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Physiological and Anatomical Properties of Some Fabaceae Plants Growing in Soil Contaminated by Nickel and Leads

A Dissertation submitted to the
Faculty of Science and Health at Koya University
as a Partial fulfillment for the Degree of
Doctor of Philosophy of Science in Biology / Plant physiology

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"In the name of Allah, the Most Gracious, the Most Merciful "

هَذَا مِنْ فَضْلِ رَبِّي

This is By The Grace Of My Lord

"Great truth of God "

Surah An-Naml Ayat (40).

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Hereby I Dr.Ikbal Muhammed Gharib state that this thesis as entitled physiological and anatomical properties of some fabaceae plants growing in soil contaminated by nickel and leads was prepared under my supervision at the department of Biology, the Faculty of Science & Health at Koya University by **Sargul Ahmed Khudhur** as a partial fulfillment for the degree of Doctor of Science (Ph.D) in Plant physiology.

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- *My brothers and sisters with my love;*
- *My friends with my best wishes; and*
- *Those who taught and supported me.*

Sargul

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List of Abbreviations

AA	Ascorbic acid	**TFC**	Total flavonoid content
BNF	Biological nitrogen fixation	**TGC**	Total glycoside content
$CaCO_3$	Calcium carbonate	**TPC**	Total phenolic content
CAT	Catalase	**TSC**	Total saponin content
Chl a	Chlorophyll a	**TSTC**	Total steroid content
Chl b	Chorophyll b	**TTC**	Total tannins content
CO_3	Carbon trioxide	**TTEC**	Total terpenes content
DBH	Diameter at breast height	**W**	Watt (Heating power)
DPX	Destrin plastisizer xylene		
ds/m	Decisiemens per metre		
EC	Electrical conductivity		
FAA	Formalin-acetic-acid-alcohol		
H_2O_2	Hydrogen peroxide		
H_2SO_4	Sulfuric acid		
HCO_3	Bicarbonate		
HMs	Heavy metals		
K_2HPO_4	Dipotassium phosphate		
KH_2PO_4	Potassium dihydrogen phosphate		
KNO_3	Potassium nitrate		
MB	Methylene blue		
N.D.	Not Detected		
nm	Nanometer		
Na_2CO_3	Sodium carbonate		
Ni	Nickel		
$NiCl_2$	Nickel chloride		
NR	Nitrate reductase		
O.M	Organic matter		
Pb	Lead		
$PbCl_2$	Lead chloride		
pH	Power of hydrogen		
POD	Peroxidase		
Pr	Proline		
ROS	Reactive oxygen species		

RPM	Rotary per minute		
TAC	Total alkaloid content		
TC	Total carotenoid		
TCHO	Total carbohydrate		
TPr	Total protein		

Summary

This study was conducted in an open field in Koya from March 25th to August 20th, 2021. The objective of the present study was to investigate the phytoremediation of soil contamination with nickel (Ni), lead (Pb), and their interactions on the seed performance, morphological, physiological, phytochemical, and anatomical characteristics of three forest tree species belonging to the Fabaceae family: *Gleditsia triacanthos*, *Leucaena leucocephala*, and *Robinia pseudoacacia*. The healthy legume seeds of these species were cultivated on March 25th, 2021. A factorial experiment with a completely randomized design and three replications was used for each species to study the effects of mixing the soil with different concentrations (0, 15, 30, and 45 mg.kg soil^{-1}) of two heavy metals: nickel chloride ($NiCl_2$) and lead chloride ($PbCl_2$), and also their interactions on each species separately. These concentrations were denoted as Ni_0, Ni_{15}, Ni_{30}, and Ni_{45} for the first salt, and Pb_0, Pb_{15}, Pb_{30}, and Pb_{45} for the second.

The results revealed that neither Ni nor Pb nor their interactions had a significantly effect on the percentage of *G. triacanthos* seed germination. However, plant leaf area exhibited a significant increase at Ni15 and Ni30, as well as P15, P30, and P45, while non significantly affecting the other vegetative growth characteristics compared to the control. On the other hand, *L. leucocephala* appeared to be more tolerant to increasing concentrations of Ni and Pb metals. In the case of *R. pseudoacacia*, Ni application resulted in a significant increase in root diameter for all treatments, in addition the nodules dry matter at 15, Ni30 and Ni45. Pb application, on the other hand, caused a significant increase in plant leaf area. No root nodules were observed in the *G. triacanthos* species. The results suggest that the inhibitory effects of Ni and Pb elements depend on their concentration, the type of metal, and the plant species. It was found that low levels of nickel chloride are necessary for plant growth, while increasing concentrations inhibit seed germination and plant growth may even lead to toxicity. However, the tolerance efficiency varies depending on the plant species. In conclusion, all the species studied were affected by the addition of Ni and Pb to the soil, which had an effect on seed and

seedling performance. *L. leucocephala* demonstrated greater tolerance to Ni and Pb heavy metals compared to the other two species, with higher concentrations, especially 45 $mg.kg^{-1}$, having more pronounced effects on the characteristics examined.

The study examined the effects of soil contamination with nickel and lead, as well as their interactions, on various physiological parameters. The results showed that at a concentration of 45 $mg.kg^{-1}$, chlorophyll a and chlorophyll b levels increased in *G. triacanthos, L. leucocephala,* and *R. pseudoacacia,* while total carotenoid levels decreased. Heavy metals were found to have negative effects on the content and functionality of photosynthetic pigments. The reduction in pigment photosynthesis, resulting from the decreased absorption of essential mineral nutrients, indirectly contributed to plant chlorosis. Comparing the responses of *G. triacanthos, L. leucocephala,* and *R. pseudoacacia* to nickel and lead, it was observed that *G. triacanthos* and *L. leucocephala* were less affected than *R. pseudoacacia*. The values of chlorophyll a and total carotenoid followed the magnitude order: *G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*, while for chlorophyll b, the order was *L. leucocephala* > *G. triacanthos* > *R. pseudoacacia.*

In terms of enzymatic and non-enzymatic antioxidants, as well as nitrate reductase (NR) enzyme activity, the study examined the effects of nickel, lead, and their interactions on above enzyme *G. triacanthos, L. leucocephala,* and *R. pseudoacacia.* The results revealed a significant increase in peroxidase enzyme activity and a significant decrease in catalase enzyme activity, proline, and total carbohydrate content in the leaves of three species with increasing concentrations of nickel and lead, except for total carbohydrate content, which increased only in *L. leucocephala*. Ascorbic acid and total protein content increased significantly with increasing concentrations of nickel and lead in all the studied species. NR enzyme activity decreased significantly in *L. leucocephala* and *R. pseudoacacia*, while it increased significantly in *G. triacanthos.* In conclusion, there were general increases or decreases in the content of certain antioxidants in the leaves of all species studied. However, there were also some species-specific differences, indicating different mechanisms of tolerance to heavy metal stress among these species.

The effects of nickel (Ni), lead (Pb), and their interactions on the macro, micro, and non – essential heavy metal elements in the shoots, roots, and soils, as well as the physical and chemical properties of the soil after planting, were observed to decrease some parameteries at concentrations of 15, 30, and 45 $mg.kg^{-1}$ for the three species studied. According to this study,

the highest accumulation of heavy metals in plant organs and species as follows: shoots > roots > soils, and *G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*.

The effects of Nickel (Ni), Lead (Pb), and their interactions on the qualitative and quantitative total content of various phytochemicals (phenolic, flavonoid, glycoside, alkaloid, steroid, terpene, tannin, and saponin) were observed using three different solvent extraction methods (methanol, water, and ethyl acetate). The results indicated that the methanol solvent extraction yielded higher values for all the qualitative and quantitative phytochemicals, except for the total saponin content, which was only higher when using water as the solvent for *G. triacanthos, L. leucocephala,* and *R. pseudoacacia* leaves. The extraction of qualitative and quantitative phytochemicals was influenced by factors such as solvent polarity, the ratio of solvent to plant materials, particle size of the materials, extraction method, and temperature. The phytochemicals were found to be directly dependent on the developmental stage of the plant, the specific parts of the plant used, and the solvents employed for extraction and isolation. Minerals play a diverse role in the metabolism of medicinal plants, and their presence in varying degrees can have various effects on plant metabolism. These effects can be both severe and varied depending on the abundance or scarcity of these minerals.

The applications of nickel (Ni), lead (Pb), and their interactions on certain anatomical characteristics of plants, such as leaves, petioles, stems, roots, as well as the number, length, and width of stomata, were found to increase at concentrations of 30 and 45 $mg.kg^{-1}$ in seedlings of *G. triacanthos, L. leucocephala, and R. pseudoacacia.*

INTRODUCTION

Chapter One
Introduction

The honey locust (*Gleditsia triacanthos*), white lead tree (*Leucaena leucocephala*) and black locust (*Robinia pseudoacacia*) are three fabaceae trees, easily cultivated species, planted as landscape in Iraq and Kurdistan region; they are important for carbon restoration, soil stabilization and landfills, and wastelands, re-vegetation; they produce high levels of biomass and enhance tolerate, and can accumulate high levels of heavy metals in their aboveground parts. These plants are used for phytoremediation and decrease regeneration costs. They can live in many stressed environmental conditions except frost. These species are fast growing, can reach maturity and produce seeds during 6-7 months. These species produce phytochemicals or pharmacological activity compounds which are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Sharma *et al.,* 2022; Ssenku *et al.,* 2017).

The subject of heavy metals nowadays has attracted the attention of scholars worldwide, due to their risks to human and the ecosystem by polluting soil, water, and food, where soil is the media for seed germination, and plants growth and development (Deswal and Laura, 2018). Soil contamination of heavy metals due to many activities such as; industrial waste, fertilizers, pesticides, irrigation with wastewater, gasoline, paint, mining waste and many other resources. The two heavy metals that are found most frequently in contaminated sites are lead (Pb) and nickel (Ni). According to several authors, the toxicity of Pb and Ni could result in oxidative cellular damage when they produce reactive oxygen species (ROS). Antioxidant enzymes can therefore lessen or stop the toxic effects of ROS brought on by metal stress (Sharma *et al.,* 2023; Nyiramigisha *et al*., 2021; Kumari and Amarnath, 2021).

In small amounts, Ni is necessary for seed germination and normal plant growth and development. However, at high concentrations, Ni becomes toxic and causes changes at the physiological, biochemical, and cellular levels that cause severe damage to plants. Ni is one of the 17 essential mineral elements for plants. The most prevalent symptoms of Ni poisoning in plants include impairments in photosynthesis, mitotic activity, sugar transfer, and plant development. Some farmland has been rendered unusable for growing crops, fruits, and vegetables due to extremely high soil Ni concentrations (Ahmad *et al*.,2009). Pb is highly toxic heavy metal, which is also called protoplasmic poison and it is a hazardous pollutant for environment (Mehboob *et al.,* 2018). Although it is not a necessary elements for plants, it has

a negative impact on a variety of physiological processes, including preventing seed germination, preventing root formation, delaying seedling growth, reducing mineral nutrition, these effects may lead to a cell death at high concentrations, and may kill the plant (Fazal 2015; Sharma and Dubey, 2005). The primary methods used by roots to absorb Ni and Pb include passive diffusion and/or active transport, depending on the kind of plant, the acidity of the soil, the presence of other metals, and the concentration of Ni^{2+} in the soil solution. Pb is generally taken up by passive diffusion, mostly through intercellular spaces of the apoplast, following the water movement within the plant (Kun *et al.,* 2021; Álvarez *et al.,* 2019; Joseph *et al.,*2018).

As a result of an imbalance between the ROS defense system and the antioxidant defense system in plants, which results from heavy metals, high light intensity, high temperature, air pollution, soil salinity, drought, and biotic stress, plants experience oxidative stress. Plants create ROS as by products of a variety of metabolic processes, including respiration and photosynthesis. The build up of ROS disrupts physiological processes normally occurring and damages biomolecules, cells, and tissues (Sachdev *et al.,* 2021; Ali *et al*., 2020). Antioxidants are chemicals that are found in plants at lower concentrations than oxidizable substrates and that significantly slow down or stop oxidation. Carotenoids and ascorbic acid are examples of non-enzymatic antioxidants, whereas catalase (CAT) and peroxidase (POD) are the two main enzyme-based antioxidants. While non-enzymatic antioxidants work by stopping free radical chain reactions to prevent cell damage, enzymatic antioxidants work by dissolving and expelling free radicals from the cells. Antioxidant defense is present everywhere in nature, and plants manufacture it to defend themselves against a variety of biotic and abiotic challenges (Ren *et al.,* 2021; Santos-Sánchez *et al.,* 2019; Kartoori *et al.,* 2018).

Species of the fabaceae family are a rich source of phytochemicals including phenols, flavonoids, glycosides, alkaloids, tannins, terpenoids, steroids and saponins, which exhibit a variety of health benefits, especially anti-cancer properties. A major source of medication is plants, which have a wide range of biological effects, including antioxidant, antibacterial, similar antifungal properties. The majority of the global population relies primarily on plants for their primary healthcare and nearly 25% of conventional medications. Plant-based medicines are readily available, inexpensive, efficient, safe, and rarely have negative effects. All around the world, medicinal plants are a source of significant economic value. Minerals play a variety of roles in the metabolism of medicinal plants (Usman *et al.,* 2022; Saio *et al.,* 2015; Mishra *et al.,* 2012).

As plants is subject to a variety of demanding environmental conditions over the course of their lifetimes that have a negative impact on their growth and developmental processes. Under situations of heavy metal stress, growing plants experience a variety of alterations, affecting their leaf, petiole, stem, and root systems (Saraiva *et al.*, 2020; Gao *et al.*, 2019; Amari *et al.*, 2017). Numerous variables, including an element's properties, the type of soil, the species of plant, and the organ of the plant, affect the uptake and transport of heavy metals in plants. Since the root is the first organ to come into touch with the soil, it is in charge of absorbing and moving ions and water. The principal organ of photosynthesis is the leaf, which is a crucial procedure for supplying energy to keep all plant tissues' physiological functions operating. Reduced water and mineral intake by roots due to anatomical and morphological changes compromises the capacity of plant growth (Pandey *et al.*, 2022; Yadav *et al.*, 2021). A decrease in cell division that resulted in an increase in cell wall thickness and/or an imbalance in the activity and concentration of phytohormones like auxin in roots exposed to heavy metals may be the cause of a reduction in root growth (Hamza *et al.*, 2020).

Nowadays, a variety of remediation, including physical, chemical and biological remediation (microbial remediation and phytoremediation) approaches have been developed to reclaim heavy metal-polluted soils. The phytoremediation uses plants to extract and transport elemental pollutants in the soil, stabilizing soil fertility and removing heavy metals from the soil, which is considered to be the most efficient and environmentally friendly method for remediation of heavy metal-contaminated soils. (Sharma *et al.*, 2023; Yan *et al.*, 2020).

These two elements (Ni and Pb) were selected because so few studies where conducted about them, specially using both of them together on our study plant species.

The aim of this study is to investigate the effects of Ni and Pb elements as soil pollutants, heavy metals on seed performance, morphological, physiological, phytochemical and anatomical properties of *G. triacanthos, L. leucocephala and R. pseudoacacia* as three forest species belonging to fabaceae family and enable these species to realize phytoremediation in Kurdistan region-Iraq.

REVIEW
OF
LITERATURE

Chapter Two

Review of literature

2.1 Botanical, economical, climatic requirments and cultivation of *G. triacanthos, L. leucocephala and R. pseudoacacia* species

2.1.1 Botanical description

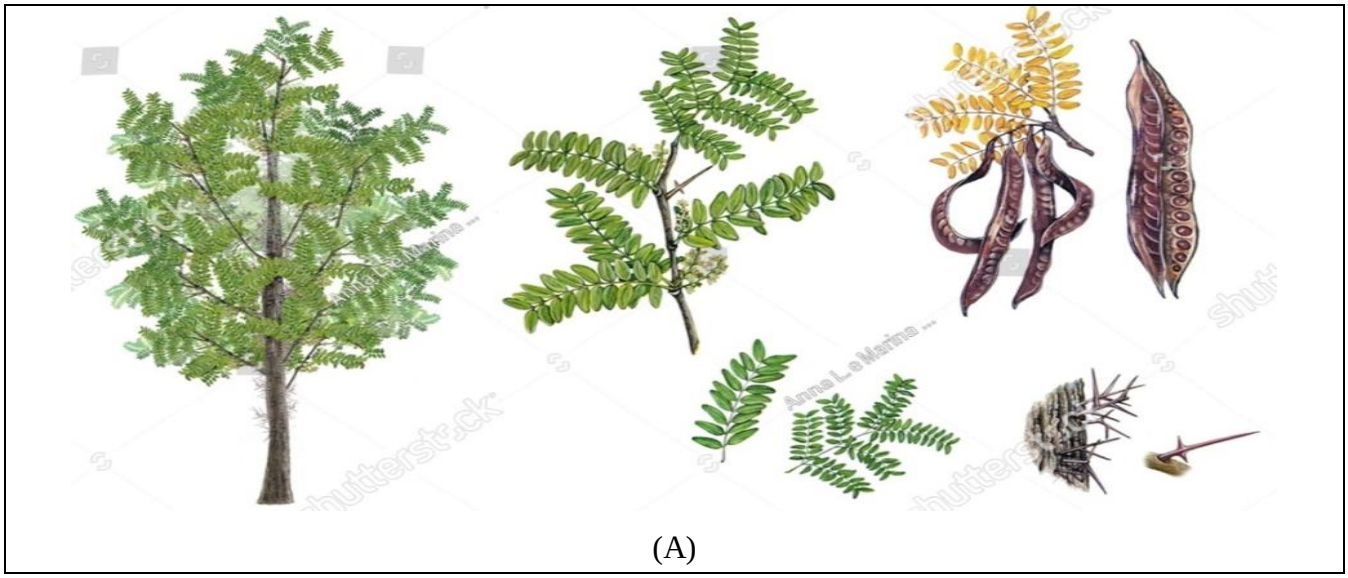
The honey locust called *Gleditsia triacanthos* is a deciduous tree belonging to fabaceae family. Genus *Gleditsia* comprises 14 species which can reach a height of 15-25 m and 0.5-1 m diameter. The bark of trees is reddish-brown to black, scaly, ridged, and frequently covered in clusters of huge, branched thorns. Trees also have a short bole and an open, narrow, or spreading crown. Its extensive root system is branching and has a robust taproot. The arrangement of the leaves is alternating, single or double pinnate. Those single-compound plants emerge early on short or dwarf branches, bear 14–30 leaflets on a central stalk that is 15–20 cm long, and are preformed in buds. During the growing season, those doubly compound plants neoform and produce 4–7 pairs of branches that each resemble a single doubly compound leaf. Leaflets are 25–40 mm long, and they are widest near the base. They have rounded tips that occasionally have very little teeth. Flowers are tiny, 5 mm wide, regular, and greenish-white. On the same tree, frequently on different branches, are the male and female blooms. Furthermore, there can be perfect blossoms. Fruit pods that are 15–40 cm long, flat, curved, or twisted, and have leathery husks fall during the winter without opening. Seeds are bean like; with a hard, impermeable seed coat; 0.5-1.5 cm long, dark brown, smooth (Dana *et al.*, 2022 ; Heuzé *et al.*, 2018).

White lead tree i.e. *Leucaena leucocephala* was known as a miracle tree because of its worldwide success as a long lived, the fast growing, nitrogen fixing tree/shrub. It creates dense thickets and grows up to 10 m tall throughout the cleared areas. An evergreen is a species of Leucaena. The compound pinnate leaves of *L. leucocephala* are linear-lanceolate, 8-15 mm long, 2-4.5 mm wide, slightly asymmetric, acute at tip, linear-oblong to weakly elliptic, glabrous except on margins that are rounded to obtuse at base. Pinnular rachis are generally 5-10.2 cm long and are bipinnate with 6-8 pairs of pinnae bearing 9-20 pairs leaflets. The axillary globose head's paired inflorescences have a diameter between 12 and 20 mm, a peduncle length between 2 and 3 cm, and several flowers are produced. After the seed germination period of 4

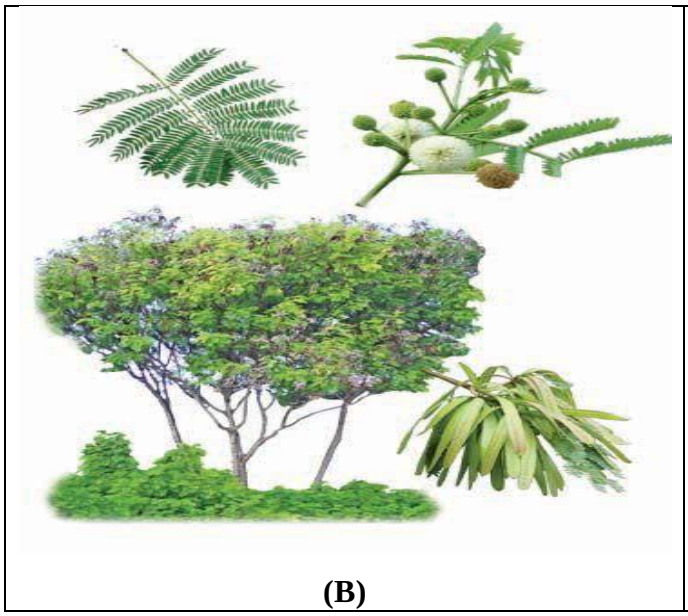
to 6 months, *L. leucocephala* begins to blossom. The flowering season often lasts two years or is seasonal. The spherical, whitish flower heads, which grow on young branches that are actively growing, have a diameter of 2 to 2.5 cm, 100 to 180 flowers per head, and 2 to 6 in each group of leaf axils. Each flower head produces 5 to 20 pods that are 11 cm to 19 cm long, 15 mm to 21 mm wide, linear-oblong in shape, flat, with 8 to 18 seeds, mid-to orange-brown in color, glabrous and faintly glossy in white silky hairs, papery, and opening along both borders. The testa, which is firm, dark brown, and oriented transversely in the pod, is between 6.7 and 9.6 millimeters in length and between 4 and 6.3 millimeters in width. The fruits start to produce a lot of fruit after the first year. The seeds are tiny (8 mm long), glossy, flat, teardrop-shaped, dark brown, and covered in a thin but reasonably resistant seed coat. Per kilogram, there are around 17,000–21,000 seeds. (Hamad and Anwer, 2021; Septina and Rivai, 2020).

Black locust called *Robinia pseudoacacia* is a medium size tree, 25 m tall, 60 cm dbh; trunk irregular; crown open, irregular; branches short, brittle; the persistent stout spines on young shoots are found on mature wood; the smooth bark becomes reddish-brown and deeply furrowed with age. The leaves are alternating, pinnate, deciduous, and have 7–19 leaflets on a central stalk that is 20–30 cm long. Each leaf has two spines at the base, and the leaflets are oval, 30–50 mm long, dull green, bristle-tipped, and smooth-margined. At the end of a new branch, spectacular, white, pea-like, fragrant flowers appear in loose, drooping clusters approximately 14 cm long. The fruit is a flat, elongated pod that is 7 to 10 cm long. It has a thin-walled, silky husk that is dark to reddish-brown, numerous on a central stalk, and stays on the tree throughout the winter. Beginning around age 3, seed crops start to occur every 1-2 years, with an abundant crop every 2-3 years. *R. pseudoacacia* is similar to other secondary forest legumes that grow quickly (Hallmann, 2020).

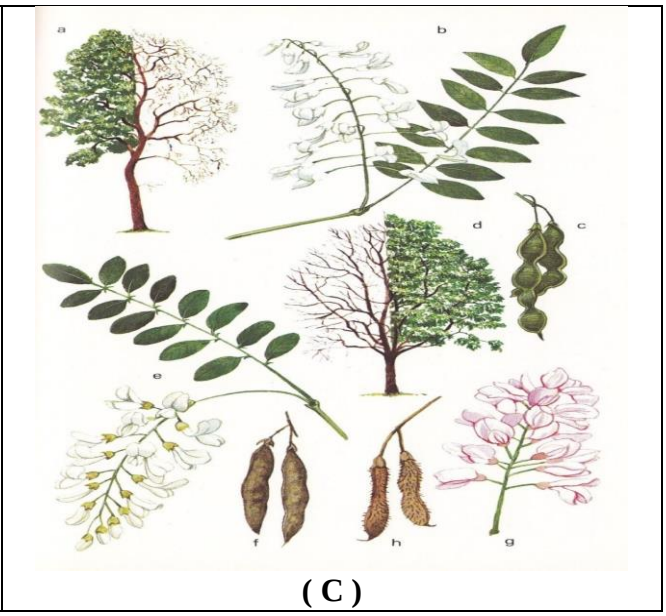
Seeds of *G. triacanthos, L. leucocephala and R. pseudoacacia* ripen in late summer and early autumn and pods are shed from September to April in Kurdistan Region and Iraq (Mustafa, 2009).



(A)



(B)



(C)

Fig 2.1*. Botanical description of (A) G. triacanthos, (B) L. leucocephala and (C) R. pseudoacacia* species

2.1.2 Economical values

Gleditsia species is planted for soil erosion control and for wind breaks as shade and ornamental trees, livestock feed and wood. *Gleditsia* plant have been widely used in traditional chinese medicine. The fruits and thorns are used for treating apoplexy, headache, productive cough, asthma and suppurative skin diseases. It was reported that *G. triacanthos* seed extracts can be used as a natural source of phenolic compounds and as antioxidants; In addition extracts of *Gleditsia* plant possess important pharmacological activities in treating rheumatoid arthritis, as anti-mutagenic, anticancer and they have significant cytotoxic activity against different cell lines (Dana *et al.*, 2022).

L. leucocephala has multipurpose uses, such as generation of firewood, timber, greens, fodder and green manure; It also to provide shade and control soil erosion. *L. leucocephala* is a plant rich in chemical compounds which is distributed in every part of the plant. Due to its numerous pharmacological qualities, it has been utilized for therapeutic purposes. Studies have revealed that this plant contains a variety of secondary metabolites, including alkaloids, tannins, flavonoids, saponins, and glycosides. It is employed as an abortifacient, contraceptive, and treatment for stomach aches in conventional medicine. The antimicrobial, anthelmintic, antibacterial, anti-proliferative, anti-diabetic, cancer-preventive, diuretic, anti-inflammatory, antioxidant, antitumor, antihistaminic, nematicide, pesticide, antiandrogenic, hypocholesterolemic, and hepatoprotective properties of this substance have all been used in medicine (Deivasigamani, 2018).

R. pseudoacacia has been introduced worldwide as a commercially important multipurpose tree because of its adaptability to environmental stresses, its rapid growth and the hardness of its wood. Mostly decorative trees were planted there. One of the most significant sources of honey production is black locust blossoms. They could be a good source of several bioactive components in the diet of consumers. Flowers with a variety of hues contain a lot of bioactive substances. Different phytochemical profiles were seen depending on the flower hue, with yellow and orange flowers showing higher concentrations of carotenoids and flavonoids and purple, violet, and blue flowers showing larger amounts of anthocyanins. The primary goal of flower coloring is to increase the flowers' appeal to pollinators. The most prevalent polyphenol components are quercetin and its glucosides, epigallocatechin and ferulic acid. On the other hand, flowers can provide the human diet with polyphenol chemicals. A higher intake of polyphenols can lower cardiovascular risk and prevent certain types of cancer (Orwa *et al.*, 2010).

2.1.3 Climatic requirement

G. triacanthos within the natural range, a large amount of variation exists in both climate and soil conditions. *G. triacanthos* occurs naturally in humid and subhumid climate regions; it grows naturally to 760 m, but has been planted from sea level to 1500 m in temperate latitudes will grows to above 2500 m in subtropical highlands. It doesn't grow in the shadow and only establishes in open areas. *G. triacanthos* is tolerant to salinity and drought. Mean yearly temperatures are 15–24 °C and mean annual precipitation is 510–1520 mm. Although it also thrives on saline soils, the best growth is seen in deep soils (pH 6-8) in wet, alluvial floodplains.

It frequently fails on shallow soils and performs poorly on gravelly or heavy clay soils (Heuzé *et al.*, 2018).

L. leucocephala is essentially a tropical species requiring warm temperatures for optimum growth and has poor cold tolerance and significantly reduced growth during cool winter months in subtropical areas. Only subhumid or humid regions with moderately long dry seasons (up to 6-7 months) support its growth. It performs best on calcareous soils but may also be found on salty soils and on alkaline soils up to pH (8); it is not tolerant of acid soils or waterlogged circumstances. Altitude (0-1500), max. 2100 m, mean annual temperature is 25-30 °C, and its mean annual rainfall is 650-3000 mm. Known to be intolerant of soils with low pH, low phosphorus, low calcium, high salinity, high aluminium saturation, and waterlogging, *L. leucocephala* has frequently failed in these environments (Zayed *et al*., 2018).

R. pseudoacacia species is distribution optimum in sub-mediterranean to warm continental climates. The trees are pioneers on disturbed soils or burned sites. It dominates early forest regeneration in many native forest stands. The tree does not tolerate waterlogging or shade. Altitude is over 800 m, mean annual temperature should be 35 - 40 °C, mean annual rainfall should be 1000-1500 mm. Soil type is found on a wide range of soils but does well on calcareous, well-drained loams with pH range of 4.6-8.2 , (Hallmann, 2020).

