb, the contamination has reached the same distance as Zn for Gremda and Tunis roads while for Manzel Chaker road, the contamination did not exceed three meter in the vicinity of the road. For Cu, only the first site near Gremda road exceeded the threshold. On the other hand, no site has been contaminated by Cr. To better study the metal contamination of the soils; two contaminations 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 50m from the road. Moderate contamination; up to 10m, from Manzel Chaker road and 3m from Tunis road. For Pb, Gremda soil is moderately polluted up to 50m 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 50m from Gremda road, 3m from Manzel Chaker road and was low for all sites of Tunis road. Indeed, studies by Fakayode and Olu-Owolabi (2003) indicated that Pb emissions can reach a distance of 50 m

For Zn, the use of CF showed a considerable contamination up to a distance of 50m, moderate at a distance of 3m from Manzel chaker road and up to 50m 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 $Zn > Pb > Cu > Cr$.

Lead, like other metals, persists in the soil for many years and is not biodegradable. Tunisia has implemented the use of unleaded gasoline since 2001 but the Pb in the soil is expected to remain longer. In addition, it seems difficult to explain the contamination of the soil by this element. This may be due to a persistent old contamination or to the use of contraband fuels containing Pb. Indeed, around 60% of informal trade with Algeria concerning petroleum products (CCET 2017). Smuggling of petroleum products (petrol and diesel) with Libya and Algeria is estimated at 1 billion liters per year (IPEMED 2017). Around one million cubic meters (m³) per year, or a quarter of the national consumption of fuel, gasoline and especially diesel fuel, comes from smuggling with Algeria and Libya (Haddad, 2016). The study published by the World Bank in December 2013, entitled "Estimation of informal trade across Tunisian land borders", estimates the volume of smuggled fuels from Algeria to a quarter of national consumption. Algerian gas contained Pb until 2015. Likewise, studies carried out by the Tunisian Institute of Strategic Studies (ITES) at the end of 2015 estimated the quantities of smuggled fuels coming from Algeria and Libya at 26% of national consumption, or a first approximation of 900,000 tonnes in 2015.

For hydrocarbon contamination, we noted that the highest levels (1650mg/kg) are recorded in 1m from Manzel Chaker road; then in 5m site, we recorded a reduction of 33.3% in hydrocarbon content. In the same distance (3m), in the vicinity of Tunis and Gremda roads, the contents recorded represent 41% and 38% respectively of the contents recorded in the soils of Manzel Chaker

In the same region, the analyzes of hydrocarbons in soil of Sfax showed values ranging from zero to 134 ppm (baklouti et al. 2017). In other hand the study of Hentati et al (2013) showed a content between zero to 11.63% in the soil of Sfax region

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 50m from the road. On the other hand, the pH is alkaline for Tunis and Manzel Chaker roadsoils. 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 depends on the pH of the soil (Myung et al., 1997). The increase of soil acidity lead to the elevation of the bioavailability of metals (Chlopecka 1996). Organic matter levels exceed 1% only near Manzel Chaker road. While for the sites of Tunis and Gremda roads the contents does not exceed 7% and 8% respectively. Indeed, the availability of metals in the soil-plant system depends on soil pH and organic matter concentration (Chlopecka 1996; Myung et al. 1997). The organic part of the soil also has a great affinity for metal cations through the presence of ligands or functional groups which can form complexes with metals.

Therefore, the low content of organic matter increases solubility 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 Manzel Chaker road are characterized by an enrichment in Ca²⁺ with values approaching 1.5% (1MC). Increased Ca²⁺ concentrations could reduce heavy metal solubility (Calile et al., 2004; Zhou et al., 2020). The K⁺ and Na⁺ contents are similar between the different sites studied. The variation of clay contents between the three sites can affect availability of the metals which may be more available near Gremda and Tunis roads. The clay soil retains a high amount of metals (Pikuła et al., 2021). The bioavailable fraction of anthropogenic heavy metals, such as Cu, Pb and Zn, decreased with decreasing soil particle size such as clay particle (Liu et al 2018).

Effect of contamination on seed germination

The percentage and speed of germination of the seeds of the two studied species are illustrated in figures 2 and 3. For Gremda road, the percentage of tomato germination was 12% at 3m from the road. For the same distance of Tunis and Manzel Chaker roads, the reduction of germination percentage was 14.4% and 70% respectively. The percentages of reduction in tomato germination on 3m soils compared to the control sites were 81%, 68.7% 22.3% for Gremda, Tunis and Manzel chaker roads respectively. These reduction percentages become 77%, 37% and 17% at 15m from Gremda, Tunis and Manzel chaker roads respectively. 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 of tomato. The effect 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 (Vernay et al, 2009). While, for organic pollutants, Baker (1971) and Adam et al (2002) proved that petroleum hydrocarbons can form a film on the seed, preventing the entry of oxygen and water.

For cucumber, the results obtained showed that, in 3m away from Gremda road, the germination was 50.7%; while it is in the order of 64% and 70.7% at the same distance from Tunis and Manzel Chaker roads respectively. The germination percentage reduction of cucumber germinated on 3m from Gremda, Tunis and Manzel Chaker roads compared to the control soil were 25% 7% 16% respectively. We note that the metal contamination of the gremda soil and the hydrocarbon contamination of Manzel Chaker soil affected the cucumber germination less

than tomato germination. Studies by Munzuroglu and Geckil (2002) 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.

Sharma et al (2010) who showed that the effect of Zn on the germination of several plant species from 100 mg/kg. On the other hand, according to Habtamu et al. (2013), Zn with 500 µg/g can reduce the germination percentage of tomato.

Monitoring of aerial part elongation of tomato and cucumbers for 43 days is illustrated in the figure 3.

The tomato growth on Gremda road soils showing the absence of metal contamination effect on tomato aerial growth. On the other hand, on the soils of Manzel Chaker road, we notice a reduction of 51% in the aerial elongation of tomatoes on 1m away the road compared to the control soil (1000 m). The high hydrocarbon content recorded at 1m from Manzel Chaker road (1650 mg/kg) affected the aerial part elongation of the tomato. The hydrocarbons can participate in the inhibition of cell division of the leaf (Henner 1999). Tesar et al (2002) noticed a 96% reduction in ryegrass biomass after 30 days of growth on soil contaminated with 25 g/kg of petroleum hydrocarbons

For the cucumber we notice that on the three roadsoils, aerial growth did not affect with distance from the studied roads, so unlike the tomato, the contamination of the soils of Manzel Chaker by hydrocarbons has no impact on aerial growth of cucumber which shows resistance to metal contamination of gremda soils and organic contamination of Manzel Chaker soils

The use of principal component analysis (Fig 4) showed the intervention of heavy metals, especially Pb and Zn in the percentage and speed of tomato germination. On the other hand, this analysis showed no effect of Cu and Cr on the germination of this species. On the other hand, this analysis showed the absence of effect of heavy metals on cucumber germination.

