

Research paper

The phytoremediator capacity of *Quercus cerris* L. against heavy metals contamination in the Ezer forest, Lebanon

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Abstract. The uncontrolled rise in heavy metal pollution due to anthropogenic activities has become a global environmental concern. Lebanon’s Ezer forest, dominated by *Quercus cerris* L., is exposed to vehicular activity along its northern edge due to an unpaved public road and intensive recreational activities in its center. This study aimed to determine the concentrations of metals in *Q. cerris* leaves and soil across three distinct forest stands: S1 and S2 (polluted sites) and Ctrl (control site). Cadmium, chromium, lead, and aluminium were extracted via the wet digestion method and analyzed by Atomic Absorption Spectrometry. Additionally, hydrogen peroxide levels, the total carbohydrate and phenolic content in *Q. cerris* leaves, as well as the activity of antioxidant enzymes, were measured. The results revealed that Ezer forest is at risk of heavy metal contamination, with a significant increase in hydrogen peroxide levels at the polluted sites compared to the Ctrl site. The total carbohydrate and phenolic content was notably reduced at the polluted sites, while peroxidase and catalase activity increased, indicating that *Q. cerris* has developed adaptive mechanisms to cope with oxidative stress. National strategies to raise public awareness and reduce heavy metal contamination in the forest are urgently needed to protect the rare *Q. cerris* species.

Key words: total carbohydrates, phenols, antioxidant enzymes.

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Introduction

Environmental pollution with heavy metals is one of the serious problems in the contemporary world. Heavy metals are defined as metallic elements with an atomic

number over 20 and a density of more than 5 g/cm³ (Liu *et al.*, 2013). They are known to pose a potential threat to all living organisms by contaminating water, soil, food crops, and the surrounding atmosphere (Emamverdian *et al.*, 2015).

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They are naturally found throughout the earth's crust. However, most environmental contamination by heavy metals results from anthropogenic activities such as industrial emissions, fossil fuel combustion, mining, coal burning, and improper agricultural practices (Tchounwou *et al.*, 2012). Among these, industries and vehicles remain the chief causes of heavy metal pollution (Shahid *et al.*, 2017; Türtscher *et al.*, 2017).

There are two types of heavy metals found in soils: the essential micronutrients, namely cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), molybdenum (Mo), and zinc (Zn) and the non-essential elements with an unidentified biological and physiological function such as mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), aluminium (Al) and lead (Pb) (Shahid *et al.*, 2017; Rai *et al.*, 2019).

The essential elements play parts in various fundamental processes of plants, including plant growth and development, chlorophyll biosynthesis, nucleic acids synthesis, sugar metabolism, redox homeostasis, and nitrogen fixation (Emamverdian *et al.*, 2015; Singh *et al.*, 2016; Morkunas *et al.*, 2018). Yet, at elevated concentrations, both essential and non-essential metals may induce severe toxicity in plants such as chlorosis, disruption of protein activity, DNA damage, water imbalance, alteration of nutrient assimilation, generation of reactive oxygen species (ROS), and senescence that ultimately cause plant death (Singh & Kalamdhad, 2011; Emamverdian *et al.*, 2015; Singh *et al.*, 2016). Metals at toxic levels are also well-known for their capability to attack the photosynthetic machinery and hinder photosynthesis (Singh *et al.*, 2016). Heavy metals exert their toxic effects on plants via diverse mechanisms. These comprise the direct production of reactive oxygen species through Fenton-like reactions, activation of NADPH oxidases that catalyze the reduction of molecular oxygen into hydroxyl radical, inactivation of oxidative stress response enzymes re-

sponsible for the detoxification of ROS, or inactivation of essential proteins by interacting with their sulfhydryl group. Heavy metals can also attack plants by displacing essential metals from their specific binding sites and thus disrupting the cellular metabolism (Singh *et al.*, 2016).

ROS are highly reactive molecules generated in several cellular compartments such as chloroplasts, mitochondria, and peroxisomes. Depending on their concentration in plants, ROS behave like a double-edged sword. At low concentrations, ROS act as second messengers in a variety of cellular processes including stomatal closure, root gravitropism, programmed cell death, and conferment of tolerance to plants against various environmental stresses. Nevertheless, at high concentrations, ROS cause oxidative damage to lipids, proteins, and nucleic acids (Sharma *et al.*, 2012). In plants, ROS exist as free radicals and/or non-radical molecules. Free radicals include superoxide anions ($O_2^{\cdot-}$) and hydroxyl radicals ($\cdot OH$), while non-radical molecules principally include singlet oxygen (1O_2) and hydrogen peroxide (H_2O_2) (Saed-Moucheshi *et al.*, 2014). The latter is constantly generated as a by-product of diverse metabolic reactions. However, under environmental stress, H_2O_2 levels rise, referring to the exposure of plants to oxidative stress. To alleviate the negative impacts of oxidative stress and increased ROS levels, plants develop defensive mechanisms to protect themselves. The mechanisms of detoxification of ROS in plant cells can be divided into enzymatic and nonenzymatic actions (Hasan *et al.*, 2011). Phenolic compounds, also referred to as phenols, are a class of secondary metabolites that play a key role in protecting against oxidative stress by directly scavenging ROS or inhibiting lipid peroxidation by trapping lipid alkoxyl radicals. Moreover, they act as metal chelators during heavy metals stress. Their hydroxyl and carboxyl groups and the nucleophilic character of the aromatic ring make phe-

nols able to strongly bind metals (Sharma *et al.*, 2012). This antioxidant activity complements the function of oxidative stress response enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX) (Hartmann & Asch, 2019).

Under stressful circumstances, SODs constitute the first barrier against ROS. They catalyze the dismutation of oxygen radicals into molecular oxygen and hydrogen peroxide (Das & Roychoudhury, 2014). CAT, APX, and GPX cooperate to eliminate excess H_2O_2 both during normal metabolism and under adverse conditions. GR is a flavoprotein oxidoreductase that reduces glutathione disulfide (GSSG) to glutathione (GSH) with the use of NADPH as a reducing agent, thus sustaining the redox status of the cell. In chloroplast, GSH and GR are engaged in the scavenging of H_2O_2 produced by the Mehler reaction (Sharma *et al.*, 2012).

The most reliable and economical approach for detecting heavy metal pollution is to exploit soil samples and plant material as biomonitors. Indeed, the deposition of heavy metals from air pollution is primarily on the soil surface (Shahid *et al.*, 2017). Heavy metals can also be taken up by plants through their roots and translocated through an active mechanism to their upper organs. Furthermore, airborne heavy metals emitted from street dust and vehicles can readily attach themselves to the foliar surface and thus perturb several biochemical and physiological processes in plants.

In Lebanon and other developing nations, environmental contamination with noxious metals represents a serious issue of concern. This is mainly due to the transport sector, bad quality fuel consumption, inadequate waste management, and lack of powerful environmental policies.

The Ezer forest, situated in the northern part of Lebanon, is well characterized by the abundance of a rare tree species *Quercus cerris*, which is used as an effec-

tive biomonitor of airborne pollution. The northern side of the forest is bordered by an unpaved public road experiencing elevated vehicular movement. Furthermore, the middle of the forest is witnessing intensive camping activities in addition to the entry of different types of vehicles that threaten the well-being of fauna and flora in the forest.

To the best of our knowledge, there are no studies on environmental contamination by heavy metals in the Ezer forest. Therefore, the present study was mainly designed to determine heavy metal concentrations in *Quercus cerris* leaves and soil sampled from experimental and Ctrl sites within the forest. The effects of heavy metal pollution on various factors in *Quercus cerris* leaves, including the oxidative stress marker H_2O_2 , total carbohydrates, and phenolic levels, as well as the enzymatic antioxidant activities, were also evaluated.

