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Some Enzymatic and Non-enzymatic Antioxidants Response under Nickel and Lead Stress for Some Fabaceae Trees

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***Abstract*—This study investigates the effects of soil contamination by nickel and lead on some enzymatic and non-enzymatic antioxidants in addition to the nitrate reductase (NR) enzyme activity for Gleditsia triacanthos*, Leucaena leucocephala, and Robinia pseudoacacia* plant species. The results of this study show a significant increase in peroxidase enzyme activity and a significant decrease in catalase enzyme activity, proline, total carotenoids, and total carbohydrate content of leaves of the three species with increasing the concentration of Ni and Pb except for the total carbohydrate, which increased only for *L. leucocephala* species. Each NR enzyme activity and ascorbic acid content are increased significantly with increasing the concentration of Ni and Pb for *G. triacanthos, L. leucocephala,* and on the contrary, they decreased significantly for *R. pseudoacacia* species. From the result, we can conclude a general increase or decrease in leaves content of some antioxidants content for all the species, whereas there is some peculiarity according to the plant species regarding other contents, which in turn reflects different mechanisms of these species to tolerant heavy metal stress.**

***Index Terms*—Ascorbic acid, Catalase, Heavy metals, Nickel and Lead, Nitrate reductase, Peroxidase, Proline.**

I. Introduction

Soils can be contaminated with heavy metals through industrial waste, gasoline, paint, mining waste, fertilizers, pesticides, animal fertilizers, irrigation of wastewater, and other sources. Lead (Pb) and nickel (Ni) can be considered the most commonly found heavy metals in contaminated sites. Several authors have reported that the toxicity of Pb and Ni could produce oxidative cellular damage when generating reactive oxygen species (ROS), which in turn leads to several detrimental effects on plant cells, so antioxidant enzymes can reduce or prevent the toxic effects of ROS induced by metal stress. (Nyiramigisha, et al., 2021; Kumari and Mishra, 2021 and Álvarez, et al., 2019).

ROS are usually produced due to heavy metals, high light intensity, high temperature, biotic stress, air pollutants, soil salinity, and drought, the imbalance between the ROS and antioxidants defense system in plants creates oxidative stress in the plants. ROS are produced in plants as by-products during many metabolic reactions, such as photosynthesis and respiration. The accumulation of ROS leads to disturbances in normal physiological processes and leads to damage to biomolecules, cells, and tissues. Disturbance in the ratio of pro-oxidant and anti-oxidant leads to potential damage to the cells and tissues. Disturbances in the normal redox state of cells can cause toxic effects. However, ROS has a role in signal transduction pathways by cellular antioxidant machinery, which involves detoxifying enzymes and non-enzymatic antioxidant compounds which can control toxic ROS. ROS are produced from molecular oxygen as a result of normal cellular metabolism. (Sachdev, et al., 2021; Ali, et al., 2020; Das and Aryadeep, 2014).

Antioxidants are the substances that are present in plants at the lower concentrations compared to that of oxidizable substrates, significantly delaying, or preventing the oxidation of substrates. Antioxidants such as carotenoids and ascorbic acid (AA) are non-enzymatic in nature whereas catalase (CAT) and peroxidase (POD) are the major enzymatic antioxidants. Enzymatic antioxidants function by breaking down and removing the free radicals from the cells while non-enzymatic antioxidants work by interrupting free radical chain reactions thus protecting cells from damage. The presence of antioxidant defense is universal in nature, and plants produce it to protect themselves from the ultraviolet light of the sun and ROS generated during photosynthesis that would cause irreparable damage to the plant tissues. Although the mechanisms of antioxidant defenses are changed from plant species to another, the existence of antioxidant defense is general (Ren, et al., 2021; Santos-Sánchez, et al., 2019; Kartoori, et al., 2018).