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 sugars and proline are illustrated in figure 5

On the soil 3m away from Gremda road, the chl a and Chl b contents of tomato recorded a reduction of 81% 53% compared to those recorded in the control site. The percentages decrease away from the road. On 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 on the soils of Tunis road. So, we see that metal contamination of gremda soil affects the chlorophyll uptake of tomato. For cucumber, on 3m away from Gremda road, metal contamination contribute to the reduction in Chl a content with 20% compared to the control site while the Chl b content was reduced by 24%. Over the distance from the Tunis road, a small reduction in Chl a content which does not exceed 1% compared to the control site, while there is a reduction of 3% in chl b. On the other hand, on the soil 3m away from the Manzel Chaker road, the chl a and chl b content of cucumber were reduced by 24% and 9% respectively. The reduction in chl a and Chl b contents for tomato continu on the Gremda road up a distance of 10m while on the Manzel Chaker road, the reduction in chlorophyll assimilation affects only the two first sites.

The reduction occurred in chlorophyll of tomato which is mainly related to its biosynthesis (Stobart et al 1985). The negative effect of metals on tomatoes is also demonstrated by Baszynski et al (1988). Recently, an inhibiting effect of metals has been observed in other species like cucumber (Malik et al., 1992). The effect of metals on plastid pigments depends on leaf age and plant development (Barcelo et al., 1988). Chlorophyll concentrations could be lowered by activating enzymatic degradation in metal-stressed plants (Farid et al., 2013). Studies by Baruah et al (2014) 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.

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 3m, 43% at 10m and 33% at 50m 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 3m 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 Manzel chaker 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 leaves when the tomato are germinated on soil 3 and 10m away respectively from the control site (1000m) then the contents approach those recorded in the site witness. Soluble sugars in tomato leaves grown under the stress of Cu²⁺, Zn²⁺ and Cd²⁺ was reduced by 57.5% (Saeed et al., 2013). 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 (Nayek et al., 2010). The decline of sugars content was related to the loss of photosynthetic pigments and the high energy needs of plants due to oxidative stress and toxicity response (John et al., 2008; Nayek et al., 2010).

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 (Alia and Saradhi, 1991). 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 (0.287µg/g Ms) is similar to that recorded in the control site (0.288µg/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 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.

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 (Tantrey and Agnihotri, 2010). On the other hand, Nayek et al (2010) reported another source for proline, other than road pollution, which is wastewater.

Biochemical response

Tomato leaves grown at sites near Gremda road 1G, 2G and 3G showed an increase in peroxidase 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 Saeed et al (2013). An increase in catalase activity in tomato plants was observed when Cd²⁺ was enriched the soil (Gratao et al., 2006). Similarly, ascorbate peroxidase activity was induced due to Cd²⁺ stress in *Bechmeria nivea* (Liu et al., 2007). The production of protective enzymes in plants has been considered as a defense mechanism to reduce oxidative stress (Liu et al., 2007). Oxidative stress, induced by the accumulation of Cd²⁺ in plant cells, is manifested by increased accumulation of H₂O₂ (Liu et al., 2007; Israr et al., 2011).

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 oraganic stress. Cargnelutti et al (2006) showed that cucumber can resist pollution of 500 µM HgCl₂. Likewise, studies by Gonçalves et al (2006) 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.

Conclusion

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 contributes to the modification of the biochemical and physiological factors of two endemic species in the Sfax region. After this study we conclude an aspect of remarkable resistance of cucumber to metallic and organic pollutants that can be exploited soon

References

405 Aazami MA, Rasouli F, Ebrahimzadeh A (2021) Oxidative damage, antioxidant mechanism and gene
 406 expression in tomato responding to salinity stress under in vitro conditions and application of iron and zinc oxide
 407 nanoparticles on callus induction and plant regeneration. Aazami et al. BMC Plant Biology. *BMC Plant Biol* **21**,
 408 597. <https://doi.org/10.1186/s12870-021-03379-7>
 409 Adam G, Duncan H (2002) Influence of diesel fuel on seed germination. *Environmental Pollution* **120**(2):363-
 410 370. [https://doi.org/10.1016/S0269-7491\(02\)00119-7](https://doi.org/10.1016/S0269-7491(02)00119-7)
 411 Adam G, Gamoh K, Morrison DG, Duncan H (2002) Effect of alcohol addition on the movement of petroleum
 412 hydrocarbon fuels in soil. *Science of the Total Environment*, **286**(1-3), pp. 15-25.
 413 Aebi H (1984) Catalase in vitro, *Methods Enzymol.* **105** 121–126
 414 Ahmad RZ, Khalid R, Aqeel M, Ameen F, Li CJ (2020) Fungal endophytes trigger *Achnatherum inebrians*
 415 germination ability against environmental stresses. *South African Journal of Botany* **134**, 230–236.
 416 doi:10.1016/j.sajb.2020.01.004
 417 Akbar KF, Hale WHG, Headley AD, Athar M (2006) Heavy metal contamination of roadside soils of northern
 418 England. *Soil Water Res.*, **4**, 158–163.
 419 Alatawi A, Wang X, Maqbool A, Saleem MH, Usman K, Rizwan M, Yasmeen T, Arif MS, Noreen S, Hussain
 420 A, Ali S (2022) S-Fertilizer (Elemental Sulfur) improves the phytoextraction of cadmium through *Solanum*
 421 *nigrum* L. *International Journal of Environmental Research and Public Health* **19**, 1655.
 422 doi:10.3390/ijerph19031655
 423 Alia P and Saradhi P P (1991), Proline accumulation under heavy metal stress. *J. Plant Physiol.* **138**: 554-558
 424 Alkio M, Tabuchi TM, Wang XC, Colón-Carmona A (2005) Stress responses to polycyclic aromatic
 425 hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. *J. Exp. Bot.*
 426 **56**, 2983–2994
 427 Baker JM (1971). Growth stimulation following oil pollution, in *Ecological Effects of Oil Pollution on Littoral*
 428 *Communities*, E. B.Cowell, ed. Barking U.K. Applied Science Publishers.
 429 Baklouti S, Ahmadi R, Sedik M, Bougi M, Rasheed MA , Srinivasa Rao P.L , Hasan SZ, Ksibi M (2017)
 430 Surface geochemical prospection for hydrocarbons in the oriental platform; the case of Guebiba oilfield, Sfax
 431 region, Tunisia. *Journal of Petroleum Science and Engineering*. Volume 159. Pages 830-840.
 432 Barcelo J, Vazquez M, Poschenrieder C (1988) Structural and ultrastructural disorders in cadmium-treated
 433 bush bean plants (*Phaseolus vulgaris* L.). *New Phytologist*. **108**: 37–49.
 434 Baruah P, Saikia RR, Baruah PP, Deka S (2014) Effect of crude oil contamination on the chlorophyll content and
 435 morpho-anatomy of *Cyperus brevifolius* (Rottb.) Hassk. *Environmental Science and Pollution*
 436 *Research* volume 21, pages12530–12538
 437 Baszynski T, Tukendorf M, Ruszkowska M, Skorzynska E and Maksymiec W (1988) Characteristics of the
 438 photosynthetic apparatus of copper non-tolerant spinach exposed to excess copper. *J. Plant Physiol.* **132**: 708-
 439 713.
 440 Ben Abdallah F., Boukhris, M., 1990. Action des polluants atmospheriques sur la vegetation de la region de
 441 Sfax (Tunisie). *Pollut. Atmos.* **127**, 292 – 297
 442 Bo S, Mei L, Tongbin C, Yuanming, Z, Yunfeng, X, Xiaoyan L and Ding, G (2009) Assessing the health risk of
 443 heavy metals in vegetables to the general population in Beijing, China. *J. Environ. Sci.* **21**: 1702-1709.
 444 Calile N, Swanwick S, Zhao FJ, McGrath SP (2004) Arsenic hyperaccumulation by *Pteris vittata* from arsenic
 445 contaminated soils and the effect of liming and phosphate fertilisation. *Environ. Pollut.* **132**, 113–120.
 446 [CrossRef] [PubMed]
 447 Cargnelutti D, Tabaldi LA, Spanevello RM, Jucoski GO, Battisti V, Redin M, Linares CEB, Dressler VL, Érico
 448 Flores EM, Nicoloso FT, Morsch VM, Schetinger MC (2006) Mercury toxicity induces oxidative stress in
 449 growing cucumber seedlings. *Chemosphere*, Volume 65, Issue 6, Pages 999-1006, ISSN 0045-6535.
 450 Chance B, Maehly AC (1955) Assay of catalases and peroxidases. *Meth. Enzym* **2**, 764–775.
 451 Cheng S (2003) Effect of heavy metals on plants and resistance mechanism. *Environ. Sci. Poll. Res.* **10**: 256-
 452 264.
 453 Chlopecka A (1996) Assessment of form of Cd, Zn and Pb in contaminated calcareous and gleyed soils in
 454 Southwest Poland." *Science of the Total Environment* **188**.2-3: 253-262.
 455 Claire LC, Adriano DC, Sajwan KS, Abel SL, Thoma DP and Driver JT (1991) Effects of selected trace metals
 456 on germinating seeds of six plant species. *Water Air Soil Poll.* **59**: 231-240.
 457 Dachs J, Eisenreich SJ (2000) Adsorption onto Aerosol Soot Carbon Dominates Gas-Particle Partitioning of
 458 Polycyclic Aromatic Hydrocarbons *Environ. Sci. Technol.* **34**, **17**, 3690–3697
 459 Dar MI, Naikoo MI, Khan FA, Rehman F, Green ID, Naushin F, Ansari AA (2017) An introduction to reactive
 460 oxygen species metabolism under changing climate in plants. In *Reactive Oxygen Species and Antioxidant*
 461 *Systems in Plants: Role and Regulation under Abiotic Stress*. https://doi.org/10.1007/978-981-10-5254-5_2