Material and Methods

Leaves sampling and preparation

The aerial parts of *Quercus cerris* were collected from the Ezer forest (34° 28' 15" N, 36° 12' 25" E) situated in Fneidiq village in Akkar governorate at an elevation of 1300–1500 meters above sea level. The Ezer forest, expanding over an area of 922,267 square meters, is distinguished by the spread and density of *Quercus cerris* species that reach more than 30 meters in height. The area under study has a Mediterranean climate, which is characterized by a hot summer that can reach its highest temperature of 32°C during July and cold and snowy winter where the temperature can drop to 2°C.

Three sampling areas were chosen to carry out this study (Figure 1). The public roadside, labeled as S1, has been taken as a “very polluted” site. The center of the forest (S2), which is considered a recreation zone, is chosen as a “medium polluted” site, while the western side of the Ezer for-

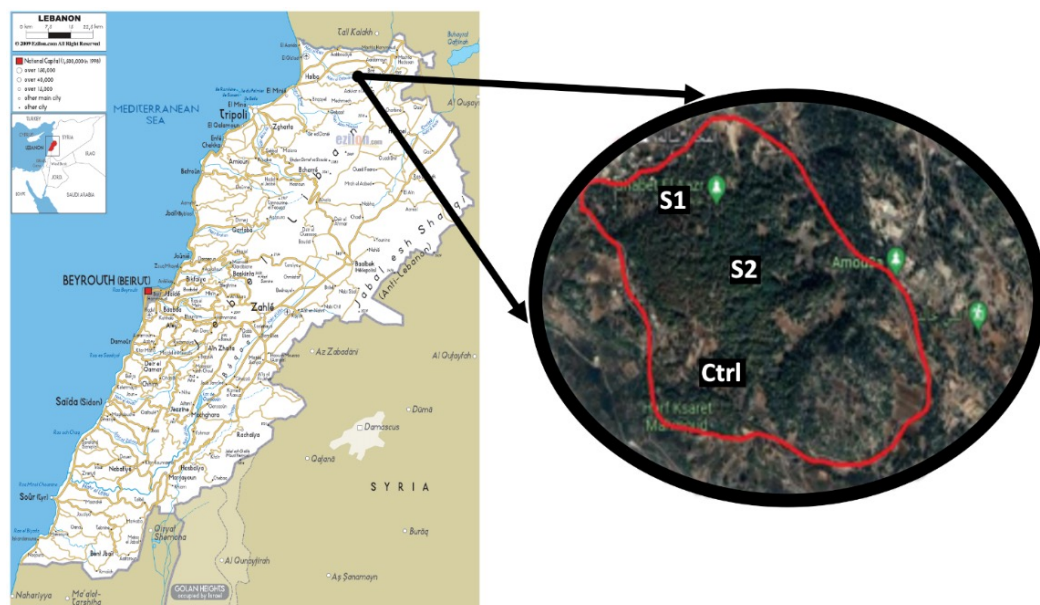


Figure 1. Geographical location of the Ezer forest and distribution of the studied sites; S1: very polluted site, S2: moderate polluted site and Ctrl: control site (Ezilon Maps, 2015; Google Earth, 2024).

est, approximately distant from the two spots, is selected as a control site (Ctrl). According to Fneidik municipality, the Ctrl site is not surrounded by residential buildings, factories or waste dumps, indicating a lack of human settlements and industrial activities.

Matured *Quercus cerris* leaves were gathered from experimental and Ctrl spots on the same day each month, spanning from June to October 2020. Samples were placed in ice boxes and transported to the laboratory of the Lebanese Agricultural Research Institute (LARI), Fanar, for analysis. Some of the fresh leaves were preserved in a freezer in the laboratory until the activity of antioxidant enzymes was analyzed within 24 hours of their sampling. Concurrently, the rest of the leaves were oven-dried at 70°C and grounded to powder.

Soil sampling

Soil samples were collected from each site from the base of the trees to be sampled at

a depth of 35 cm, kept in labeled polyethylene bags, and brought to the laboratory where they were air-dried and sieved with the use of a special soil grinder until analysis (Wao *et al.*, 2014).

Soil and leaves digestion and heavy metal determination

The determination of heavy metals in leaves and soil was done based on the acid wet digestion method (Uddin *et al.*, 2016). Briefly, 0.5 g of dried and grounded samples were digested with 30 ml of a mixture (10:1:4) of nitric acid – sulfuric acid – perchloric acid in a Kjeldahl digester at 250°C for 2 hours. The digested samples were then diluted to 100 ml, filtrated, and subjected to analysis by atomic absorption using iCE 3000 series AA spectrometers (Thermo Fisher Scientific). All samples were carried out in three replicates and mean values were noted for each determination.

Total carbohydrates estimation

Total carbohydrates were estimated using the anthrone colorimetric method (Yemm & Willis, 1954). 20 mg of dried grounded leaves were extracted with 2.5 ml (80%) ethanol. After that, 5 ml of 1.1% hydrochloric acid was added to the extract and heated in a boiling water bath for 30 minutes. The dilution to 10 ml was then completed with distilled water. Next, 1 ml of the mix was pipetted into test tubes with 5 ml anthrone reagent (0.2 g anthrone in 100 ml (72%) sulfuric acid). The solution was then heated for 11 minutes in a boiling water bath, followed by spectrophotometric reading at 630 nm after a brief cooling. The concentration of carbohydrates was evaluated from a starch calibration curve.

Hydrogen peroxide determination

Hydrogen peroxide content was estimated as described in the method of Karataş *et al.* (2014). 500 mg of fresh leaves were extracted with 5 ml of trichloroacetic acid and then centrifuged at 3000 g for 15 minutes. 0.5 ml of the supernatant was then transferred to a test tube and mixed with 1 ml of potassium iodide (1M) and 0.5 ml of potassium phosphate buffer (10 mM). Finally, the absorbance of the sample was read spectrophotometrically at 390 nm against a blank. The concentration of hydrogen peroxide was calculated from a predetermined H_2O_2 standard curve.

Phenolic compounds estimation

Total phenolic content was determined with the Folin-Ciocalteu method (Khatoon *et al.*, 2013). This assay is based on the reduction of samples containing polyphenols by the Folin-Ciocalteu reagent and subsequent formation of a blue-colored complex. For total phenolic compounds determination, 100 mg of dried and grounded leaves were added to 5 ml of 80% ethanol and incubated in a water bath at 30°C for 30 minutes. After incubation, separation of the phase by centrifugation was done at 3000 g for 10 minutes. Pellet was re-ex-

tracted using 2.5 ml of 80% ethanol. After that, 0.1 ml of the extract was mixed for 8 minutes with 5 ml of reagent A (25 ml Folin-Ciocalteu reagent diluted with 250 ml distilled water). Then, 3.5 ml of reagent B (57.5 g sodium carbonate in 500 ml distilled water) were added, and the mixture was incubated at 40°C for 1 hour in a water bath. The absorbance was read at 765 nm against a blank. The total phenolic content was calculated from a p-coumaric acid calibration curve.

Enzymatic scavengers' assays

50 g of fresh leaves were homogenized in a chilled mortar and pestle with quartz sand for grinding and 2 ml of ice-cold ethylenediaminetetraacetic acid (EDTA) phosphate buffer (7 pH) containing (0.1 M) K_2HPO_4 and (0.1 M) EDTA as the extraction medium. The homogenate was then centrifuged, and the supernatant was collected for enzyme's assays (Hartmann & Asch, 2019).

The activity of SOD was determined spectrophotometrically by measuring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) by the enzyme. 0.1 ml of enzyme extract and aside a control sample were added to a reaction mixture each containing 0.05 ml of (1.3 μ M) riboflavin, (13 μ M) methionine, (63 μ M) nitroblue tetrazolium, (0.05 M) sodium carbonate, and 0.9 ml of distilled water and incubated for 10 minutes at 25°C under fluorescent lamp illumination. After incubation, absorbance at 560 nm was recorded. One unit of SOD activity is considered as the amount of enzyme that inhibited 50% of NBT reduction (Panda, 2012).