Gleditsia triacanthos, Leucaena leucocephala, and Robinia pseudoacacia plant species are planted as landscapes in the Iraqi Kurdistan region, and they are important for carbon sequestration, soil stabilization and re-vegetation of

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landfills, mining areas, and wastelands, in biotherapy and landscaping. These plants produce high levels of biomass, grow rapidly, are easily cultivated, and most importantly, tolerate and accumulate high concentrations of heavy metals in the aboveground parts. These plants have numerous inherent characteristics that can be exploited to enhance phytoremediation and lower the cost of regeneration. These species can survive in severe environmental conditions with the exception of heavily frosted conditions and occur in a wide range of ecological settings. They are fast-growing species, capable of reaching maturity in 6–7 months to produce a vast amount of seeds that can germinate into numerous seedlings to carry on further remediation of the polluted site. They are endowed with high proficiency for nitrogen fixation through nodule formation and can substantially revitalize microbial mass and micro-bioactivities to pave the way for the re-establishment of self-sustaining plant communities over the polluted sites (Ssenku, et al., 2017).

One of the 14 essential mineral elements for plants is nickel which is required in small amounts for healthy growth and development. Higher concentrations of Ni in plant cells result in alterations at the physiological, biochemical, and cellular levels leading to severe damage to plants. The most common symptoms of Ni^{2+} toxicity in plants are inhibition of photosynthesis and mitotic activities, inhibition of sugar transport, and reductions in plant growth. Extremely high soil Ni concentrations have left some farmland unsuitable for growing crops, fruits, and vegetables.

In general, the value between 0.5 and 5 $mg \cdot kg^{-1}$ of plant dry weight is the accepted Ni content in plant tissues whereas, for soil is vary between 5 and 150 $mg \cdot kg^{-1}$ dry weight. Total uptake of Ni by plants are depend on many factors including soil solution concentration of Ni^{2+}, the metabolism of plant, soil solution acidity, exist of other metals, and soil contain of organic matter. Regarding lead it is not considered as essential element, also, for living organisms it is relatively considered as unavailable because they are immobe in the soil solution and their transport through the short transport system from roots to plants is very limited. In uncontaminated soil the concentration of Pb is varied within the range of 10–50 $mg \cdot kg^{-1}$; however, its concentration can be expected to range from 30 to 100 $mg \cdot kg^{-1}$ in soil with low-level contamination (Kacálková, et al., 2014; Vatansever, et al., 2010). Ni and Pb uptake by plant roots is not mediated by the same mechanisms. Ni uptake is mainly carried out by roots through a passive diffusion and/or active transport. Pb is generally taken up through the root system through passive diffusion, mainly in the apoplast through the intercellular spaces, following the water movement within the plant (Amari, et al., 2017).

This study aimed to investigate the effects of Ni and Pb elements as soil polluted heavy metals on changes in some enzymatic and non-enzymatic antioxidants in addition to nitrate reductase (NR) in leaves of *G. triacanthos, L. leucocephala, and R. pseudoacacia* as three forest tree species belong to fabaceae family.

II. Materials and Methods

A. Plant Materials and Growth Conditions

The ripe seed pods of *G. triacanthos, L. leucocephala,* and *R. pseudoacacia* were collected on March 2, 2021, in Koya University- Campus (Koya city, Erbil, Iraq, located at 44°38 E, 36°4N and 570 m of altitude above sea level). The pods were collected directly from 10 years aged, with straight bales and well-formed trees by climbing the trees manually. The pods were soaked in ordinary (tap) water at room temperature for 24 h. A factorial, completely randomized design experiment with three replicates was conducted under the ambient environmental conditions with the temperature that varied from (14°C to 39°C) and relative humidity from 19% to 64%.

The healthy plant seeds were cultivated on 25 March 2021. Seeds were sown (2.5 cm depth) in black polyethylene bags (12 cm diameter and 25 cm height) filled with 3 kg of loamy soil mixed with Ni (0, 15, 30 or 45 mg $NiCl_{2.}$ kg^{-1} soil) that was denoted as Ni_0, Ni_{15}, Ni_{30}, and Ni_{45} or Pb (0, 15, 30, or 45 mg $PbCl_2$ kg^{-1} soil) that denoted as Pb_0, Pb_{15}, Pb_{30}, and Pb_{45} and their interactions. (Abdullah, et al., 2018; Olorunmaiye, 2019 and Dirr and Heuser, 1987). The bags were suitably watered with ordinary (tap) water to provide possible uniform soil moisture conditions. 70 days after seed germination 3rd–4th fully matured leaves (Fig. 1) were taken to estimate some enzymatic and non-enzymatic antioxidants in addition to NR enzyme, as follows:

B. Extraction Some Enzymatic and Non-enzymatic Antioxidants and NR Enzyme Activity in the Leaves of the Studied Plant

In fresh leaf samples, the activity of the following enzymes was measured; POD enzyme activity was estimated according to the method described by (Nezih, 1985), where the absorbance was read at 420 nm and the activity of POD [$\mu g.g^{-1}$ fresh weight] was calculated. The activity of the CAT enzyme was estimated according to the method of Aebi (1974), where the absorbance was read at 240 nm using a UV-spectrophotometer (GENESYS 10 UV Spectrometer), Thermo Electron Corporation, USA), and the activity of CAT [microgram. $gram^{-1}$] was calculated. Although NR enzyme is not an antioxidant, we measure it because has an important role in the Fabaceae family species used in the study, the activity of this enzyme was measured by preparing enzyme extract using the method (Ahmad, et al., 2010), for this enzyme the absorbance was read at 540 nm using a spectrophotometer (PD-303) and the activity of NR [$\mu M.l^{-1}$] was calculated. AA [$g.\ l^{-1}$] was determined by the method described by Elbsheer (2018). The Pr [$\mu g.ml^{-1}$] content in fresh leaf samples was determined by the method of Bates, Waldron, and Tears, (1973). The absorbance of the toluene layer was read at 520 nm, using a spectrophotometer (PD-303). The amount of total carotenoids (TC) [$mg.\ g^{-1}$ fresh weight] was estimated according to the method of Lichtenthaler and Wellburn (1983) and total carbohydrates (TCHOs) [%] were determined by the method of Joslyn (1970).

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C. Statistical Analysis

The experiment was conducted according to a factorial completely randomized design. Each treatment was replicated 3 times and ten bags were considered as an experimental unit. Treatment means were compared by the analysis of variance using the SAS Statistical program. Duncan's multiple range test ($\alpha \leq 0.05$) was used for comparing treatment means (Al-Mohammadi and Al-Mohammadi, 2002).

III. Results and Discussion

The results shown in Tables I-III indicated that the effect of Ni and Pb was significant on some leaves

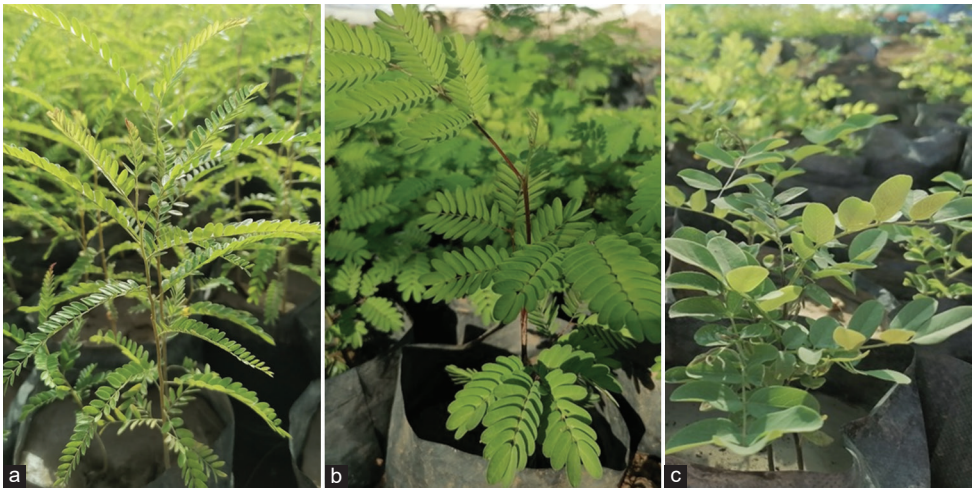

Fig. 1. Three month old of (a) *Gleditsia triacanthos*, (b) *Leucaena leucocephala,* and (c) *Robinia pseudoacacia* species.