Elloumi N, Ben Abdallah F, Mezghanni I, Boukhris M (2003) Accumulation du Pb par quelques espèces végétales cultivées au voisinage d'une fonderie de plomb à Sfax. *Pollution atmosphérique*, 178,285-295.

Fakayode SO, Olu-Owolabi BI (2003) Heavy metal contamination of roadside topsoil in Osogbo, Nigeria: its relationship to traffic density and proximity to highways. *Environ Geol* 44:150–157.

Farid M, Muhammad S, Sana E, Shafaqat A, Muhammad Z, Muhammad Asif H (2013) Morphological, physiological and biochemical responses of different plant species to Cd stress. *International Journal of Chemical and Biochemical Sciences*. 3. 53-60.

Gawryluk A, Stępniewska A, Lipińska H (2022) Effect of soil contamination with polycyclic aromatic hydrocarbons from drilling waste on germination and growth of lawn grasses. *Ecotoxicol Environ Saf* 236:113492. <https://doi.org/10.1016/j.ecoenv.2022.113492>

Gonçalves JF, Becker AG, Cargnelutti D, Tabaldi LA, Pereira LB, Battisti V, Spanevello RM, Morsch VM, Nicoloso FT, Schetinger MRC (2006) Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings Braz. *J. Plant Physiol.*, 19(3):223-232

Gorsuch JW (1991) Plants for toxicity assessment, ASTM Committee E-47 on biological effects and environmental fate. p. 401

Govarthanan M, Selvankumar T, Mythili R, Srinivasan P, Ameen F, AlYahya SA, Kamala-Kannan S (2019) Biogreen remediation of chromium-contaminated soil using *Pseudomonas* sp. (RPT) and neem (*Azadirachta indica*) oil cake. *International Journal of Environmental Science and Technology* 16, 4595–4600. doi:10.1007/s13762-018- 2136-6

Gratao PL, Gomes-Junior RA, Delite FS, Lea PJ and Azevedo RA (2006) Antioxidant stress responses of plants to cadmium. In *Cadmium Toxicity and Tolerance in Plants*; Khan, N. A. and Samiullah, Eds.; Narosa Publishing House: New Delhi, 1-34.

Habtamu A, Derara A, Tesfaye F (2013) Effect of copper and zinc on seed germination, phytotoxicity, tolerance and seedling vigor of tomato (*Lycopersicum esculentum* L. cultivar Roma VF). *Int J Agric Sci Res* 2(11):312–317

Haider FU, Ejaz M, Cheema SA, Khan MI, Zhao B, Liqun C et al (2021) Phytotoxicity of petroleum hydrocarbons: sources, impacts and remediation strategies. *Environ Res* 197:111031. <https://doi.org/10.1016/j.envres.2021.111031>

Håkanson L (1980) An ecological risk index for aquatic pollution control: A sedimentological approach. *Wat Res* 14: 975–1001.

Han XF (2007) Trace element pollution in arid zone soil. In: *Biogeochemistry of Trace Elements in Arid Environments*; Brain, J. A. and Jack, T. T. Eds.; Environ. Poll. 13, Springer, The Netherlands, 267-287.

Hattab S, Chouba L, Kheder M, Mahouachi T, Boussetta H (2009) Cadmium- and copper- induced DNA damage in *pisumsativum*, roots and leaves as determined by the comet assay. *Plant Biosyst*;143:S6. doi: 10.1080/11263500903187035. [[CrossRef](#)] [[Google Scholar](#)]

Hattab S, Dridi B, Chouba L, Kheder MB and Boussetta H (2009) Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metal stress. *J. Environ. Sci.* 21: 1552-1556.

Henner P, Schiavon M, Druelle V, Lichtfouse E (1999) Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. *Organic Geochemistry*, 30, 963-969.