Catalase activity was assayed using the method of titration. 1 ml of enzyme extract was mixed in a conical flask with 3 ml of 0.1 M potassium phosphate buffer (pH 7) and 1 ml of 100 μ M H_2O_2 . The mixture was then incubated at 25°C for 1 minute. Catalase is responsible for catalyzing the decomposition of H_2O_2 into O_2 and H_2O . The reaction was stopped by adding 10 ml

of 2% sulfuric acid. A control set titration was also prepared without the addition of the enzyme extract to the reaction mixture. The remaining H_2O_2 was titrated with 0.02 N potassium permanganate till a faint pink color was obtained.

The assay of glutathione reductase was carried out based on the reduction of GSSG to GSH with NADPH as the electron donor (Khaleghi *et al.*, 2019). 1 ml of a solution consisting of 0.2 M Tris-HCl (pH 7.6), 3 mM EDTA, 8.4 mM NADPH, and 50 mM GSSG

was added to 50 μL of enzyme aliquot. The absorbance was then measured using a UV spectrophotometer at 340 nm against a blank without the enzyme extract.

The measurement of ascorbate peroxidase activity was carried out according to the method of Panda (2012). To 3 ml of reaction mixture (0.2 M Tris-HCl buffer, 50 mM ascorbic acid, and 0.5 mM H_2O_2), 30 μL of plant extract was added at 25°C. The decrease in absorbance at 290 nm for one minute was then recorded.

Table 1. Temporal variations of heavy metals concentrations in *Quercus cerris* leaves and soil samples.

Heavy metals (ppm)	Site/ Month	June	July	August	September	October	P-value (temporal variation) ^a
[Cd] leaves	Ctrl	0.02±0.00	0.03±0.00	0.07±0.00	0.19±0.04	0.30±0.07	<0.001
	S1	0.23±0.01	0.61±0.02	0.67±0.04	0.75±0.09	0.96±0.01	<0.001
	S2	0.16±0.01	0.52±0.03	0.58±0.02	0.66±0.09	0.78±0.03	<0.001
[Cd] soil	Ctrl	0.16±0.04	0.09±0.07	0.19±0.03	0.77±0.03	0.86±0.03	<0.001
	S1	0.18±0.06	0.62±0.16	0.75±0.04	0.66±0.14	1.07±0.07	<0.001
	S2	0.18±0.01	0.57±0.02	0.63±0.04	0.69±0.15	1.61±0.04	<0.001
[Cr] leaves	Ctrl	0.13±0.01	0.12±0.00	0.13±0.00	0.12±0.01	0.12±0.01	>0.05
	S1	0.12±0.01	0.12±0.01	0.12±0.01	0.12±0.00	0.13±0.00	>0.05
	S2	0.12±0.03	0.12±0.00	0.12±0.00	0.12±0.02	0.13±0.02	>0.05
[Cr] soil	Ctrl	1.26±0.03	1.19±0.10	1.25±0.02	1.23±0.06	1.28±0.00	>0.05
	S1	1.23±0.07	1.18±0.05	1.22±0.01	1.25±0.01	1.25±0.04	>0.05
	S2	1.27±0.01	1.24±0.08	1.2±0.04	1.25±0.02	1.26±0.03	>0.05
[Pb] leaves	Ctrl	6.95±0.53	5.99±0.15	4.85±0.69	10.38±0.35	4.49±0.22	<0.001
	S1	7.74±0.22	7.13±0.25	6.42±0.44	12.35±0.59	7.94±0.64	<0.001
	S2	7.73±0.57	7.97±0.23	7.91±0.88	13.16±0.25	8.40±0.28	<0.001
[Pb] soil	Ctrl	28.76±0.79	41.60±2.45	29.90±1.08	36.51±5.18	33.32±0.87	<0.001
	S1	28.91±0.75	54.11±2.98	46.06±3.34	45.44±1.94	42.50±1.22	<0.001
	S2	43.58±0.44	55.56±3	86.46±2.84	62.33±2.01	42.00±2.97	<0.001
[Al] leaves	Ctrl	0.93±0.07	0.48±0.03	0.83±0.05	0.6±0.03	0.13±0.01	<0.001
	S1	0.94±0.04	1.02±0.03	0.90±0.02	0.83±0.02	1.03±0.02	<0.001
	S2	0.93±0.15	1.05±0.09	0.93±0.03	0.78±0.08	0.86±0.07	0.017
[Al] soil	Ctrl	35.36±0.63	26.87±1.31	26.26±1.48	25.58±0.67	26.63±0.63	<0.001
	S1	34.31±1.98	31.81±0.76	31.82±0.66	35.16±1.26	33.13±0.53	0.006
	S2	36.02±2.16	33.72±2.14	30.45±1.19	32.43±0.68	33.06±1.46	0.007

Data are represented as a mean of 3 samples ± SD. ^aP-value for the comparison between months. Significant P-values at probability levels < 0.05 are in bold.

Guaiacol peroxidase activity was assayed by adding 30 μ L of the enzyme extract to 3 ml of a reaction mix containing 50 mM sodium acetate buffer (pH 7), 25 mM guaiacol, and 25 mM H_2O_2 . One unit of GPX is defined as the amount of enzymes that cause an absorbance increase of 0.01 per minute at 470 nm (Li, 2003).

Statistical data analysis

All experiments were performed thrice, and the results were presented as mean $\pm$ standard deviation. The significant differences between the means of the Ctrl site and each of the polluted sites were determined by using the ANOVA test, which were considered significant at p -value < 0.05 . One-way ANOVA was also applied to determine the significant impact of months on the studied parameters in each sampling site.

Results

Cd, Cr, Pb, and Al concentrations in *Quercus cerris* leaves and soil from the three investigated sites are presented in Figures 2 and 3, respectively. Cd recorded its highest concentrations in roadside *Quercus cerris* leaves, followed by the S2 site, and then the Ctrl area. The Cd content in the leaves increased progressively at the three sites throughout the studied months and reached its greatest levels in October ($p < 0.001$) (Figure 2a; Table 1). Cd concentrations in the soil sampled from the three different sites ranged from 0.1 to 1.62 ppm (Figure 3a). Cd scored its greatest levels in the polluted sites in almost all months with an exception in June and September, when there was no significant difference between the Ctrl and polluted sites. In July and August, S1 recorded the highest levels, while

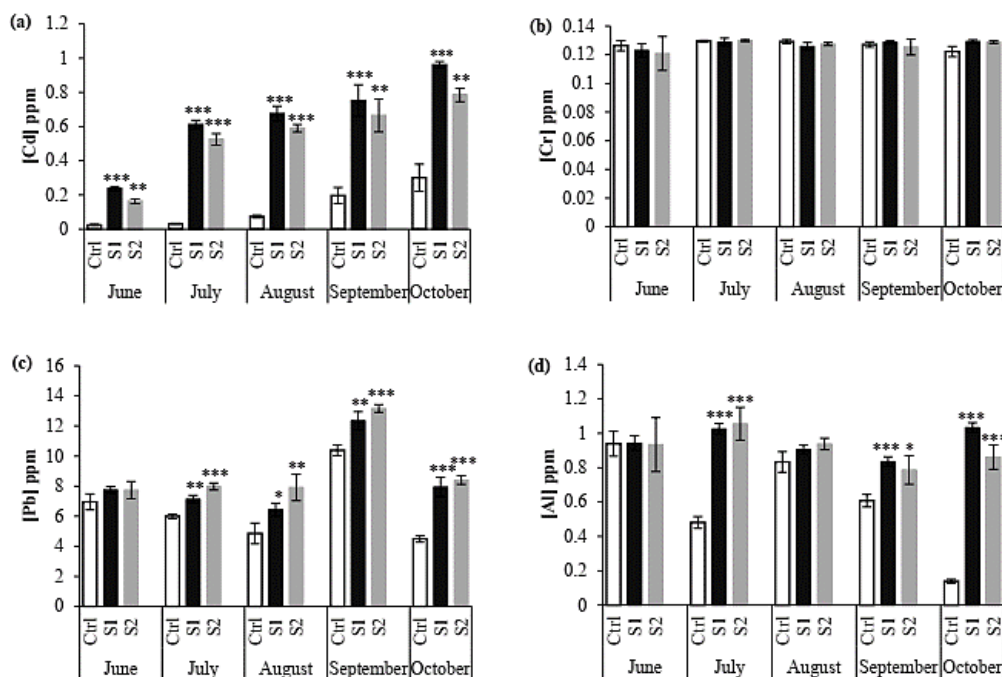


Figure 2. Heavy metals concentrations (a): Cd, (b): Cr, (c): Pb, (d): Al in *Quercus cerris* leaves sampled from polluted and control sites (Significant at: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