Table I
Effects of Nickel, Lead, and their Interactions on Some Enzymatic, Non-enzymatic Antioxidants and Nitrate Reductase Activity of *Gleditsia triacanthos* Plant Leaves

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants			
	Catalase (microgram. Gram^{-1})	Peroxidase (microgram. gram^{-1})	Nitrate Reductase (micromole.L^{-1})	Ascorbic Acid (gram.L^{-1})	Proline (microgram.ml^{-1})	Total Carotenoids (mg. g^{-1} fresh weight)	Total Carbohydrate (%)
Ni concentration (mg.kg^{-1} Soil)							
Ni 0	327.5^{a}	1692.5^{b}	0.17^{c}	1.50^{d}	55.37^{a}	0.367^{a}	1.04^{a}
Ni 15	277.5^{b}	1873.3^{ab}	0.14^{d}	2.19^{c}	44.50^{b}	0.363^{b}	0.66^{c}
Ni 30	194.2^{c}	1699.2^{b}	0.18^{b}	2.77^{a}	37.34^{c}	0.362^{b}	0.76^{b}
Ni 45	139.2^{d}	2030.8^{a}	0.20^{a}	2.30^{b}	35.74^{c}	0.361^{b}	0.30^{d}
Pb concentration (mg.kg^{-1} Soil)							
Pb 0	269.2^{a}	1837.5^{a}	0.18^{ab}	3.22^{a}	48.70^{a}	0.377^{a}	0.60^{b}
Pb 15	194.2^{c}	1738.3^{a}	0.14^{c}	1.49^{c}	43.42^{b}	0.370^{b}	0.80^{a}
Pb 30	236.33^{b}	1804.2^{a}	0.18^{b}	2.63^{b}	39.30^{c}	0.354^{c}	0.78^{a}
Pb 45	238.3^{b}	1915.8^{a}	0.19^{a}	1.49^{c}	41.52^{bc}	0.351^{c}	0.58^{b}
Interactions between Ni and Pb							
Ni0 Pb0	496.7^{a}	1710.0^{b}	0.20^{d}	3.94^{c}	63.35^{a}	0.387^{a}	1.25^{b}
Ni0 Pb15	240.0^{d}	1753.3^{b}	0.17^{e}	1.41^{h}	56.96^{b}	0.370^{b}	0.54^{d}
Ni0 Pb30	216.7^{d}	1593.3^{b}	0.20^{d}	0.53^{j}	51.23^{bc}	0.357^{c}	1.48^{a}
Ni0 Pb45	356.7^{b}	1713.3^{b}	0.11^{g}	0.13k	49.92^{c}	0.353^{c}	0.87^{c}
Ni15 Pb0	303.3^{c}	1826.7^{ab}	0.14^{f}	1.63^{g}	49.46^{c}	0.370^{b}	0.41^{de}
Ni15 Pb15	200.0^{de}	1960.0^{ab}	0.17^{e}	2.66^{e}	45.88^{c}	0.370^{b}	0.99^{c}
Ni15 Pb30	363.3^{b}	1736.7^{b}	0.08^{h}	1.97^{f}	37.69^{fg}	0.357^{c}	1.18^{b}
Ni15 Pb45	243.3^{d}	1970.0^{ab}	0.17^{e}	2.50^{e}	44.92^{cde}	0.350^{c}	0.06^{g}
Ni30 Pb0	136.7^{f}	1873.3^{ab}	0.22^{c}	4.41^{b}	42.46^{def}	0.370^{b}	0.31^{e}
Ni30 Pb15	210.0^{d}	1493.3^{b}	0.14^{f}	0.94^{i}	34.04^{g}	0.370^{b}	1.17^{b}
Ni30 Pb30	216.7^{d}	1926.7^{ab}	0.21^{c}	3.09^{d}	34.96^{g}	0.353^{c}	0.29^{ef}
Ni30 Pb45	213.3^{d}	1503.3^{b}	0.14^{f}	2.66^{e}	37.89^{fg}	0.350^{c}	1.28^{b}
Ni45 Pb0	140.0^{f}	1940.0^{ab}	0.17^{e}	2.91^{d}	39.54^{efg}	0.380^{a}	0.41^{de}
Ni45 Pb15	126.7^{f}	1746.7^{b}	0.07^{h}	0.72^{j}	36.81^{fg}	0.370^{b}	0.51^{d}
Ni45 Pb30	150.0^{fe}	1960.0^{ab}	0.23^{b}	4.91^{a}	33.27^{g}	0.350^{c}	0.16^{fg}
Ni45 Pb45	140.0^{f}	2476.7^{a}	0.33^{a}	0.66^{j}	33.35^{g}	0.350^{c}	0.10^{g}