Ilyas N, Shoukat U, Saeed M, Akhtar N, Yasmin H, Khan W, Iqbal S (2021) Comparison of plant growth and remediation potential of pyrochar and thermal desorption for crude oil-contaminated soils. *Sci Rep* 11:2817. <https://doi.org/10.1038/s41598-021-82243-y>

Iseri OD, Korpe DA, yurtcu E, Sahin FI, Haberal M (2011) Copper-induced oxidative damage, antioxidant response and genotoxicity in *Lycopersicum esculentum* Mill. and *Cucumis sativus*. *Plant Cell Rep* 30:1713–1721. DOI 10.1007/s00299-011-1079-x

Javed MT, Tanwir K, Abbas S, Saleem MH, Iqbal R, Chaudhary HJ (2021) Chromium retention potential of two contrasting *Solanum lycopersicum* Mill. cultivars as deciphered by altered pH dynamics, growth, and organic acid exudation under Cr stress. *Environmental Science and Pollution Research* 28, 27542–27554. doi:10.1007/s11356-020-12269-8

John R, Ahmad P, Gadgil K and Sharma S (2008) Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ.* 54: 262-270

Kavouras H, Iebkhiri L, Iebkhiri M (1999). bioaccumulation of heavy metals in tomato and lettuce fertilized by the sludge of a treatment plant. *Bull. So. Phar. bordeaux*:77-92.

515 Kumar V, Kothiyal NC (2011) Distribution behavior of polycyclic aromatic hydrocarbons in roadside soil at
516 traffic intercepts within developing cities. *Int. J. Environ. Sci. Tech.*, 8 (1), 63-72, Winter 2011 ISSN: 1735-
517 1472.

518 Liu G, Wang J, Liu X, Liu X, Li X, Ren Y, Wang J, Dong L (2018) Partitioning and geochemical fractions of
519 heavy metals from geogenic and anthropogenic sources in various soil particle size fractions. *Geoderma*, Volume
520 312, Pages 104-113, ISSN 0016-7061, <https://doi.org/10.1016/j.geoderma.2017.10.013>.

521 Liu H, Weisman D, Ye YB, Cui B, Huang YH, Colón-Carmona A, Wang ZH (2009) An oxidative stress
522 response to polycyclic aromatic hydrocarbon exposure is rapid and complex in *Arabidopsis thaliana*. *Plant Sci.*
523 176, 375– 382.

524 Liu Y, Wang X, Zeng G, Qu D, Gu J, Zhou M and Chai L (2007) Cadmium-induced oxidative stress and
525 response of the ascorbate-glutathione cycle in *Beckhamia nivea* (L.) Gaud. *Chemosphere*, 69: 99-107

526 Malik D, Sheoran I, Singh R (1992) Carbon metabolism in leaves of cadmium treated wheat seedlings. *Plant*
527 *Physiology and Biochemistry*. 30: 223–229.

528 Maliszewska K B., Smreczak, B., 2000. Ecotoxicological activity of soils polluted with polycyclic aromatic
529 hydrocarbons (PAHs)-effect on plants. *Environ. Technol.* 21, 1099–1110.

530 Masakorala K, Yao J, Guo H, Chandankere R, Wang J, Cai M et al (2013) Phytotoxicity of long-term total
531 petroleum hydrocarboncontaminated soil a comparative and combined approach. *Water Air Soil Pollut*
532 224:1553. <https://doi.org/10.1007/s11270-013-1553-x>

533 McCready R M, Guggolz J, Silveira V, Owens HS (1950) Determination of starch and amylose in vegetables.
534 *Anal. Chem.* 22: 1156–1158. doi:10.1021/ac60045a016

535 McGrath SP, Cunliffe CH (1985) A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Pb, Cr, Co
536 and Mn from soils and sewage sludges. *J Sci Food Agric* 36:794–798

537 Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. *Curr. Opin. Plant Biol.* 3: 153-
538 162.

539 Mehla N, Sindhi V, Josula D, Bisht P, Wani SH (2017) An introduction to antioxidants and their roles in plant
540 stress tolerance. In *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under*
541 *Abiotic Stress*. https://doi.org/10.1007/978-981-10-5254-5_1

542 Mezghanni I (2001) Etude de l'accumulation du fluor et du cadmium chez certains végétaux dans la région du
543 Sfax. Effet de la synthèse de deux protéines de défense : les β , 1, 3-glucanases et les chitinases, thèse, *faculté des*
544 *sciences de Sfax*, 125 p

545 Monteiro M, Santos C, Soares AMVM and Mann RM (2008) Does subcellular distribution in plants dictate the
546 trophic bioavailability of cadmium to *Porcellio dilatatus* (Crustacea, Isopoda)? *Environ. Toxicol. Chem.* 27:
547 2548-2556.

548 Monteiro M, Santos C, Soares AMVM and Mann RM (2009) Assessment of biomarkers of cadmium stress in
549 lettuce. *Ecotoxicol. Environ. Safety*, 72: 811-818.

550 Muller, G (1969) Index of geo-accumulation in sediments of the Rhine River. *Geol. J.* 1969, 2, 108–118.

551 Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptile and
552 hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Arch Environ Contam Toxicol* 43:203–213

553 Myung C, Jung I T (1997) Environmental contamination and seasonal variation of metals in soils, plants and
554 waters in the paddy fields around a Pb/Zn mine in Korea, *Science of The Total Environment*, Volume 198,
555 Issue 2, Pages 105-121, ISSN 0048-9697, [https://doi.org/10.1016/S0048-9697\(97\)05434-X](https://doi.org/10.1016/S0048-9697(97)05434-X).

556 Nabulo G, Oryem O.H, Diamond M (2006) Assessment of lead, cadmium, and zinc contamination of roadside
557 soils, surface films, and vegetables in Kampala City, Uganda. *Environ. Res.* 2006, 101, 42–52.

558 Nakano Y, Asada K (1981) Hydrogen peroxidase is scavenged by ascorbate-specific peroxidase in spinach
559 chloroplasts. *Plant Cell Physiol.* 22: 867–880.

560 Nasibi F, Kalantari KM, Zahra Manzari Tavakoli ZM (2020) Effects of Hydrogen Sulfide on Cold-Induced
561 Oxidative Damage in *Cucumis sativus* L. *International Journal of Horticultural Science and Technology* Vol. 7,
562 No. 3; pp 199-211 Print ISSN: 2322-1461 Online ISSN: 2588-3143 DOI: 10.22059/ijhst.2020.284285.301

563 Nayek S, Gupta S, Saha RN (2010) Metal accumulation and its effects in relation to biochemical response of
564 vegetables irrigated with metal contaminated water and wastewater. *J. Hazard. Mat.* 178: 588-595.

565 Ndiokwere CL (1984) A study of heavy metal pollution from motor vehicle emissions and its effect on roadside
566 soil, vegetation and crops in Nigeria. *Environ. Pollut. B Chem. Phys* , 7, 35–42.

567 Oguntimehin I, Eissa F, Sakugawa H (2010) Negative effects of fluoranthene on the eco-physiology of tomato
568 plants (*Lycopersicon esculentum* Mill). *Chemosphere* 78, 877–884.