2.1.4 Cultivation

Gleditsia is propagated by seed, pre-soaked for 24 hours in warm water and then sown in spring in a greenhouse. If the seed has swollen, then it can be planted; if not, then soak it in warm water for an additional 24 hours. When filing away a portion of the seed coat, take be careful not to harm the embryo if this does not work. The seed should then expand after additional soaking. The seed should germinate in 2 to 4 weeks at 20°C once it has swelled. Prick the seedlings out into separate, deep pots as soon as they are strong enough to handle them, then plant them in their final locations in the summer. For their first few outdoor winters, provide the plants with some protection from the cold (Tognetti *et al.,* 2019).

Leucaena can be planted by seed or 'bare stem' seedlings. Seeding rates of 1-2 kg/ha at depths of 2-3 cm are usually recommended in rows 3-10 m apart. Freshly harvested *leucaena* often has a high degree of hard seed due to an impermeable waxy coat which must be broken before the seed will imbibe water and germinate. Scarification to break this dormancy usually involves treatment with hot water (boiling water for 4 second) or acid (concentrated sulphuric acid for

5-10 min). Select ripe dried pods for their seeds; these can either be sown directly or in pots. Rows of seeds can be planted, spaced 50-100 cm apart (width according to planned plant height), (Shelton *et al.,* 1998).

Robinia reproduction is primarily vegetative, although it can also reproduce by seed. It sprouts from the roots and forms clones, particularly in sandy soils. In response to harm, it also readily grows from stumps. Black locust matures and grows quickly; some trees might start producing seed at six years old. Heavy seed yields are produced every one to two years. The seeds' outer layer is dense and impenetrable. However, this species also depends on sexual reproduction due to its hermaphrodite blooms, which are pollinated by bees, and its abundant seed output. Late summer and early autumn are when seeds ripen, and September through April is when pods shed their contents. Pods holding seeds remain on the crown of the plant throughout the winter. They may open on the tree or may be dispersed far from the mother plant (Giuliani *et al.,* 2015).

2.2. Nickel and lead as heavy metals

Ni is one of the 17 necessary minerals for plants, needed in minute levels for normal growth and development. Ni concentrations in plant cells that are higher cause physiological, biochemical, and cellular changes that severely harm plants. The most typical signs of Ni toxicity in plants include decreased plant growth, obstruction of sugar transport, and inhibition of photosynthesis and mitotic activity. Some farmland is no longer viable for growing crops, fruits, or vegetables due to extremely high Ni concentrations in the soil. Generally, accepted Ni values in plant tissues are between 0.5-5 $mg \cdot kg^{1}$ of dry weight. Soil total Ni contents vary between 5-150 $mg \cdot kg^{-1}$ dw. The overall uptake of Ni by plants depends on the concentration of Ni in soil solution, plant metabolism, the acidity of soil or solution, the presence of other metals, and organic matter composition. Lead is not an essential element and is considered relatively unavailable for living organisms due to immobilization in the soil and limited transport from roots to plants. The concentration of total Pb in uncontaminated soil fluctuated within the range of 10-50 $mg \cdot kg^{-1}$; however, in soil with low-level contamination, Pb concentration can be expected to range from 30 to100 $mg \cdot kg^{-1}$ (Kacálková *et al.,* 2014 and Vatansever *et al.,* 2010).

2.2.1. Heavy metals definition and sources

Worldwide, dealing with heavy metals toxicity is a major challenge for agricultural scientists because they are in soil environment have become a leading health concern, especially for plants, humans and animals (Haseeb *et al.*, 2022). Heavy metals are non-degradable by any biological or physical process and are persistent in the soil for a long period, which pose a long-term threat for the environment. According to their role in biological systems, heavy metals can be grouped as essential and non-essential. Essential heavy metals including Fe, Zn, Cu, Mn, Mo and Ni, are needed for physiological and biochemical processes throughout plant life cycle, but when present in excess, they may be hazardous. Non-essential heavy metals like Pb are extremely poisonous and have no known function in plants and may cause environmental pollution and seriously affect a variety of physiological and biochemical processes in crop plants and reduce agricultural productivity (Kafle *et al.*, 2022; Álvarez *et al.*, 2019).

According to Khalid *et al.* (2017) soil contamination with potentially (eco) toxic heavy metals is ubiquitous around the globe. Concentration of these heavy metals in soil has increased drastically over the past three decades, thus posing risk to the environment and human health. Some technologies have long been in use to remediate the dangerous heavy metals. Conventional remediation methods for heavy metals are generally based on physical, chemical and biological approaches, which may be used in combination with one another to clean-up heavy metal contaminated soils to an acceptable and safe level.

Sachan *et al.* (2017) compared that HMs toxicity has an unavoidable hazard to environment and public health due to their increasing contamination and accumulation in atmosphere which ultimately pass to the living beings via the route of food chain. Heavy metals are increasing quickly in soil and water by weathering of rocks and anthropogenic activities and are now posing a serious health risk both to people and plants. Among them Ni is a controversial element because of debate on its essentiality or non-essentiality in plants, while at a higher level it affects all cellular and metabolic processes and causes retardation of germination, competition with other essential metal ions, osmotic imbalance, alteration of many enzymatic activities, disruption of cell structure and wilting, reduced photosynthetic activity, oxidative stress etc. Plants also possess some natural and stress-induced strategies to cope up with Ni excess/toxicity. These strategies include growth regulators, antioxidative enzymes, amino acids as osmoprotectant and chelation of Ni with metalloproteins and metallothionins.

According to Khalid *et al.* (2016), excessive build-up of heavy metals in agricultural soils results in increased heavy metal uptake by food crops and vegetables, which in turn may induce serious health risks to human beings. Heavy metals are reported to cause several disorders in humans including cardiovascular diseases, cancer, cognitive impairment, chronic anemia,

damage of kidneys, nervous system, brain, skin, and bones. Owing to potential harmful effects associated with heavy metal exposure, there is a global concern to ensure that the heavy metal content of agricultural soil and the crops grown on these soils do not exceed the allowable regulatory limits. Additionally, people are becoming more aware of the effects that heavy metal contaminated soils on human and environmental health, resulting in the improvement and development of technologies for clean-up of heavy metal contaminated areas. Contrasting to organic contaminants, heavy metals are somewhat unique by the fact that they are highly resistant to either biologically or chemically induced degradation.

2.2.2. Effects of nickel and lead on some seed performace, morphological, physiological and anatomical characteristics of plants

2.2.2.1 Seed performance

Pavlova *et al.* (2018) found that the effect of Ni on seeds of *Alyssum markgrafii* and *A. murale,* an obligate and a facultative Ni hyperaccumularors respectively, distributed on the Balkans. Seeds collected from natural populations of the species in Albania were germinated with standard solutions of 0.5, 1, 2, 4, 6, 8 mM Ni as $NiCl_2.6H_2O$ in distilled water and compared with germinated seeds in distilled water. The results demonstrated that Ni had an impact on germination process and differences were discovered not only between the species but also between the populations. The germinability decreased with elevation of Ni concentrations. The relative frequency of germination in *A. markgrafii* was more spread out through time. The seeds of *A. murale* displayed greater homogeneity and higher synchrony of germination. The seeds of *A. murale* were less sensitive (or tolerant to) when treated with higher metal concentrations and germination was less influenced compared to *A. markgrafii*. Lower concentrations of Ni enhanced hypocotyl elongation but inhibited root elongation in both species. The roots were more sensitive to Ni compared to the hypocotyl. The abnormalities at early stage of seed germination found in both species disturbed the normal growth of the seedlings.

Sharifi-Rad (2017) carried out a study to assess the phytotoxicity of Ni on seed germination of *Hyssop* (*Hyssopus officinalis* L.); The experiments were performed in different aqueous concentrations 50, 100 and 150 μM of aforementioned heavy metals over the period of 14 sequential days. The results demonstrated that heavy metals negatively impact the normal growth of plants by reducing seed germination. The harmful effects of chosen heavy metals on seed germination can be organized by the grade order of inhibition. These results indicated a

model system for varying concentrations of heavy metals for their phytotoxicity effects and also for the seeds' ability to negate the toxicity impacts of heavy metals in various types of irrigation waters and soils. Shweti and Verma, (2018) found that the Ni is an essential micronutrient for seed germination at low concentration. When it is present in large concentrations in the soil and in plants, it becomes toxic. However, the effectiveness of tolerance varies depending on the variety of plant. The response of Ni on nine wheat (*Triticum aestivum L.)* cultivars. The seeds of wheat cultivars were germinated in petriplates lined with whatman filter paper No.1 and treated with various concentration of Ni (5, 10, 25, 50 and 75) mg/L respectively and distilled water was used as control. The germination percentage was recorded at ten days after soaking. The lower concentration (5 mg/L) of Ni has no adverse effect on germination, while the higher concentration decreased the germination. The result indicates that the low amount of Ni is required for wheat cultivars. Whereas, the increasing level of Ni cause toxicity.

Bezini *et al*. (2019) evaluated the effect of Pb on germination of *Medicago arborea* seeds. Solutions of four concentrations 25, 50, 75 and 100 ppm of Pb were used. The following germination indices: Final germination percentage (FGP), Mean daily germination (MDG), Mean germination time (MGT) were estimated. The results showed that FGP, MDG were significantly affected by heavy metal stress. In contrast, the increase of applied heavy metal dose resulted in prolongation of MGT, and therefore, in significant increase of its value. It should be noted that *M. arborea* seeds were able to germinate even at 100 ppm, which is a concentration higher than critical limits for agricultural soils and irrigation water. This shows that M. arborea may be considered as a moderately tolerant species, at least during the germination phase, to metal stress and as a candidate with acceptable potential for phytoremediation. Kabira *et al.* (2018) tested that the effects of Pb on seed germination of *Leucaena leucocephala* where increase in concentration of metal treatment from 25 to 100 ppm, significantly ($p<0.05$) reduced germination percentage which was more prominent for Pb treatments as compared to control.

Ismail *et al.*(2013) reported that the toxicity effects of Pb on germination of three tree species; *Thespesia populneoides*, *Leucaena leucocephala* and *Delonex regia* were evaluated. The results illestrated that dose response of heavy metals were inversely proportional to germination. The percentage germination of *L. leucocephala* was least affected by lead toxicity while scoring the best germination response among the three tree species. Exposure high concentration (125ppm) to Pb inhibited of *D. regia* while much higher reduction was observed for *L. leucocephala*. Furthermore, the phytotoxicity and tolerance index confirmed that *D.*

regia appeared to be the most tolerant species whereas, *T. populneoides* and *L. leucocephala* were moderately tolerant and less tolerant species respectively against the Pb treatment. Shafiq *et al.* (2008) found that seed of *Leucaena leucocephala* were grown under laboratory conditions at 25, 50, 75 and 100 ppm of Pb. Increasing the concentration of lead to 75 ppm, significantly ($p<0.05$) decreased seed germination as compared to control. The results of the study suggest that due to better metal tolerance indices there is a possibility of growing *L. leucocephala* in areas contaminated with Pb.

Deswal and Laura (2018) observed the seed germination percentage was affected only at relatively higher concentrations of Ni and Pb. While at the lower concentration of the heavy metals 1 mM for Ni and Pb there was only delay in the germination. Ertekin *et al.*(2020) conducted a study to determine the effects of different doses (0, 100, 200, 400 and 800) mg L^{-1} of Ni and Pb on seed germination of sorghum. In the research, germination rate and mean germination time were measured during germination to determine the impacts of heavy metals. The results indicated that both germination time and mean were adversely affected by heavy metals. Increasing heavy metal doses negatively affected all investigated properties.

2.2.2.2. Morphological characterestics

Patra *et al.* (2019) reported that the Ni affects growth and the morphology of plants. The mobility of Ni in the environment and the consequent contamination in soil and water is of great concern. Also, the detrimental effects of excessive Ni on plant growth have been well known for many years. Toxic impacts of Ni on plants include alterations in the growth of roots, stems and leaves. Total dry matter production was significantly affected by Ni and also causes deleterious effects on plant physiological processes. Faizy *et al.* (2022) found the influence of Ni fertilizer at 0, 50, 100 and 150 $mg.kg^{-1}$ concentrations on the vegetative part of *Trigonell afoenum-graecum*. The results showed that the heavy metal 50 $mg.kg^{-1}$ of Ni with control has a significant impact on the height of plant.

Shweti and Verma (2018) found that the Ni is an essential micronutrient for plant growth at low concentration. It becomes poisonous when the concentration is high in the soil and in plants. However the effectiveness of tolerance depends on the plants species. The response of Ni on nine wheat (*Triticum aestivum* L.) cultivars with various concentrations of Ni 5, 10, 25, 50 and 75 mg/L respectively and distilled water was used as control. Seedling growth and weight were recorded at ten days after soaking. The lower concentration (5 mg/L) of Ni has no adverse effect on shoot and root length and weight, seedling growth while the higher

concentration decreased the seedling growth, shoot and root length and weight. The result indicates that the low amount of Ni is required for wheat cultivars, whereas, the increasing level of Ni reduced plant growth and cause toxicity. Batool (2018) studied the bioaccumulation of Ni in chickpea (*Cicer arietinum L.*) and its impact on the growth. Ni treatment was applied in solution form 25, 50, 100 and 150 mg.$L^{-1}$to the soil. A significant decreasing trend in shoot length, number of branches, number of leaves and biomass yield was observed for all treatments as compared to control. Accumulation of Ni in plant shoots was gradually increased with increasing concentrations of Ni application. Ertekin *et al.*(2020) conducted a study to determine the effects of different doses 0, 100, 200, 400 and 800 mg L^{-1} of Ni and Pb on seedling growth of sorghum. In the study, root fresh weight and shoot fresh weight were measured during seedling growth to determine the affects of heavy metals. The results demonstrated that seedling growth properties was adversely affected by heavy metals. Increasing heavy metal doses negatively impacted all investigated properties. Anwar *et al.* (2022) illustrated the Pb produced significant (p<0.05) effects on various growth parameters of *Albizia lebbek and Prosopis julifora* number of leaves, leaf area and biomass of both seedlings exhibited significant (p<0.05) decrease when treatments of lead were increased from 20 ppm to 30 ppm. Lead treatments of 25, 50 and 75 ppm were also carried out in pot experiment. Pb treatments at 75 ppm demonstrated much reduction in growth as compared with control. The rise in absorption of Pb directly effects shoot growth and root length is also reduced. However at 25 ppm shoot growth of *A. lebbeck* was better than control while shoot growth of *P. juliflora* was reduced. The shoot length was much decreased by increase in concentration of Pb. The seedlings grown in control soils have better growth as compared with Pb treated soils. The increase in concentration of Pb reduced the seedling size. The seedlings which were treated with the 25 ppm and 50 ppm were almost of same size but 75 ppm caused much reduction in the seedlings growth. In both cases Increased concentration of Pb reduced the dry weight. General fallouts of different limits showed that Pb is a toxic element, which caused much reduction in growth of both *A. lebbeck* and *P. juliflora.*

Nas and Ali (2018) investigated that some heavy metals are easily absorbed and build up in many areas of a plant while being neither a necessary element nor playing any part in cell metabolism. The pH, particle size, cation exchange capacity, root exudation, and various other physical and chemical characteristics all play a major role in controlling lead uptake. In plants, a high concentration of heavy metals, such as lead, can result in a variety of hazardous symptoms, including growth retardation (stunted growth). Kabira *et al.* (2018) confirmed that the effects of Pb on seedling growth of *Leucaena leucocephala* were increase in concentration

of metal treatment from 25 to 100 ppm, significance ($p<0.05$). Seedling root and shoot length, seedling size, root/shoot ratio, seedling fresh and dry weights all declined significantly ($p<0.05$) as compared to control treatment. Seedlings growth of *L. leucocephala* gradually reduced with an increase in concentrations of Pb as compared to control. Mehboob *et al.* (2018) noticed in an experiment the effects of different concentrations (0, 20, 40, 60, 80 and 100) ppm of Pb on growth performance of wheat (*Triticum aestivum*) as compared to control. Pb treatment in the form of Pb acetate at 100 ppm completely inhibited the seedling growth of *T. aestivum* as compared to control. Pb treatment at 20 ppm concentration produced significant reduction in shoot length as compared to control. At various Pb concentrations, root development, an important growth characteristic, was found to be significantly inhibited. The treatment of Pb at 20 ppm produced significant ($p<0.05$) reduction in seedling dry weight as compared to control. Ismail *et al.* (2013) reported the toxicity effects of Pb on the root length and dry biomass of three tree species; *Thespesia populneoides*, *Leucaena leucocephala* and *Delonex regia*. The results revealed that dose response of heavy metals were inversely proportional to root growth and dry biomass. Exposing high concentration (125ppm) Pb inhibited the root growth of *D. regia,* while much higher reduction was observed for *L. leucocephala.*

Fahr *et al.* (2013) showed that Pb affects plants primarily through their root systems. Plant roots quickly respond either by the production and deposition of callose, creating a barrier that prevents Pb entering through the uptake of large amounts of Pb and its sequestration in the vacuole accompanied by changes in root growth and branching pattern or by its translocation to the aboveground parts of plant in the case of hyperaccumulators plants. Pb has no biological function but can cause morphological, physiological, and biochemical dysfunctions in plants. Mami *et al.* (2011) conducted a study in order to evaluate two tomato (*Solanum lycopersicum*) varieties (Barakat and Local tomato) response to Pb in northern of Iran. Five doses (0, 0.001, 0.01, 0.1 and 1) % of Pb was investigated. Results found that the reaction of varieties to different heavy metal compounds and those doses is varied and Barakat variety has greater resistance in more indices. Also, heavy metal kinds and different doses of these compounds affected some growth indices. Kabir *et al.* (2010) found that the effects of Pb on root, shoot and seedling length, leaf area, number of leaves, seedling dry weight (root/shoot) and leaf area ratios of *Thespesia populnea L*. were determined in greenhouses under natural environmental conditions with and without phytotoxic metal ions at (5, 10, 15, 20 and 25) µmol/l. Pb treatments have a strong influence on the growth and development of *T. populnea* by reducing

significantly ($P < 0.05$) all the above parameters. Pb treatment at 5–25 μmol/l produced significant ($P < 0.05$) effects on seedling and root length and seedling dry weight of *T. populnea,* while Pb treatment at 10–25 μmol/l produced significant ($P < 0.05$) effects on shoot length, number of leaves and leaf area as compared to control. Tolerance in *T. populnea* seedling at 25 μmol/l of Pb treatment was lowest as compared to all other treatments.

Oladele *et al.*(2017) reported that the seedling of bambara nut (*Vigna subterranea*) irrigated with various concentrations of $Pb(NO_3)_2$ (100,150 and 200) $mg.kg^{-1}$ show that a negative relationship existed between the different metal concentrations in the soil and the growth parameters (stem height, root length, leaf area, fresh and dry weight) compared to control experiment. Malar *et al.* (2014) conclude that the effect of Pb toxicity on physiological and biochemical changes in water hyacinth *(Eichhornia crassipes*) seedlings. The plant growth was significantly inhibited (50 %) at 1000 mg/L Pb concentration. Huiying *et al*. (2012) reported that perennial ryegrass *(Lolium perenne*), as one of the widely used turfgrass and forage species, has a potential for bioremediation. Ryegrass seedlings were subjected to 0, 0.5 and 3.2 mM of $Pb(NO_3)_2$ for 7 days in a hydroponic system maintained in a greenhouse. Both root and shoot growths were inhibited by Pb compared with the control. Kadiry (2009) studied the lead Pb accumulation and distribution and its effects on the growth of *Solanum melongena* seedlings which were grown under pot culture conditions in a glasshouse with (75, 150 and 300) $mg.\ L^{-1}$. Growth characterestics such as root elongation, plant height; fresh and dry biomass of root and shoot; and leaf area were studied. The reference values from the control treatment were normal values for this plant growing in alkaline soils. When compared to low-level Pb treatment, the greatest amount of Pb generally reduced the growth characteristics.

2.2.2.3. Physiological characteristics

2.2.2.3.1. Photosynthetic pigments

Batool (2018) studied the effects of Ni toxicity on photosynthetic pigments of Chickpea (*Cicer arietinum*). Ni was applied to the soil as solution 0, 25, 50, 100 and 150 mg. l^{-1}. A significant decrease in carotenoid contents was observed with increasing concentrations of Ni application. The plants also showed a similar declining pattern, which may be explained by reduced pigment concentrations and reduced photosynthetic activity. Pant *et al.*(2020) discovered that plants that grow in Pb-contaminated soils can absorb the heavy metal and transport it to different areas both intracellularly and extracellularly. Pb poisoning can have an impact on a plant's overall health since it interferes with fundamental physiological and metabolic functions including photosynthesis.

Deswal and Laura (2018) observed that the heavy metals, Ni and Pb quantitative accumulation occurred in the order leaves. The heavy metals lowered the total carotenoids' quantity, and the reduction in total carotenoids depended on concentration. SaiKachout *et al.* (2015) conducted a study on the elements Ni and Pb to annual atriplex (*A. hortensis* and *A. rosea*) to phytoremediate soils polluted with these heavy metals. The soils, which contained up to 1674 mg Ni and 1334 mg Pb/kg^{-1} were sampled around metal contaminated site in southwest of France. Some heavy metals accumulated in the plants may have reached toxic levels on the pigment contents Significant increases in chlorophyll content were observed in leaves for atriplex varieties; on the other hand the carotenoid content was also reduced when exposed to high concentrations of polluted soil. Singh *et al.* (2012) carried out a study on the effect of Pb and Ni on chlorophyll a, b, carotenoids of black gram (*Vigna mungo L.*) seedlings were evaluated under 10, 50 and 100 μM concentration. These concentrations significantly affected chlorophyll, carotenoid of Black gram as compared to control. Pb and Ni at 10 μM concentration resulted in less significant effect on chlorophyll, a, b and carotenoids, while higher concentrations 50 and 100 μM significantly reduced carotenoid contents of the seedlings. Abdalla and Mahmoud (2008) carried out an experiment in the open field at station of vegetable on the effect of Pb and Ni added in combinations as thawing acetate salts to the soil mixture of six-months-old transplants of *Acalypha wilkesiana*., *Asclepias curassavica*, *Dodonaea viscosa* and *Tabernaemontana divaricate.* The chemical analysis of the obtained the results showed that carotenoids contents in the leaves of the studied shrubs were reduced with increasing heavy metal concentrations.

2.2.2.3.2 Enzymatic and non – enzymatic antioxidants

Baran and Ekmekci (2021) studied the antioxidant defence of safflower species (*Carthamus oxyacantha M. Bieb.* and *Carthamus tinctorius L*. exposed to Ni 0, 0.50, 0.75 and 1.00 mM. Both species might certainly resist harmful Ni poisoning if their own defense mechanisms, including antioxidant enzymes, were better upregulated. POD activities among them increased with rising Ni concentrations. The POD activities of both species were most prominent and consistently increased in toxic Ni levels and maght protect them from negative effect of H_2O_2. El-Amier *et al.*(2019) showed that the effect of Ni on the biochemical and physiological processes in *P. sativum* seedling which was grown in Hoagland solution treated with (100 and 200) M of $NiCl_2$ for 72 h in the growth chamber. Proline was induced due to the toxicity of Ni concentration. The activities of antioxidant enzyme catalase was induced under the treatments of the metals. Sirhindi *et al.* (2016) evaluated the physio-biochemical attributes, antioxidant enzyme activity in soybean (*Glycine max* L.) plants subjected to Ni stress, where it showed an increase in total protein 22.58 % respectively, under Ni toxicity over the control. The activities of peroxidase (POD) increases by 28.22 %, respectively, over the control in Ni treated seedlings. Pompeu *et al.*(2008) noticed that Ni at high concentrations can lead to production of ROS resulting in oxidative damage at the cellular level. They investigated the antioxidative responses of *Nicotiana tabacum* to Ni 0.075 and 0.75 mM over a 72 h period with special attention to potential alterations in CAT. Analysis revealed that CAT activity plays a major role in the early response to Ni induced oxidative stress, particularly when the Ni concentration used was low. De Macedo *et al.* (2016) reported that Ni is needed by plants in tiny doses for optimal growth, especially legumes because of its function in N metabolism. Soybean plants inoculated with *Bradyrhizobium japonicum* were cultivated in soil polluted with five Ni rates 0.0, 0.1, 0.5, 1.0, and 10.0 mg. dm^{-3} Ni. The NR activity was not affected indicating good biological nitrogen fixation for all plants. The reddish color of the nodules increased with Ni rates confirms the good BNF due to Ni availability.

Zainab *et al.* (2021) observed in a study that the oxidative stress occurs when crop plants are exposed to extreme abiotic conditions that Pb to the excessive production and accumulation of ROS. Drought, high temperatures, heavy metals, salinity, and ultraviolet (UV) radiation are only a few of the harsh abiotic factors or stresses that result in crop production and quality losses. ROS are highly reactive entities that can destroy molecules, metabolites, and organelles in plants by obstructing several metabolic processes until cell death occurs. To detoxify ROS and defend themselves from oxidative damage, plants have developed defensive mechanisms

that involve the generation of antioxidants. Staszak *et al.* (2020) who evaluated that plant physiological and biochemical processes can be severely harmed by Pb. The degree of a metal's toxicity depends on its chemical form. Pb in concentrations of 12.5 mM and 25 mM on pine (*Pinus sylvestris*) seed germination. Redox changes occur in the cell as a result of the high levels of ROS. The antioxidant cycle's effective non-enzymatic activity predominated in the early stages of germination processes, while in the late stages, enzymatic activity was observed in the presence of Pb compounds. Seneviratne *et al*. (2017) revealed that heavy metal Pb contamination in soils can impact various biochemical processes in plants, including antioxidant production, protein mobilization. Whenever heavy metals are present, nutrient sources are constrained. Furthermore, with heavy metal stress, protein content can be seen to increase. The amino acid proline is crucial for cellular metabolism. Additionally, under heavy metal stress, the production of heat shock proteins has been noted. Zengin and Munzuroglu (2005) evualated an experiment on the effect of lead on the content of ascorbic acid in 17-day-old bean seedlings (*Phaseolus vulgaris*) grown in Hoagland solution with various concentrations of Pb. Control heavy metal-treated plants were grown for 10 days in Hoagland solution. The amount of ascorbic acid in ten-day-old main leaves was measured. After 10 days, main leaves showed a considerable rise in ascorbic acid content along with rising levels of heavy metals. The plants that had been exposed to Pb heavy metals had the greatest impact on the ascorbic acid content. Khan *et al.* (2017) showed that proteins the sequence of amino acids determines each protein's unique 3-dimensional structure and its specific function such as catalysis of biochemical reactions, mechanical support and immune protection.

Bielen *et al.* (2013) reported that plants growing on soils contaminated with excess levels of metals Pb and Ni experience a disturbance of the cellular redox balance, which leads to an increase of ROS. Even though the increased ROS levels can cause cellular damage, controlled levels play an important role in modulating signaling networks that control physiological processes and stress responses. Under stress conditions like exposure to excess metals as well as under non-stress settings, plants regulate ROS levels via their antioxidative defense system. A well-known and significant part of the plant's antioxidant system is ascorbate (AsA). It can directly and indirectly lower ROS as the principal antioxidant. Additionally, AsA plays a crucial part in physiological processes, some of which are hampered by too much metal. Abdalla and Mahmoud (2008) conducted a sudy in the open field at station of vegetable on the effect of lead Pb and nickel Ni added in combinations as thawing acetate salts to the soil mixture of six-months-old transplants of *Acalypha wilkesiana, Asclepias curassavic*, *Dodonaea viscosa* and *Tabernaemontana divaricate*. The results indicated total carbohydrates

contents in the leaves of the studied shrubs were decreased with increasing heavy metal concentrations.

2.2.2.3.3 Elements concentration

Baran and Ekmekci (2021) studied safflower species (*Carthamus oxyacantha* and *Carthamus tinctorius*) exposed to Ni 0, 0.50, 0.75 and 1.00 mM. Where the accumulation of Ni was higher in roots than in stem and leaves for both species. Bislimi *et al.* (2021) showed that in contaminated site, the mean level of all the metals in soil and different parts (root and leaf) of the plant were found to be significantly ($p<0.01$) higher than the uncontaminated site.

Palowsk *et al.* (2016) who evaluated that concentrations of Pb in leaves of *Robinia pseudoacacia* from several sites (three industrial cities and two rural villages) in southern Poland. The lowest concentrations of Pb was found in one of the industrial cities in the area. On the other hand, the concentrations of Pb was similar in the rural areas when compared to the concentrations observed in the cities. The high level of metal contamination of air in rural areas may be a result of the long-range transport of emissions. The leaves of *R. pseudoacacia* are good bioindicators of metal contamination of air in towns and cities with different traffic intensities and within the surroundings of industrial plants and railway tracks. *R. pseudoacacia* was also used for the first time to assess the long-range transport of emissions.

Malar *et al.* (2014) concluded that the effect of Pb toxicity on physiological and biochemical changes in water hyacinth *(Eichhornia crassipes*) seedlings. Accumulation of Pb was higher in root than in shoot tissues. The maximum level of Pb accumulation was noticed in roots (5.45 %) followed by petiole (2.72 %) and leaf tissues (0.66 %). Hamza *et al*. (2020) illustrated heavy metal accumulation of *Juncus rigidus,* from different regions of the Basrah province in Southern of Iraq. Specifically, the concentrations of lead and nickel were determined in the roots and leaves of the plant. The results indicated that the highest accumulation of the heavy metal was recorded for Pb 12.50± 3.58 $mg.kg^{-1}$ and then in Ni < 0.30 $mg.kg^{-1}$. As well as, Pb concentrations in *J. rigidus* varied in different locations and parts of the plant from undetectable in control to 12.66 and 9.80 $mg.kg^{-1}$ in leaves and roots respectively from station 1 and 10.76 and 9.50 $mg.kg^{-1}$ in station 2.