*Means followed by the same letters with in columns are not significantly different at $P \leq 0.05$ according to the Duncan test. Ni: Nickel, Pb: Lead

Table II
Effects of Nickel, Lead and their Interactions on Some Enzymatic, Non-enzymatic Antioxidants, and Nitrate Reductase Activity of *Leucaena leucocephala* Plant Leaves

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants			
	Catalase (microgram.gram^{-1})	Peroxidase (microgram.gram^{-1})	Nitrate Reductase (micromole.L^{-1})	Ascorbic Acid (gram. L^{-1})	Proline (microgram.ml^{-1})	Total Carotenoids (mg. g^{-1} fresh weight)	Total Carbohydrate (%)
Ni concentration (mg.kg^{-1} Soil)							
Ni 0	200.83^{a}	1366.7^{b}	0.17^{c}	0.74^{d}	75.05^{a}	0.377^{a}	0.22^{c}
Ni 15	133.33^{b}	1535.8^{b}	0.14^{d}	2.93^{a}	58.0^{d}	0.366^{b}	0.23^{c}
Ni 30	132.50^{b}	1801.7^{a}	0.18^{b}	1.66^{c}	66.55^{b}	0.363^{b}	0.45^{a}
Ni 45	131.67^{b}	1989.2^{a}	0.20^{a}	2.03^{b}	60.05^{c}	0.363^{b}	0.37^{b}
Pb concentration (mg.kg^{-1} Soil)							
Pb 0	195.8^{a}	1659.2^{a}	0.18^{ab}	1.08^{c}	63.80^{c}	0.368^{b}	0.31^{b}
Pb 15	150.0^{b}	1644.2^{a}	0.14^{c}	1.05^{c}	70.42^{a}	0.370^{ab}	0.30^{b}
Pb 30	131.7^{bc}	1644.2^{a}	0.18^{b}	2.07^{b}	60.50^{d}	0.372^{a}	0.26^{b}
Pb 45	120.8^{c}	1745.8^{a}	0.19^{a}	3.16^{a}	63.92^{b}	0.358^{c}	0.40^{a}
Interactions between Ni and Pb							
Ni0 Pb0	310.0^{a}	1143.3^{e}	0.20^{d}	0.16^{n}	87.04^{b}	0.390^{a}	0.38^{bcd}
Ni0 Pb15	220.0^{b}	1520.0^{cde}	0.17^{e}	0.22^{n}	71.15^{f}	0.370^{bc}	0.06^{h}
Ni0 Pb30	133.3^{c}	1320.0^{de}	0.20^{d}	1.84^{f}	82.54^{d}	0.377^{b}	0.19^{fgh}
Ni0 Pb45	140.0^{c}	1483.3^{cde}	0.11^{g}	0.72^{l}	59.46^{h}	0.370^{bc}	0.23^{efg}
Ni15 Pb0	200.0^{b}	1510.0^{cde}	0.14^{f}	1.03^{jk}	31.15^{k}	0.360^{d}	0.10^{gh}
Ni15 Pb15	113.3^{cd}	1560.0^{cde}	0.17^{e}	1.47^{h}	84.73^{c}	0.370^{bc}	0.36^{cde}
Ni15 Pb30	133.3^{c}	1560.0^{cde}	0.08^{h}	1.09^{ij}	24.23^{l}	0.370^{bc}	0.06^{h}
Ni15 Pb45	86.7^{d}	1513.3^{cde}	0.17^{e}	8.13^{a}	91.85^{a}	0.363^{cd}	0.39^{bcd}
Ni30 Pb0	136.7^{c}	1836.7^{bcd}	0.22^{c}	0.34^{m}	70.50^{f}	0.360^{d}	0.23^{efg}
Ni30 Pb15	150.0^{c}	1960.0^{abc}	0.14^{f}	1.59^{g}	69.73^{f}	0.370^{bc}	0.50^{ab}
Ni30 Pb30	113.3^{cd}	1853.3^{bcd}	0.21^{c}	2.09^{e}	48.27^{j}	0.370^{bc}	0.56^{a}
Ni30 Pb45	130.0^{cd}	1556.7^{cde}	0.14^{f}	2.63^{d}	77.69^{e}	0.350^{e}	0.52^{ab}
Ni45 Pb0	136.7^{c}	2146.7^{ab}	0.17^{e}	2.78^{c}	66.50^{g}	0.360^{d}	0.51^{ab}
Ni45 Pb15	116.7^{cd}	1536.7^{cde}	0.07^{h}	0.91^{k}	56.08^{i}	0.370^{bc}	0.27^{def}
Ni45 Pb30	146.7^{c}	1843.3^{bcd}	0.23^{b}	3.25^{b}	86.92^{b}	0.370^{bc}	0.21^{fg}
Ni45 Pb45	126.7^{cd}	2430.0^{a}	0.33^{a}	1.16^{i}	30.69^{k}	0.350^{e}	0.48^{abc}