569 Pagotto C, Rémy N, Legret M, Le Cloirec P (2001) Heavy metal pollution of road dust and roadside soil near a
570 major rural highway, *Environmental Technology* 22.

Pardo-Castro JL, Perdomo-Rojas MC, Benavides-López de Mesa JL (2004) Efecto de la adición de fertilizantes inorgánicos compuestos en la degradación de hidrocarburos en suelos contaminados con petróleo [Effect of the addition of compound inorganic fertilizers on the hydrocarbon degradation in soils polluted by petroleum]. *Nova* 2(2):40-49. <https://doi.org/10.22490/24629448.6>

Pariyar S, Eichert T, Goldbach HE, Hunsche M, Burkhardt J (2013) The exclusion of ambient aerosols changes the water relations of sunflower (*Helianthus annuus*) and bean (*Vicia faba*) plants. *Env. Exp. Bot.* 88, 43e52.

Pati S , Ghadei A , Arzoo A , Nayak SK , Mohapatra A , Satapathy KB (2014) Physiological responses induced by chromium+6 toxicity to *Cucumis sativus* L. and *Macrotyloma uniflorum* Lam. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*.e-ISSN: 2319-2402.

Pikuła D, Wojciech S (2021) Effect of the Degree of Soil Contamination with Heavy Metals on Their Mobility in the Soil Profile in a Microplot Experiment" *Agronomy* 11, no. 5: 878. <https://doi.org/10.3390/agronomy11050878>

Pauwels JM, Van Ranst E, Verloo M, Mvondo ZA (1992) Manuel de laboratoire de pédologie: Méthodes d'analyse de sol et des plantes. Equipement, gestion des stocks de verreries et produits chimiques. Publication agricole, 28, 250.

Qin T (1994) Effect of cadmium, lead and their interactions on the physiological and biochemical characteristics of *Brassica chinensis*. *Acta Ecologica Sinica*, 14: 46-50.

Ramana Rao MV, Weindorf D, Breitenbeck G, Baisakh N (2012) Differential expression of the transcripts of *Spartina alterniflora* Loisel (smooth cordgrass) induced in response to petroleum hydrocarbon. *Molecular Biotechnology* 51(1):18-26. <https://doi.org/10.1007/s12033-011-9436-0>.

Saeed A, Sohail M , Rashi N, Iqbal M (2013) Effects of heavy metals toxicity on the biochemical response in tomato plants grown in contaminated silt-soil. *Bangladesh J. Sci. Ind. Res.* 48(4), 229-236

Serbaji M (2000) Use of a multi-source GIS for understanding and integrated management of the coastal ecosystem of the Sfax region (Tunisia). Dissertation, University of Tunis.

Sharma S, Sharma P, Datta SP, Gupta V (2010) Morphological and biochemical response of *Cicer arietinum* L. var. pusa-256 towards an excess of zinc concentration. *Life Sci J* 7(1):95–98.

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

Stobart AR, Griffiths WT, Ameen-Bukhari J, Shewood RP (1985) The effect of Cd(II) on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum*. 63: 293-298

Sverdrup LE, Krogh PH, Nielsen T, Kjær C, Stenersen J (2003) Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pratense*), ryegrass (*Lolium perenne*), and mustard (*Sinapis alba*). *Chemosphere* 53, 993– 1003.

Tantrey MS, Agnihotri RK (2010), Chlorophyll and proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatments. *Res. J. Agri. Sci.* 1: 119- 122.

Tesar M, Reichenauer TG, Sessitsch A (2002) Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. *Soil Biology and Biochemistry*, Volume 34, Issue 12. Pages 1883-1892, ISSN 0038-0717, [https://doi.org/10.1016/S0038-0717\(02\)00202-X](https://doi.org/10.1016/S0038-0717(02)00202-X).

Vernay P, Austruy A A , Gauthier CM, Hitmi A (2009) Germination and functioning of the photosynthetic system of plants as bioindicators of soil pollution. *Study and Management of Soils*, Volume 16, 3/4. Volume 120, Issue 2. Pages 363-370, ISSN 0269-7491, [https://doi.org/10.1016/S0269-7491\(02\)00119-7](https://doi.org/10.1016/S0269-7491(02)00119-7).

Walkley A, Black I A (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29-37.

Xishan L, Wang C, Li N, Gao Y, Ju Z, Liao G, Deqi Xiong D (2021) Combined Effects of Elevated Temperature and Crude Oil Pollution on Oxidative Stress and Apoptosis in Sea Cucumber (*Apostichopus japonicus*, Selenka)" *International Journal of Environmental Research and Public Health* 18, no. 2: 801. <https://doi.org/10.3390/ijerph18020801>.

Xishan L, Zou Y, Xuan H, Yang W, Liao G, Wang C, Xiong D (2022) Impact of Physically and Chemically Dispersed Crude Oil on the Antioxidant Defense Capacities and Non-Specific Immune Responses in Sea Cucumber (*Apostichopus japonicus*)" *Journal of Marine Science and Engineering* 10, no. 10: 1544. <https://doi.org/10.3390/jmse10101544>

Yan X, Shi WZ, Zhao WJ, Luo NN (2014) Impact of aerosols and atmospheric particles on plant leaf proteins. *Atmos. Environ.* 88, 115–122.

Youssef N, Elshahed MS, McInerney MJ (2009) Microbial processes in oil fields: culprits, problems, and opportunities. *Advances in Applied Microbiology* 66:141-251. [https://doi.org/10.1016/S0065-2164\(08\)00806-X](https://doi.org/10.1016/S0065-2164(08)00806-X)

Yusuf M, Almehri AS , Alnajjar AJN , Alam P , Elsayed N , Khalil R , Hayat S (2021) Glucose modulates copper induced changes in photosynthesis, ion uptake, antioxidants and proline in *Cucumis sativus* plants. *Carbohydrate Research*, Volume 501, 108271, ISSN 0008-6215, <https://doi.org/10.1016/j.carres.2021.108271>.

Zhang F, Yan X, Zeng C, Zhang M, Shrestha S, Devkota LP, Yao T (2012) Influence of traffic activity on heavy metal concentrations of roadside farmland soil in mountainous areas. *Int. J. Environ. Res. Public Health* , 9, 1715–1731.