Kacálková *et al.* (2014) studied effects of the Ni and Pb on maize (*Zea mays*), sunflower (*Helianthus annuus*), willow (*Salix smithiana*), and poplar (*Populus nigra*) and the realtionship between the contaminants in soil and in plants. The total and available soil metal concentrations in soil were investigated. Only a low portion of risk elements were available for plants (6 % Ni and 1.3 % Pb). Ni and Pb showed a similar trend to element accumulation where the highest

amount was found in plant roots, higher in herbs than in trees (5.04 mg Ni · kg^{-1}, and 7.76 mg Pb · kg^{-1}). Results also indicated that translocation of Ni and Pb from roots to aboveground biomass of willow and poplar was low (89-98 % of risk elements was retained in roots). The highest translocation from plant roots to aboveground biomass of maize and sunflower was found in the case of Pb (76 %).

Stancovic *et al*. (2009) suggested that the heavy metals Ni and Pb in leaves of the species *Paulownia elongata* growing under urban and sub-urban conditions with the comparison to the concentration of these elements in leaves of the species *Paulownia elongata* and *Paulownia fortunei* showed average nickel concentrations of 2.7 μg/g on the experimental plot in Bela Crkva, Serbia, twice as low as the concentrations measured under extreme urban conditions (6.62 μg/g, or 4.54 μg/g) in the immediate vicinity of suburban traffic lines. In as much as *Paulownia elongata* endures urban conditions well, it can be recommended for cultivation in parks, tree alleys, and wind-protection zones along urban and regional traffic lines.

Nas and Ali (2018) who investigated that heavy metal is neither essential element nor have any role in the process of cell metabolism but is easily absorbed and accumulated in different parts of a plant. The lead uptake is mainly regulated by pH, particle size, and cation exchange capacity of the soil, root exudation and by different other physical and chemical parameters. The high concentration of the heavy metals such as lead can cause a number of toxic symptoms in plants that may be retardated in growth (Stunted growth).

Kadiry (2009) carried out a study on the lead accumulation and spread and its impacts on growth and nutrient content, *Solanum melongena* seedlings were grown in pot culture conditions in a glasshouse with 75, 150, and 300 mg. $litre^{-1}$. Growth parameters and mineral elements Ca, Mg, K, P, Fe, Zn, Cu, and Mn where tested. For this plant, which was grown in alkaline soils, the control treatment yielded usual values. Comparing the high-level Pb treatment to the low-level Pb treatment, all mineral elements were typically blocked from being taken up. Abdalla and Mahmoud, (2008) conducted a study in the open field at station of vegetable on the effect of Pb and Ni added in combinations as thawing acetate salts to the soil mixture of six-months-old transplants of *Acalypha wilkesiana*, *Asclepias curassavica*, *Dodonaea viscosa* and *Tabernaemontana divaricate*. The results indicated that N, P and K contents in the leaves of the studied shrubs were decreased with increasing heavy metal concentrations.

2.2.2.4 Phytochemical concentration

Usman *et al.* (2022) noticed that the phytochemicals of the fabaceae family have industrial and pharmacological importance. This family is a big source of phytochemicals, namely, flavonoids, saponins, alkaloids and phenolic acids, which have an anti-cancer property and the use of these phytochemicals is increasing over time.

Ushie *et al*. (2022) evaluated that the extracts of the leaves were prepared by soaking 100 g of the sample in 250 ml ethyl acetate for 72 hours with frequent agitation. The phytochemical screening of *Thaumatococcus danielli* was undertaken through controlled experiments. The results showed that flavonoids, alkaloids, steroids, terpenes, tannins, glycosides and saponins are present in the ethyl acetate leaf extracts. Quantitative analysis showed the order of the concentrations in $mg.g^{-1}$ as follows: tannins: (4.644); flavonoid: (1.830); phenols: (0.756); alkaloids: (0.578); and saponins: (0.440).

Islam *et al*. (2021) utilized water, methanol and ethyl acetate solvent extraction methods to perform a phytochemical screening of *Tragia involucrata* leaves, where they found that methanol demonstrated the higher number of secondary metabolites compared to ethyl acetate. Methanol contains phenols, alkaloids, flavonoids, tannins, saponins and terpenoids. Because of diverse biological functions, these metabolites have particular interest in drug discovery research and thus advance the medicinal research field.

Septina *et al*. (2020) and Deivasigamani (2018) demonstrated that *L. leucocephala* has been used in various traditional medicinal systems to treat multiple human diseases and this plant has been reported to contain numerous secondary metabolites of leaves like phenols, alkaloids, cardiac glycosides, flavonoids, saponins, tannins and triterpenoids.

Kaloo *et al.* (2018) and Bhat *et al.* (2015) in their studies used *R. pseudoacacia* and different solvent extracts to detect the presence of active constituents like alkaloids, flavonoids saponins, tannins and phenols. However, the positive results for the detection of flavonoids, tannins and phenols were obtained and the maximum yield of ethyl acetate extract of plant leaves namely 38.23 % was obtained**.**

Rajkumar *et al*. (2015) showed that preliminary phytochemical screening of methanolic extracts of *Garcinia imberti* revealed the presence of various bioactive components like alkaloids, flavanoids, steroids, glycosides, phenols, saponins, terpenoids and tannins in the leaf. The quantitative analysis of phytochemicals of the extracts showed the presence of high amount of tannins (0.92 ± 0.23 $mg.g^{-1}$) and alkaloids (0.83 ± 0.48 $mg.g^{-1}$) in leaf extract. The study concluded that the methanolic extracts of *Garcinia imberti* leaves posse. antioxidant activity

due to the presence of significant amount of phenolic compounds which are the major contributors of antioxidant activity.

Mishra *et al.* (2012) illustrated that minerals have a diversified role in medicinal plant metabolism. Severity or scarcity of these causes multifarious effects in plant metabolism. Due to the manufacture of bioactive compounds (secondary metabolites), medicinal plants acquire resistance to a variety of diseases brought on by fungi, bacteria, viruses, mycoplasma, insects, and pests. The concentration of these minerals of both group i.e. activators or inhibitors present in the soil play a vital role in secondary plant metabolism. Minerals also play a major role in the reproduction of these medicinally important plants. Alkaloids, flavonoids, glycosides, phenolic compounds, and tannins, among other bioactive molecules of medical relevance, are created through various biosynthetic pathways of plants and are a boon to the urban, hilly, and distant populations of each country. Thus, biosynthesis of these bioactive molecules in a plant system are widely dependent on the availability of mineral elements in the soil for example N in nitrates and ammonium salts, phosphorus (P) in phosphates, calcium (Ca), magnesium (Mg) and potassium (K) in their salts as sulfates or chlorides, sulphur (S) in sulphates, iron (Fe) in ferrous or ferric salts (more readily from ferrous salts), manganese (Mn) in magnanimous salts, boron (B) in borates, copper (Cu) and zinc (Zn) in their salts, and molybdenum (Mo) in molybdates.

2.2.2.5 Anatomical studies

Pishchik *et al.* (2021) reported that the Ni affects on the physical barriers, such as plants cell walls and thick cuticles, which reduce the elevated levels of Ni entrance. Saraiva, *et al.*, (2020) concluded that soybean plants under high Ni concentrations. Ni 0 and 200 μM. Ni excess provoked damage to root and leaf structures, causing anatomical disorders in these tissues. For leaf tissue, significant increases in palisade (11%) and spongy parenchyma (29%) were detected in plants sprayed and exposed to Ni, which were intrinsically related to stomatal density and stomatal functionality.

Chaudhari *et al.*(2016) placed *Pisum sativum L.* seeds in petri dishes and allowed to grow for 15 days in different concentrations i.e. 100 ppm, 200 ppm and 300 ppm of Pb as [$Pb(NO_3)_2$] and Ni as [$NiCl_2$]. Higher concentrations (300 ppm) of Pb exhibit certain anatomical abnormalities like abnormal vascular system and enlarge cortical cells. However, no specific abnormalities were observed with Ni doses.

Musa *et al.* (2019) reported that anatomical differences found in *Luffa cyclindrical* and *Amaranthus viridis* grown on the dumpsite is an indication of the HMs and also determine the distribution of HMs on the dumpsite. The results showed the HMs detected in the dumpsite soil at three spots were significantly higher than the control. Anatomical changes in the leaves epidermis such as irregular shape of the epidermis, decrease in quality and stomata size were all witnessed in the dumpsite plant as against the control. Hence, it is likely that all detected differences in the epidermal structures of the test plant grown on dumpsite soil were caused by the high level of HMs present in the dumpsites and therefore suggested that, changes in epidermal structures of *L. cyclindrical* and *A. viridis* grown on poluted soils is an indication of HMs such as Pb present.

Amari *et al.* (2017) showed that concentrations of heavy metals in soil seldom reach a level sufficient to cause osmotic disturbances in plants. Heavy metals influence water delivery to the shoot due to inhibition of transpiration as they decrease the size of the leaves and the thickness of the lamina, reduce intercellular spaces, affect the density of stomata and decrease their aperture. Stomata closure is induced by direct interaction of toxic metals with guard cells and/or as a consequence of the early effects of metal toxicity on roots and stems. An excess of both essential and non-essential metals induces ion stress in plants and causes multiple direct or indirect effects, which concern practically all physiological functions. Stomata function is important on the physiology, adaptation and productivity of plants, and adaptation ability of the plants is closely associated with transpiration and photosynthesis process occurred in their leaves, the number and distribution of the stomata in unit leaf area have an important role in these processes by adjusting CO_2, O_2 and moisture exchange between the leaves and the atmosphere. Some studies mentioned the important role for stomata number per area in net photosynthesis products and vegetative growth in some peach varieties. Stomata play an essential role in the regulation of gas exchange in flowering plants and are distributed throughout the aerial epidermis. In leaves, the pattern of stomata distribution is highly variable between species but is regulated by a mechanism that maintains a minimum of one cell spacing between stomata. The environment also has significant effects on stomata development. In a number of species both light intensity and CO_2 concentrations have been shown to influence the frequency at which stomata develop on leaves (Casson and Gray, 2008; Brownlee, 2001).

Azmat *et al.* (2009) revealed the hypothesis that variations in leaf anatomy and morphology reflect their adaptability to the environmental stress. A self defense mechanism system related the surface of leaves for the detoxification of Pb was observed in *Phaseolus mungo* and *Lens*

culinaris through light microscopy in leaves. Increase in number of stomata in the adaxial (upper) leaf surface of both species seems to constitute an important morphological mechanism for survival that allows this species to maintain good photosystem II efficiency during the stress. The importance of the increase in the number of stomata in relation with the absorption of CO_2 with increase in creatine kinase (CK) enzymatic activity, creatine, glucose and reducing sugars ($p<.001$) for both species under the metal stress were observed.

Kadiry (2009) examined an experiment on the lead (Pb) accumulation and spread and its effects on growth and nutrient content, *Solanum melongena* seedlings were grown in pot culture conditions in a glasshouse via 75, 150 and 300 mg. litre^{-1}. A negative effect was determined by Pb concentration on stomatal parameters such as stomata length and width in this study. The highest level of Pb generally inhibited the stomata parameters as compared with the low-level Pb treatment.

2.2.2.6 Soil phytoremediation

Kafle *et al.* (2022) investigated that the current intensive agriculture practices and industrialization, pollution of natural resources like land with heavy metals, organic pollutants, radionuclides, pesticides, and fertilizers has become a major concern. Phytoremediation is a cost-effective and environmentally friendly technique that utilizes plants to immobilize, uptake, reduce toxicity, stabilize, or degrade the compounds that are released into the environment from different sources. Studies have found that plants can be used for the remediation of heavy metals, organic pollutants, radionuclides, antibiotics, and pesticides. Despite being used for many years, phytoremediation is still a relatively new technology. Diverse plants remediate different pollutants at different rates through one or multiple mechanisms. Given the low-cost of phytoremediation compared to conventional technology and sustainability associated with plants and use of renewable energy, phytoremediation can be a reliable solution for a sustainable and economical remediation of soil from the organic and inorganic pollutants. Solomou *et al.* (2022) reported that use of contaminated soils in food production imposes the need for the reduction in heavy metals concentrations, using various techniques, in order to eliminate the toxic effects of pollution and ensure safety in the consumption of agricultural products. Many hyperaccumulating plants have been identified that can be used in soil cleansing, enhancing the applicability and replicability of the method. The selection of the appropriate plant species is based on their specific physiological

characteristics to remove undesirable elements from the soils and, in certain cases, there is a preference for use of non-native species. However, because they have the potential to seriously affect the environmental and ecological dynamics of the regional plant communities, such species may exhibit invasive characteristics, posing high uncertainties and hazards in the preservation of local ecosystems, particularly in the Mediterranean region. Since native plants are better adapted, have no impact on the regional ecological balance, and may be used without violating any laws, their use is often more advantageous.

Nedjimi (2021) evaluated that toxic metal contamination of soil is a major environmental hazard. The use of heat treatment, electroremediation, soil replacement, precipitation, and chemical leaching are all examples of chemical treatments for heavy metal decontamination that are typically very expensive and inapplicable to agricultural fields. To clean up polluted surroundings, numerous methods are employed. One of these is phytoremediation, which relies on the use of hyper-accumulator plant species that can withstand significant concentrations of hazardous HMs in the air, water, or soil. Such a strategy uses green plants to remove, degrade, or detoxify toxic metals. Five types of phytoremediation technologies have often been employed for soil decontamination: *phytostabilization, phytodegradation, rhizofiltration*, *phytoextraction* and *phytovolatilization*. Traditional phytoremediation techniques have certain drawbacks when used on a big scale; therefore using genetic engineering techniques like transgenic transformation, to increase the effectiveness of plants as candidates for HMs decontamination, nanoparticle addition, phytoremediation supported with phytohormones, and plant growth-promoting bacteria inoculation have all been used. Al-Heety *et al.* (2021) showed that heavy metals' accumulation in the leaves of urban plants as a biological indicator of soil pollution. The plant leaf and soil samples were collected from 51 sites adjacent to power generators in Ramadi-Iraq. Six common plant species, namely, *Albizia lebbeck*, *Conocarpus lancifolius*, *Dodonaea viscosa*, *Eucalyptus camaldulensis*, *Ficus microcarpa*, and *Ziziphus spina-christi* were selected. The highest heavy metal content was found in *Conocarpus viscosa* leaves followed by_ *Dodonaea viscosa*. These two plants have a significant difference ($p< 0.05$) in the heavy metal content compared to other plants. The total average of plant heavy metal concentration was as follows: Ni 7.55 and Pb 6.49 mg per kg. Concentrations of heavy metals in leaves have the trend as Ni> Pb. A high concentration of nickel, and lead are found in the plant leaves compared to the Food and Agriculture Organization-World Health Organization (FAO/WHO) permissible values.

Yan *et al.* (2020) investigated the mechanisms of how heavy metals taken up and translocated in plants are described, and the detoxification strategies (avoidance and tolerance) adopted by plants in response to heavy metal. The recent advances in developing phytoremediation techniques, including the strategies to improve heavy metal bioavailability, tolerance, and accumulation. There are series of processes involved in accumulation of heavy metal in plants, including heavy metal mobilization, root uptake, xylem loading, root-to-shoot transport. Heavy metal mostly exists as insoluble form in soil, which is not bioavailable to plants.

MATERIALS
AND
METHODS

Chapter Three

Materials and methods

3.1 Plant materials and growth conditions

Three tree species of fabaceae family: *G. triacanthos, L. leucocephala and R. pseudoacacia* were selected because of rere of studies about them where classified by Dr. Serwan T. Al-dabbagh, Field Crops and Medicinal Plants Department., College of Agricultural Engineering Sciences, Salahaddin University – Erbil (Townsend and Guest, 1974). The healthy legume seeds (figure 3.1) were collected from the campus of Koya University, Iraqi Kurdistan Region on March 2nd, 2021. By manually climbing the 10 year old, straight-baled, well-formed trees, the mature pods were directly harvested.

At a private field in Koya city located at 44º38 E, 36 º4N and 570 m of altitude above sea level, a factorial experiment with a complete randomized design with three replications were used for each species to study the effects of concentrations 0, 15, 30 and 45 $mg.kg^{-1}$ soil of two heavy metals nickel chloride ($NiCl_2$) and lead chloride ($PbCl_2$) and their interactions on each species separately.

Seeds were soaked in ordinary water at room temperature for 24 hours as described by Shah Asif and Rather (2018) for *G. triacanthos*; Olorunmaiye *et al.* (2019) for *L. leucocephala;* and Roman *et al.* (2022) for *R. pseudoacacia*, the planting of seeds on March 25th, 2021 were done (2.5 cm in depth) using black polyethylene bags (sized 10x10x 30 cm) filled with 3 kg of loamy soil that was well mixed with the required concentrations of $NiCl_2$ or $PbCl_2$, and which were denoted as Ni0, Ni15, Ni30 and Ni45 for the first salt and Pb0, Pb15, Pb30 and Pb45 for the second salt respectively, with their interactions.

Each experimental unit consists of 10 polyethylene bags. The pots were kept under polyethylene then and saran fabric (figure 3.2) and the pots were watered when needed with tap water to provide a possible uniform soil moisture conditions.

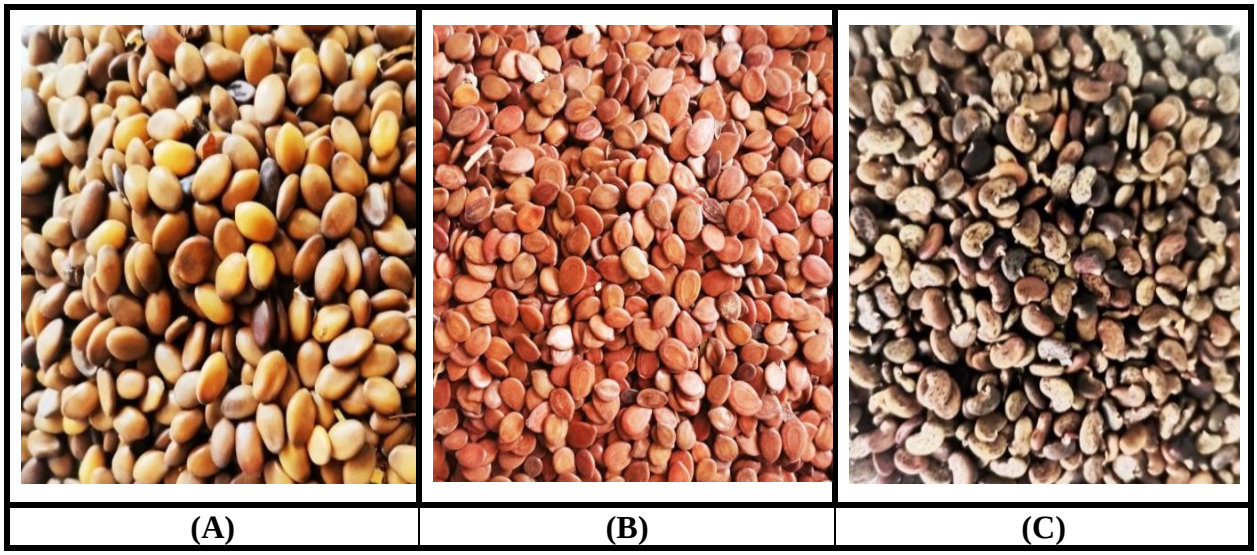


Figure 3.1. *The seeds of (A) G. triacanthos, (B) L. leucocephala and (C) R. pseudoacacia species.*

Fig 3.2. *(A) G. triacanthos,(B) L. leucocephala and (C) R.pseudoacacia seedling four months aged.*

3.2 Meteorological data

Maximum and minimum temperature, the relative humidity and the amount of rain fall in the open field during the planting season are shown in Table (3.1), as recorded by Agro-Meteorological Station in Koya.

Table 3.1. Maximum and minimum temperature, the relative humidity and the amount of rain fall during the growing season.

Month (2021)	Air Temp. C°		Relative Humidity%		Rain fall (mm)
	Maximum	Minimum	Maximum	Minimum	
March	26	4	83	29	24.3
April	39	10	85	18	9.6
May	43	19	50	16	4.3
June	47	22	38	13	0.0
July	48	25	29	12	0.0
August	48	23	33	14	0.0
September	45	22	35	15	0.0
October	39	13	67	18	0.0

3.3. Studied characteristics

3.3.1. Germination percentage (%) and velocity (Days)

Ahmadloo *et al*. (2012) described seed germination (G) formula, as shown below:

$$\mathrm{G}(\%) = \frac{\mathrm{L}}{\mathrm{S}} * 100$$

G (%) = Germination percentage

L = Total number of emerged seedlings

S = Number of planted seeds

Ranal and Santana (2006) designed a formula of velocity of germination as following:

$$\mathrm{M}(\mathrm{days}) = \sum_{i=1}^{n} N_i G_i \; / \; \sum_{i=1}^{n} G_i$$

where:

M: velocity of germination (days)

G: number of seedlings emerged on the day of observation

N: number of days counted from the day of sowing until the day of observation.

Each of the following parameters were taken for three plants for each experimental unit on Augast 2021 (4 months after seed germination)

3.3.2. Vegetative growth (Khudhur, 2019 and Al-Barzinji *et al.*, 2015):

3.3.2.1. Plant height (cm): It was measured by metric tapeline from the point at stem attachment with soil to the apical point of the main shoot.

3.3.2.2 Number of leaves plant^{-1}

3.3.2.3 Stem diameter (cm): It was measured by vernier caliper instrument.

3.3.2.4 Length of main root (cm): After washing the root by tap and distilled water, length of main root measured from the point of root attachment with soil to the end point of the main root by using metric tapeline.

3.3.2.5 Root diameter (cm): It was measured by vernier caliper instrument.

3.3.2.6 Leaf area (cm^2): Plant leaf area calculated by the image J software described by (Easlon and Bloom, 2014).

3.3.2.7 Fresh weight of shoot (g): The shoot of fresh plants were weighted by sensitive balance.

3.3.2.8 Dry weight of shoot (g): The shoot of plants were oven dried to constant weight at 65°C .

3.3.2.9 Fresh weight of root (g): The root of fresh plants were weighted by sensitive balance.

3.3.2.10 Dry weight of root (g): The roots of plants were oven dried to constant weight at 65°C.

3.3.2.11 Shoot dry matter (%): Calculated by the following formula, as described by Al-Sahaf (1989).

$$\text{Shoot dry matter (\%)} = \frac{\text{Shoot dry weight}}{\text{Shoot fresh weight}} * 100$$

3.3.2.12 Root dry matter (%): The following formula was used to calculate the percentage of the root dry matter, as described by Al-Sahaf (1989).

$$\text{Root dry matter (\%)} = \frac{\text{Root dry weight}}{\text{Root fresh weight}} * 100$$

3.3.2.13 Number of nodules plant^{-1}

3.3.2.14 Fresh weight of nodules (g): The nodule were weighted by sensitive balance.

3.3.2.15 Dry weight of nodules (g): The nodules were oven dried to constant weight at 65°C.

3.3.2.16 Nodule dry matter (%): Calculated by the following formula, as described by Al-Sahaf (1989).

$$\text{Nodule dry matter (\%)} = \frac{\text{Nodule dry weight}}{\text{Nodule fresh weight}} * 100$$

3rd and 4th fully grown leaves were collected 70 days after seed germination to make the following estimations

3.3.3 Estimation of the photosynthetic pigments content: Chlorophyll a, b and total carotenoids (mg/g fresh weight)

The amount of chlorophyll a, b and total carotenoids were estimated using method 80% acetone as mentioned by Lichtenthaler and Wellburn (1983). Mixture ratio was 50 ml: 1 g sample, where 0.4g of fresh leaves were mixed with 20 ml 80% acetone then grinded by mortar and pestle and filtered by filter paper. The extraction placed in a 25 ml glass vial (closed dark bottle), to prevent evaporation and avoid photo-oxidation of pigments, and then read the results by spectrophotometer at 663, 646 and 470 nm wave lengths. Chlorophyll a, chlorophyll b and total carotenoids were calculated as follows:

Chl a = (12.21*A663) - (2.81*A646)
Chl b = (20.13*A646) - (5.03*A663)
TC = (1000*A470 - 3.27*Chl a – 104*Chl b)/229

where, A is Absorbance, Chl a = chlorophyll a (mg/L), Chl b = chlorophyll b (mg/L) and TC = total carotenoids (mg/L).

For converting the concentration from mg/L to mg/g fresh weight, each value is multiplied by (extraction volume / sample weight) *1000.

3.3.4. Extraction and activity of enzymatic antioxidants

To determine the activity of the antioxidant enzymes: POD and CAT samples of the leaves were crushed 1 g of fresh weight after cutting them with a clean knife into small pieces were frozen, the ground to a powder with mortar and pestle with 10 ml of (0.1M) potassium phosphate organized at cold pH 7.8 after filtering using a centrifuge at 1000 RPM for 10 minute. The process was carried out under cold condition (4°C). Fresh leaves were kept in ice during the course of homogenization. The extracts were prepared to analysis (Pitotti *et al*., 1995).

3.3.4.1 Peroxidase (POD) enzyme activity (μgg^{-1})

The activity of POD enzyme was determinated according to the method described by Nezih (1985), as clarified below

Material and used solutions

A- Guaicaol solution: Prepare by mixing 1.36 ml of guaicaol in a volumetric flask and then complete volume to 250 ml using distilled water.

B- Hydrogen peroxide - H_2O_2 solution at a concentration of 0.1%: Prepare by taking a volume of 0.4 ml of 30 % H_2O_2 and completing to 120 ml by using distilled water

Procedure

A. Mix 1 ml of H_2O_2 solution with 1 ml of guaicaol solution (reaction mixture).

B. The enzyme activity was estimated by adding 2 ml of the reaction mixture in the cuvette, then 0.1 ml of the sample was added and the change is followed up absorption of light every 30 seconds for a period of 3 minutes at a wavelength of 420 nm by spectrophotometer (model 721-2000, China). Blank was prepared in the same way without a sample.

Calculation

The activity of the POD enzyme was calculated by:

$$\text{POD Activity (unit.ml}^{-1}) = \frac{\frac{\Delta \text{ Optical Density Reading}}{\Delta\, Time}}{0.1 \text{ X } 0.01}$$

where: 0.1: volume of the sample, 0.01: one unit of enzyme (the amount of enzyme that increases in light absorption (they are 0.01 units per minute at a wavelength of 420 nm).

3.3.4.2 Catalase (CAT) enzyme activity (μgg^{-1})

The activity of the CAT enzyme was estimated by a UV-V is spectrophotometer according to Aebi (1974) method, which uses the amount of change in absorbance at 240 nm , (30 mM) of hydrogen peroxide and (50 mM) of phosphate buffer has a pH of (7).

Solutions used

- **Solution A:** prepared by dissolving 1.742 g of K_2HPO_4 in the distilled water after completing the volum to 200 ml by using distilled water.
- **Solution B:** prepared by dissolving 1.3608 g of KH_2PO_4 in the distilled water and completing the volum to 200 ml by using distilled water.
- **50 mM phosphate buffer (pH = 7) solution**: Prepare by adding a limited amount of solution B to 50 mL of solution A until it reaches pH 7.
- **30 mM of H_2O_2 solution:** prepared by taking 0.34 ml of 30 % H_2O_2 and completing the volume to 100 ml by using the buffer solution.

Procedure

Mix 0.1 ml of sample extract with 1.9 ml of buffer solution and then 1 ml of hydrogen peroxide solution added; At that moment the reaction will start, the materials mixed well with gentle knocks on the walls of test tubes, then read at 240 nm by UV spectrophotometer (Model: Agilent Technologies Cary Series UV-Spectrophotometer) using quartz cuvettes and optical density reading were taken every 30 seconds for 3 minutes. Blank was prepared in the same way without a sample.

Calculation

The activity of the CAT enzyme was calculated by:

$$\text{CAT Activity } (\text{unit.ml}^{-1}) = \frac{\frac{\Delta \text{ Optical Density Reading}}{\Delta \, Time}}{0.1 \text{ X } 0.01}$$

since 0.1: volume of the sample, (0.01: one unit of enzyme) the amount of enzyme that increases in light absorption (they are 0.01 units per minute at a wavelength of 240 nm).