*Means followed by the same letters with in columns are not significantly different at $P \leq 0.05$ according to the Duncan test. Ni: Nickel, Pb: Lead

antioxidants for *G. triacanthos, L. leucocephala,* and *R. pseudoacacia* species, where the activity of POD and NR were increased with increasing Ni and Pb concentrations for the three species, except NR enzyme activity for *R. pseudoacacia* which decreased with increasing the elements concentration. The highest activity of POD (2030.8, 1989.2, and 2418.3 microgram. gram^{-1}) for the three species, respectively, and (0.20 micromole.l^{-1}) NR activity for each of G. triacanthos and *L. leucocephala* was recorded from 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations, whereas the lowest activities (1692.5, 1366.7, and 1767.5 microgram. gram^{-1} and 0.17 micromole.l^{-1}) were recorded in the control treatment, same results were obtained from each (Bartkowiak, Lemanowicz, and Lamparski, 2000; Matraszek, 2008). Whereas the activity of CAT enzyme decreased in the three species by increasing the concentrations to 45 mg.kg^{-1} of Ni and Pb as compared to the control treatment, these results were confirmed by Yan, et al. (2008) and Dey, et al. (2007) who found a decrease in CAT enzyme activity with increasing the concentration of Ni and Pb. The interactions between concentrations of Ni and Pb also had significant effects on all the study parameters for different plants Hussain, et al. (2020) and Andresen, Edgar, and Hendrik (2018). The differences between the species response to Ni and Pb may be due to the uptake of heavy metals from the soil solution depending on species, form and concentration of metal, the soil or nutrient solution acidity and organic matter composition. (Amari, et al., 2017). Pb and Ni are considered as a dangerous pollutant of plants, because they affect many biological processes such as photosynthesis, carbohydrate synthesis, and cell wall by decrease of exchange between inner and outer. (Taha, et al., 2008). Plant exposure to higher concentrations of heavy metals can increase the production of (ROS) and change antioxidant response (Shahid, et al., 2015; Gratão et al., 2005). Antioxidants are the first line of defense against the damages caused by free radicals and are critical for the optimum health of plant cells. Plant antioxidants play a significant role in assisting plant development through a wide variety of mechanisms and functions (Rajput, et al., 2021). In fact, these enzymes are key components in preventing the oxidative stress in plants as the activity of one or more of these enzymes are generally increased in plants when exposed to stressful conditions (Das, et al., 2014). In recent years, a new role for ROS has been identified: the control and regulation of biological processes, such as growth and development. The use of ROS as signaling molecules by plant cells suggests that, during the course of evolution, plants were able to achieve a high degree of control over ROS toxicity and are now using ROS as signaling molecules. (Mittler, et al., 2004).