Zhou JM , Jiang Z C, Qin XQ, Zhang LK , Huang QB, Xu G L (2020) Effects and Mechanisms of Calcium Ion Addition on Lead Removal from Water by *Eichhornia crassipes*. *Int. J. Environ. Res. Public Health*, 17, 928; doi:10.3390/ijerph17030928

Table 1 The metals and hydrocarbons content in the studied soil

	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 ^{***}

646

647

648

649

650

651

652

653

654

655

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

	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 ±0,08	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

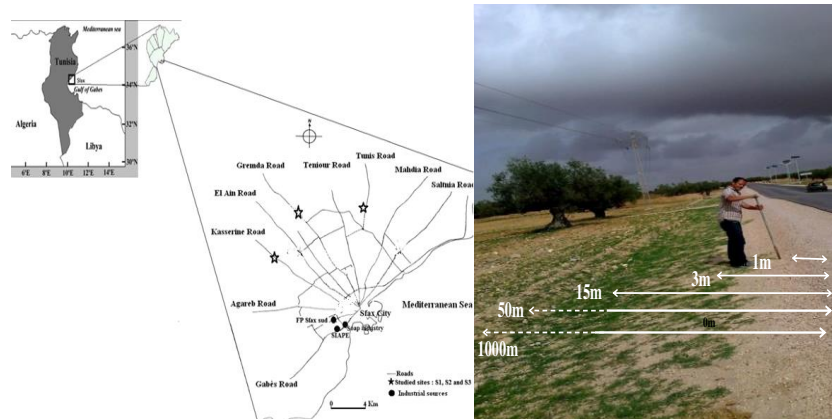


Fig.1 study area

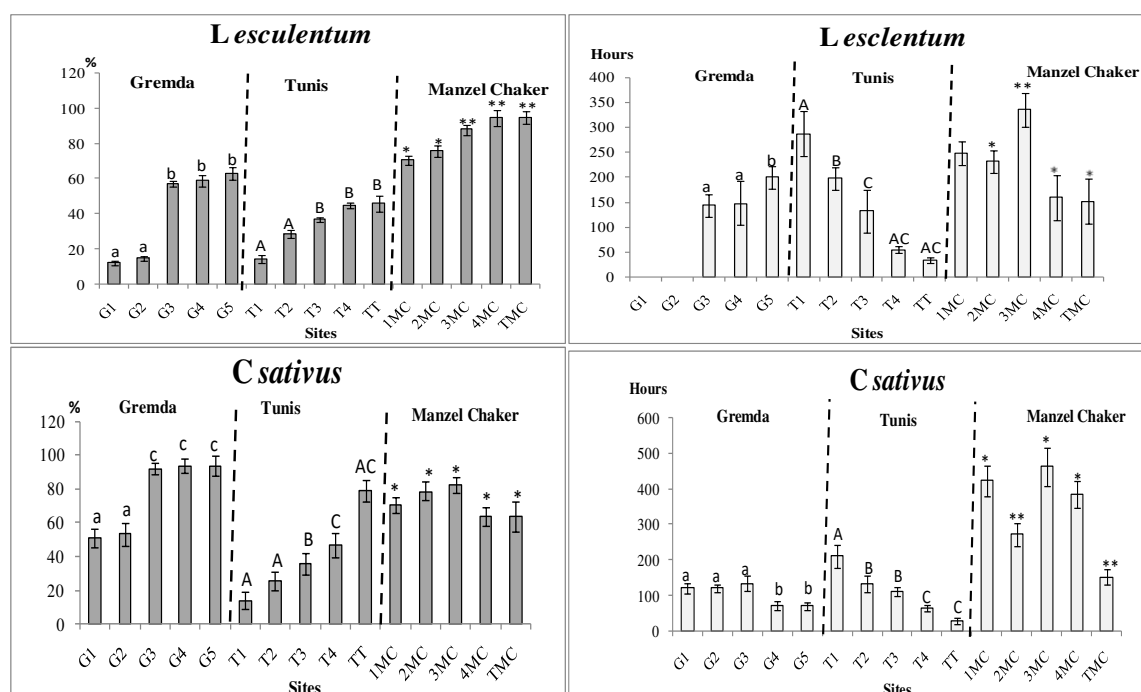
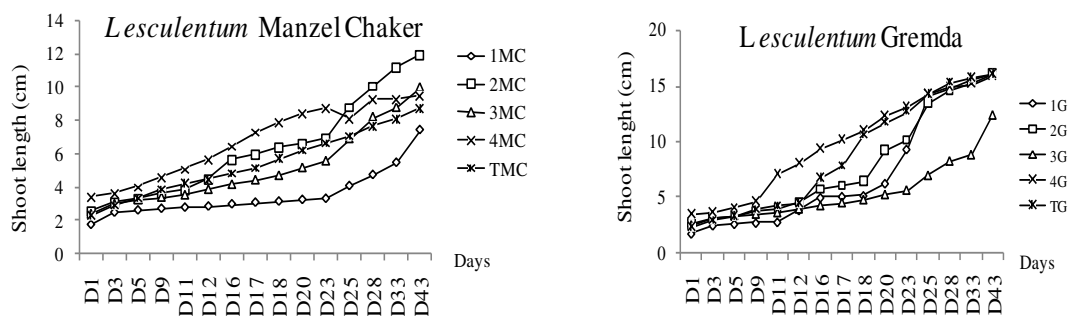


Fig 2 Capacity and speed of germination of the studied species on contaminated and control soils for the three studied areas



b/4

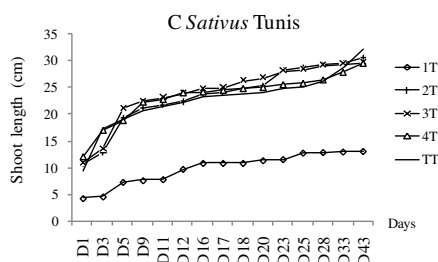
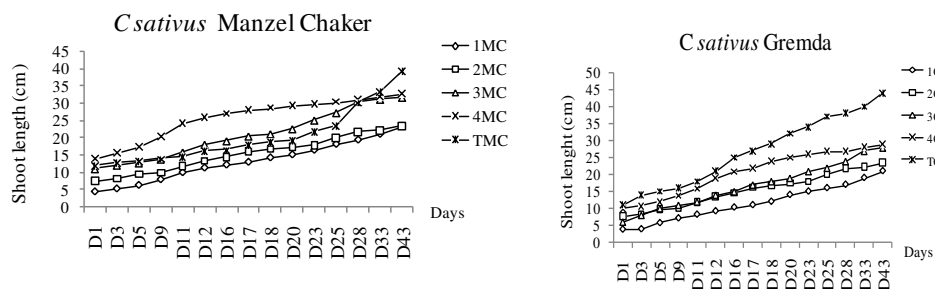
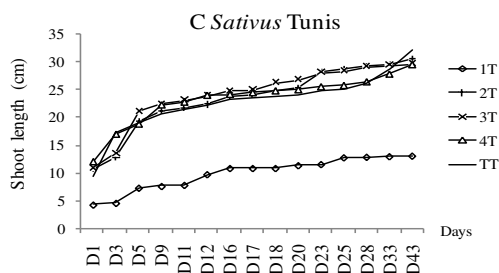


Fig 3 elongation of aerial part of the studied species

Variables (axes F1 et F2 : 76,02 %)