3.3.4.3 Nitrate reductase (NR) enzyme activity (μmL^{-1})

The NR activity of studied plant species was done according to the procedure of Ahmad *et al.* (2010). Fresh leaves weighting 0.25 g were taken and cut it into 2 mm slices using mortar and pestle and ice – cold incubation medium that containing 3 ml of (0.05M) potassium phosphate buffer (pH 7.8) and 3 ml of (0.4M KNO_3) solution. Then, two times tubes of the mixtures sample were evacuated with a vacuum pump for 5 min. Then the mixture placed in a water bath at 35ºC for 75 min under dark conditions. At the end of the period, tubes were kept in boiling water bath 100 ºC for 5 min to stop the enzyme activity and complete leaching of the nitrite in the medium. After that 0.2 ml of the filtered sample from reaction mixture was taken and added 1 ml of solution A and 1 ml of solution B. [Solution A: 1.0 % sulphanilamide in (1N – HCl). Solution B: 0.025% N-(1-Napthyl)-ethylene diammonium dichloride (NEDD) in double distilled water were added]. Then, allowed for 30 min until the pink colour produced due to diazotization, after which the volume was made up to 6 ml with double distilled water. The ratio of material to medium was (1:6) w/w. The absorbance was read by UV-Vis spectrophotometer at 540 nm (Model DU 640B, Beckman, USA). The stander curve was prepared by using $NaNO_2$ or KNO_2 solution.

3.3.5 Extraction of non-enzymatic antioxidants

3.3.5.1 Ascorbic acid (AA) content (gL^{-1})

A. Preparation of solutions

- Stock solution of ascorbic acid:

Containing (0.1 $molL^{-1}$) of ascorbic acid was prepared by dissolving appropriate amount (0.4 g) of ascorbic acid in distilled water and stored in a glass stopper bottle at 4C˚ in the dark. Solutions of variable concentrations were prepared by diluting the stock standard solution in water before use.

- Methylene blue solution (MB):

(0.0004 mol dm^{-3}) was prepared by dissolving 0.0126 g of methylene blue in 100 ml distilled water.

B. Preparation of samples

2 ml of glacial acetic acid was added to 2.5 g of leaves sample which were coarsely powdered. The mixture was stirred for about 20 minutes and rapidly filtered using Buchner funnel, transferred into100 ml volumetric flask and diluted to the mark with distilled water. The samples were then read by spectrophotometer.

C. Procedure

The spectrophotometric study was carried out by UV/VIS spectrophotometer (model 721-2000, made in China) to determine the amounts of ascorbic acid in the samples. Fifty microliters of a sample solution was mixed with 125 µl of MB (0.0004 mol dm^{-3}) solution and diluted up to 10 ml with distilled water. The absorption was measured at 665 nm. Results were expressed in mg of ascorbic acid per 100 g of the dry sample (Elbsheer, 2018).

3.3.5.2 Proline (Pr) content (µgml^{-1})

Estimation of the concentration of proline in fresh leaves according to the method of Bates *et al.* (1973). A weight of 0.5 g was taken from the fresh leaves and placed in a porcelain mortar and pestle, and crushed well after adding 10 ml of sulfosalicylic acid (3 %, w/v) dissolving (3 g of sulfosalicylic acid in 100 ml distilled water), then separated by centrifuge at 2000 RPM for 10 minutes, taking 2 ml of the plant extract and 2 ml of glacial acetic acid was added and 2 ml of ninhydrin reagent which was prepared by mixing (1.25 g of niniadrine with 30 mL of glacial acetic acid and 20 mL of (6 M) H_3PO_4 phosphoric acid) and the plant leave mixture on a quiet fire with stirring until melting and the appearance of yellow color; Then the test tubes were placed in a water bath at a temperature of 100 °C for an hour (1 h), the appearance of the red color, and leave the mixture on the room temperature for 2 minutes, and adding 4 ml of toluene, and then it was shaked until separated the red layers, then 3 ml of the upper layer was colored with red (containing proline), then it was measured by optical spectrophotometer wavelength at 520 nm and has three replicates for each parameter. The blank contained only the toluene. These readings were compared with standard curve of pure proline.

The standard curve of proline

The standard curve of proline prepared according to the method of Bates *et al.* (1973) using a concentration, extracted from pure proline 0, 10, 30, 50, 70, 90, 110 and 130 µgml^{-1}. After that, 2 ml of each dilution i.e pure proline; was taken and 2 ml of each of the glacial acetic acid and 2 ml ninhydrin were added to it, then put it in water bath 100 °C for 60 minutes. Cool the solution and add 4 ml of toluene and mixing using the test tube stirrer for one minute. Then reading was done with the spectrophotometer at a wavelength of 520 nm, the standard curve of proline comparing with the reading of samples.

3.3.5.3. Total carbohydrates (TCHO) %

0.1 g of dry leaves were mixed with 50 ml of distilled water, and then it was put in a water bath at 80°C for 30 minutes. Then centrifuged at 2000 RPM for 10 min., After filtaration, the

volume of the extract is reduced and placed in a bottle. Then 1 ml of the extract was mixed with 1 ml phenol (5 %), Mix well, then 5 ml of concentrated sulfuric acid (H_2SO_4) and 10 ml of distilled water were added. Then the samples were measured by spectrophotometer at 488 nm (Thiele and Palsson, 2010).

For finding the carbohydrate concentration, the standard curve was obtained by taking 50 mg glucose and 50 mg fructose in 1 liter of distilled water, then 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/L stock solution was prepared. Like the samples, 1 ml of these dilutions was mixed with 1 ml of phenol (5 %) well and then 5 ml of concentrated sulfuric acid (H_2SO_4) were added; Spectra were observed at 488 nm to extract the relationship between concentrations and optical spectrophotometer intensity readings (Al-Hayani, 2015). The percentage of total carbohydrates was determined by the following formula:

$$\text{Total carbohydrate (\%)} = \frac{\text{Concentration from standerd curve} * \text{Dilution}}{1000 * 1\text{ ml} * \text{sample weight}} * 100$$

3.3.6 Estimation of macro, micro elements and some heavy metals in the plants shoot, root and soil

Leaves were oven dried at 65° C for 72 hours untill weight fixed and ground by electrical grinder, then stored in an airtight container with proper labeling prior to use. They were subjected for analysis to determine the mineral contents of each plant and soil samples (Ryan *et al.*, 2001). Analysis of elements for the different parts of studied plant species and soils were done at the laboratories of the Department of Environment, Salahaddin University by using X-ray fluorescence (XRF) spectroscopy (NEX CG, Rigaku, USA) which is a well-established analytical technique for qualitative and quantitative elemental evaluation. It is a multi-elemental, simultaneous technique and additionally a non-destructive tool (Haschke, 2014).

Method analysis for some nutrient contents

Some other selected elements, chemical and physical properties of the soils were determined of Agriculture Consultancy and Agricultural Research Center/ Ainkawa / Erbil.

Total phosphorus (%): Spectrophotometer at 882 nm was used for determination of total phosphorus content after digested samples, as described by Menage and Pridmore (1973).

Total magnisium (%): Total magnisium content was estimated from digested samples by atomic absorption spectrophotometer at 405 nm, as mentioned by Chapman and Pratt (1978).

Total sulphate (%): Total sulphate was estimated after digested samples by using turbidimetric method by spectrophotometer at 420 nm as described by Gupta (2004).

Total organic nitrogen and protein (%): Total organic nitrogen content was determined after digested dry samples by kjeldahl method. The value of nitrogen obtained was multiplied by 6.25 leading to its conversion to crude protein (Usman *et al.*, 2018; Ryan *et al.*, 2001):

$$\% N = 14 \times VA \times N \times W/100$$

Then,

$$\% \text{ Crude Protein} = 6.25 \times \%N$$

where :

14 = Atomic weight of nitrogen

VA = Volume of acid which used for titration

N = Normality of HCl

W= Weight of the sample (g)

Table 3. 2. Some chemical and physical properties of the study soil before planting.

Soil properties		Units
EC	0.3	ds/m

pH		8.2	-----
O.M		0.6	%
CO_3		N.D	
HCO_3		0.03	
$CaCO_3$		44.6	
Macro elements			
N		0.000199	
P		0.002532	
K		0.61	
Mg		0.00333	
Ca		9.94	
SO_4		0.000922	
Microelements			
Cu		0.0054	
Fe		0.490	
Mn		0.0679	
Zn		0.0065	
Ni		0.022	
Non – essential elements			
Pb		0.0009	
Rb		0.0026	
Cd		0.00016	
Ag		0.029	
As		0.00034	
Ba		0.405	
Particle Size Distribution	**Clay**	22.1	
	Sand	26.8	
	Silt	51.9	
Soil texture		Silty loam	

3.3.7 Determination of qualitative and quantitative of some phytochemical constituents of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves

The qualitative and quantitative of some phytochemicals for the studied plants were done on the laboratories of Protein Research Center, Shahid Beheshti University, Tahran, Iran. As described by Ushie *et al.*(2022), Lahare *et al*. (2021) and Islam *et al.* (2021), as follows:

The leaves of the plant were properly washed in tap water and rinsed in distilled water. The rinsed leaves were hot air-dried for 3 days. The dried leaves of each plant were pulverized using pestle and mortar to obtain a powdered form which was stored in airtight glass containers at 4°C until used. 10 g of powdered sample was soaked in distilled water, methanol and ethylacetate 200 ml, 100 ml and 100 ml separately for 24 hrs at room temperature (over night 20 – 23 °C). The extracts were then filtered and concentrated to a final volume of 50 ml and prepared to qualitative phytochemical analysis.

3.3.7.1 Qualitative determination of the phytochemical contents

Preliminary qualitative phytochemical screening was carried out following standard protocols.

- **Ferric chloride test for phenols:** The test solutions were treated with 3–4 drops of 0.1 % (v/v) ferric chloride. Presence of phenols/ phenolic compounds were confirmed by brownish green or blue color.
- **Ferric chloride test for flavonoids:** The test solution was mixed with a few drops of ferric chloride solution and the presence of flavonoids compounds were confirmed by an intense red color.
- **Keller-kilani test for glycosides:** Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of glycosides.
- **Wagners test for alkaloids:** Filtrate was treated with wagners reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- **Liebermann burchard test for steroids:** The test solution was treated with a few drops of acetic anhydride (Liebermann Burchard) solution and mixed properly. Concentrated sulphuric acid was added from the sides of the test tube. Presence of steroids was confirmed by the formation of a brown ring at the junction of two layers and the green color of the upper layer.
- **Salkowski test for terpenoids:** Few drops of concentrated sulphuric acid was slowly added to the test solution and shaken properly and let stand at room temperature. Presence of terpenoids was confirmed by a reddish brown colour.
- **Test for tannins:** Crude extract was mixed with 2 ml of 2 % solution of $FeCl_3$. A blue-green or blue- black or greenish brown coloration indicated the presence of tannins.

- **Test for saponins:** Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously for 30 seconds. The formation of stable foam (1 cm height) even after 30 minutes was taken as an indication for the presence of saponins.

3.3.7.2 Quantitative determination of the phytochemical content(mg/g dry weight):

Extraction method:

1- 5 g of each plant dry powder mixed to 50 mL of extracting solvent water / methanol / ethyl acetate separately.

2- Extract using 500 W sonication bath for 2 h

3- Filtered through 0.45 µm filter paper

4- Dried using freeze drier at -193 °C

5- Prepare exact concentration (1000 ppm)

6- Analyses using individual methods.

Calibration curve preparation:

Table 3. 3. Aliquot amount of standards prepared for each phytochemical:

Examination Category	Standard compound	Concentration range	Detection wavelength (nm)
Phenolic content	Gallic acid	0.5 – 110 mg/g	765
Flavonoid content	Quercetin	1 – 25 mg/g	415
Glycoside content	Glucose	0.1-100 mg/g	490
Alkaloid content	Quinine	0.005 – 5 mg/g	720
Steroid content	B-sitosterol	0.001 – 2 µg/g	565
Terpene content	Pernol	0.05 – 20 µg/g	550
Tannin content	Tannic acid	0.05 – 10 µg/g	490
Saponin content	Solanine	1 – 100 %	-

A. Determination of total phenolic content (TPC)

The total phenolic content was determined for individual extracts using the Folin–Ciocalteu method. Briefly, 50 µL of extract solution or gallic acid standard was mixed with 150 µL of 10 % (w/v) Folin–Ciocalteu reagent. After 5 minutes, 50 µL of Na_2CO_3 (75 %) was subsequently added to the mixture and incubated at 50 °C for 10 minutes with intermittent agitation. Afterwards, the sample was cooled and the absorbance was measured utilizing a UV Spectrophotometer at 765 nm against a blank without extract. The outcome data were

expressed as mg/g of gallic acid equivalents in milligrams per gram (mg /g) of dry extract as shown below in Fig. 3.3 and Table 3.4.

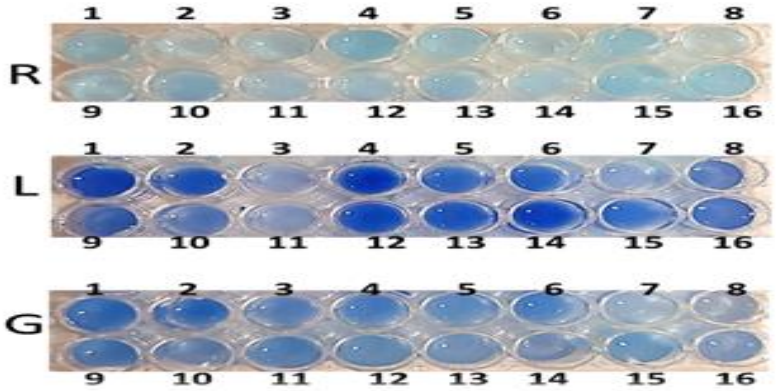


Fig 3.3. *Total phenolic content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.4. The absorbance of different concentrations of gallic acid used for preparing standard curve for total phenolic contents.

	Gallic acid mg	Abs. 765 nm
Abs. 765 nm	**0.5**	0.3
	2	0.36
	10	0.54
	30	0.87
	70	1.3
	110	1.9

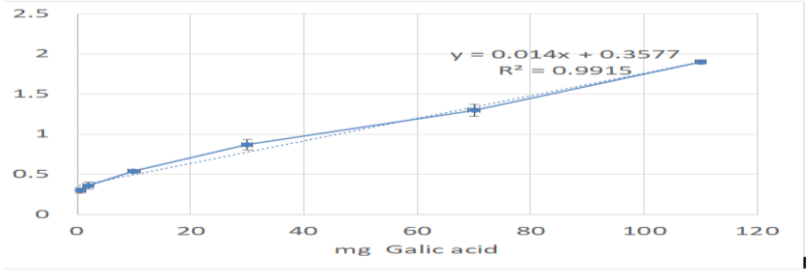


Fig 3.4. *Standard curve for estimating phenolic conent.*

B. Determination of total flavonoids content (TFC)

The flavonoid contents of individual extracts were measured as follows: aliquot of 50 µL of extract solution or quercetin were mixed with 20 µL of 10 % (w/v) $AlCl_3$ solution in methanol 20 µL (1 M) potassium acetate and 100 mL distilled water. The mixture was incubated for 30 min at room temperature followed by the measurement of absorbance at 415 nm against the blank. The outcome data were expressed as mg/g of quercetin equivalents in milligrams quercetin per gram (mg /g) of dry extract as shown below in Fig. 3.5 & Table 3.5.

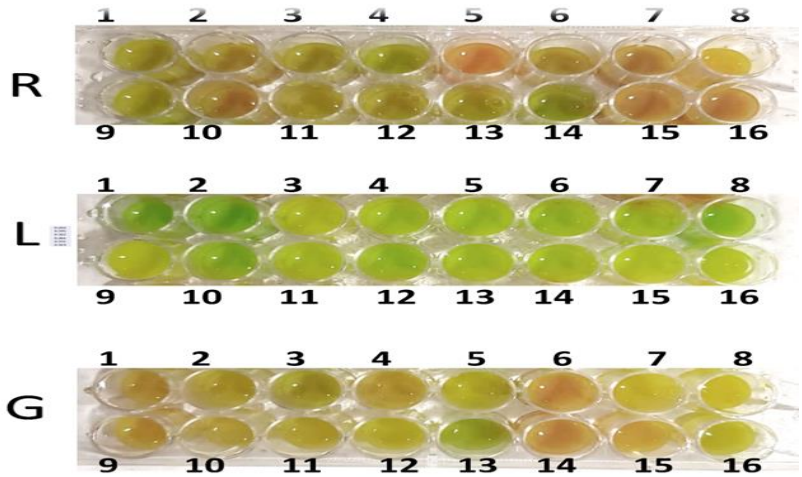


Fig 3.5*. Total flavonoid content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.5. The absorbance of different concentrations of quercetin used for preparing standard curve for total flavonoid contents.

	Quercetine mg	Abs. 415 nm
Abs. 415 nm	1	0.05
	5	0.21
	10	0.37
	15	0.59
	20	0.78
	25	1.03

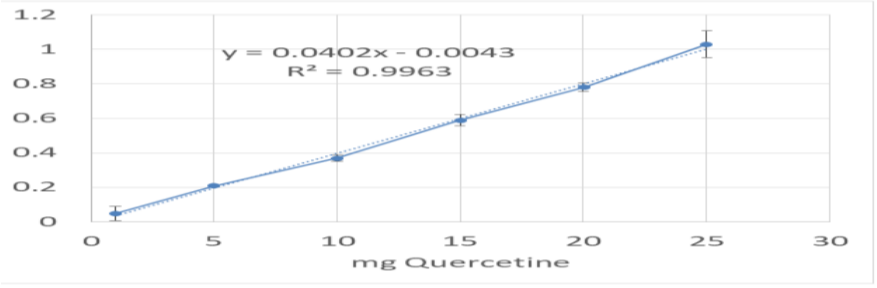


Fig 3.6. *Standard curve for estimating flavonoid content*

C. Determination of total glycosides content (TGC)

Add 50 µl of 80 % phenol solution (80 % phenol by weight) to 100 µL of sample/standard. Add 20 µl concentrated sulphuric acid in a stream. Stand 10 min at 90 °C ben-marry bath. Read absorbance an 490 nm as shown below in Fig. 3.7 & Table 3.6.

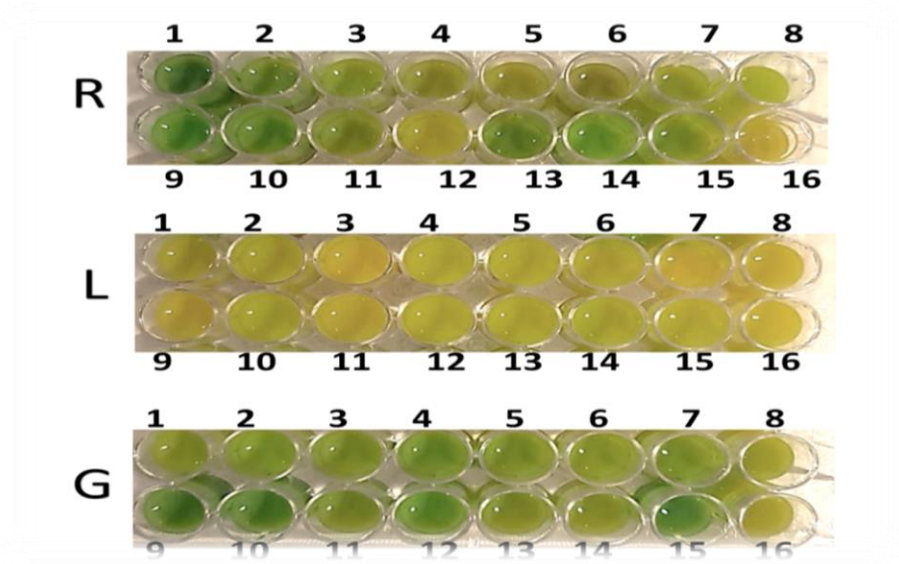


Fig 3.7. *Total glycoside content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.6. The absorbance of different concentrations of glucose used for preparing standard curve for total glycoside contents.

Glucose mg	Abs. 490 nm
0.1	0.119
1	0.218
5	0.261
10	0.319
50	0.781
100	1.301

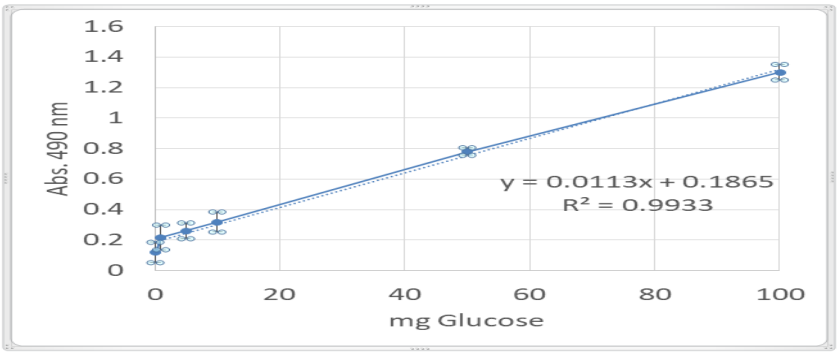


Fig 3.8. *Standard curve for estimating glycoside conten*

D. Determination of total alkaloids content (TAC): (Wagner's test)

100 µL of extract/standard was taken and placed into a 48 well plate. Then 100 µL of potassium mercuric iodide solution (Wagner's test reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids. The measurement of reddish-

brown precipitate signifies the existence of alkaloids positive result absorbance at 720 nm against the blank as shown below in Fig. 3.9 & Table 3.7.

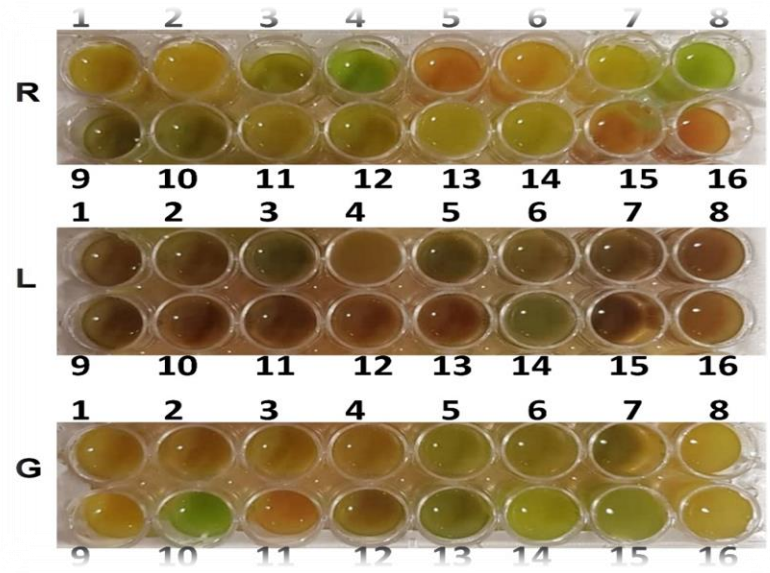


Fig 3.9. *Total alkaloid content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.7. The absorbance of different concentrations of quinine used for preparing standard curve for total alkaloid contents.

Quinines mg	Abs. 720 nm
0.005	0.35
0.05	0.53
0.3	0.67
0.5	0.72
1	0.8
5	2.65

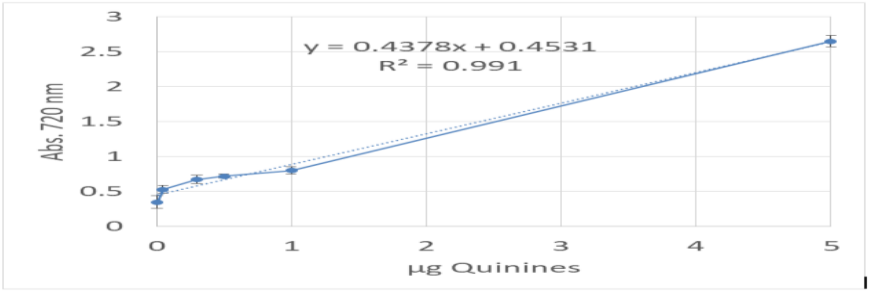


Fig 3.10. *Standard curve for estimating alkaloid conten*

E. Determination of total steroids content (TSTC): (Liebermann-Burchard test)

100 µL of extract/standard was mixed to 100 µL chloroform. Add 30 µL of acetic anhydride and then 30 µL of concentrated H_2SO_4 and mix carefully. After the reaction is finished, the

concentration of the steroid can be measured using spectrophotometry at 565 nm as shown below in Fig. 3.11 & Table 3.8.

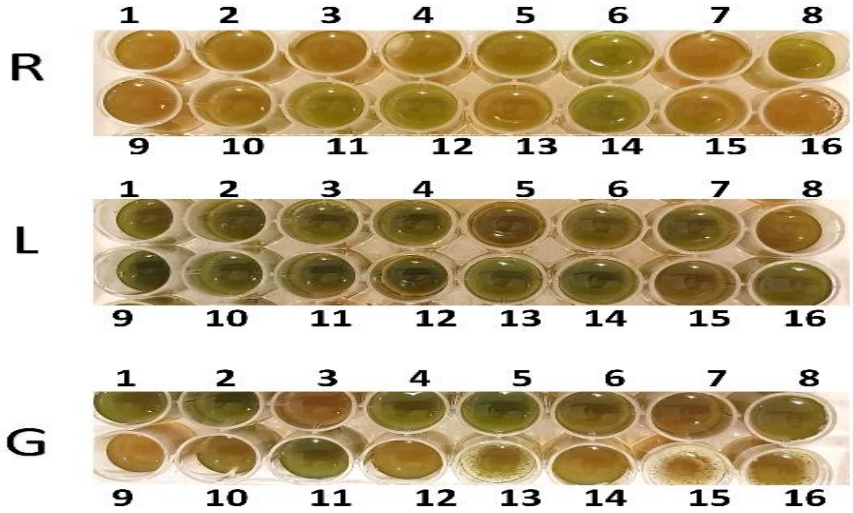


Fig 3.11. *Total steroid content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.8. The absorbance of different concentrations of b-sitosterol used for preparing standard curve for total steroid contents.

β-sitostrole µg	Abs. 565 nm
0.001	0.005
0.005	0.05
0.05	0.1
0.5	0.22
1	0.41
2	0.78

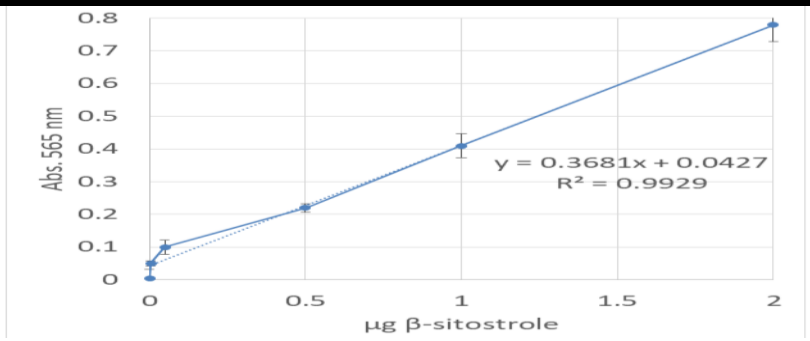


Fig 3.12. *Standard curve for estimating steroid content*

F. Determination of total terpenes content (TTEC): (Salkowski test)

100 µL of methanolic and ethyl acetate extract/ standard solution was mixed to 100 µL of chloroform and keep at room temperature for 15 min. then 100 µL sulphonic acid (60 %) was

added and reddish-brown solution was measure at 550 nm as shown below in Fig. 3.13 & Table 3.9.

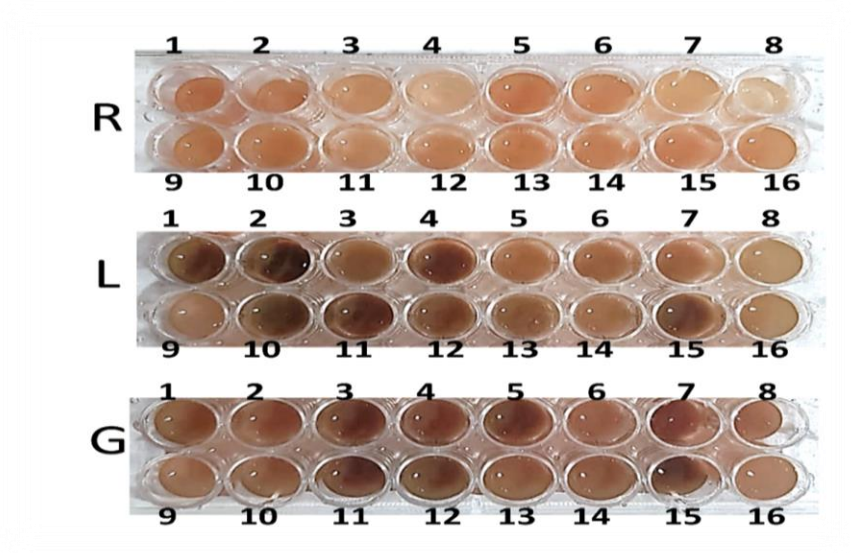


Fig 3.13. *Total terpene content by methanol extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.9. The absorbance of different concentrations of pernol used for preparing standard curve for total terpene contents.

Pernol mg	Abs. 550 nm
0.05	0.003
0.5	0.014
1	0.054
5	0.198
10	0.471
20	0.959

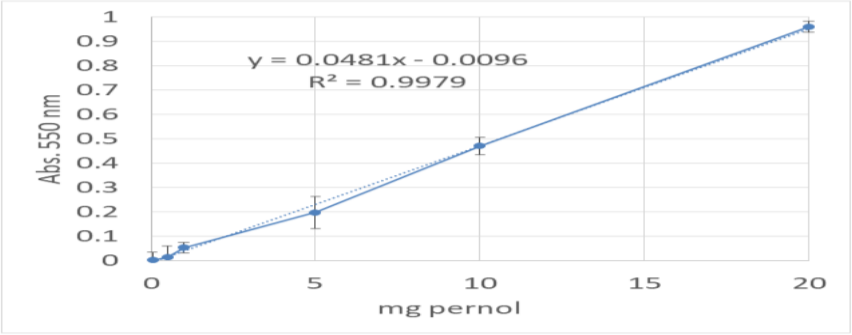


Fig 3.14. *Standard curve for estimating terpenes content*

G. Determination of total tannins content (TTC): (Braemer test)

100 µL of methanolic and ethyl acetate extract/ standard solution was mixed to 70 µL of alcoholic ferric chloride solution (50 w/v) and kept at 40 °C for 30 min. The tannins content was measured at 490 nm as shown below in Fig. 3.15 & Table 3.10.