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Table III
Effects of Nickel, Lead, and their Interactions on Some Enzymatic, Non-enzymatic Antioxidants, and Nitrate Reductase Activity of *Robinia pseudoacacia* Plant Leaves

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants			
	Catalase (microgram. gram^{-1})	Peroxidase (microgram. gram^{-1})	Nitrate Reductase (micromole.L^{-1})	Ascorbic Acid (gram. L^{-1})	Proline (microgram.ml^{-1})	Total Carotenoids (mg. g^{-1} fresh weight)	Total Carbohydrate (%)
Ni concentration (mg.kg^{-1} Soil)							
Ni 0	285.83^{a}	1767.5^{b}	0.127^{a}	0.77^{c}	51.52^{a}	0.380^{a}	1.22^{b}
Ni 15	255.00^{b}	2291.7^{a}	0.112^{b}	0.91^{b}	46.82^{b}	0.370^{b}	1.33^{a}
Ni 30	174.17^{c}	2390.0^{a}	0.124^{a}	1.31^{a}	45.14^{c}	0.370^{b}	0.77^{d}
Ni 45	150.00^{d}	2418.3^{a}	0.093^{c}	0.35^{d}	40.75^{d}	0.370^{b}	0.93^{c}
Pb concentration (mg.kg^{-1} Soil)							
Pb 0	226.7^{a}	1914.2^{b}	0.102^{c}	1.38^{a}	58.94^{a}	0.373^{a}	0.94^{b}
Pb 15	209.2^{a}	2084.2^{b}	0.143^{a}	0.89^{b}	43.58^{b}	0.373^{a}	1.15^{a}
Pb 30	208.3^{a}	2688.3^{a}	0.099^{c}	0.52^{c}	38.25^{c}	0.373^{a}	1.23^{a}
Pb 45	220.8^{a}	2180.8^{b}	0.112^{b}	0.54^{c}	43.46^{b}	0.373^{a}	0.93^{b}
Interactions between Ni and Pb							
Ni0 Pb0	360.0^{a}	1543.3^{d}	0.083^{i}	2.16^{b}	49.38^{ef}	0.380^{a}	1.70^{a}
Ni0 Pb15	250.0^{b}	1626.7^{d}	0.170^{b}	0.28^{hi}	49.31^{ef}	0.380^{a}	0.97^{cde}
Ni0 Pb30	276.7^{b}	2090.0^{bcd}	0.126^{de}	0.53^{f}	48.30^{f}	0.380^{a}	1.18^{b}
Ni0 Pb45	256.7^{b}	1810.0^{cd}	0.130^{cd}	0.09^{i}	58.85^{c}	0.380^{a}	1.03^{bcde}
Ni15 Pb0	270.0^{b}	1583.3^{d}	0.120^{e}	0.19^{hi}	63.31^{b}	0.370^{b}	0.94^{de}
Ni15 Pb15	246.7^{b}	2150.0^{bcd}	0.130^{cd}	0.81^{e}	39.84^{h}	0.370^{b}	1.15^{bc}
Ni15 Pb30	250.0^{b}	3243.3^{a}	0.060^{j}	1.00^{d}	41.46^{h}	0.370^{b}	1.58^{a}
Ni15 Pb45	253.3^{b}	2190.0^{bcd}	0.136^{c}	1.63^{c}	35.96^{i}	0.370^{b}	1.64^{a}
Ni30 Pb0	156.7^{cd}	2146.7^{bcd}	0.106^{fg}	2.91^{a}	51.24^{de}	0.370^{b}	0.45^{g}
Ni30 Pb15	186.7^{c}	2736.7^{ab}	0.180^{a}	1.53^{c}	43.54^{g}	0.370^{b}	0.89^{e}
Ni30 Pb30	153.3^{cd}	2620.0^{abc}	0.110^{f}	0.47^{fg}	40.88^{h}	0.370^{b}	1.09^{bcd}
Ni30 Pb45	200.0^{c}	2056.7^{bcd}	0.100^{gh}	0.34^{gh}	51.62^{d}	0.370^{b}	0.64^{f}
Ni45 Pb0	120.0^{d}	2383.3^{bcd}	0.100^{fgh}	0.25^{hi}	71.69^{a}	0.370^{b}	0.68^{f}
Ni45 Pb15	153.3^{cd}	1823.3^{cd}	0.090^{h}	0.97^{de}	41.54^{h}	0.370^{b}	1.58^{a}
Ni45 Pb30	153.3^{cd}	2800. 0^{ab}	0.100^{gh}	0.093^{i}	22.35^{k}	0.370^{b}	1.06^{bcde}
Ni45 Pb45	173.3^{c}	2666.7^{abc}	0.080^{i}	0.093^{i}	27.42^{j}	0.370^{b}	0.41^{g}