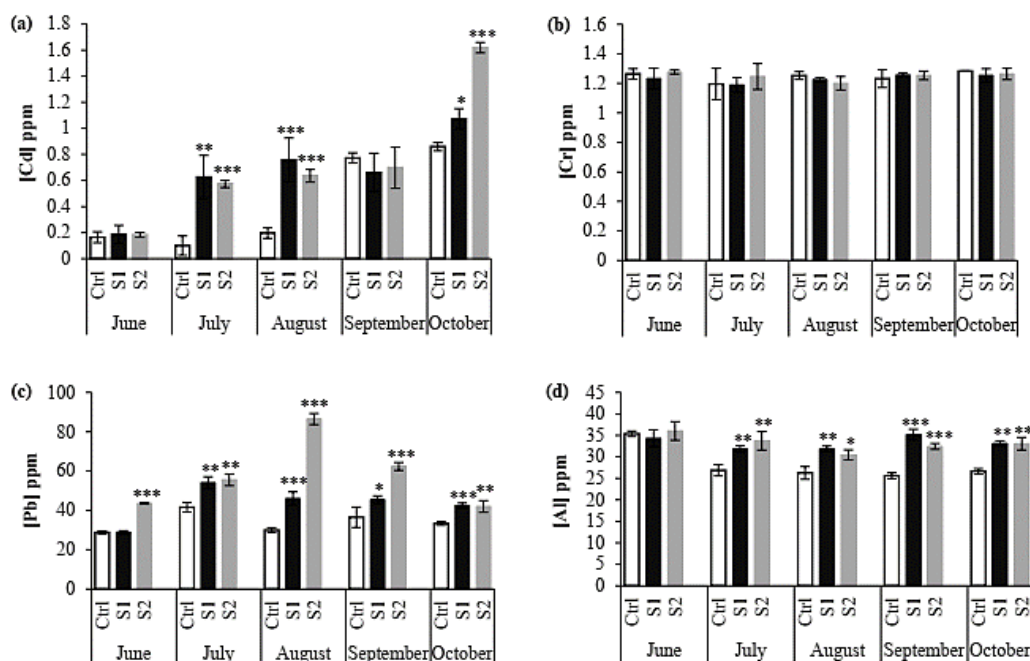


Figure 3. Heavy metals concentrations (a): Cd, (b): Cr, (c): Pb, (d): Al in soil sampled from polluted and control sites (Significant at: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

in October S2 surpassed the S1 site. Cd was found to score the highest concentrations at the three sites in October when compared to the previous months (Table 1).

On the other hand, the findings of this study showed that there was no significant difference between the concentrations of Cr in leaves and soil samples in polluted and control areas (Figures 2b–3b). Additionally, Cr maintained its levels in leaves and soil from the three sites throughout the study ($p > 0.05$) (Table 1). As for Pb, the results revealed a significant difference in Pb concentrations between sampling sites and periods in both *Quercus cerris* leaves and soil samples (Table 1). The levels of Pb in *Quercus cerris* leaves collected from experimental sites were higher than those from the Ctrl site. The highest levels of Pb were observed throughout the studied months at the S2 site. It is worth noting that Pb levels notably increased in the three studied sites in September compared to the previous months (Figure 2c). Pb also noted its

highest levels in soil sampled from the S2 site, followed by S1, and then the Ctrl site. The maximum increase was observed in August at the S2 site, where it surpassed the Ctrl site by 57% increment (Figure 3c). Finally, from this study, an elevation in Al content in *Quercus cerris* leaves was recorded at the polluted sites over the experimental months, except for June and August, when no considerable difference in Al levels was noted between the three chosen sites. In July, the S2 site recorded the greatest Al levels, followed by S1. In September and October, S1 surpassed the other sites (Figure 2d). Similarly to the leaf samples, Al levels in soil did not show any noteworthy difference between sampling sites in June. However, in the following four experimental months, the Al levels in soil collected from polluted environments were greater than those from the control area. During these months, S1 had the highest levels, except for July when the S2 site surpassed S1 (Figure 3d).

Carbohydrates are osmoprotectants with ROS scavenging activities. In the current study, the uppermost recorded levels of carbohydrates were at the control site (Figure 4). A stark difference is shown between the polluted and Ctrl sites. Carbohydrate levels decreased from Ctrl to S2 to S1. Besides, carbohydrate levels changed markedly over the months at each site ($p < 0.01$ at S1 and $p < 0.001$ at Ctrl and S2 sites) (Table 2).

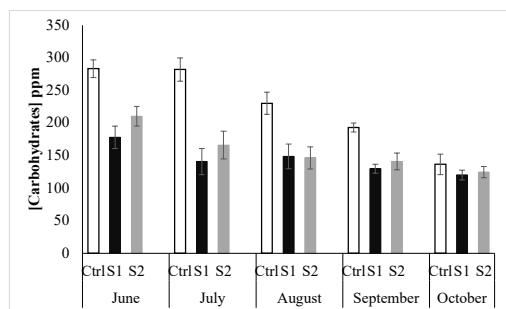


Figure 4. Variation of the total carbohydrate content in the different sites during the study period (Significant at: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Hydrogen peroxide levels in *Quercus cerris* leaves are drawn in Figure 5. The first remark to note is that the highest levels of hydrogen peroxide were recorded in the polluted sites. The data obtained for H_2O_2 ranged from 20.62 to 27.40 ppm, 23.28 to 46.04 ppm, and 28.92 to 46.04 ppm in the Ctrl, S1, and S2 sites, respectively.

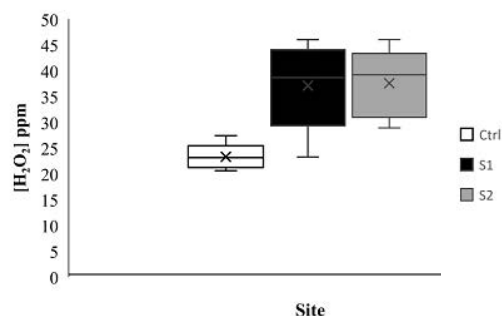


Figure 5. Variation of hydrogen peroxide levels in the three studied areas.

Total phenolic compounds levels were determined in *Q. cerris* leaves from the three studied sites (Figure 6). Total phenolic contents in *Q. cerris* leaves from the polluted sites were considerably lower than at the Ctrl site. In addition to the spatial variations, significant differences among the months were observed ($p < 0.001$ for the three sites) (Table 2).

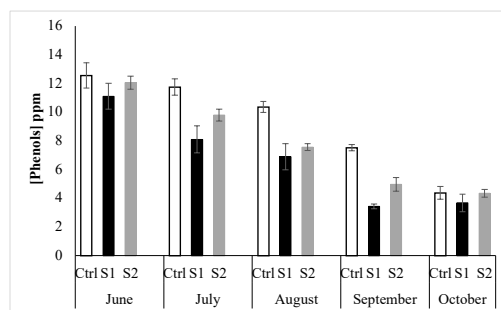


Figure 6. Variation of the total phenolic content in the three selected sites (Significant at: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Activities of enzymatic scavengers are presented in Figure 7. In June, SOD activity was found to be higher in S1 and S2 sites than in the Ctrl site. However, in July, August, and October, the Ctrl site surpassed the polluted sites (Figure 7a). As for catalase, peroxidases, and glutathione reductase, they recorded their highest activities in polluted sites S1 and S2 in almost all the months (Figures 7b;7c;7d;7e). These results are consistent with those observed for H_2O_2 . In fact, the latter noted its highest levels in the polluted sites. Along with spatial variation, the antioxidant enzymes displayed temporal variation (Table 2). Peroxidase activities were found to remarkably increase in the three investigated sites in September and October compared to the previous months ($p < 0.001$).