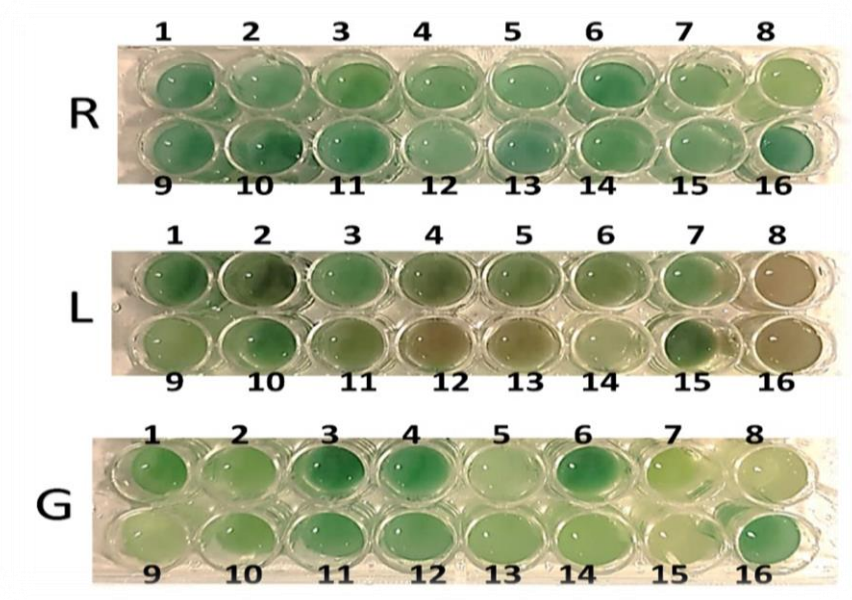


Fig 3.15. *Total tannin content by methanol of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

Table 3.10. The absorbance of different concentrations of tannic acid used for preparing standard curve for total tannin contents.

Tannic acid µg	Abs. 490 nm
0.05	0.112
0.1	0.141
0.5	0.181
2	0.259
5	0.478
10	0.98

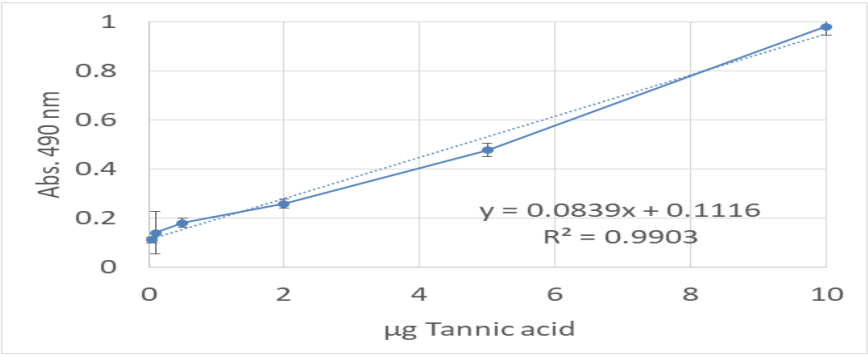


Fig 3.16. *Standard curve for estimating tannins content*

H. Determination of total saponins content (TSC)

1 mL of 80 % aqueous extract/standard solution was heated at 55 °C for 1 h in a water bath. The mixture was filterated, and transferred to 10 ml of ethanol. The volume of this mixture was reduced to 5 ml by boiling in a hot water bath. Diethyl ether was added, and it was shaken vigorously in a separating funnel; the aqueous layer was removed for purification by repeating the steps described above. Total saponin content in the extracts was assessed by standard procedures as shown below in Fig. 3.17.

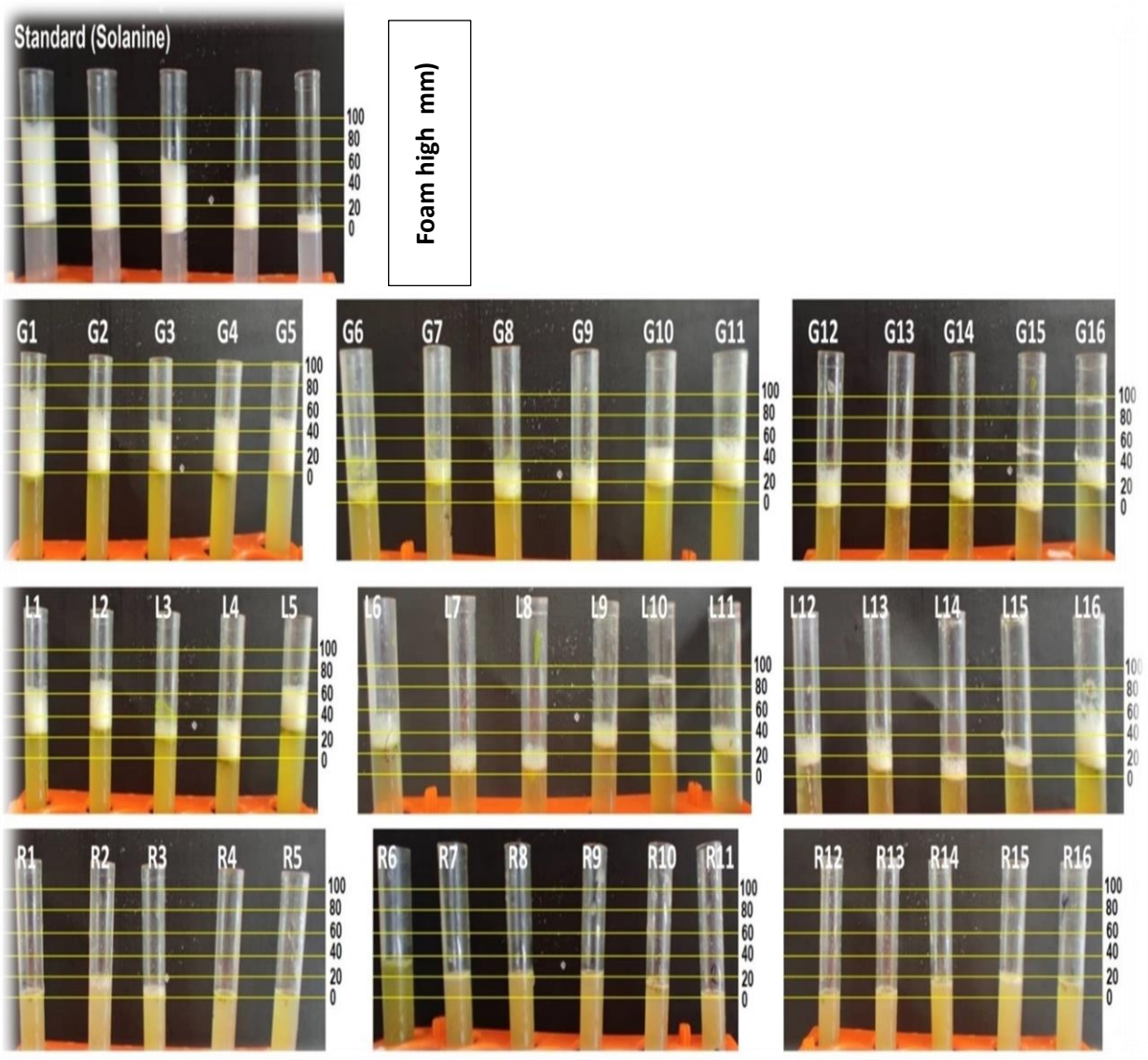


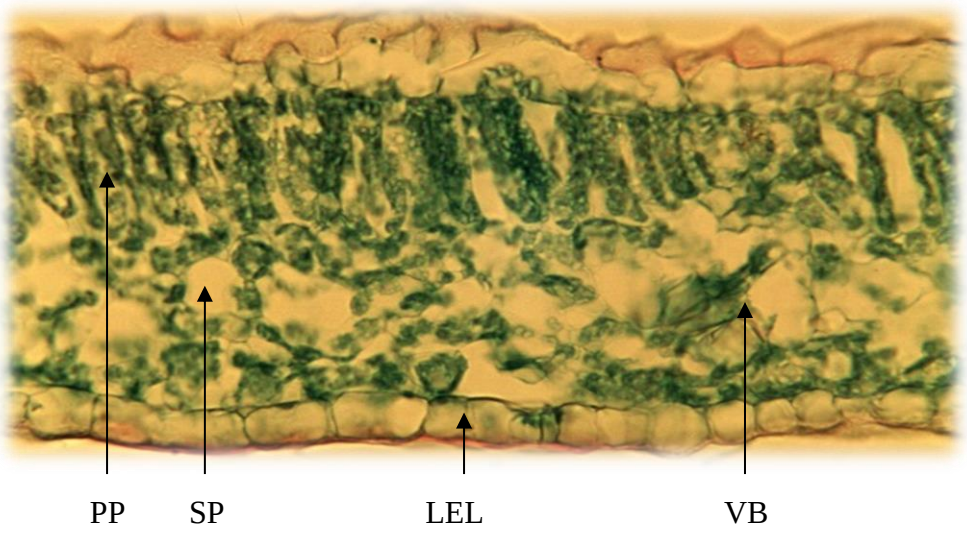
Fig 3.17. *Total saponin content by water extraction of G. triacanthos (G), L. leucocephala (L) and R. pseudoacacia (R) species (1. Ni0Pb0, 2. Ni0Pb15, 3. Ni0Pb30, 4. Ni0Pb45, 5. Ni15Pb0, 6. Ni15Pb15, 7. Ni15Pb30, 8. Ni15Pb45, 9. Ni30Pb0, 10. Ni30Pb15, 11. Ni30Pb30, 12. Ni30Pb45, 13. Ni45Pb0, 14. Ni45Pb15, 15. Ni45Pb30, 16. Ni45Pb45) mg.kg-^{1} soil.*

3.3. 8 The anatomy of the studied plant species

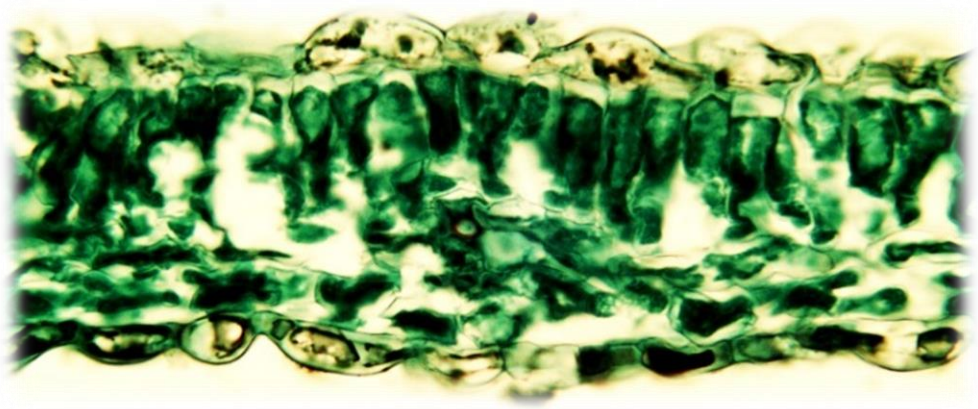
Plant materials of the studied species were collected for preparation of the tissue sections by paraffin methods as follow:

3.3.8.1 Paraffin method

The plant tissues (leaf, petiole, stem and root) cut into small pieces of approximately 1-2 cm and the pieces of samples have been mixed in Formalin-Acetic-Acid-Alcohol (FAA) solution, prepared as a mixture of 90 ml of (70 % alcohol, 5ml of glacial acetic acid, 5 ml of formalin and 10 ml distill water) for 24 hrs. After that, the samples have been dehydrated using series concentrations of alcohol (95 %, 100 % and 100 %), for 95 % (1 hr), then by 100 % (3 - 4 hours), and repeated by 100 % concentration (3 - 4 hours); Then, the samples were placed in xylene for 3 - 4 hrs (Twice). After that the samples were embedded twice in a mixture of xylene and paraffin (1:1) at 60°C for 30 min., then they were transferred to pure paraffin and left at 60°C overnight. Following that the preparation of paraffin blocks were made and sections were prepared with the thickness of 8 micrometer using the rotary microtome (Bright, MIC). The sections were put in the water bath (25°C), were put it on the slides, and then they put in the oven 60°C for afew minutes to melt the paraffin and then they were dried by special instrument. The sections were then stained using safranin (1 %) and fast green or light green (1 %). Finally the sections were mounted by Destrin Plastisizer Xylene (DPX); The samples were prepared and observed under light microscope (DM 300, Leica Microsystems, China) with camera attached using Analysis Image with Analysis Software, the magnification for each part separetly (Najmaddin and Saeed, 2020), as it is clear in the following figures;



(A)



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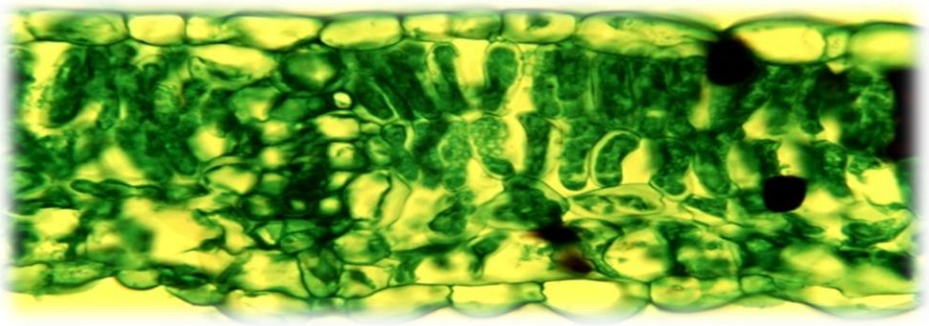
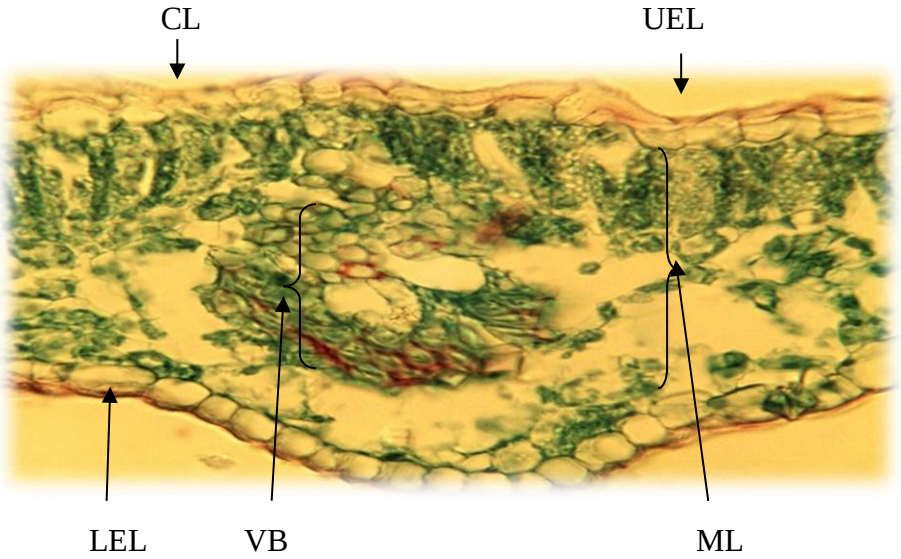
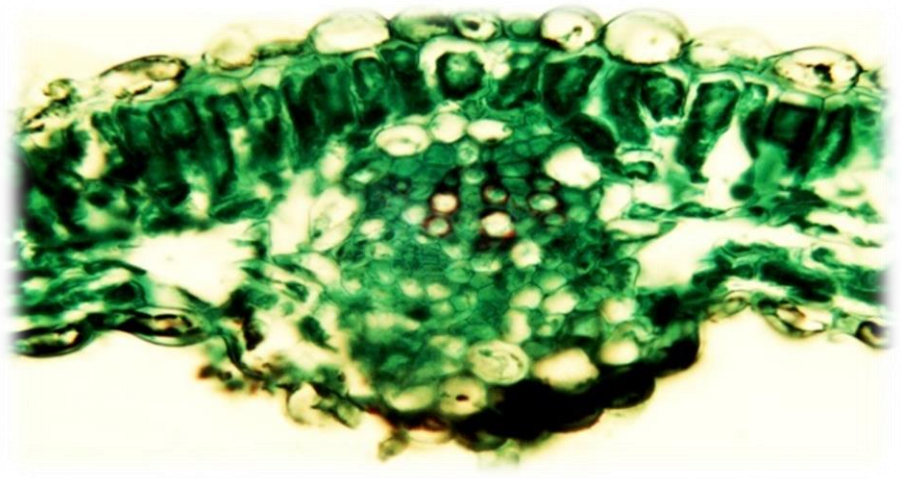


(C)

Fig 3.18: *Leaf lamina cross section at 40x of (A). G. triacanthos; (B). L. leucocephala and (C). R. pseudoacacia, (CL)Cuticle layer, (UEL)Upper epidermis layer, Mesophyll layer((PP) palisade parenchyma and (SP)spongy parenchyma), (LEL) Lower epidermis layer and (VB)Vascular bundle (phloem and xylem).*



(A)



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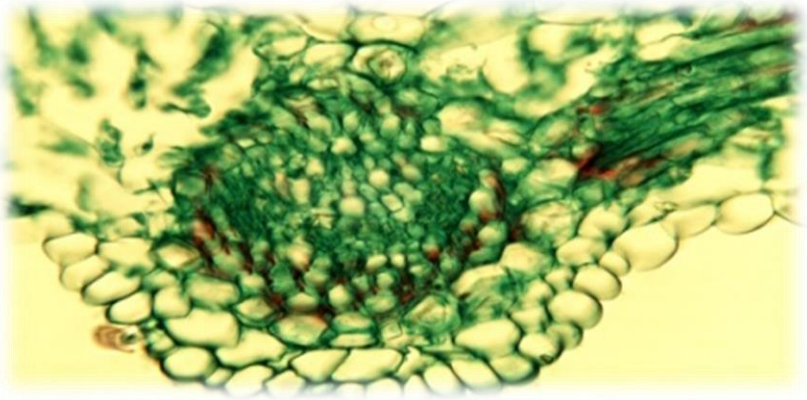
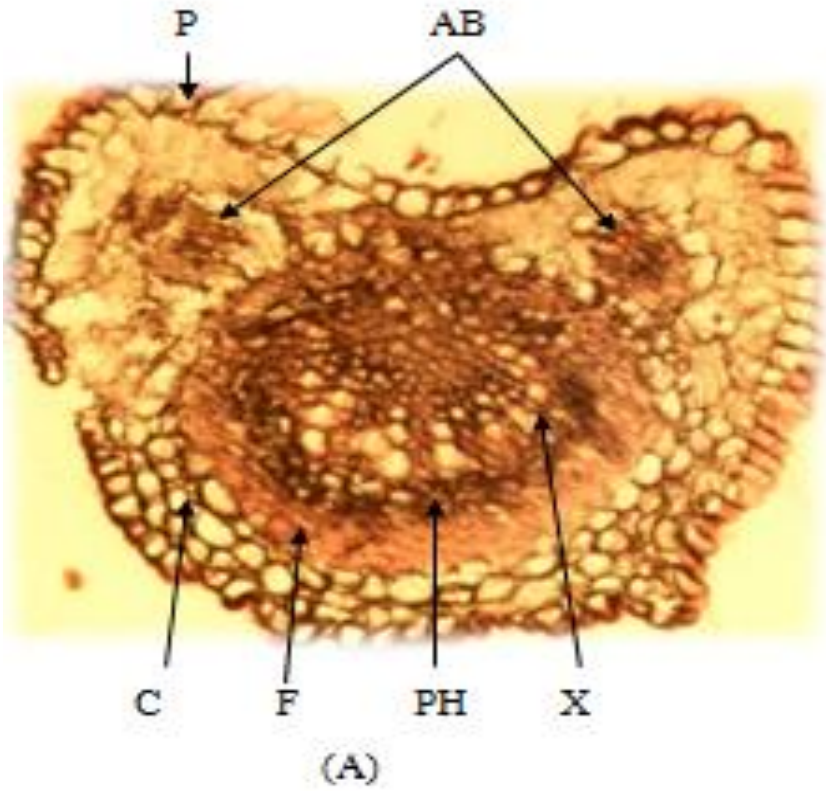
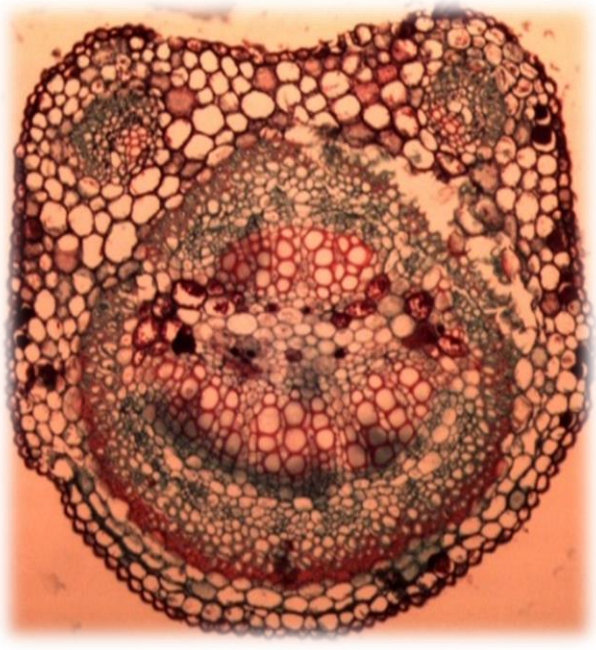
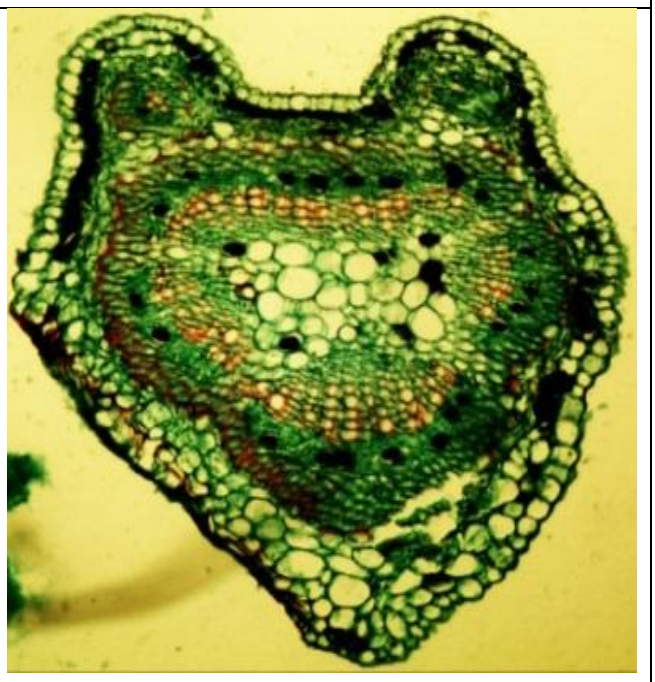


(C)

Fig 3.19: *Leaf midrib cross section at 10x of (A). G. triacanthos; (B). L. leucocephala and (C). R. pseudoacacia, (ECL) Cuticle layer, (UEL)Upper epidermis layer, (LEL)Lower epidermis layer, (VB) Vascular bundle (phloem and xylem) and (ML)Mesophyll layer (palisade land spongy).*

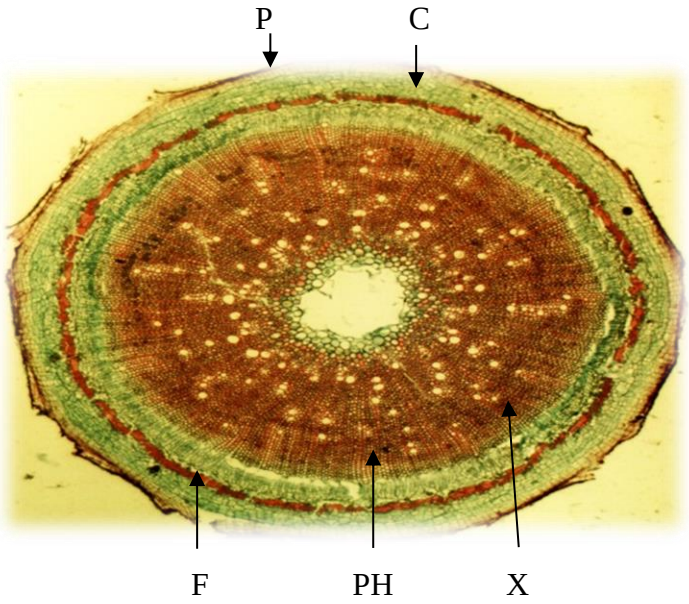






(B) **(C)**

Fig 3.20: *Petiole cross section at 10x of (A). G. triacanthos; (B). L. leucocephala and (C). R. pseudoacacia, (P)Periderm, (AB) Accessory bundles, (C)Cortex , (F) Fiber, (PH) Phloem and (X) Xylem.*



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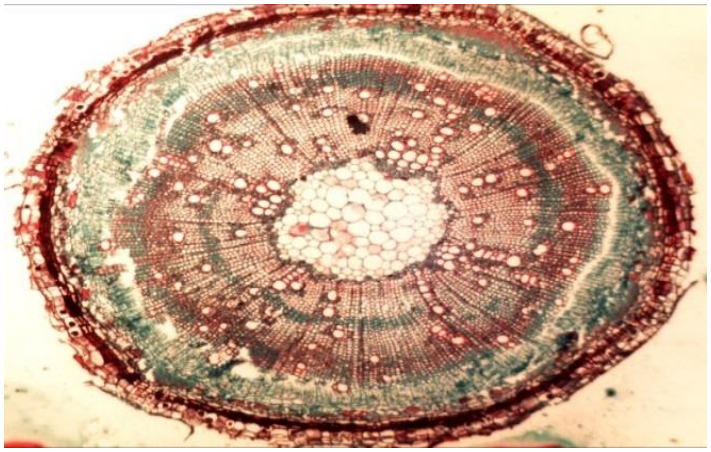
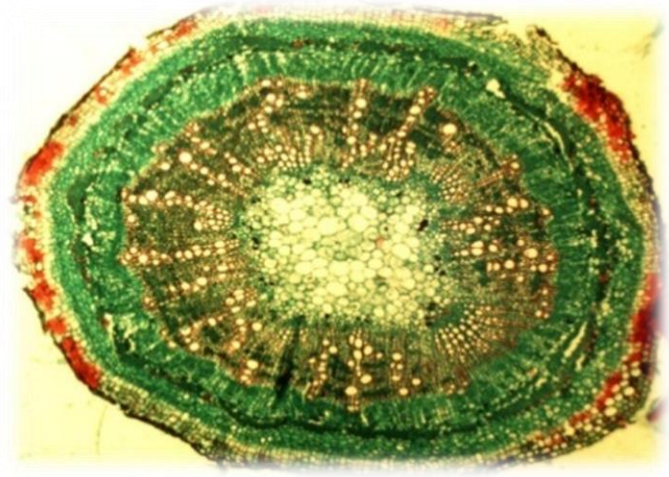
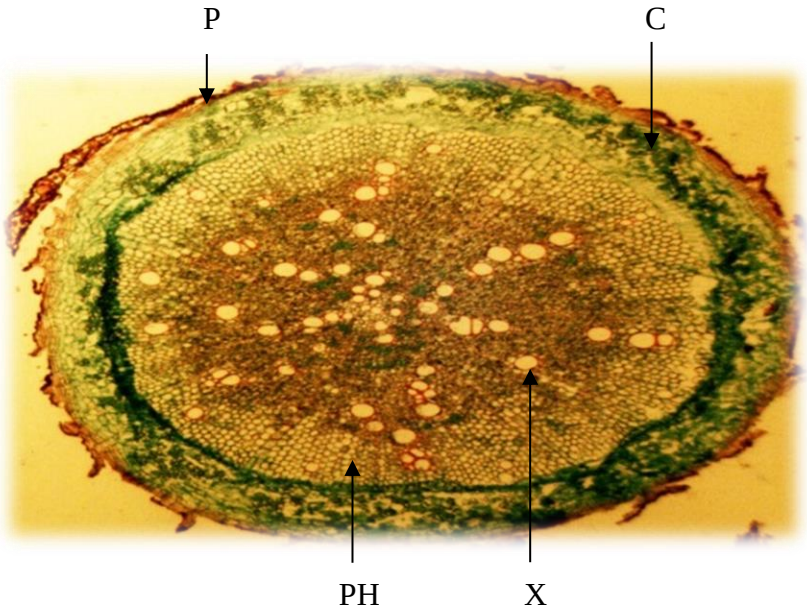


(B)

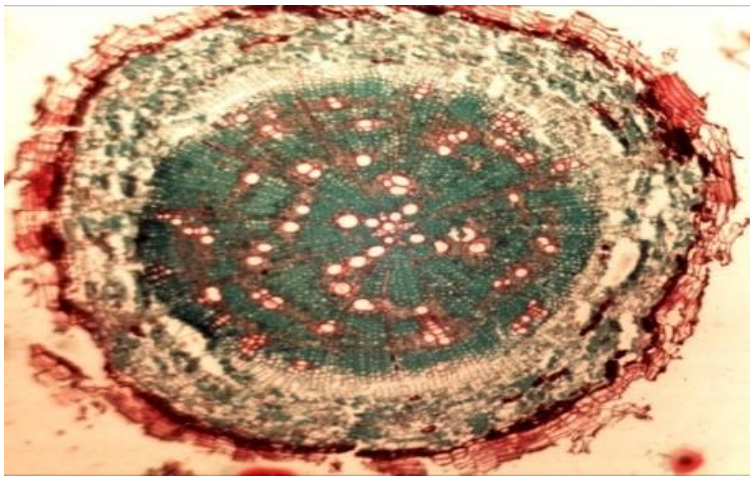


(C)

Fig 3.21: *Stem cross section at 4x of (A). G. triacanthos; (B). L. leucocephala and (C). R. pseudoacacia, (P)Periderm , (C) Cortex, (F)Fiber, (PH) Phloem and (X) Xylem.*



(A)



(B)

(C)

Fig 3.22: *Root cross section at 4x of (A). G. triacanthos; (B). L. leucocephala and (C). R. pseudoacacia, (P) Periderm , (C) Cortex, (PH) Phloem and (X) Xylem.*

3.3.8. 2 Determination of stomata characteristics of the studied plant species

On July 10, 2021, 3rd - 4th full matured healthy leaves were taken and kept in polythene bags. The lasting impressions method was used to leaf epidermal peel slides. In this method, at least one square centimeter on leaf surface was painted by a thick patch of clear nail polish. Allowing the nail polish to dry completely then taped a piece of clear cellophane tape to the dried nail polish patch by carton sealing tape. Then, peeling out the nail polish patch by pulling a corner of the tape and the finger nail polish along with the leaf peel. This leaf impression which was taped on slides and labeled as adaxial and abaxial surfaces then the samples were observed under a light microscope (DM 300, Leica Microsystems, China) with camera attached using Analysis Image with Analysis Software. (Rai and Mishra, 2013).