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. Ni: Nickel, Pb: Lead

Results of the study showed in Tables I-III the effects of Ni and Pb and their interactions on some non-enzymatic antioxidants for G. triacanthos, *L. leucocephala,* and *R. pseudoacacia* leaves. It shows that proline (Pr) and TC were decreased with increasing the concentration of Ni and Pb for the three species. TCHO decreased with increasing concentration for only G. triacanthos *and R. pseudoacacia,* but not for *L. leucocephala where* the TCHO increased with increased the elements concentration, same results were confirmed by Mame and Al-Rashed (2021); Singh, et al. (2012) and Tzvetkova and Kolarov (1996). The AA content increased with increasing the concentration of Ni and Pb for G. triacanthos *and L. leucocephala* but not for *R. pseudoacacia where it* decreased with increasing the elements concentrations (Bielen, et al., 2013; Gad, El-Sherif, and El-Gereedly, 2007). However, at high concentrations it is actually harmful to plants, and leads to programmed cell death (Gill and Tuteja, 2010), Ni and Pb may accumulate at such low concentrations in the leaves, so it was not detected. In this work, we assumed that each of *G. triacanthos*, *L. leucocephala,* and *R. pseudoacacia* plants may be better adapted and are tolerant to heavy metal stress conditions. However, highest concentration of Ni and pb reduced photosynthetic attributes, decreased pigment contents, the activity of non-enzymatic antioxidants AA, Pr, TC, and TCHO, in association with relatively high concentrations of Ni and Pb, where stresses increase the amount of ROS, thereby changes the formation and imbalance the contents of photosynthetic pigments, and non-enzymatic antioxidants. Plants increase antioxidant defense mechanisms under abiotic stresses such as drought, excessive watering, extreme temperatures (cold, frost, and heat), salinity, UV-B radiation and mineral toxicity, to alleviate oxidative damage. Different responses of antioxidants are stimulated to avoid ROS damages to cellular constituents, as well as to sustain normal growth and development. Excess levels of ROS are damaging to the plant; thus, to remain the balance of cellular redox, both non-enzymatic and enzymatic systems are motivated to repair the toxic levels of ROS. The balance between the producing and eliminating the ROS is persistent by enzymatic and non-enzymatic antioxidants. In plants, the appearance of many antioxidant enzymes is correlated positively with high levels of tolerance against the abiotic stresses, so, the increase in the activities of enzymes are closely related to the decrease in oxidative damage. The activation of some enzymes leads to plant protection against oxidative damage. Thus, in plants a complex enzymatic system has developed to scavenge extra ROS also, to protect them from the oxidative stress (Gull, Lone, and Wani, 2019; Caverzan, Casassola, and Brammer, 2016)

IV. Conclusions and Recommendations

The contamination of soil with heavy metals is a serious problem that affects the physiological process in plants including antioxidants defense systems. From this study, it is concluded that each of *G. triacanthos*, *L. leucocephala,* and *R. pseudoacacia* plant species were a response to soil increase in Ni and Pb heavy metals concentration like each other regarding the decrease in the CAT enzyme activity and each of Pr, and TC content, also they sharing the increase in POD activity, whereas *R. pseudoacacia* species unlike the two other species in decreasing the activity of NR enzyme and AA content with the increase in Ni and Pb concentrations. However, *L. leucocephala* is unlike the two other species in increasing CHO content with increasing the concentration of Ni and Pb elements. On the other hand, this study indicated that *L. leucocephala* was revealed to be more tolerant to Ni and Pb stress than other plant species. It can be recommended to conduct genetic studies regarding the responses to heavy metal stress to understand the differences between the same family species at the gene level.

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