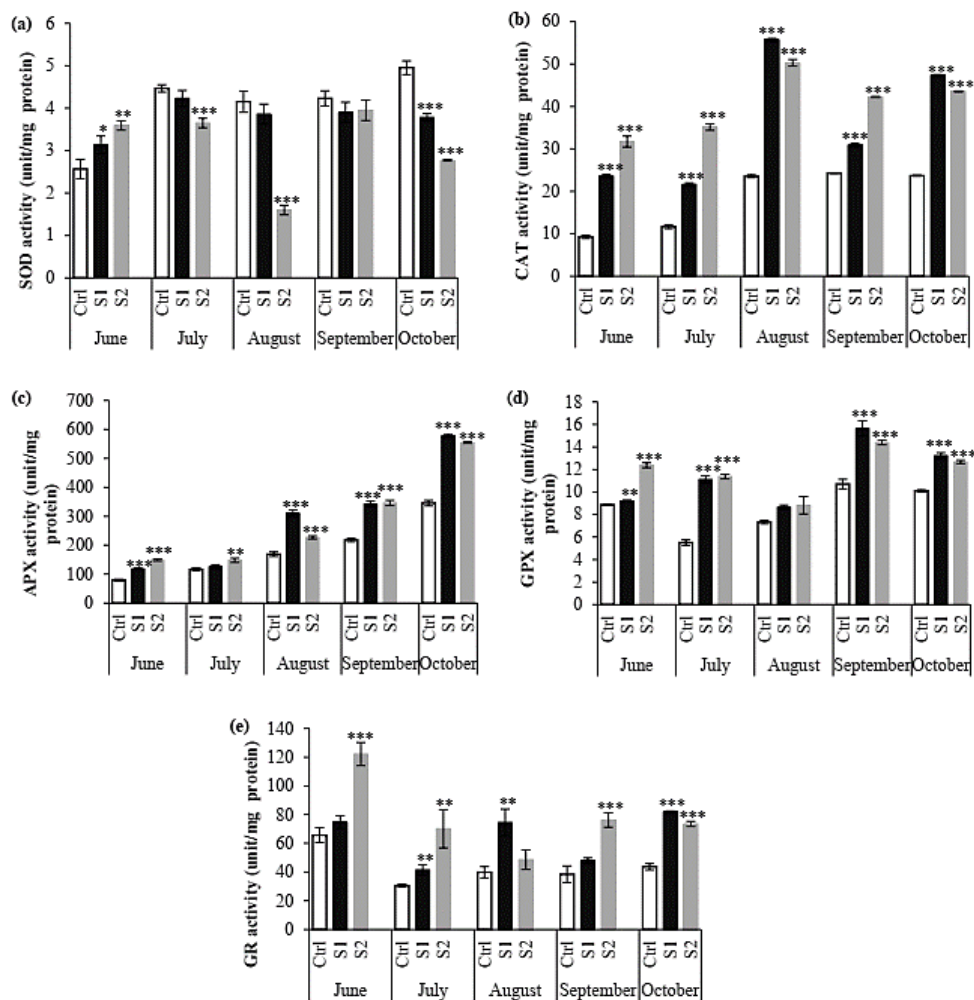


Figure 7. Antioxidant enzymes activity of *Quercus cerris* leaves sampled from polluted and control sites; (a): SOD, (b): catalase, (c): ascorbate peroxidase, (d): guaiacol peroxidase and (e): glutathione reductase (Significant at: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Table 2. Temporal variations of enzymatic and nonenzymatic scavenger activities in *Quercus cerris* leaves.

Bio-chemical parameters	Site/Month	June	July	August	September	October	<i>P</i> -value (temporal variation) ^a
Carbo-hydrates (ppm)	Ctrl	283.42 ± 13.71	282.17 ± 17.83	230.37 ± 16.91	193.22 ± 6.83	136.61 ± 15.73	<0.001
	S1	177.91 ± 17.91	140.93 ± 20.05	148.74 ± 18.94	129.97 ± 6.85	120.25 ± 7.59	0.003
	S2	210.50 ± 15.05	166.18 ± 21.18	146.4 ± 17.22	140.98 ± 12.91	124.60 ± 8.74	<0.001
Phenols (ppm)	Ctrl	12.55 ± 0.88	11.74 ± 0.57	10.36 ± 0.39	7.52 ± 0.22	4.37 ± 0.44	<0.001
	S1	11.10 ± 0.90	8.10 ± 0.95	6.89 ± 0.91	3.43 ± 0.16	3.67 ± 0.61	<0.001
	S2	12.05 ± 0.45	9.79 ± 0.41	7.56 ± 0.24	4.96 ± 0.47	4.35 ± 0.27	<0.001
SOD (unit/mg protein)	Ctrl	2.57 ± 0.23	4.47 ± 0.08	4.47 ± 0.25	4.23 ± 0.18	4.95 ± 0.16	<0.001
	S1	3.14 ± 0.21	3.14 ± 0.24	3.86 ± 0.23	3.90 ± 0.24	3.79 ± 0.09	<0.001
	S2	3.59 ± 0.11	3.65 ± 0.11	1.60 ± 0.11	3.95 ± 0.24	2.77 ± 0.02	<0.001
CAT (unit/mg protein)	Ctrl	9.26 ± 0.40	11.65 ± 0.43	23.56 ± 0.34	24.29 ± 0.07	23.69 ± 0.11	<0.001
	S1	23.62 ± 0.34	21.67 ± 0.35	55.73 ± 0.32	30.94 ± 0.36	47.38 ± 0.19	<0.001
	S2	31.73 ± 1.29	35.15 ± 0.76	50.30 ± 0.78	42.24 ± 0.12	43.55 ± 0.13	<0.001
GPX (unit/mg protein)	Ctrl	8.87 ± 0.08	5.51 ± 0.23	7.32 ± 0.15	10.71 ± 0.42	10.10 ± 0.10	<0.001
	S1	9.22 ± 0.05	11.12 ± 0.33	8.66 ± 0.18	15.66 ± 0.66	13.23 ± 0.26	<0.001
	S2	12.37 ± 0.22	11.37 ± 0.21	8.82 ± 0.77	14.39 ± 0.20	12.67 ± 0.194	<0.001
APX (unit/mg protein)	Ctrl	79.56 ± 2.86	116.44 ± 5.40	168.88 ± 7.74	217.73 ± 5.78	345.33 ± 9.57	<0.001
	S1	118.55 ± 2.07	125.91 ± 6.78	310.49 ± 11.19	343.11 ± 8.66	577.72 ± 4.27	<0.001
	S2	149.00 ± 3.75	147.89 ± 7.23	226.80 ± 5.27	345.89 ± 9.02	554.62 ± 2.77	<0.001
GR (unit/mg protein)	Ctrl	65.68 ± 5.17	30.56 ± 0.96	39.90 ± 3.91	38.43 ± 5.83	43.70 ± 2.21	<0.001
	S1	74.73 ± 4.40	41.55 ± 3.63	74.58 ± 9.22	47.91 ± 1.93	81.96 ± 0.59	<0.001
	S2	122.16 ± 8.01	70.15 ± 13.33	48.64 ± 6.80	76.24 ± 5.02	73.55 ± 1.77	<0.001

Data are represented as a mean of 3 samples ± SD. ^a*P*-value for the comparison between months. Significant *P*-values at probability levels < 0.05 are in bold.

Discussion

Certain metals like micronutrients are deemed to be essential for normal plant growth and metabolism. Other non-essential metals such as Cd, Cr, Al, and Pb can be absorbed and accumulated by plants, thus inducing severe toxicity even at low

applied levels. They may originate from various sources. Among these, vehicles are responsible for considerable emissions of toxic metals into the atmosphere. These metals are released via various processes like fossil fuel burning, brake lining, tire wear, corrosion of metallic parts, and road surface degradation. Once emitted in am-

bient air, metals can travel long distances or attach to dust particles and disturb vital physiological and metabolic processes in plants (Shahid *et al.*, 2017).