3.3.8.2.1 Number of stomata

Slides of leaf impression was examined under 40x by light microscope. Numbers of appeared stomata on lens field were counted for each adaxial and abaxial leave surface.

3.3.8.2.2 Length and width of stomata

Length and width of stomata guard cells of adaxial and abaxial leaf surfaces were measured in micron with scaled ocular lens.

Statistical analysis

The experiment was conducted according to a factorial complete randomized design (F_2 - CRD). Whrere the element type Ni and Pb considered as first factor, whereas the concentrations were considered as the second factor. Ten bags were used as one experimental unit, with three replications of each treatment. Treatment means were compared by the analysis of variance test (ANOVA) using the SAS statistical program. Duncan`s multiple range ($\alpha \leq 0.05$) was used for comparing between treatment means and their interactions (Al-Mohammadi and Al-Mohammadi, 2002).

RESULTS

And

DISCUSSIONS

Chapter Four

Results

4.1 Effects of nickel, lead and their interactions on seed performance and some vegetative growth of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species

4.1.1 Effects of nickel, lead and their interactions on seed performance and some vegetative growth of *G. triacanthos*

Results shown in table 4.1 found that neither Ni nor Pb, and their interactions non significantly effects the percentage of *G. triacanthos* seeds germination, whereas the seeds were germinated earlier with increasing Ni and Pb concentrations compared to the control treatment. The germination velocity decreased at application 45 $mg.kg^{-1}$ Ni and Pb respect. Also for the interactions where the control treatment seeds emerged later (47.9 days) significantly compared to all other interactions except the treatment Ni30Pb15.

For the vegetative growth parameters, the control treatment did not differ significantly with the treated plants for plant height, stem diameter, and the percentage of shoot and root dry matter. Plant leaf area significantly increased at both Ni15, Ni30 as compared to control while decreased significantly at Ni 45. Regarding Pb effects on the studied vegetative growth characteristics, it is found that it had no significant effects on each of stem diameter, plant leaves number, and the percentage of shoot and root dry matter content, whereas increasing Pb concentration to 45 $mg.kg^{-1}$ decreased plant height significantly to 27.2 cm compared to 31.4 and 29.5 cm for the 15 $mg.kg^{-1}$ and the control treatment. Using Pb at 15 $mg.kg^{-1}$ concentration increased plant leaf area significantly to 21.9 cm^2, whereas increasing the concentration to 30 $mg.kg^{-1}$ and 45 $mg.kg^{-1}$ decreased this parameter, which is still significantly higher than the control treatment. Using high concentration of Pb element caused non-significant increase in root length, while they caused a significant decrease in root diameter which reached 0.29 and 0.32 cm compared to the control treatment (0.38 cm). The interactions between Ni and Pb effects on the vegetative growth differ according to the studied characteristics, where only the interactions of 0 and 30 $mg.kg^{-1}$ Ni with different Pb concentrations increased the plant height and plant leaves number significantly in comparison with the control treatment, whereas differences between the control treatment and most other interactions did not differ

significantly regarding the plants stems diameter. Interactions of 15 and 30 mg.kg^{-1} Ni with different Pb concentrations increased the plant leaf area significantly compared to the control treatment, whereas, the interactions of 45 mg.kg^{-1} Ni cause a significant decrease in this characteristic. Dry matter percentage of each of shoot and root was affected non-significantly by each of Ni and Pb application in comparison with the control plants. Regarding root length most interactions of Ni decreased them significantly. Most of the interactions were not differed significantly except the significant increase in root diameter which reached 0.43 cm for each of N0Pb15, N15Pb0, and Ni15Pb45 treatments respectively. With regard to the characteristics of the vegetative growth, root nodules are not formed in Gleditsia plant genus (Figure 4.1).

4.1.2. Effects of nickel, lead and their interactions on seed performance, some vegetative growth and nodules of *L. leucocephala*

Results in table 4.2 show that each of Ni, Pb and their interactions significantly effects the percentage and velocity of seed germination, and some growth parameters for *L. leucocephala* seedling. It is clarified that increasing Ni concentration causes a significant decrease in the percentage of seed germination, where the highest value (38.4%) was recorded for the control, whereas the low value (22.3%) was recorded for the high concentration (45 mg.kg^{-1}). Also for Pb application the highest germination percentage (34.3%) was recorded in the control treatment, while a lower germination percentage (26.8%) was recorded for the 30 mg.kg^{-1}. Ni application increased velocity of seed germination (42.5%) was recorded for 30 mg.kg^{-1}, while a lower velocity of germination (20.1%) was recorded to the control treatment. High concentration of Pb (45 mg.kg^{-1}) decreased the velocity of germination to 21.3 days compared to the control. Ni application significantly increased plant height at all concentrations, while increased plant leaf area significantly at Ni15, Ni45 and shoot dry weight percentage at Ni30. Both Ni30 and Ni45 significantly increased root dry matter percentage and nodules number as compared to control, whereas they did not have a significant effect on the stem diameter, plant leaves number, root length, root diameter and nodules dry matter percentage. At the same time Pb had no significant effects on each of plant height, stem diameter, root and nodules dry matter percent, whereas it decreased significantly plant leaves number, shoot dry matter, root length and root diameter, exception plant leaf area and plant nodules number were increased significantly with high concentration. Interactions between the highest concentrations levels of Ni and Pb decreased the percentage of seed germination significantly and some vegetative characterestics such as plant leaves number, root length. On the other hand, the velocity of

germination, plant height, plant leaf area, roor diameter, root dry matter, plant nodules number were significantly increased with two elements at high concentrations (Table 4.2).

4.1.3. Effects of nickel, lead and their interactions on seed performance, some vegetative growth and nodules of *R. pseudoacacia*

The results of table 4.3 show that Ni application to the soil significantly effects studied characteristics for seeds performance and the growth of seedling, and nodules of *R. pseudoacacia*. An exception comprises the percentage of root dry matter, where the differences were not significant. Increasing concentration of Ni lead to a decrease in the percentage of seed germination significantly to 16.4% for the highest Ni concentration (45 $mg.kg^{-1}$) compared with the control treatment (57.5%). However, this decrease was offset by a significant decrease in days for seed germination with increasing the concentration to reach 24.8 days for the 45 $mg.kg^{-1}$ soil treatment compared with the control plants (40.1 days). Regarding the effects of Pb on seed performance, it is shown that there were non-significant differences between Pb concentrations with the control treatment in respect to the percentage and velocity of seed germination. For the interaction between Ni and Pb, there were significant effects on the percentage and velocity of seed germination, and it is appear that the interaction of low concentrations of each of Ni and Pb (15 $mg.kg^{-1}$) gave the best combination (74.7 % and 26.2 days) for the percentage and velocity of seed germination. Regarding the vegetative growth and nodules, Ni application significantly increased root diameter and nodules dry mater percentage at all treatments as compared with control, whereas it decreased plant height, stem diameter, plant leaves number, plant leaf area, shoot dry matter, root length and plant nodules number as compared to the control treatment. There were non-significant differences between the control with most Pb concentrations especially for the plant height, stem diameter, plant leaves number, percentage of shoot and root dry matter, root diameter, and percentage of nodules dry matter, whereas plant leaf area significantly increased at all treatments and the highest value was by adding Pb at concentration Pb15. With regard to the nodules number there was significant differences between all Pb treatments, where the highest value found in the 30 $mg.kg^{-1}$, whereas the lowest value was in 45 $mg.kg^{-1}$ treatment. Most interactions occur between Ni and Pb concentrations on the vegetative growth and nodules parameters; effects non-significantly compared with the control plants in respect to plant height, plant stem diameter, root dry matter and plant leaf area, whereas interactions of high concentrations of Ni (30 and 45 $mg.kg^{-1}$) cause a significant decrease in plant leaves number, percentage of shoot

dry matter, root length, and nodules number, on the other hand these interactions increased the root diameter significantly compared to the control treatment plants (Table 4.3).

Table 4.1. Effects of nickel, lead and their interactions mean of seed performance and some vegetative growth of G. triacanthos species.

Treatments	Germination		Plant Height (cm)	Stem diameter (cm)	Plant Leaves Number	Plant Leaf Area (cm^2)	Shoot Dry Matter (%)	Root Length (Cm)	Root diameter (cm)	Root Dry Matter (%)
	Percent (%)	Velocity Days)								
Nickel con. ($mg.kg^{-1}$ soil)										
Ni 0	50.1 a	37.1 a	28.9 ab	0.28 ab	14.8 a	17.7 b	64.5 a	26.0 a	0.36 a	71.1 a
Ni 15	54.0 a	35.9 a	27.7 b	0.25 b	12.5 b	25.7 a	57.0 a	20.9 b	0.33 ab	82.8 a
Ni 30	48.2 a	33.8 a	30.9 a	0.31 a	15.4 a	26.4 a	50.8 a	24.3 a	0.34 ab	77.6 a
Ni 45	55.7 a	29.7 b	29.6 ab	0.28 ab	14.1 a	8.3 c	54.3 a	25.8 a	0.30 b	77.5 a
Lead con. ($mg.kg^{-1}$ soil)										
Pb 0	54.8 a	36.2 a	29.5 ab	0.28 a	14.1 a	16.9 c	55.8 a	23.9 ab	0.38 a	76.1 a
Pb 15	55.7 a	35.1 a	31.4 a	0.29 a	13.6 a	21.9 a	60.7 a	23.3 b	0.34 ab	75.9 a
Pb 30	48.1 a	34.1 ab	28.9 bc	0.28 a	14.1 a	19.7 b	57.0 a	24.4 ab	0.29 c	77.1 a
Pb 45	49.4 a	31.2 b	27.2 c	0.28 a	14.9 a	19.6 b	53.0 a	25.4 a	0.32 bc	79.7 a
Interaction between Ni and Pb concentrations										
Ni0 Pb0	53.0 a	47.9 a	25.1 def	0.27 abc	11.4 de	14.2 cd	64.7 a	26.0 a-e	0.33 bc	65.7 ab
Ni0 Pb15	38.0 a	38.4 bc	34.1 a	0.33 a	15.2 abc	22.7 b	64.8 a	23.0 d-g	0.43 a	66.7 ab
Ni0 Pb30	49.0 a	36.5 cd	24.1 ef	0.23 bc	16.2 ab	16.3 c	65.8 a	30.0 a	0.37 ab	78.7 ab
Ni0 Pb45	60.3 a	25.5 g	32.3 ab	0.30 ab	16.1 ab	16.3 c	62.9 a	25.2 b-f	0.30 bcd	73.1 ab
Ni15 Pb0	49.7 a	36.9 c	28.7 b-e	0.27 abc	14.9 a-d	32.0 a	43.4 a	21.2 fg	0.43 a	78.8 ab
Ni15 Pb15	63.7 a	28.1 d-g	30.0 a-d	0.27 abc	11.1 e	23.0 b	64.4 a	19.3 g	0.27 cd	83.0 ab
Ni15 Pb30	51.0 a	34.9 c-f	29.7 a-d	0.26 abc	12.0 cde	25.3 b	55.8 a	22.0 efg	0.17 e	76.7 ab
Ni15 Pb45	51.7 a	35.3 cde	22.2 f	0.20 c	12.1 cde	22.5 b	39.4 a	21.1 fg	0.43 a	92.6 a
Ni30 Pb0	56.0 a	28.2 d-g	34.2 a	0.30 ab	15.2 abc	11.5 de	66.3 a	21.4 fg	0.37 ab	85.3 ab
Ni30 Pb15	55.0 a	46.0 ab	31.5 ab	0.30 ab	14.4 a-e	33.0 a	54.3 a	23.0 c-f	0.37 ab	80.5 ab
Ni30 Pb30	41.7 a	38.5 bc	32.3 ab	0.30 ab	15.1 abc	31.0 a	57.0 a	23.1 c-g	0.33 bc	82.7 ab
Ni30 Pb45	40.0 a	31.0 c-g	25.6 c-f	0.33 a	16.7 a	30.3 a	50.3 a	29.0 ab	0.30 bcd	61.7 b
Ni45 Pb0	60.7 a	31.6 c-g	30.0 a-d	0.27 abc	14.9 a-d	10.0 ef	48.8 a	27.1 a-d	0.37 ab	74.6 ab
Ni45 Pb15	66.0 a	27.8 efg	30.2 abc	0.27 abc	13.7 a-e	9.0 ef	59.3 a	27.2 abc	0.30 bcd	73.4 ab
Ni45 Pb30	50.7 a	26.5 fg	29.8 a-d	0.33 a	13.0 b-e	6.0 f	49.6 a	22.7 efg	0.30 bcd	70.5 ab
Ni45 Pb45	45.7 a	32.9 c-g	28.7 b-e	0.27 abc	14.7 a-d	8.0 ef	59.3 a	26.1 a-e	0.23 de	91.4 a

* Means follow ed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.2. Effects of nickel, lead and their interactions mean of seed performance, some vegetative growth, and nodules of L. leucocephala species.

Treatments	Germination		Plant Height (cm)	Stem diameter (cm)	Plant Leaves Number	Plant Leaf Area (cm^2)	Shoot Dry Matter (%)	Root Length (cm)	Root diameter (cm)	Root Dry Matter (%)	Plants Nodules Number	Nodules Dry Matter (%)
	Percent (%)	Velocity (Days)										
Nickel con. ($mg.kg^{-1}$ soil)												
Ni 0	38.4 a	20.1 c	40.0 b	0.40 a	9.0 a	29.6 b	53.1 b	32.2 ab	0.55 a	73.0 b	5.50 c	57.6 a
Ni 15	33.3 ab	21.1 c	45.3 a	0.38 a	9.3 a	34.6 a	61.4 ab	30.0 b	0.50 a	81.8 ab	5.33 c	61.7 a
Ni 30	29.8 b	42.5 a	44.7 a	0.38 a	9.7 a	27.2 c	70.8 a	32.0 ab	0.54 a	84.0 a	9.25 a	60.3 a
Ni 45	22.3 c	24.5 b	44.1 a	0.37 a	9.3 a	35. 4 a	65.3 ab	34.2 a	0.50 a	85.4 a	8.41 b	50.8 a
Lead con. ($mg.kg^{-1}$ soil)												
Pb 0	34.3 a	26.7 b	43.1 ab	0.38 a	9.5 a	30.1 b	72.0 a	34.7 a	0.55 a	79.8 a	6.33 c	56.4 a
Pb 15	32.3 ab	33.7 a	45.5 a	0.40 a	10.1 a	31.7 b	68.8 a	31.2 b	0.54 ab	82.2 a	8.33 a	67.6 a
Pb 30	26.8 b	26.5 b	44.5 a	0.39 a	9.8 a	30.1 b	55.6 b	31.6 ab	0.51 ab	83.2 a	7.25 b	55.3 a
Pb 45	30.3 ab	21.3 c	40.9 b	0.36 a	8.1 b	34.8 a	54.1 b	30.9 b	0.49 b	78.9 a	6.58 bc	51.1 a
Interaction between Ni and Pb concentrations												
Ni0 Pb0	37.3 abc	18.6 de	37.3 bc	0.40 ab	10.5 ab	24.0 e	60.4 a-d	44.0 a	0.53 bc	50.0 c	3.3 g	48.5 a
Ni0 Pb15	33.3 a-d	27.7 c	45.5 a	0.47 a	9.3 a-d	32.4 bc	67.7 abc	30.5 b-d	0.53 bc	89.0 a	6.7 d-f	77.9 a
Ni0 Pb30	38.3 ab	19.1 de	43.0 a	0.37 ab	10.4 ab	28.5 cd	30.5 e	30.2 b-d	0.67 a	87.7 a	6.7 d-f	52.1 a
Ni0 Pb45	44.7 a	15.0 e	33.9 c	0.37 ab	6.3 e	33.5 ab	53.7 b-e	24.1 d	0.47 bc	65.3 bc	5.3 f	52.0 a
Ni15 Pb0	32.3 a-e	24.8 cd	45.0 a	0.37 ab	8.5 cd	36.3 ab	75.6 abc	30.9 bcd	0.57 ab	90.1 a	6.3 ef	54.7 a
Ni15 Pb15	29.0 b-f	20.9 de	45.4 a	0.37 ab	9.8 a-d	32.5 bc	85.8 a	28.1 bcd	0.53 bc	76.5 ab	7.3 cde	73.1 a
Ni15 Pb30	27.0 b-g	19.3 de	45.5 a	0.43 ab	10.2 a-c	32.7 bc	50.2 cde	25.9 cd	0.43 c	73.7 ab	5.0 f	66.3 a
Ni15 Pb45	44.7 a	19.7 de	45.0 a	0.37 ab	8.9 bcd	36.9 ab	34.0 de	35.9 b	0.47 bc	86.7 a	2.7 g	52.9 a
Ni30 Pb0	44.7 a	43.0 b	46.5 a	0.37 ab	9.8 a-d	25.9 de	82.7 ab	28.9 bcd	0.53 bc	90.1 a	9.0 abc	74.0 a
Ni30 Pb15	44.7 a	58.3 a	44.9 a	0.37 ab	11.0 a	24.6 de	58.4 a-e	32.2 bc	0.57 ab	81.5 ab	10.7 a	62.7 a
Ni30 Pb30	16.3 fg	39.7 b	44.8 a	0.40 ab	9.3 a-d	23.1 e	79.6 abc	35.2 b	0.50 bc	82.1 ab	8.3 bcd	63.8 a
Ni30 Pb45	13.7 g	29.1 c	42.4 ab	0.37 ab	8.8 bcd	35.0 ab	62.5 a-d	31.4 bc	0.57 ab	82.0 ab	9.0 abc	40.8 a
Ni45 Pb0	23.0 c-g	20.6 de	43.3 a	0.37 ab	9.5 a-d	34.1 ab	69.0 abc	34.9 b	0.57 ab	89.0 a	6.7 def	48.5 a
Ni45 Pb15	22.3 d-g	30.0 c	46.1 a	0.40 ab	10.1 a-d	37.5 a	63.4 a-d	34.1 b	0.53 bc	81.6 ab	8.7 bc	56.7 a
Ni45 Pb30	25.7 b-g	28.0 c	44.8 a	0.37 ab	9.3 a-d	36.1 ab	62.2 a-d	34.9 b	0.43 c	89.2 a	9.0 abc	39.0 a
Ni45 Pb45	18.3 efg	20.6 de	42.2 ab	0.33 b	8.2 d	33.7 ab	66.4 abc	32.8 bc	0.47 bc	81.6 ab	9.33 ab	59.0 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.3. Effects of nickel, lead and their interactions mean of seed performance, some vegetative growth, and nodules of R. pseudoacacia species.

Treatments	Germination		Plant Height (cm)	Stem diameter (cm)	Plant Leaves Number	Plant Leaf Area (cm^2)	Shoot Dry Matter (%)	Root Length (Cm)	Root diameter (cm)	Root Dry Matter (%)	Plants Nodules Number	Nodules Dry Matter (%)
	Percent (%)	Velocity (Days)										
Nickel con. ($mg.kg^{-1}$ soil)												
Ni 0	57.5 a	40.1 a	28.33 a	0.40 a	16.65 a	31.71 a	67.98 a	34.20 a	0.26 c	69.46 a	10.67 a	49.43 b
Ni 15	55.8 a	28.8 b	25.55 b	0.36 a	12.88 b	28.46 b	48.66 b	30.20 b	0.33 b	66.46 a	9.42 b	64.37 a
Ni 30	53.7 a	25.2 c	23.30 c	0.32 b	8.63 c	28.33 b	63.43 ab	28.83 b	0.34 b	71.60 a	8.25 c	64.30 a
Ni 45	16.4 b	24.8 c	24.11 bc	0.30 b	8.40 c	25.21 c	48.26 b	25.1 c	0.40 a	70.38 a	4.67 d	62.20 a
Lead con. (mg.kg-1 soil)												
Pb 0	47.67 ab	29.5 ab	26.41 a	0.35 a	11.46 b	24.54 d	63.1 a	31.67 a	0.34ab	70.23 a	9.33 b	58.99 ab
Pb 15	54.42 a	31.0 a	22.78 b	0.31 b	11.61 ab	32.50 a	62.83 a	28.38 b	0.31 b	73.61 a	6.67 c	53.35 b
Pb 30	38.1 b	28.3 b	25.51 a	0.34 ab	13.50 a	29.54 b	56.48 ab	29..33 b	0.35 a	68.23 a	11.75 a	67.45 a
Pb 45	43.3 ab	30.0 ab	26.58 a	0.36 a	10.00 b	27.13 c	45.96 b	28.90 b	0.32 ab	65.83 a	5.25 d	60.50 ab
Interaction between Ni and Pb concentrations												
Ni0 Pb0	15.3 b	38.9 bc	27.5 b	0.37 bc	16.23 a	28.0 d-g	71.7 ab	34.7 ab	0.23 e	69.63 a	13.3 a	44.1 d
Ni0 Pb15	17.7 b	45.1 a	25.6 bc	0.37 bc	15.23 a	36.0 a	65.9 a-d	37.5 a	0.23 e	69.27 a	10.0 cd	49.1 cd
Ni0 Pb30	21.7 b	35.7 cd	27.0 b	0.37 bc	18.47 a	32.3 bc	72.7 ab	31.0 bcd	0.30 cde	65.80 a	12.3 ab	52.1 bcd
Ni0 Pb45	11.0 b	40.5 b	33.8 a	0.47 a	16.67 a	30.5 b-e	61.7 a-d	33.1 bc	0.27 de	73.13 a	6.7 ef	52.5 bcd
Ni15 Pb0	56.0 a	32.3 ef	25.6 bc	0.43 ab	11.13 b	25.3 fg	42.2 bcd	32.1bcd	0.30 cde	68.90 a	10.0 cd	72.3 ab
Ni15 Pb15	74.7 a	26.2 fgh	25.0 bcd	0.30 cd	16.00 a	32.8 ab	60.7 a-d	31.0 bcd	0.37 bc	81.03 a	7.0 ef	58.2 bcd
Ni15 Pb30	25.7 b	29.1 ef	25.9 bc	0.33 cd	16.87 a	27.7 efg	53.0 a-d	29.1 de	0.37 bc	57.00 a	14.0 a	69.6 abc
Ni15 Pb45	72.3 a	27.6 fgh	25.6 bc	0.37 bc	7.53 b	28.0 d-g	35.8 d	28.6 de	0.30 cde	58.90 a	6.0 ef	57.3 bcd
Ni30 Pb0	58.3 a	21.5 i	25.6 bc	0.30 cd	8.90 b	28.8 c-f	65.1 ac	30.0 cde	0.37 bc	65.13 a	8.0 de	63.0 bcd
Ni30 Pb15	65.3 a	28.7 efg	21.9 cde	0.30 cd	7.77 b	29.7 b-e	82.6 a	23.7 fg	0.30 cde	67.60 a	6.3 ef	45.0 d
Ni30 Pb30	53.7 a	24.7 ghi	21.1 de	0.37 bc	9.43 b	29.5 b-e	64.3 a-d	30.3cde	0.40 ab	91.83 a	13.7 a	86.1 a
Ni30 Pb45	52.7 a	25.8 fgh	24.7 bcd	0.30 cd	8.43 b	25.3 fg	41.8 bcd	31.3bcd	0.30 cde	63.80 a	5.0 fg	63.1 bcd
Ni45 Pb0	61.0 a	25.5 fgh	27.5 b	0.30 cd	9.57 b	16.0 h	70.2 abc	29.9 cde	0.47 a	77.27 a	5.3 fg	56.6 bcd
Ni45 Pb15	60.0 a	23.9 hi	18.7 e	0.27 d	7.43 b	31.5 bcd	42.1 bcd	21.3 g	0.33 bcd	78.53 a	3.3 g	61.2 bcd
Ni45 Pb30	51.3 a	23.7 hi	28.1 b	0.30 cd	9.23 b	28.7 c-f	36.0 cd	26.5 ef	0.33 bcd	58.27 a	6.7 ef	62.0 bcd
Ni45 Pb45	51.0 a	25.8 fgh	22.1cde	0.30 cd	7.33 b	24.7 g	44.6 bcd	22.6 fg	0.40 ab	67.47 a	3.3 g	69.1 abc

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa

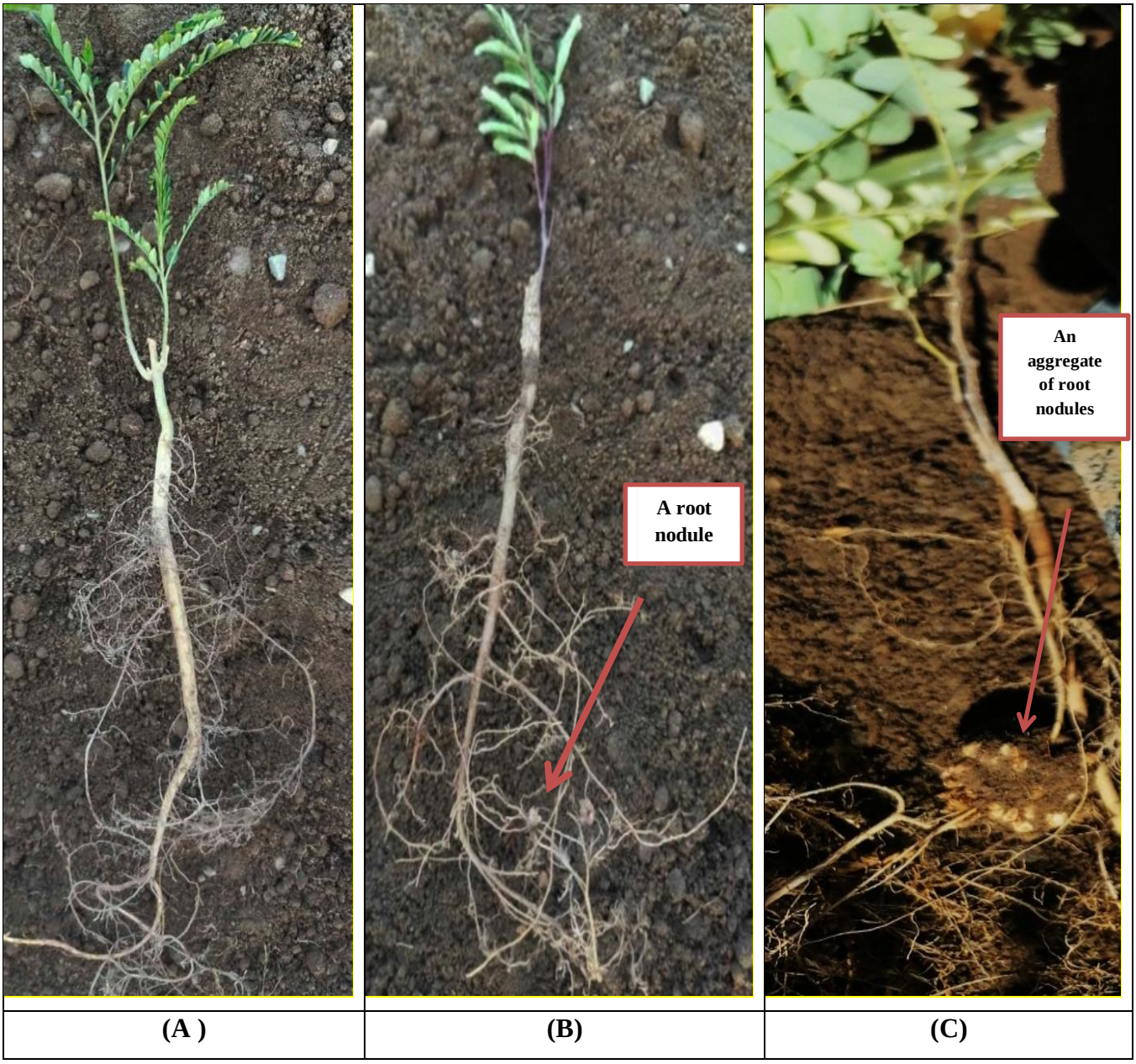


Fig 4.1 : *The nodules statuse of (A) G. triacanthos, (B) L. leucocephala and (C) R. pseudoacacia species*

4.2 Effects of nickel, lead and their interactions on physiological characterestics

4.2.1 Photosynthetic pigments

Results in the table 4.4 show that the effect of Ni, Pb and their interactions were significant on some photosynthetic pigments for the studied species (chlorophyll a, chlorophyll b and total carotenoids). The Chl a and Chl b were increased with increasing Ni concentrations for *G. triacanthos* and *L. leucocephala* species. The highest value of the Chl a, Chl b (1.04 and 0.84) mg/g fresh weight and (1.03 and 0.93) mg/g fresh weight for two plant species (*G. triacanthos* and *L. leucocephala*) were recorded from 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment, while the lowest values (1.02 and 0.75) mg/g fresh weight and (1.021 and 0.82) mg/g fresh weight were recorded in the control treatment. The Chl a and Chl b were increased with increasing Pb concentrations for *G. triacanthos* and *L. leucocephala* from 30 and 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment, except chl b of *G. triacanthos* species was decreased with increasing to 15 and 30 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment. The interactions between Ni and Pb concentrations had significant effects on Chl a and Chl b for these plant species and increased with increasing Ni and Pb concentrations. Inaddition, the effect of Ni, Pb and their interactions were non significant on Chl a, b for *R. pseudoacacia*.