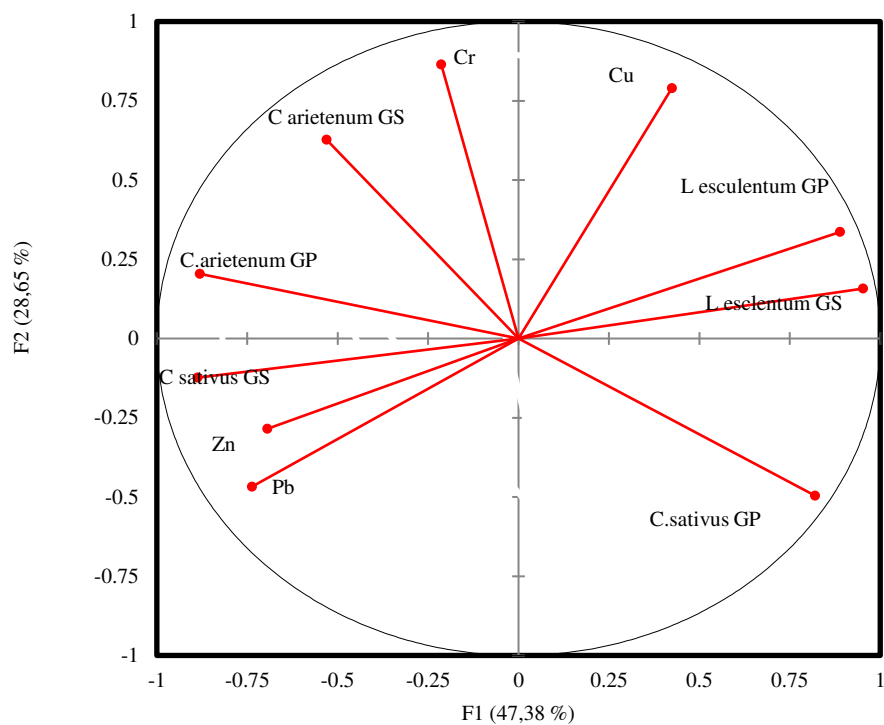
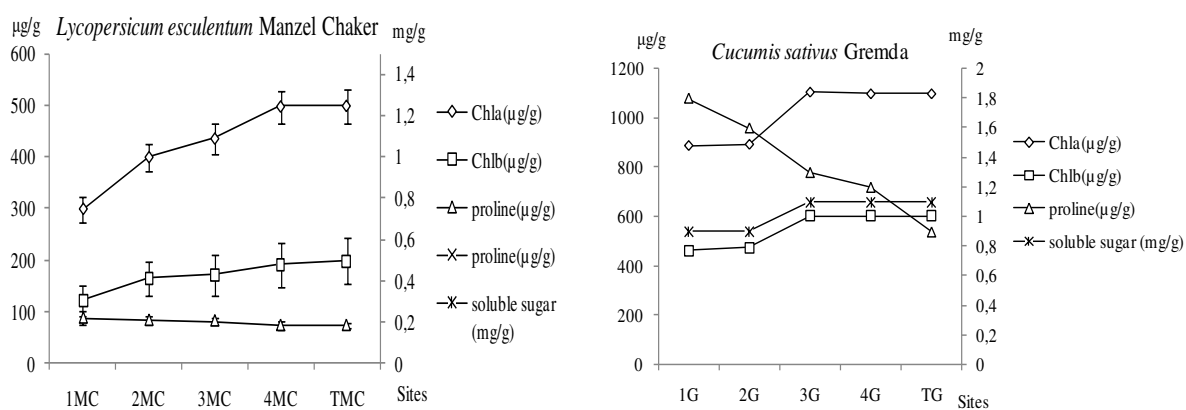


Fig 4 2D projection of variables according to factors 1 (explaining 47, 38 % of variability) and 2 (explaining 28.65% of variability) where: GS germination speed, GP germination perce



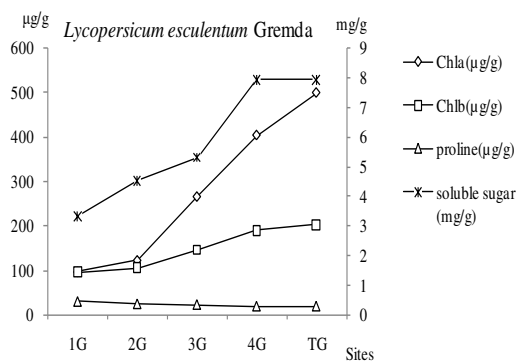
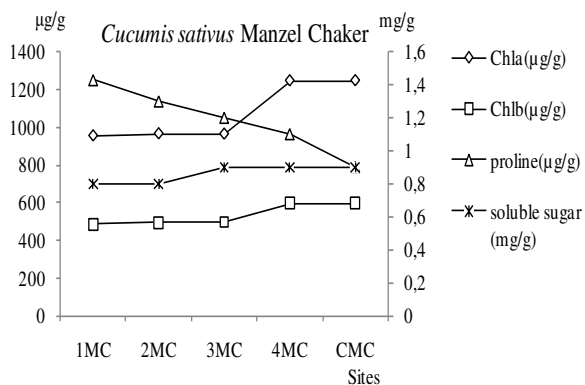
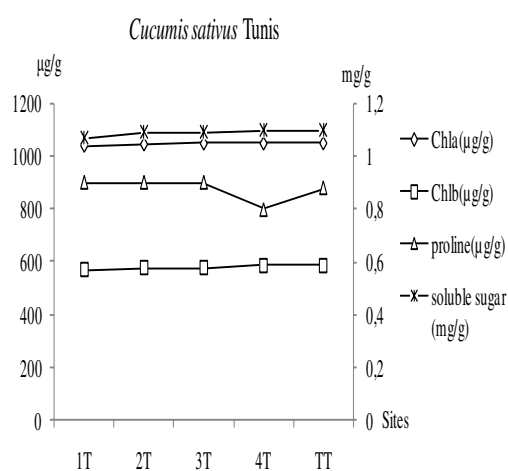
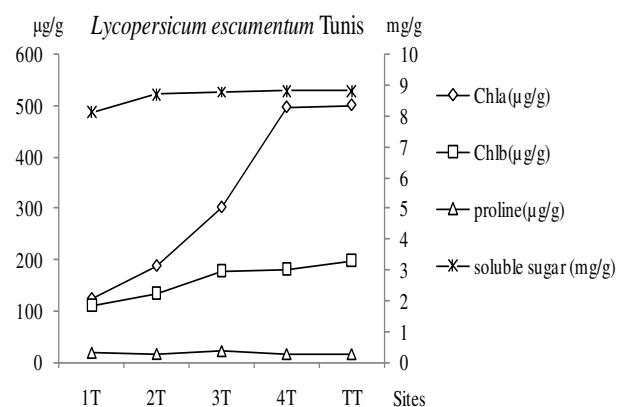