Cd has no vital biological or physiological functions but is potentially toxic even at very low concentrations. The toxicity of Cd derives from its ability to decrease the photosynthetic pigment contents, depress the quantum yield of PSII, and decrease enzymes' activities by reacting with their sulfhydryl group (Ojekunle *et al.*, 2014). In the current study, Cd levels in *Q. cerris* leaves were found to be significantly increased at polluted sites when compared to the Ctrl site. Depending on the distance between the trees and the unpaved road, the levels of Cd in leaves varied: the highest Cd levels were in the roadside trees, followed by S2, and then trees from the Ctrl site. The acceptable limit of Cd in plants according to FAO/WHO (2001) is 0.2 ppm, but Cd exceeded this value in almost all samples collected from the polluted sites throughout the study. Furthermore, even samples from the Ctrl site exhibited worrying levels, exceeding the acceptable limit in October with a value of 0.3 ± 0.07 ppm. Skrynetska *et al.* (2018) indicated that Cd could be absorbed by the tree from the soil or directly taken up by the tree's foliar system from the air and falling dust. Additionally, once in the air, Cd can travel and reach places distant from the source of emission. The main sources of Cd in the environment are the burning of fossil fuel, incineration of garbage, and dumping of plastic waste (Ojekunle *et al.*, 2014). Similarly, Cd recorded its greatest levels in the soil from the polluted sites in July, August, and October. Despite these variations, Cd levels in the Ctrl (ranging from 0.09 to 0.86 ppm), S1 (0.18 to 1.07 ppm), and S2 (0.18 to 1.61 ppm) sites remained within the critical standard established by the FAO/WHO (2001), which is 3 ppm across all studied months. On the other hand, it is worth mentioning that Cd contents in leaves and soil samples from three sites in-

creased over months ($p < 0.001$) and scored their uppermost levels in October, indicating a potential risk of Cd accumulation in soil and *Quercus cerris* leaves and showing an increase in environmental pollution in autumn when vehicular traffic and recreational activities notably increased.

As for Cr, this metal is well recognized as an inhibitory element that impedes plants' growth and development. It is commonly found in soil as Cr(III) and Cr(VI). The latter derives its toxicological properties from its capability to traverse cellular membranes and oxidize key biomolecules (Singh & Kalamdhad, 2011). Additionally, the accumulation of excessive Cr can decrease the translocation of a broad array of nutrients, including P, Ca, K, Mn, and Mg from the roots to the aerial parts of plants (Emamverdian *et al.*, 2015). The permissible limits of Cr in soil by FAO/WHO (2001) is 100 ppm. Moreover, chromium concentrations within the range of 5 to 30 ppm in plants can result in reduced plant yield. The levels of Cr in the three studied sites were below these target values.

As for Pb, the findings of this research study showed that the Ezer forest is the most endangered by Pb contamination. The levels of Pb in *Q. cerris* leaves ranged from 4.49 to 10.38 ppm, 6.42 to 12.35 ppm, and 7.73 to 13.16 ppm in Ctrl, S1, and S2 sites, respectively. The S2 site, experiencing recreational activities and vehicle parking, exhibited the highest levels of Pb during the study period, followed by the S1 site. The FAO/WHO's (2001) permissible value for Pb in plants (0.3 ppm) was exceeded at almost all the spots throughout the experimental months. Pb levels in soil were also significantly higher at polluted sites S1 (28.91 to 54.11 ppm) and S2 (42.01 to 86.46 ppm) compared to the Ctrl site (28.76 to 41.6 ppm). Despite this increase in Pb levels at the polluted sites, they still remained below the maximum permissible limit set by the FAO/WHO (2001), which is 300 ppm throughout the study. Although Pb exists naturally at

low concentrations in the soil, its highest concentrations found in the environment are due to manmade activities, especially from its usage in gasoline and exhaust burning (Wao *et al.*, 2014). The elevated Pb content at the S2 site may be attributed to vehicle parking, the usage of leaded petrol, lighting of fires, and burning of garbage. Very low Pb concentrations in soils may produce noxious effects on plants, such as the inhibition of photosynthesis, respiration, and mitosis processes, while high Pb levels may reduce soil productivity (Singh & Kalamdhad, 2011).

Al toxicity has been reported in plants grown in acid soil with pH values less than 5.5 along with Al concentrations ranging from 2 to 3 ppm. The danger of Al lies in its ability to inhibit root growth, induce root damage, and alter the photosynthetic apparatus performance. In the Ezer forest, Al scored the greatest levels in *Quercus cerris* leaves and soil from the polluted sites in almost all months, reaching 35.16 ± 1.26 ppm in S1 site and 36.02 ± 2.16 ppm in S2 site. Compared to the critical levels of Al for plants (2–3 ppm), these values significantly exceeded the acceptable range, indicating a concerning level of contamination in the study area. This increase in Al levels at the polluted sites could be attributed to the deposition of road dust containing substantial amounts of heavy metals such as Pb, Zn, and Al, as well as inappropriate garbage disposal practices, like the throwing of beverage cans on the ground at the recreational site (S2) (Khan & Strand, 2018). Al is also found in wheels, electrical wiring, and vehicle engine parts. The results of this study align with the findings of other researchers. Onder *et al.* (2007) reported high toxic levels of Pb in soil and Cd and Cr metals in grass growing along roadsides with elevated traffic activity. Hour *et al.* (2020) also noted an increase in Cd, Cr, and Al levels in roadside *Urginea maritima* (L.) Baker plants when compared with those from the Ctrl area situated 800 meters away from the road.

Carbohydrates act as osmolytes to preserve osmotic balance and maintain cellular turgor. It has been reported that carbohydrate accumulation varies depending on the sensitivity of plant species to environmental pollution. Research has demonstrated that tolerant species accumulate carbohydrates under polluted conditions, while less resistant species show lower accumulation (Agbaire, 2016). In the studied area, the carbohydrate content of the leaves diminished as the distance from the road decreased. The reduction in the total carbohydrate content could be attributed to increased respiration rate and decreased CO₂ fixation. Carbohydrates are directly linked to photosynthesis, so the fluctuations of the carbohydrates concentrations reflect the performance of the photosynthetic apparatus. The findings of this study correlate with those of Tzvetkova & Kolarov (1996). They reported a considerable decrease in the total sugar content of *Quercus cerris* leaves exposed to heavy metal pollution when compared with those from the non-polluted site. They suggested that the negative impact of heavy metals on carbohydrate metabolism could be due to the interaction of heavy metals with the reactive center of the Calvin cycle's key enzyme ribulose 1,5-bisphosphate carboxylase. On the other hand, carbohydrate levels were also found to experience seasonal variations. ANOVA tests revealed significant decreases in the three sites during fall months ($p < 0.001$ at Ctrl and S2 site and $p < 0.01$ at S1 site). This decline could be explained by the fact that generally during autumn, daylight hours become shorter and temperature drops, leading to a decrease in the photosynthesis process (Gennu, 2021). Furthermore, during fall, deciduous trees undergo senescence that is characterized by the translocation of nutrients, particularly carbohydrates, from the leaves to the other tissues like stems and roots for storage. As nutrients are relocated for storage, the leaves experience a decline in their carbohydrate content (Da Silva *et al.*, 2014).