The content of TC were decreased in the three plant species by increasing the concentrations to 45 $mg.kg^{-1}$ of Ni and Pb as compared to the control treatment. TC were decreased with increasing Ni concentrations from 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species. The highest value of TC (0.37, 0.38 and 0.38) mg/g fresh weight were recorded in the control treatment for three plant species. The lowest value of TC (0.36, 0.36 and 0.37 mg/g fresh weight) for *G. triacanthos, L. leucocephala and R. pseudoacacia* respectively were recorded from 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment. TC were decreased with increasing Pb concentrations for two plant species to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. The interactions between concentrations of Ni and Pb also had significant effects on TC for *G. triacanthos, L. leucocephala and R. pseudoacacia* as same as for each element individually.

Table 4.4. Effects of nickel, lead and their interactions mean of some photosynthesis pigments of the studied species.

Treatments	*G. triacanthos*			*L. leucocephala*			*R. pseudoacacia*		
	Chl_a	**Chl_b**	**TC**	**Chl_a**	**Chl_b**	**TC**	**Chl_a**	**Chl_b**	**TC**
	mg /g fresh weight								
Nickel con. ($mg.kg^{-1}$ soil)									
Ni 0	1.02 b	0.75 b	0.37 a	1.021 b	0.82 c	0.376 a	0.97 a	0.32 a	0.38 a
Ni 15	1.03 ab	0.76 b	0.36 b	1.028 ab	0.93 a	0.365 b	0.97 a	0.29 a	0.37 b
Ni 30	1.04 a	0.83 a	0.36 b	1.033 a	0.90 ab	0.362 b	0.96 a	0.31 a	0.37 b
Ni 45	1.03 ab	0.84 a	0.36 b	1.033 a	0.89 b	0.362 b	0.96 a	0.28 a	0.37 b
Lead con. ($mg.kg^{-1}$ soil)									
Pb 0	1.06 b	0.87 a	0.38 a	1.06 b	0.90 b	0.367 b	0.97 a	0.28 a	0.37 a
Pb 15	0.93 c	0.62 c	0.37 b	0.91 c	0.77 c	0.370 ab	0.97 a	0.31 a	0.37 a
Pb 30	1.08 a	0.79 b	0.35 c	1.08 a	0.94 a	0.371 a	0.97 a	0.31 a	0.37 a
Pb 45	1.08 a	0.91 a	0.35 c	1.08 a	0.95 a	0.358 c	0.97 a	0.30 a	0.37 a
Interaction between Ni & Pb con.									
Ni0 Pb0	1.01 c	0.75 cde	0.39 a	1.01 b	0.77 c	0.39 a	0.970 ab	0.29 ab	0.38 a
Ni0 Pb15	0.93 d	0.64 ef	0.37 b	0.92 c	0.72 c	0.37 bc	0.970 ab	0.33 a	0.38 a
Ni0 Pb30	1.08 ab	0.77 b-e	0.36 c	1.07 a	0.87 b	0.38 b	0.973 a	0.32 ab	0.38 a
Ni0 Pb45	1.08 ab	0.83 abc	0.35 c	1.08 a	0.93 a	0.37 bc	0.97 ab	0.32 ab	0.38 a
Ni15 Pb0	1.07 ab	0.86 abc	0.37 b	1.07 a	0.94 a	0.36 d	0.97 ab	0.25 b	0.37 b
Ni15 Pb15	0.92 d	0.56 f	0.37 b	0.89 e	0.87 b	0.37 bc	0.97 ab	0.30 ab	0.37 b
Ni15 Pb30	1.05 b	0.67 def	0.36 c	1.07 a	0.95 a	0.37 bc	0.97 ab	0.31 ab	0.37 b
Ni15 Pb45	1.08 ab	0.93 a	0.35 c	1.08 a	0.96 a	0.36 cd	0.97 ab	0.32 ab	0.37 b
Ni30 Pb0	1.07 ab	0.90 ab	0.37 b	1.07 a	0.95 a	0.36 d	0.97 ab	0.29 ab	0.37 b
Ni30 Pb15	0.93 d	0.67 def	0.37 b	0.91 cd	0.74 c	0.37 bc	0.97 ab	0.33 ab	0.37 b
Ni30 Pb30	1.09 a	0.81 a-d	0.35 c	1.07 a	0.96 a	0.37 bc	0.97 ab	0.34 a	0.37 b
Ni30 Pb45	1.08 ab	0.94 a	0.35 c	1.08 a	0.96 a	0.35 e	0.97 ab	0.27 ab	0.37 b
Ni45 Pb0	1.07 ab	0.95 a	0.38 a	1.07 a	0.95 a	0.36 d	0.97 ab	0.28 ab	0.37 b
Ni45 Pb15	0.93 d	0.60 f	0.37 b	0.90 de	0.73 c	0.37 bc	0.96 b	0.27 ab	0.37 b
Ni45 Pb30	1.08 ab	0.90 abc	0.35 c	1.08 a	0.96 a	0.37 bc	0.97 ab	0.27 ab	0.37 b
Ni45 Pb45	1.08 ab	0.93 a	0.35 c	1.08 a	0.96 a	0.35 e	0.97 ab	0.31 ab	0.37 b

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.2.2 Enzymatic and non- enzymatic antioxidants

4.2.2.1 Enzymatic antioxidants

Results in the tables 4.5, 4.6 and 4.7 indicate that the effect of Ni, Pb and their interactions were significant on some of the enzymatic antioxidants such as CAT, POD and NR of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* fresh leaves. Where the activity of POD was increased with increasing Ni concentrations to 45 $mg.kg^{-1}$ $NiCl_2$ for three studied species. The highest activity of POD (2030.8, 1989.2 and 2418.3) μgg^{-1} were obtained from increasing Ni concentrations to 45 $mg.kg^{-1}$ as compared with the control treatment for the three studied species, while the lowest activities of POD (1692.5, 1366.7 and 1767.5) μgg^{-1} were obtained from the control of three species. The activity of POD was significantly increased with increasing Pb concentrations to 45 $mg.kg^{-1}$ $PbCl_2$ concentrations only for *R. pseudoacacia*, whereas it non - significant for other species. The interactions between Ni and Pb elements on the activity of POD was recorded from increasing Ni and Pb concentrations to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of the studied species.

The activity of NR which increased with increasing the Ni concentrations to 45 $mg.kg^{-1}$ of *G. triacantho*, whereas the NR enzyme activity decreased with increasing the Ni concentrations to 30 and 45 $mg.kg^{-1}$ of *L. leucocephala* and *R. pseudoacacia*. The highest activity of NR (0.20) μML^{-1} was recorded from 45 $mg.kg^{-1}$ as compared to the control treatment of *G. triacanthos* species, while the lowest activities of NR (0.17) μML^{-1} was obtained from the control of *G. triacanthos*. Whereas the highest activity of NR (0.14 and 0.12) μML^{-1} were recorded from the control treatment for *L. leucocephala* and *R. pseudoacacia*, the lowest activities of the NR activity (0.11 and 0.095) μML^{-1} were obtained from 45 $mg.kg^{-1}$ as compared to the control of two species. The activity of NR significantly increased with increasing Pb concentrations to 15 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ for *R. pseudoacacia*, whreas NR activity significantly decreased with increasing Pb concentrations to 30 $mg.kg^{-1}$ $PbCl_2$ for *L. leucocephala* and *G. triacanthos*. The interactions between Ni and Pb elements on the activity of NR were obtained from increasing Ni and Pb concentrations to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia*.

The activity of CAT was significantly decreased with increasing Ni, Pb and their interactions to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of three studied species. The highest activity of CAT (327.5, 200.83 and 285.83) μgg^{-1} were recorded from the control treatment of three studied species, while, the lowest activities (139.2, 131.67 and 150.0) μgg^{-1} were recorded from 45 $mg.kg^{-1}$ $NiCl_2$ concentrations of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species respectively.

4.2.2.2 Non- enzymatic antioxidants

As displayed in the tables 4.5, 4.6 and 4.7 results show that the effects of Ni and Pb and their interactions were significant on some of the non-enzymatic antoxidants such as Pr, AA and CHO of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* leaves. They show that proline (Pr) significantly decreased with increasing the concentrations of Ni to 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment of three studied species. The highest value of Pr (55.37, 75.05 and 51.52) μgml^{-1} were recorded from the control treatment of the three species, while the lowest values (35.74, 58.5 and 40.75) μgml^{-1} were obtained from 15 and 45 $mg.kg^{-1}$ as compared to the control treatment of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species. The Pr decreased with increasing the concentrations of Pb to 45 $mg.kg^{-1}$ $PbCl_2$ concentrations for the studied species, except the Pr increased with increasing Pb concentrations to 45 $mg.kg^{-1}$ $PbCl_2$ for *L. leucocephala* species. The interaction between Ni and Pb had significant effect and decreased on the Pr for three studied species at 45 $mg.kg^{-1}$. The AA increased significantly with Ni application for three studied species. The highest value of AA (2.30, 2.03 and 1.31) gL^{-1} were recorded from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment for three studied species, while the lowest values (1.50, 0.74 and 0.77) gL^{-1} were recorded from the control for three species. Whereas the AA decreased with increasing Pb concentration for *G. triacanthos* and *R. pseudoacacia*, except it increased significantlty for *L. leucocephala.* The interaction between Ni and Pb had significant effect and decreased on the AA for *G. triacanthos* and *R. pseudoacacia* at 45 $mg.kg^{-1}$, except it increased for *L. leucocephala* at 45 $mg.kg^{-1}$. The CHO decreased with increasing Ni, Pb and their interaction to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ for studied species, except for the CHO which increased with increasing Ni, Pb and their interactions to 45 $mg.kg^{-1}$ only for *L. leucocephala* species. The highest value of CHO (1.04 and 1.22) % were obtained from the control for *G. triacanthos* and *R. pseudoacacia* species. While the lowest value of CHO (0.29 and 0.93) % were obtained from 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment of *G. triacanthos* and *R. pseudoacacia* species.

Table 4.5. Effects of nickel , lead and their interactions mean of some enzymatic, non-enzymatic antioxidants and nitrate reductase activity of G. triacanthos leaves.

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants		
	Catalase (μgg^{-1})	**Peroxidase** (μgg^{-1})	**Nitrate Reductase** (μML^{-1})	**Ascorbic Acid** (gL^{-1})	**Proline** (μgml^{-1})	**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	1.04 a
Ni 15	277.5 b	1873.3 ab	0.14 d	2.19 c	44.50 b	0.66 c
Ni 30	194.2 c	1699.2 b	0.18 b	2.77 a	37.34 c	0.76 b
Ni 45	139.2 d	2030.8 a	0.20 a	2.30 b	35.74 c	0.29 d
Pb concentration (mg.kg-1 Soil)						
Pb 0	269.2 a	1837.5 a	0.183 ab	3.22 a	48.70 a	0.60 b
Pb 15	194.2 c	1738.3 a	0.138 c	1.49 c	43.42 b	0.80 a
Pb 30	236.33 b	1804.2 a	0.182 b	2.63 b	39.30 c	0.78 a
Pb 45	238.3 b	1915.8 a	0.189 a	1.49 c	41.52 bc	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	1.25 b
Ni0 Pb15	240.0 d	1753.3 b	0.17 e	1.41 h	56.96 b	0.54 d
Ni0 Pb30	216.7 d	1593.3 b	0.20 d	0.53 j	51.23 bc	1.48 a
Ni0 Pb45	356.7 b	1713.3 b	0.11 g	0.13 k	49.92 c	0.87 c
Ni15 Pb0	303.3 c	1826.7 ab	0.14 f	1.63 g	49.46 c	0.41 de
Ni15 Pb15	200.0 de	1960.0 ab	0.17 e	2.66 e	45.88 c	0.99 c
Ni15 Pb30	363.3 b	1736.7 b	0.08 h	1.97 f	37.69 fg	1.18 b
Ni15 Pb45	243.3 d	1970.0 ab	0.17 e	2.50 e	44.92 cde	0.06 g
Ni30 Pb0	136.7 f	1873.3 ab	0.22 c	4.41 b	42.46 def	0.31 e
Ni30 Pb15	210.0 d	1493.3 b	0.14 f	0.94 i	34.04 g	1.17 b
Ni30 Pb30	216.7 d	1926.7 ab	0.21 c	3.09 d	34.96 g	0.29 ef
Ni30 Pb45	213.3 d	1503.3 b	0.14 f	2.66 e	37.89 fg	1.28 b
Ni45 Pb0	140.0 f	1940.0 ab	0.17 e	2.91 d	39.54 efg	0.41 de
Ni45 Pb15	126.7 f	1746.7 b	0.07 h	0.72 j	36.81 fg	0.51 d
Ni45 Pb30	150.0 fe	1960.0 ab	0.23 b	4.91 a	33.27 g	0.16 fg
Ni45 Pb45	140.0 f	2476.7 a	0.33 a	0.66 j	33.35 g	0.10 g

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.6. Effects of nickel, lead and their interactions mean of some enzymatic, non-enzymatic antioxidants and nitrate reductase activity of L.leucocephala leaves.

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants		
	Catalase (μgg^{-1})	Peroxidase (μgg^{-1})	Nitrate Reductase (μML^{-1})	Ascorbic Acid (gL^{-1})	Proline (μgml^{-1})	Total Carbohydrate (%)
Ni concentration (mg.kg^{-1} Soil)						
Ni 0	200.83 a	1366.7 b	0.14 b	0.74 d	75.05 a	0.22 c
Ni 15	133.33 b	1535.8 b	0.19 a	2.93 a	58.0 d	0.23 c
Ni 30	132.50 b	1801.7 a	0.13 c	1.66 c	66.55 b	0.45 a
Ni 45	131.67 b	1989.2 a	0.11 d	2.03 b	60.05 c	0.37 b
Pb concentration (mg.kg-1 Soil)						
Pb 0	195.8 a	1659.2 a	0.14 b	1.08 c	63.80 c	0.31 b
Pb 15	150.0 b	1644.2 a	0.18 a	1.05 c	70.42 a	0.30 b
Pb 30	131.7 bc	1644.2 a	0.12 c	2.07 b	60.50 d	0.26 b
Pb 45	120.8 c	1745.8 a	0.14 b	3.16 a	64.92 b	0.40 a
Interactions between Ni and Pb						
Ni0 Pb0	310.0 a	1143.3 e	0.10 g	0.16 n	87.04 b	0.38 bcd
Ni0 Pb15	220.0 b	1520.0 cde	0.19 c	0.22 n	71.15 f	0.06 h
Ni0 Pb30	133.3 c	1320.0 de	0.09 g	1.84 f	82.54 d	0.19 fgh
Ni0 Pb45	140.0 c	1483.3 cde	0.20 b	0.72 l	59.46 h	0.23 efg
Ni15 Pb0	200.0 b	1510.0 cde	0.15 e	1.03 jk	31.15 k	0.10 gh
Ni15 Pb15	113.3 cd	1560.0 cde	0.24 a	1.47 h	84.73 c	0.36 cde
Ni15 Pb30	133.3 c	1560.0 cde	0.20 b	1.09 ij	24.23 l	0.06 h
Ni15 Pb45	86.7 d	1513.3 cde	0.17 d	8.13 a	91.85 a	0.39 bcd
Ni30 Pb0	136.7 c	1836.7 bcd	0.19 bc	0.34 m	70.50 f	0.23 efg
Ni30 Pb15	150.0 c	1960.0 abc	0.12 f	1.59 g	69.73 f	0.50 ab
Ni30 Pb30	113.3 cd	1853.3 bcd	0.09 g	2.09 e	48.27 j	0.56 a
Ni30 Pb45	130.0 cd	1556.7 cde	0.12 f	2.63 d	77.69 e	0.52 ab
Ni45 Pb0	136.7 c	2146.7 ab	0.11 f	2.78 c	66.50 g	0.51 ab
Ni45 Pb15	116.7 cd	1536.7 cde	0.16 e	0.91 k	56.08 i	0.27 def
Ni45 Pb30	146.7 c	1843.3 bcd	0.09 g	3.25 b	86.92 b	0.21 fg
Ni45 Pb45	126.7 cd	2430.0 a	0.08 h	1.16 i	30.69 k	0.48 abc

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.7. Effects of nickel, lead and their interactions mean of some enzymatic , non-enzymatic antioxidants and nitrate reductase activity of R. pseudoacacia leaves.

Treatments	Enzymatic Antioxidants and Nitrate Reductase			Non-Enzymatic Antioxidants		
	Catalase (μgg^{-1})	**Peroxidase** (μgg^{-1})	**Nitrate Reductase** (μML^{-1})	**Ascorbic Acid** (gL^{-1})	**Proline** (μgml^{-1})	**Total Carbohydrate** (%)
Ni concentration ($mg.kg^{-1}$ Soil)						
Ni 0	285.83 a	1767.5 b	0.128 a	0.77 c	51.52 a	1.22 b
Ni 15	255.00 b	2291.7 a	0.112 b	0.91 b	45.14 c	1.33 a
Ni 30	174.17 c	2390.0 a	0.124 a	1.31 a	46.82 b	0.77 d
Ni 45	150.00 d	2418.3 a	0.095 c	0.35 d	40.75 d	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	0.94 b
Pb 15	209.2 a	2084.2 b	0.143 a	0.89 b	43.58 b	1.15 a
Pb 30	208.3 a	2688.3 a	0.099 c	0.52 c	38.25 c	1.23 a
Pb 45	220.8 a	2180.8 b	0.112 b	0.54 c	43.46 b	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	1.70 a
Ni0 Pb15	250.0 b	1626.7 d	0.170 b	0.28 hi	49.31 ef	0.97 cde
Ni0 Pb30	276.7 b	2090.0 bcd	0.126 de	0.53 f	48.30 f	1.18 b
Ni0 Pb45	256.7 b	1810.0 cd	0.130 cd	0.09 i	58.85 c	1.03 bcde
Ni15 Pb0	270.0 b	1583.3 d	0.120 e	0.19 hi	63.31 b	0.94 de
Ni15 Pb15	246.7 b	2150.0 bcd	0.130 cd	0.81 e	39.84 h	1.15 bc
Ni15 Pb30	250.0 b	3243.3 a	0.060 j	1.00 d	41.46 h	1.58 a
Ni15 Pb45	253.3 b	2190.0 bcd	0.136 c	1.63 c	35.96 i	1.64 a
Ni30 Pb0	156.7 cd	2146.7 bcd	0.106 fg	2.91 a	51.24 de	0.45 g
Ni30 Pb15	186.7 c	2736.7 ab	0.180 a	1.53 c	43.54 g	0.89 e
Ni30 Pb30	153.3 cd	2620.0 abc	0.110 f	0.47 fg	40.88 h	1.09 bcd
Ni30 Pb45	200.0 c	2056.7 bcd	0.100 gh	0.34 gh	51.62 d	0.64 f
Ni45 Pb0	120.0 d	2383.3 bcd	0.100 fgh	0.25 hi	71.69 a	0.68 f
Ni45 Pb15	153.3 cd	1823.3 cd	0.090 h	0.97 de	41.54 h	1.58 a
Ni45 Pb30	153.3 cd	2800. 0 ab	0.100 gh	0.093 i	22.35 k	1.06 bcde
Ni45 Pb45	173.3 c	2666.7 abc	0.080 i	0.093 i	27.42 j	0.41 g

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.2.2.3 Total protein content (TPr)

Results presented in table 4.8 show that the effects of nickel, lead and their interactions were significant on the total protein contents of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* seedlings, except for the root of *R. pseudoacacia* which was non – significant. Total protein were highly affected by contamination with heavy metals. It shows that total protein contents of the roots increased with increasing the concentration of Ni from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment for *G. triacanthos* and *L. leucocephala* species. The total protein contents non-significantly for *R. pseudoacacia* roots, whereas for the shoot it increased significantly with increasing the concentration of Ni to 30 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment. The highest value of the TPr contents of the roots of *G. triacanthos* and *L. leucocephala* (2.848 and 2.334) gkg^{-1} were recorded from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations, while the lowest value (1.692 and 1.434) gkg^{-1} were recorded in 15 $mg.kg^{-1}$ treatment and the control. The highest value of the total protein contents for shoot of *R. pseudoacacia* (1.975 gkg^{-1}) were recorded from 30 $mg.kg^{-1}$ $NiCl_2$ concentration, while the lowest value (1.427 gkg^{-1}) were recorded to 15 $mg.kg^{-1}$ $NiCl_2$ concentration. The concentration of TPr contents in the roots and shoots were highly affected by soil elements for various species, the lowest protein content was noted for *R. pseudoacacia* plants grown on soil highly contaminated with Ni and Pb heavy metals from 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to other plant species. The total protein contents in the roots and shoots for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* declined with increasing pb concentration to 15 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$, except the root of *L. leucocephala* increased at 30 $mg.kg^{-1}$ $PbCl_2$, also the root of *R. pseudoacacia* which was no significant. The interactions between concentrations of Ni and Pb also had significant effects on the three species on the total protein contents, except the root of *R. pseudoacacia* which was non significant.

Table 4.8. Effects of nickel, lead and their interactions mean of shoot and root total protein contents for the studied species.

Treatments	*G. triacanthos*		*L. leucocephala*		*R. pseudoacacia*	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
	gkg^{-1} dry weight					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	2.940 a	1.848 bc	2.451 b	1.434 c	1.731 b	1.952 a
Ni 15	2.834 a	1.692 c	2.968 a	1.489 c	1.427 c	1.914 a
Ni 30	2.234 b	2.848 a	2.417 b	2.038 b	1.975 a	1.898 a
Ni 45	2.920 a	1.998 b	2.020 c	2.334 a	1.900 ab	1.785 a
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	2.898 a	2.117 b	2.767 a	1.517 c	1.887 a	1.928 a
Pb 15	2.478 b	1.943 c	2.453 b	1.928 b	1.984 a	1.892 a
Pb 30	2.573 b	2.731 a	2.414 b	2.238 a	1.887 a	1.809 a
Pb 45	2.981 a	1.595 d	2.223 b	1.613 c	1.275 b	1.921 a
Interaction between Ni & Pb con.						
Ni0 Pb0	3.007 bcd	1.280 ef	2.560 c	1.190 fg	1.773 cd	2.047 a
Ni0 Pb15	2.617 de	1.417 ef	2.380 c	1.350 efg	2.310 ab	2.010 a
Ni0 Pb30	3.027 bcd	3.193 b	2.433 c	1.780 cde	1.853 cd	1.860 a
Ni0 Pb45	3.110 bc	1.500 ef	2.430 c	1.417 defg	0.987 e	1.890 a
Ni15 Pb0	3.297 b	3.077 bc	3.763 a	2.060 c	2.130 bc	1.893 a
Ni15 Pb15	2.717 cde	1.400 ef	2.450 c	1.540 cdef	1.617 d	1.960 a
Ni15 Pb30	2.640 de	1.563 e	2.450 c	0.960 g	0.887 e	1.887 a
Ni15 Pb45	2.683 de	0.727 h	3.210 b	1.397 defg	1.073 e	1.917 a
Ni30 Pb0	2.647 de	1.507 ef	2.390 c	1.530 cdef	2.123 bc	1.870 a
Ni30 Pb15	2.010 g	3.790 a	2.500 c	3.070 b	2.120 bc	1.913 a
Ni30 Pb30	2.410 ef	2.817 cd	2.367 c	1.960 cd	2.670 a	1.787 a
Ni30 Pb45	1.860 g	3.280 b	2.410 c	1.590 cdef	0.987 e	2.023 a
Ni45 Pb0	2.640 de	2.603 d	2.353 c	1.287 efg	1.520 d	1.900 a
Ni45 Pb15	2.557 ef	1.163 fg	2.480 c	1.750 cdef	1.890 bcd	1.683 a
Ni45 Pb30	2.213 fg	3.350 b	2.407 c	4.250 a	2.137 bc	1.703 a
Ni45 Pb45	4.270 a	0.873 gh	0.840 d	2.050 c	2.053 bc	1.853 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.2.2.4 Macro, micro and some of non-essential elements for shoots, roots and soils characterestics of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species

4.2.2.4.1 Macro, micro and some of non- essential elements for shoots and roots of *G. triacanthos* species

the results shown in tables 4.9, 4.10, 4.11, 4.12, 4.13 and 4.14 indicate that the effects of Ni and Pb and their interactions were significant on some nutrients of the shoots and roots of *G. triacanthos* seedlings. They shows that some of the elements increased with increasing the concentration of Ni and Pb from 15, 30 and 45 $mg.kg^{-1}$ as compared to the control treatment. The macro elements (P, Mg and SO_4) increased significantly with increasing the Ni concentration from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment, except for the N which decreased with increasing the Ni concentration of *G. triacanthos* shoots. The highest value of the P, Mg and SO_4 (0.00415, 0.0388 and 0.00326) % respectively were recorded in the 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment, while the lowest values (0.00249, 0.0267, 0.00268) % were recorded in the control treatment. Each of P and Mg increased significantly with increasing the Pb concentration from 30 and 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. An exception the N which decresed with increasing Pb concentration at 30 $mg.kg^{-1}$. The highest values of P and Mg (0.00330 and 0.0378) % were recorded in the 30 and 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment, while the lowest values (0.00271 and 0.0289) % were recorded in the control and 15 $mg.kg^{-1}$ treatment. The interactions between Ni and Pb concentrations had significant effects on N, P, Mg and SO_4 their concentration increased with increasing Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (See Table 4.9).

Each of Fe, Mn and Zn increased significantly with increasing the Ni concentrations for 15 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment; An exception involves the Cu which decreased with increasing the Ni concentration of *G. triacanthos* shoots. The highest values of Fe, Mn and Zn (0.0652, 0.00818 and 0.00657)% were recorded in the 15 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment, while the lowest values (0.0538, 0.0113 and 0.000537) % were recorded in the control treatment. Each of Mn and Zn were increased with increasing the Pb concentration to 30 and 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment, except the Fe which was decreased with increasing the Ni concentration. The highest values of Mn and Zn (0.00779 and 0.00642) % were recorded in the 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment, while the lowest values (0.0122 and 0.00505)

% were recorded in the control treatment. The interactions between Ni and Pb had significant effects on some micro - elements such as Cu and Zn which increased with increasing Ni and Pb concentration in the 15 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, Exception an Fe and Mn that decreased with increasing the Ni concentration, as it is clear from table 4.10.

The heavy metal element Cd was increased with increasing the Ni concentrations to 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control treatment, except the Rb that decreased with increasing the Ni concentration of *G. triacanthos* shoots. The highest value of Cd (0.000665%) was recorded in the 45 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest value (0.000217%) was recorded in the control treatment. The heavy metal elements Cd and As increased with increasing the Pb concentration to 30 mg.kg^{-1} $PbCl_2$ as compared to the control treatment, except for the Rb which decreased with increasing the Pb concentration to 15 mg.kg^{-1}. The highest values of Cd and As (0.000644 and 0.000696) % were recorded in the 30 mg.kg^{-1} $PbCl_2$ concentrations, while the lowest values of the Cd and As (0.000212 and 0.000224) % were recorded in the control treatment. The interactions between Ni and Pb concentrations had significant effects on some heavy metal elements such as Pb, Cd and As which increased with increasing Ni and Pb concentration in the 15, 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment see Table 4.11.