Fig 5 Variation of the physiological parameters in the study species

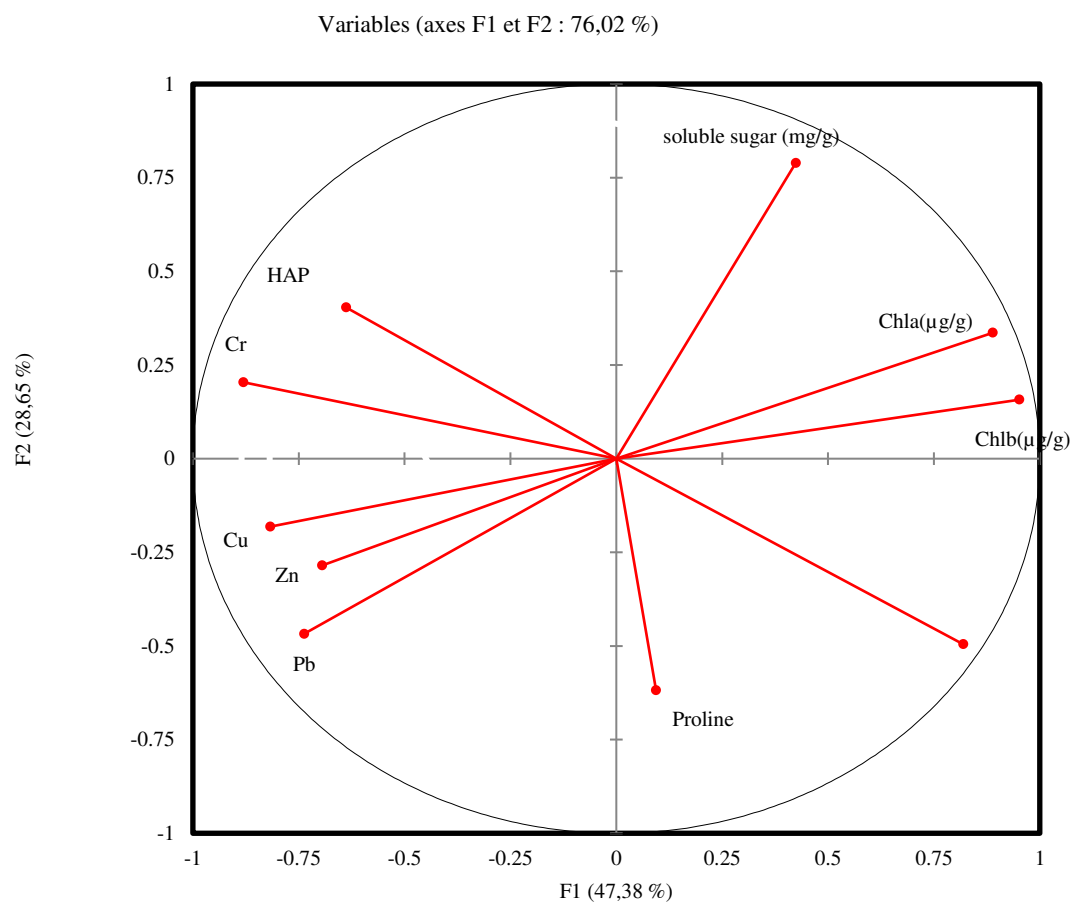


Fig 6 2D projection of variables according to factors 1 (explaining 47, 38 % of variability) and 2 (explaining 28.65% of variability) where: GS germination speed, GP germination percentage

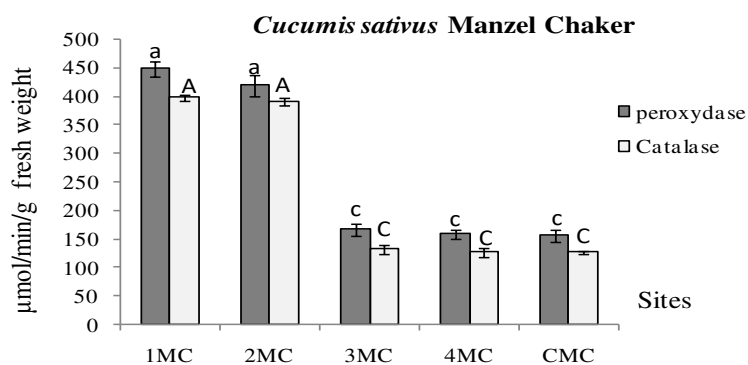
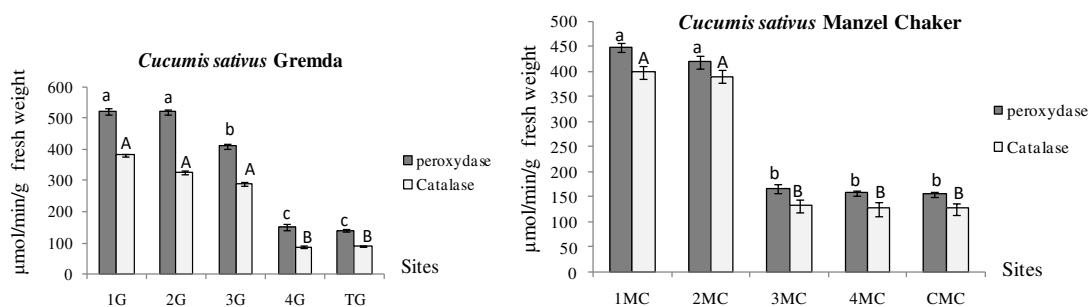
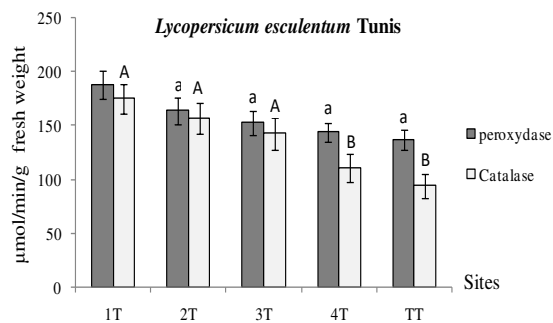
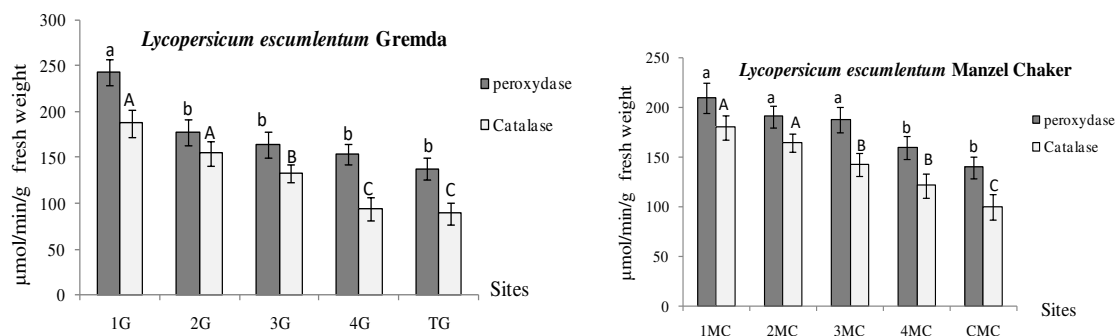


Fig 7 Catalase and peroxydase activity in the study species