Hydrogen peroxide is a non-radical reactive oxygen species. Under normal situations, it is continuously generated as a normal product in various cellular compartments. H_2O_2 is the most stable and mobile species among all ROS and thus acts as a signaling molecule facilitating several physiological mechanisms such as cell growth, photosynthesis, and photorespiration (Hossain *et al.*, 2015). Additionally, it is widely accepted that H_2O_2 imparts tolerance to plants against a broad variety of stress conditions via activating stress-sensitive genes (Christou *et al.*, 2014). Nevertheless, under severely adverse conditions, H_2O_2 can be harmfully overexpressed and ultimately inducing oxidative damage, leading to cell death (Das & Roychoudhury, 2014). In the present study, H_2O_2 levels were found to be increased in polluted sites S1 and S2 with a mean of 37.09 ± 8.67 ppm and 37.56 ± 6.71 ppm, respectively. The rise in H_2O_2 content in the polluted sites depicts the establishment of oxidative stress in *Quercus cerris* leaves in response to vehicular and human activities. Our findings correlate with Khairallah *et al.* (2018) and Gutiérrez-Martínez *et al.* (2020), who reported a considerable elevation in H_2O_2 levels in plants exposed to high contamination load.

In order to counteract the oxidative stress imposed by contamination conditions, plants have developed a well-equipped antioxidant defense system composed of enzymatic and nonenzymatic scavengers. Phenols, through their antioxidant activity, help plants withstand oxidative stress. In the present study, they showed their lowest levels in *Q. cerris* trees within the road's matrix. The decrease in phenolic content in the polluted sites indicates that atmospheric pollutants, including heavy metals, had negatively affected *Q. cerris* trees by altering their secondary metabolites synthesis. Hayat *et al.* (2021) reported that heavy metals at toxic levels can suppress genes involved in phenols and flavonoid production. Additional-

ly, significant temporal variations were observed for the total phenolic content throughout the months. According to the ANOVA tests, the total phenolic content revealed statistically significant differences among the five months, with the lowest levels recorded in the fall months across the three sites ($p < 0.001$). Studies on *Fagus sylvatica* L. leaves and other deciduous trees such as *Quercus robur* L. have shown a decline in total phenolics, hydrolyzable tannins, and flavonoids from spring to fall. This decrease in the total phenolic content is part of the natural process of autumn senescence and nutrient translocation as trees prepare for winter dormancy (Formato *et al.*, 2022).

The chief antioxidant enzymes superoxide dismutase, catalase, and peroxidases function in collaboration to get rid of the overexpressed ROS and keep the balance of the oxidative-antioxidative network. The results of enzymatic scavengers revealed that SOD activity decreased in the polluted sites in almost all months except for June. As for catalase, peroxidases, and glutathione reductase, they recorded their highest activities in polluted sites S1 and S2 in almost all the months. These results concord with the ones observed for H_2O_2 . In fact, the latter reached its highest levels in the polluted sites. Along with the spatial variations, the antioxidant enzymes displayed temporal variations. Peroxidases' activities have considerably risen in September and October in the three sites when compared with the previous months. This elevation of enzymatic scavengers' activity during autumn might not only be a response to pollution stress but could be regarded as a tolerant and adaptive response to climatic changes like the temperature drop. Li (2003) reported that peroxidase activity in many tree species changes with seasons. Many researchers reported an elevation in the antioxidant enzymes' activities in plant species subjected to pollution conditions. In a study conducted by Nadgórska-Socha *et al.* (2013), an increase was

noticed in peroxidase and catalase activities in *Vicia faba* L. plants growing in heavy metal-contaminated soil. Similarly, Rai (2016) noticed in his study a remarkable elevation of the peroxidase and catalase content in all of the studied roadside plant species exposed to atmospheric pollution stress. The results obtained in this study are in agreement with these reports as catalase and peroxidase activities, but not SOD activities, were considerably greater in the polluted sites than in the Ctrl site. Several studies reported that SOD failed to increase under pollution stress. One possible reason is that H_2O_2 interacts with SOD in a peroxidase reaction, which can lead to the oxidative modification of SOD. This modification may then interfere with the normal function of the enzyme SOD (Li, 2003). Similarly increased enzymatic activities and reduced phenolic contents have been noted by Hayat *et al.* (2021) in *Cajanus cajan* (L.) Huth exposed to heavy metals stress. It is well known that plants develop various adaptation strategies to compensate for oxidative damage resulting from unfavorable environmental conditions.

Conclusion

Vehicular emissions are a key source of heavy metal contamination, arising from the combustion of diesel and gasoline, as well as non-exhaust sources such as tire wear, brake wear, and road surface degradation. Road traffic using leaded petrol is a principal source of lead, while tires contribute to Zn, Cd, and Ni contamination. Brake linings are a significant source of Cu, and lubricants introduce Cr, Cd, Hg, Pb, Ni, and Zn into the environment. The findings of this study indicate that the Ezer forest is at risk of Cd, Pb, and Al contamination at polluted sites S1 and S2. Heavy metal contamination has induced oxidative stress, evidenced by elevated levels of oxidative stress marker H_2O_2 at the polluted sites. This stress was accompanied by

significant reductions in the total carbohydrate and phenolic content, in contrast to the Ctrl site. However, *Q. cerris* exhibited adaptive responses by increasing the scavenging activity of peroxidases and catalase to mitigate the effects of oxidative stress. Despite the high levels of toxic metals, *Q. cerris* survived and demonstrated potential as a phytoremediator. Phytoremediation, an eco-friendly and cost-effective approach, involves using plants to remove metals from polluted soils. To protect *Q. cerris*, a rare species in Lebanon, strict environmental policies are urgently needed. Measures such as promoting public transport, restricting recreational vehicle access, and limiting campfire lighting in the forest are recommended.

This research contributes to a broader understanding of plant resilience mechanisms by providing valuable insights into antioxidant enzyme activity in response to heavy metal-induced oxidative stress. The findings underscore the potential of *Q. cerris* as a phytoremediator and highlight the need for conservation efforts, contributing to global discussions on forest protection, pollution, and plant adaptation.

References

- Agbaire, P.O. 2016. Impact of air pollution on proline and soluble sugar content of selected plant species. - Chemistry and Materials Research, 8(5), 72-76.
- Christou, A., Manganaris, G.A., Fotopoulos, V. 2014. Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants via transcriptional regulation of enzymatic and non-enzymatic antioxidants. - Environmental and Experimental Botany, 107, 46-54.
- Da Silva, D., Qin, L., DeBuse, C., DeJong, T.M. 2014. Measuring and modelling seasonal patterns of carbohydrate storage and mobilization in the trunks and root crowns of peach trees. - Annals of Botany, 114(4), 643-652.
- Das, K., Roychoudhury, A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. - Frontiers in Environmental Science, 2(53), 1-13. <https://doi.org/10.3389/fenvs.2014.00053>.

- Emamverdian, A., Ding, Y., Mokherdoran, F., Xie, Y. 2015. Heavy metal stress and some mechanisms of plant defense response. – The Scientific World Journal, 2015, 756120. <https://doi.org/10.1155/2015/756120>.
- Ezilon Maps. 2015. Lebanon Map – Physical Map of Lebanon. [WWW document]. – URL <https://www.ezilon.com/maps/asia/lebanon-road-maps.html>. [Accessed 20 April 2024].
- FAO/WHO. 2001. Codex Alimentarius Commission. Food Additives and Contaminants. ALINORM 01/12A. Joint FAO/WHO Food Standards Programme. Geneva, Switzerland, FAO/WHO. 289 pp.
- Formato, M., Scharenberg, F., Pacifico, S., Zidorn, C. 2022. Seasonal variations in phenolic natural products in *Fagus sylvatica* (European beech) leaves. – Phytochemistry, 203, 113385.
- Gennu, F. 2021. The rise and fall of carbohydrates in the trees of the north. [WWW document]. – URL <https://botany.one/2021/07/the-rise-and-fall-of-carbohydrates-in-the-trees-of-the-north/>. [Accessed 23 April 2024].
- Google Earth. 2024. Ezer Forest Lebanon. [WWW document]. – URL <https://earth.google.com/web/search/ezer+forest+lebanon/@34.47072553,36.20696744,1394.75199005a,3728.2941193d,35y,0h,0t,0r/data=CigiJgokCQL7lvN5pjNAEQD7lvN5pjPAGfE4LAjlvkhAlfsnSGIKrUnAOgMKATA>. [Accessed 20 April 2024].
- Gutiérrez-Martínez, P.B., Torres-Morán, M.I., Romero-Puertas, M.C., Casas-Solís, J., Zarazúa-Villaseñor, P., Sandoval-Pinto, E., Ramírez-Hernández, B.C. 2020. Assessment of antioxidant enzymes in leaves and roots of *Phaseolus vulgaris* plants under cadmium stress. – Biotecnica, 22(2), 110–118. <https://doi.org/10.18633/biotecnica.v22i2.1252>.
- Hartmann, J., Asch, F. 2019. Extraction, storage duration, and storage temperature affect the activity of ascorbate peroxidase, glutathione reductase, and superoxide dismutase in rice tissue. – Biology, 8(4), 70.
- Hasan, S.A., Hayat, S., Wani, A.S., Ahmad, A. 2011. Establishment of sensitive and resistant variety of tomato on the basis of photosynthesis and antioxidative enzymes in the presence of cobalt applied as shotgun approach. – Brazilian Journal of Plant Physiology, 23(3), 175–185.
- Hayat, K., Khan, A., Bibi, F., Salahuddin, Murad, W., Fu, Y., Batiha, G.E., Alqarni, M., Khan, A., Al-Harrasi, A. 2021. Effect of cadmium and copper exposure on growth, physio-chemicals and medicinal properties of *Cajanus cajan* L. (Pigeon Pea). – Metabolites, 11(11), 769.
- Hossain, M.A., Bhattacharjee, S., Armin, S.-M., Qian, P., Xin, W., Li, H.-Y., Burritt, D.J., Fujita, M., Tran, L.-S.P. 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. – Frontiers in Plant Science, 6, 420. <https://doi.org/10.3389/fpls.2015.00420>.
- Houri, T., Khairallah, Y., Al Zahab, A., Osta, B., Romanos, D., Haddad, G. 2020. Heavy metals accumulation effects on the photosynthetic performance of geophytes in Mediterranean reserve. – Journal of King Saud University - Science, 32(1), 874–880.
- Karataş, I., Öztürk, L., Demir, Y., Unlükar, A., Kurunç, A., Düzdemir, O. 2014. Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. – Toxicology and Industrial Health, 30(8), 693–700. <https://doi.org/10.1177/0748233712462471>.
- Khairallah, Y., Hour, T., Osta, B., Romanos, D., Haddad, G. 2018. Biomonitoring airborne pollution: a case study of “*Urginea maritima*” species in Bentaal natural reserve-Lebanon. – Journal of Taibah University for Science, 12(6), 723–729.
- Khaleghi, A., Naderi, R., Brunetti, C., Maserti, B.E., Salami, S.A., Babalar, M. 2019. Morphological, physiochemical and antioxidant responses of *Maclura pomifera* to drought stress. – Scientific Reports, 9, 19250. <https://doi.org/10.1038/s41598-019-55889-y>.
- Khan, R.K., Strand, M.A. 2018. Road dust and its effect on human health: a literature review. – Epidemiology and Health, 40, e2018013. <https://doi.org/10.4178/epih.e2018013>.
- Khatoon, M., Islam, E., Islam, R., Rahman, A.A., Alam, A.H.M.K., Khondkar, P., Rashid, M., Parvin, S. 2013. Estimation of total phenol and *in vitro* antioxidant activity of *Albizia procera* leaves. – BMC Research Notes, 6, 121. <https://doi.org/10.1186/1756-0500-6-121>.
- Li, M.-H. 2003. Peroxidase and superoxide dismutase activities in fig leaves in response to ambient air pollution in a subtropical city. – Archives of Environmental Contamination and Toxicology, 45, 168–176.
- Liu, W.T., Ni, J.C., Zhou, Q.X. 2013. Uptake of heavy metals by trees: prospects for phytoremediation. – Materials Science Forum, 743–744, 768–781. <https://doi.org/10.4028/www.scientific.net/MSF.743-744.768>.
- Morkunas, I., Woźniak, A., Mai, V.C., Rucińska-Sobkowiak, R., Jeandet, P. 2018. The role of heavy metals in plant response to biotic stress. – Molecules, 23(9), 2320.
- Nadgórska-Socha, A., Kafel, A., Kandziora-Ciupa, M., Gospodarek, J., Zawisza-Raszka, A. 2013. Accumulation of heavy metals and antioxidant responses in *Vicia faba* plants grown on monometallic contaminated soil. – Environmental Science and Pollution Research, 20, 1124–1134. <https://doi.org/10.1007/s11356-012-1191-7>.
- Ojekunle, Z.O., Adeboje, M., Taiwo, A.G., Sangowusi, R.O., Taiwo, A.M., Ojekunle, V.O. 2014. Tree leaves as bioindicator of heavy metal pollution in mechanic village, Ogun State. – Journal of Applied Sciences and Environmental Management, 18(4), 639–644.

- Onder, S., Dursun, S., Gezgin, S., Demirbas, A. 2007. Determination of heavy metal pollution in grass and soil of city centre green areas (Konya, Turkey). – *Polish Journal of Environmental Studies*, 16(1), 145–154.
- Panda, S.K. 2012. Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. – El-Missiry, M.A. (ed.). *Antioxidant Enzyme*. London, IntechOpen, 381–400.
- Rai, P.K. 2016. Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. – *Journal of Asia-Pacific Biodiversity*, 9(1), 47–55. <https://doi.org/10.1016/j.japb.2015.10.011>.
- Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F., Kim, K.-H. 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. – *Environmental International*, 125, 365–385. <https://doi.org/10.1016/j.envint.2019.01.067>.
- Saed-Moucheshi, A., Shekoofa, A., Pessarakli, M. 2014. Reactive oxygen species (ROS) generation and detoxifying in plants. – *Journal of Plant Nutrition*, 37(10), 1573–1585. <https://doi.org/10.1080/01904167.2013.868483>.
- Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., Niazi, N.K. 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. – *Journal of Hazardous Materials*, 325, 36–58. <https://doi.org/10.1016/j.jhazmat.2016.11.063>.
- Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. – *Journal of Botany*, 2012(1), 217037. <https://doi.org/10.1155/2012/217037>.
- Singh, J., Kalamdhad, A.S. 2011. Effects of heavy metals on soil, plants, human health and aquatic life. – *International Journal of Research in Chemistry and Environment*, 1(2), 15–21.
- Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M. 2016. Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. – *Frontiers in Plant Science*, 6, 1143. <https://doi.org/10.3389/fpls.2015.01143>.
- Skrynetska, I., Ciepał, R., Kandziora-Ciupa, M., Barczyk, G., Nadgórska-Socha, A. 2018. Ecophysiological responses to environmental pollution of selected plant species in an industrial urban area. – *International Journal of Environmental Research*, 12, 255–267. <https://doi.org/10.1007/s41742-018-0088-9>.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J. 2012. Heavy metals toxicity and the environment. – *Experientia Supplementum (EXS)*, 101, 133–164.
- Türtscher, S., Berger, P., Lindebner, L., Berger, T.W. 2017. Declining atmospheric deposition of heavy metals over the last three decades is reflected in soil and foliage of 97 beech (*Fagus sylvatica*) stands in the Vienna Woods. – *Environmental Pollution*, 230, 561–573.
- Tzvetkova, N., Kolarov, D. 1996. Effect of air pollution on carbohydrate and nutrients concentrations in some deciduous tree species. – *Bulgarian Journal of Plant Physiology*, 22(1–2), 53–63.
- Uddin, A.H., Khalid, R.S., Alaama, M., Abdulkader, A.M., Kasmuri, A., Abbas, S.A. 2016. Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. – *Journal of Analytical Science and Technology*, 7, 6. <https://doi.org/10.1186/s40543-016-0085-6>.
- Wao, A.A., Khare, S., Ganguli, S. 2014. Extraction and analysis of heavy metals from soil and plants in the industrial area Govindpura, Bhopal. – *Journal of Environment and Human*, 1(2), 158–164.
- Yemm, E.W., Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. – *Biochemical Journal*, 57(3), 508–514.