Each of N, P, K, Mg, Ca and SO_4 which increased with increasing the Ni concentration for 15, 30 and 45 mg.kg^{-1} $NiCl_2$ as compared to the control treatment of *G. triacanthos* roots. The highest values of N, P, K, Mg, Ca and SO_4 (0.00456, 0.00326, 1.152, 0.0224, 1.882, 0.00087) % were recorded to 15, 30 and 45 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest values (0.00271, 0.00249, 0.656, 0.0110, 1.361, 0.00073) % were recorded in 15 mg.kg^{-1} and control treatment. Each of N, P, Mg, and SO_4 increased with increasing the Pb concentration to 30 and 45 mg.kg^{-1} $PbCl_2$ as compared to the control treatment. The highest values of N, P, Mg, and SO_4 (0.00437, 0.00308, 0.0187 and 0.00096) % were recorded in the 15 or 30 mg.kg^{-1} $PbCl_2$ concentrations, while the lowest values of N, P, Mg, and SO_4 (0.00339, 0.00278, 0.0146 and 0.00072) % were recorded in the control and 30 mg.kg^{-1} treatment. The interactions between Ni and Pb concentrations had significant effects on N, P, Mg Ca and SO_4 which increased with increasing Ni and Pb concentration in the 15 or 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment of *G. triacanthos* roots (See Table 4.12).

The elements Fe and Zn increased with increasing the Ni concentrations to 15 and 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control treatment of *G. triacanthos* roots. The highest values of Fe and

Zn (0.122 and 0.0275) % were recorded in the 15 and 45 mg.kg^{-1} $NiCl_2$ concentrations, Whereas the lowest values (0.0589 and 0.0167) % were recorded in other treatments. The micro elements (Fe) decreased significantly with increasing the Pb concentration for all $PbCl_2$ treatments as compared to the control treatment. The highest value of the Fe (0.1224 %) which was recorded in the control treatment. On the other hand, the lowest value (0.0589 %) was recorded to 30 mg.kg^{-1} $PbCl_2$ concentrations. The interactions between Ni and Pb concentrations had significant effects on Cu, Fe and Mn which increased with increasing Ni and Pb concentration in the 15 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except for the Zn which decreased with increasing the Ni and Pb concentration (See Table 4.13).

The heavy metal As increased with increasing the Ni concentrations to 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control. The highest value of (0.000441 %) was recorded in the 45 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest value (0.000308 %) was recorded in the control treatment. The Pb concentration had non- significant on the heavy metal elements of *G. triacanthos* roots. The interactions between Ni and Pb concentrations had significant effects on some heavy metal elements such as Rb and As which increased with increasing Ni and Pb concentration in the 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (Table 4.14).

Table 4.9 . Effects of nickel, lead and their interactions mean of some macro-nutrient xof G. triacanthos shoot.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00470 a	0.00249 b	0.839 a	0.0267 c	4.621 a	0.00268 b
Ni 15	0.00453 a	0.00247 b	0.975 a	0.0388 a	4.586 a	0.00287 b
Ni 30	0.00358 b	0.00415 a	0.733 a	0.0339 b	4.938 a	0.00296 b
Ni 45	0.00467 a	0.00253 b	0.764 a	0.0334 b	5.165 a	0.00326 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00464 a	0.00271 b	0.911 a	0.0295 b	5.234 a	0.00285 a
Pb 15	0.00396 b	0.00318 a	0.717 a	0.0289 b	4.720 a	0.00291 a
Pb 30	0.00412 b	0.00245 c	0.904 a	0.0366 a	4.867 a	0.00306 a
Pb 45	0.00477 a	0.00330 a	0.777 a	0.0378 a	4.489 a	0.00294 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00481 bcd	0.00222 e	1.232 a	0.0325 fg	5.273 ab	0.00242 e
Ni0 Pb15	0.00419 de	0.00237 cde	0.496 a	0.0128 k	4.149 ab	0.00245 de
Ni0 Pb30	0.00485 bcd	0.00226 de	1.054 a	0.0411 cde	4.975 ab	0.00337 ab
Ni0 Pb45	0.00497 bc	0.00312 b	0.572 a	0.0205 ij	4.087 ab	0.00246 de
Ni15 Pb0	0.00528 b	0.00232 cde	1.090 a	0.0309 fgh	4.956 ab	0.00273 bcde
Ni15 Pb15	0.00434 cde	0.00242 cde	0.635 a	0.0274 ghi	4.266 ab	0.00308 bcde
Ni15 Pb30	0.00422 de	0.00231 cde	1.122 a	0.0365 def	4.117 ab	0.00249 de
Ni15 Pb45	0.00430 de	0.00284 bc	1.052 a	0.0605 a	5.003 ab	0.00317 bc
Ni30 Pb0	0.00423 de	0.00321 b	0.672 a	0.0358 ef	6.009 a	0.00312 bcd
Ni30 Pb15	0.00323 g	0.00511 a	0.740 a	0.0494 b	5.409 ab	0.00318 bc
Ni30 Pb30	0.00386 ef	0.00306 b	0.702 a	0.0257 g-j	4.372 ab	0.00255 cde
Ni30 Pb45	0.00298 g	0.00520 a	0.816 a	0.0249 hij	3.960 b	0.00301 bcde
Ni45 Pb0	0.00422 de	0.00307 b	0.651 a	0.0187 jk	4.695 ab	0.00314 bcd
Ni45 Pb15	0.00409 ef	0.00282 bcd	1.002 a	0.0261 g-j	5.055 ab	0.00294 bcde
Ni45 Pb30	0.00354 fg	0.00220 e	0.737 a	0.0432 bcd	6.005 a	0.00384 a
Ni45 Pb45	0.00684 a	0.00205 e	0.665 a	0.0453 bc	4.905 ab	0.00312 bcd

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.10. Effects of nickel, lead and their interactions mean of some micro - nutrient of G. triacanthos shoot.

Treatments	Cu	Fe	Mn	Zn	Ni
	(%)				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.00241 a	0.0538 b	0.0113 a	0.00537 bc	0.0303 a
Ni 15	0.00254 a	0.0652 a	0.00818 b	0.00657 a	0.0343 a
Ni 30	0.00119 b	0.0588 ab	0.00846 ab	0.00451 c	0.0302 a
Ni 45	0.00274 a	0.0575 ab	0.00846 ab	0.00569 ab	0.0352 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.00219 ab	0.0666 a	0.0122 a	0.00505 b	0.0323 a
Pb 15	0.00219 ab	0.0514 c	0.00818 b	0.00483 b	0.0328 a
Pb 30	0.00155 b	0.0566 bc	0.00823 b	0.00584 ab	0.0315 a
Pb 45	0.00295 a	0.0607 ab	0.00779 b	0.00642 a	0.0336 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00256 bc	0.0913 a	0.0245 a	0.00501 c-f	0.0299 a
Ni0 Pb15	0.00263 bc	0.0407 fgh	0.00663 b	0.00496 c-f	0.0303 a
Ni0 Pb30	0.00224 bc	0.0276 h	0.00762 b	0.00613 a-e	0.0304 a
Ni0 Pb45	0.00220 bc	0.0556 def	0.00658 b	0.00539 b-f	0.0307 a
Ni15 Pb0	0.00346 ab	0.0709 cd	0.00809 b	0.00698 abc	0.0384 a
Ni15 Pb15	0.00085 c	0.0580 cde	0.00792 b	0.00426 def	0.0301 a
Ni15 Pb30	0.00081 c	0.0582 cde	0.00777 b	0.00852 a	0.0303 a
Ni15 Pb45	0.00505 a	0.0737 bc	0.00895 b	0.00651 a-d	0.0385 a
Ni30 Pb0	0.00072 c	0.0710 cd	0.00895 b	0.00458 c-f	0.0299 a
Ni30 Pb15	0.00277 abc	0.0597 cde	0.0091 b	0.00307 f	0.0301 a
Ni30 Pb30	0.00037 c	0.0527 ef	0.00889 b	0.00429 def	0.0305 a
Ni30 Pb45	0.00093 c	0.0519 ef	0.00690 b	0.00609 a-e	0.305 a
Ni45 Pb0	0.00206 bc	0.0333 gh	0.00762 b	0.00364 ef	0.0308 a
Ni45 Pb15	0.00252 bc	0.0473 efg	0.0091 b	0.00703 abc	0.0406 a
Ni45 Pb30	0.00277 abc	0.0880 ab	0.00861 b	0.00444 c-f	0.0308 a
Ni45 Pb45	0.00361 ab	0.0615 cde	0.00876 b	0.00769 ab	0.0348 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.11 . Effects of nickel, lead and their interactions mean of some non - essential heavy metals of G. triacanthos shoot.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00219 a	0.000749 b	0.000217 b	0.0255 ab	0.000259 ab	0.536 a
Ni 15	0.00281 a	0.00149 a	0.000249 b	0.0280 a	0.000195 b	0.594 a
Ni 30	0.00186 a	0.000589 b	0.000208 b	0.0179 b	0.000537 a	0.539 a
Ni 45	0.00258 a	0.000899 b	0.000665 a	0.0362 a	0.000416 ab	0.567 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00209 a	0.000937 a	0.000212 b	0.0241 a	0.000224 b	0.563 a
Pb 15	0.00237 a	0.000585 b	0.000257 b	0.0312 a	0.000204 b	0.573 a
Pb 30	0.00206 a	0.0011 a	0.000644 a	0.0262 a	0.000696 a	0.527 a
Pb 45	0.00292 a	0.00116 a	0.000226 b	0.0262 a	0.000283 b	0.573 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00205 b	0.546 a	0.000238 b	0.0269 abc	0.000243 b	0.546 a
Ni0 Pb15	0.00224 b	0.538 a	0.000188 b	0.0211 abc	0.000185 b	0.538 a
Ni0 Pb30	0.00216 b	0.525 a	0.000243 b	0.0333 abc	0.000225 b	0.525 a
Ni0 Pb45	0.00232 b	0.535 a	0.000198 b	0.0206 abc	0.000387 b	0.535 a
Ni15 Pb0	0.00192 b	0.6404 a	0.000208 b	0.0268 abc	0.000240 b	0.6404 a
Ni15 Pb15	0.00201 b	0.592 a	0.000284 b	0.0333 abc	0.000220 b	0.592 a
Ni15 Pb30	0.00271 ab	0.539 a	0.000253 b	0.0255 abc	0.000155 b	0.539 a
Ni15 Pb45	0.00461 a	0.602 a	0.000253 b	0.0265 abc	0.000165 b	0.602 a
Ni30 Pb0	0.00221 b	0.542 a	0.000198 b	0.0165 bc	0.000200 b	0.542 a
Ni30 Pb15	0.00218 b	0.536 a	0.000248 b	0.0259 abc	0.000193 b	0.536 a
Ni30 Pb30	0.00873 b	0.512 a	0.000192 b	0.0213 abc	0.001445 a	0.512 a
Ni30 Pb45	0.00219 b	0.568 a	0.000193 b	0.0082 c	0.000309 b	0.568 a
Ni45 Pb0	0.00218 b	0.523 a	0.000203 b	0.0261 abc	0.000213 b	0.523 a
Ni45 Pb15	0.00306 ab	0.626 a	0.00031 b	0.0444 ab	0.000220 b	0.626 a
Ni45 Pb30	0.00251 b	0.532 a	0.00189 a	0.0249 abc	0.00096 a	0.532 a
Ni45 Pb45	0.00258 ab	0.587 a	0.000259 b	0.0495 a	0.000273 b	0.587 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.12. Effects of nickel, lead and their interactions mean of some macro-nutrient of G. triacanthos root.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00296 bc	0.00249 b	0.656 c	0.0110 b	1.361 b	0.00073 b
Ni 15	0.00271 c	0.00262 b	0.728 bc	0.0212 a	1.882 a	0.00078 b
Ni 30	0.00456 a	0.00326 a	1.068 ab	0.0224 a	1.424 b	0.00086 a
Ni 45	0.00320 b	0.00303 a	1.152 a	0.0121 b	1.337 b	0.00087 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00339 b	0.00278 b	0.828 a	0.0161 b	1.345 a	0.00072 c
Pb 15	0.00311 c	0.00249 c	0.913 a	0.0187 a	1.687 a	0.00096 a
Pb 30	0.00437 a	0.00308 a	1.063 a	0.0146 c	1.524 a	0.00080 b
Pb 45	0.00255 d	0.00305 a	0.798 a	0.0172 b	1.345 a	0.00075 bc
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00204 ef	0.00195 e	0.876 a	0.01207 fg	1.183 b	0.00071 fg
Ni0 Pb15	0.00226 ef	0.00248 cd	0.467 a	0.00810 h	1.234 b	0.00110 a
Ni0 Pb30	0.00511 b	0.00317 ab	0.808 a	0.00813 h	1.431 b	0.00055 h
Ni0 Pb45	0.00240 ef	0.00236 de	0.471 a	0.01590 d	1.595 ab	0.00055 h
Ni15 Pb0	0.00492 bc	0.00296 abc	0.503 a	0.01680 cd	1.596 ab	0.00068 g
Ni15 Pb15	0.00224 ef	0.00136 f	0.830 a	0.02729 b	2.277 a	0.00083 cdef
Ni15 Pb30	0.00250 e	0.00319 ab	1.089 a	0.01300 ef	1.872 ab	0.00079 defg
Ni15 Pb45	0.00116 h	0.00297 abc	0.488 a	0.02790 ab	1.781 ab	0.00081 defg
Ni30 Pb0	0.00241 ef	0.00331 ab	0.721 a	0.02575 b	1.248 b	0.00073 efg
Ni30 Pb15	0.00606 a	0.00314 ab	1.314 a	0.03008 a	1.821 ab	0.00095 bc
Ni30 Pb30	0.00450 cd	0.00308 ab	1.127 a	0.01871 c	1.402 b	0.00086 cde
Ni30 Pb45	0.00525 b	0.00350 a	1.109 a	0.01530 de	1.225 b	0.00090 bcd
Ni45 Pb0	0.00417 d	0.00290 bc	1.214 a	0.01002 gh	1.351 b	0.00077 defg
Ni45 Pb15	0.00186 fg	0.00298 abc	1.041 a	0.00943 gh	1.417 b	0.00096 bc
Ni45 Pb30	0.00536 b	0.00289 bc	1.228 a	0.01903 c	1.389 b	0.00101 ab
Ni45 Pb45	0.00139 gh	0.00337 ab	1.125 a	0.00991 gh	1.183 b	0.00076 efg

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa..

Table 4.13 . Effects of nickel, lead and their interactions mean of some micro- nutrient of G. triacanthos root.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0032 ab	0.0819 b	0.0127 a	0.0167 b	0.0318 a
Ni 15	0.0031 ab	0.1224 a	0.0114 a	0.0222 ab	0.0307 a
Ni 30	0.0026 b	0.0765 b	0.0108 a	0.0169 b	0.0303 a
Ni 45	0.0035 a	0.0589 b	0.0102 a	0.0275 a	0.0333 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.0031 ab	0.1224 a	0.0114 a	0.0222 ab	0.0307 a
Pb 15	0.0026 b	0.0765 b	0.0108 a	0.0169 b	0.0303 a
Pb 30	0.0035 a	0.0589 b	0.0102 a	0.0275 a	0.0333 a
Pb 45	0.0032 ab	0.0819 b	0.0127 a	0.0167 b	0.0318 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00299 bcd	0.0898 b	0.00803 b	0.02271 a-d	0.0305 a
Ni0 Pb15	0.00304 bcd	0.0716 b	0.00903 b	0.00859 c-f	0.0306 a
Ni0 Pb30	0.00394 b	0.0779 b	0.02452 a	0.02802 ab	0.0309 a
Ni0 Pb45	0.00266 bcd	0.0887 b	0.00919 b	0.00732 ef	0.0351 a
Ni15 Pb0	0.00240 bcd	0.0901 b	0.01912 a	0.00812 def	0.03005 a
Ni15 Pb15	0.00325bcd	0.2497 a	0.00925 b	0.02074 b-f	0.0302 a
Ni15 Pb30	0.00286 bcd	0.0699 b	0.00903 b	0.0316 ab	0.0314 a
Ni15 Pb45	0.00378 bc	0.0800 b	0.00805 b	0.02853 ab	0.0309 a
Ni30 Pb0	0.00296 bcd	0.0746 b	0.00826 b	0.00859 c-f	0.0302 a
Ni30 Pb15	0.00328 bcd	0.0825 b	0.01954 a	0.00684 f	0.0302 a
Ni30 Pb30	0.00197 d	0.0825 b	0.00819 b	0.0232 abc	0.0307 a
Ni30 Pb45	0.00205 d	0.0637 b	0.00726 b	0.0293 ab	0.0302 a
Ni45 Pb0	0.006025 a	0.0615 b	0.01992 a	0.02206 b-e	0.0315 a
Ni45 Pb15	0.00279 bcd	0.0504 b	0.00642 b	0.02148 b-f	0.0303 a
Ni45 Pb30	0.00233 cd	0.0635 b	0.00779 b	0.02915 ab	0.0358 a
Ni45 Pb45	0.00303 bcd	0.0607 b	0.00679 b	0.0374 a	0.0358 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.14. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of G. triacanthos root.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00291 a	0.00094 a	0.000256 a	0.0260 a	0.000308 b	0.5736 a
Ni 15	0.00286 a	0.00142 a	0.000225 a	0.0221 a	0.000354 ab	0.5476 a
Ni 30	0.00319 a	0.00115 a	0.000202 a	0.0178 a	0.000401 a	0.5589 a
Ni 45	0.00294 a	0.00113 a	0.000253 a	0.0261 a	0.000441 a	0.5598 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00306 a	0.00102 ab	0.000215 a	0.0259 a	0.000358 ab	0.546 a
Pb 15	0.00282 a	0.00077 b	0.000241 a	0.0206 a	0.000435 a	0.583 a
Pb 30	0.00309 a	0.00136 a	0.000229 a	0.0233 a	0.000371 ab	0.587 a
Pb 45	0.00293 a	0.00149 a	0.000252 a	0.0222 a	0.000339 b	0.524 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00319 a	0.000690 c	0.0002635 a	0.0269 ab	0.000299 de	0.543 a
Ni0 Pb15	0.00310 a	0.000627 c	0.0002585 a	0.0244 ab	0.000459 bcd	0.605 a
Ni0 Pb30	0.00302 a	0.000298 c	0.0002435 a	0.0256 ab	0.000218 e	0.601 a
Ni0 Pb45	0.00231 a	0.00216 a	0.0002588 a	0.0272 ab	0.000259 de	0.546 a
Ni15 Pb0	0.00309 a	0.00189 ab	0.0002430 a	0.0256 ab	0.000308 de	0.549 a
Ni15 Pb15	0.00277 a	0.000692 c	0.000209 a	0.0163 ab	0.000420 cde	0.543 a
Ni15 Pb30	0.00325 a	0.000902 bc	0.000200 a	0.0200 ab	0.000368 cde	0.597 a
Ni15 Pb45	0.00237 a	0.00219 a	0.0002485 a	0.0263 ab	0.000319 de	0.503 a
Ni30 Pb0	0.00308 a	0.000903 bc	0.000115 a	0.0203 ab	0.000280 de	0.549 a
Ni30 Pb15	0.00300 a	0.000887 bc	0.0002430 a	0.0205 ab	0.000260 de	0.594 a
Ni30 Pb30	0.00308 a	0.00205 a	0.000209 a	0.0216 ab	0.000667 a	0.548 a
Ni30 Pb45	0.003595 a	0.000762 bc	0.0002430 a	0.0884 b	0.000399 cde	0.545 a
Ni45 Pb0	0.00287 a	0.000593 c	0.0002385 a	0.0307 a	0.000547 abc	0.543 a
Ni45 Pb15	0.00241 a	0.000857 bc	0.0002530 a	0.0211 ab	0.000603 ab	0.592 a
Ni45 Pb30	0.00305 a	0.00220 a	0.0002635 a	0.0260 ab	0.000234 e	0.604 a
Ni45 Pb45	0.00344 a	0.000850 bc	0.0002585 a	0.0265 ab	0.000380 cde	0.501 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test , and vice versa.

4.2.2.4.2 Macro, micro and some of non- essential elements for shoots and roots of *L. leucocephala* species

Results of the study shown in tables 4.15, 4.16, 4.17, 4.18, 4.19 and 4.20 indicated that the effects of Ni and Pb and their interactions were significant on some mineral elements of the shoots and roots *L. leucocephala* species. It was found that some of the elements increased with increasing the concentration of Ni and Pb from 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment. The macro elements Mg and SO_4 were increased with increasing the Ni concentration from 45 or 15 $mg.kg^{-1}$ $NiCl_2$ as compared to other treatment of *L. leucocephala* shoots, whereas the N element decreased with increasing the Ni concentration to 45 $mg.kg^{-1}$. The highest values of Mg and SO_4 macro elements (0.02695 and 0.01256) % respectively were recorded in the 45 and 15 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (0.01978 and 0.01054) % were recorded in the 15 $mg.kg^{-1}$ and control treatment. $PbCl_2$ did not affect significantly P, K, Mg and Ca elements, whereas it decreased N and SO_4 content significantly compared to the control treatment which recorded the highest values (0.00442 and 0.01183) % respectively. The interactions between Ni and Pb concentrations increased Ca concentration with increasing Ni and Pb concentration to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment, except from the N, Mg and SO_4 which were decreased with increasing the Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment. An exception Fe decreased with Ni and Pb concentration (Table 4.15).

The concentration of Fe decreased with adding $NiCl_2$ as compared to the control treatment. The highest value of the (Fe) micro element (0.015 %) was recorded in the control treatment, while the lowest value (0.008 %) which was recorded to 45 $mg.kg^{-1}$ $NiCl_2$ of *L. leucocephala* shoots. The same response appeared with adding $PbCl_2$ specially high concentrations on the Cu and Fe elements . Where the highest values of Cu and Fe (0.0027 and 0.0135) % were recorded in the control treatment, while the lowest values (0.0007 and 0.0056) % were recorded for 15 $mg.kg^{-1}$ treatments. Cu response was similar to that of Fe regarding the effects of $PbCl_2$ application. Each of Mn, Zn and Ni did not affect significantly by the $PbCl_2$ concentrations. The interactions between Ni and Pb concentrations had significant effects on Mn which increased with increasing Ni and Pb concentration for 15 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (Table 4.16).

The concentration of As element decreased with increasing the Ni concentrations to 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment of *L. leucocephala* shoots. The highest value of As (0.00031 %) was

recorded in the control treatment, while the lowest value of the As heavy metal elements (0.00019 %) was recorded in the 45 mg.kg^{-1} $NiCl_2$ concentrations, whereas Ni application had not any significant effects on Pb, Rb, Cd, Ag and Ba elements compared to the control treatment. Also the application of $PbCl_2$ did not have any significant effects on Pb, Cd, Ag and Ba concentrations, while it increased shoots content of Rb and As elements significantly compared to the control treatments. The interactions between Ni and Pb had significant effects on Rb and As which increased with increasing Ni and Pb concentration to 30 or 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (Table 4.17).

Regarding the effects of $NiCl_2$ and $PbCl_2$ on *L. leucocephala* roots, the elements N, P and Ca increased with application Ni by 30 and 45 mg.kg^{-1} $NiCl_2$ as compared to the control treatment; An exception is the Mg element which decreased with 15 and 45 mg.kg^{-1} Ni concentration as compared to the control. The highest value of N, P and Ca (0.00374, 0.00247 and 1.05) % were recorded in the 30 and 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control treatment, while the lowest values (0.00229, 0.00194 and 0.45) % respectively were recorded in the control treatments, 15 and 45 mg.kg^{-1}. The macro elements N and P increased with increasing the Pb concentration to 15 and 30 mg.kg^{-1} $PbCl_2$ as compared to the control treatment; Except the Mg element which decreased with increasing the Pb concentration. The highest value of the mineral elements Mg (0.00428) % was recorded in the control treatment, while the lowest value (0.00312) % was recorded in the the 45 mg.kg^{-1} $PbCl_2$ concentrations. The interactions between Ni and Pb concentrations had significant effects on N, P, K, Mg, Ca and SO_4 which increased with increasing Ni and Pb concentration in the 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (Table 4.18).

The micro elements (Fe) increased with adding $NiCl_2$ as compared to the control treatment, where the highest value (0.0457 %) was recorded in the 15 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control treatment (0.0313 %). Ni application did not significant effects on each of Cu, Mn, Zn and Ni elements. Also $PbCl_2$ did not significant effect on Mn, Zn and Ni elements, except the significant decreased in root contain of Cu with all $PbCl_2$ concentrations and, the significant decreased in Fe content when 15 mg.kg^{-1} $PbCl_2$ were applied. The interactions between Ni and Pb concentrations had significant effects on Fe increased with increasing Ni and Pb concentration to 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the Cu element decreased with increasing with Ni and Pb concentration to 30 mg.kg^{-1}, as shown in Table 4.19.

The heavy metal elements (Ag) increased with adding $NiCl_2$ as compared to the control treatment. The highest value of (Ag) heavy metal elements (0.222 %) was recorded in the 15 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment (0.0128 %). As element decreased with other treatment as compared to control. Each of Pb, Rb, Cd and Ba in the control treatment did not differ significantly with Ni concentration as compared to the other treatments. Pb concentration had a significant effect on Rb, Ag and As elements of *L. leucocephala* roots. Where the highest values (0.00162, 0.0232 and 0.00054) % were recorded in the control and 15 $mg.kg^{-1}$ treatment, the lowest values (0.000594, 0.0132 and 0.00022) % were recorded to 45, 15 and 30 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment. The interactions between Ni and Pb concentrations had significant effects on Rb which increased with increasing Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, exception the Ag and As decreased with increasing Ni and Pb concentration at 45 $mg.kg^{-1}$ as compared to control treatment (Table 4.20).

Table 4.15. Effects of nickel, lead and their interactions mean of some macro- nutrient of L. leucocephala shoot.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00392 b	0.00443 a	0.98 ab	0.02239 b	4.98 a	0.01054 b
Ni 15	0.00475 a	0.00412 a	1.30 ab	0.02089 b	4.82 a	0.01256 a
Ni 30	0.00386 b	0.00400 a	0.9.0 b	0.01978 b	5.36 a	0.01058 b
Ni 45	0.00323 c	0.00439 a	1.41 a	0.02695 a	5.49 a	0.01112 b
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00442 a	0.00400 a	1.28 a	0.02006 a	4.94 a	0.01183 a
Pb 15	0.00392 b	0.00445 a	1.03 a	0.02418 a	5.03 a	0.01047 c
Pb 30	0.00386 b	0.00415 a	1.02 a	0.02419 a	5.04 a	0.01152 ab
Pb 45	0.00356 b	0.00434 a	1.25 a	0.02159 a	5.63 a	0.01098 bc
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00410 c	0.00400 ab	1.26 ab	0.03074 abc	3.81 d	0.00897 g
Ni0 Pb15	0.00380 c	0.00484 ab	0.80 b	0.02011 defg	4.81 abcd	0.00905 g
Ni0 Pb30	0.00389 c	0.00481 ab	0.64 b	0.01892 efg	5.37 abcd	0.01046 efg
Ni0 Pb45	0.00389 c	0.00406 ab	1.22 ab	0.01978 defg	5.93 abc	0.01367 ab
Ni15 Pb0	0.00602 a	0.00433 ab	1.40 ab	0.01892 efg	5.24 abcd	0.01437 a
Ni15 Pb15	0.00392 c	0.00418 ab	1.29 ab	0.02955 abc	4.39 bcd	0.01139 cdef
Ni15 Pb30	0.00392 c	0.00373 b	1.14 ab	0.02153 cdef	5.35 abcd	0.01179 cde
Ni15 Pb45	0.00514 b	0.00426 ab	1.39 ab	0.01356 fg	4.30 cd	0.01269 bc
Ni30 Pb0	0.00382 c	0.00377 ab	1.23 ab	0.01481 efg	4.84 abcd	0.01153 cdef
Ni30 Pb15	0.00400 c	0.00406 ab	0.81 b	0.01156 g	5.05 abcd	0.01030 efg
Ni30 Pb30	0.00378 c	0.00401 ab	0.50 b	0.02397 bcde	5.33 abcd	0.01013 fg
Ni30 Pb45	0.00386 c	0.00417 ab	1.05 ab	0.02877 abcd	6.22 a	0.01037 efg
Ni45 Pb0	0.00376 c	0.00389 ab	1.23 ab	0.01576 efg	5.87 abc	0.01246 bcd
Ni45 Pb15	0.00397 c	0.00471 ab	1.24 ab	0.03550 a	5.88 abc	0.01115 def
Ni45 Pb30	0.00385 c	0.00406 ab	1.82 a	0.03233 ab	4.12 d	0.01369 ab
Ni45 Pb45	0.00134 d	0.00488 a	1.34 ab	0.02421 bcde	6.08 ab	0.00720 h

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.16. Effects of nickel, lead and their interactions mean of some micro- nutrient of L. leucocephala shoot.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0022 a	0.015 a	0.0054 ab	0.0033 a	0.035 a
Ni 15	0.0017 a	0.007 b	0.0043 b	0.0026 a	0.033 a
Ni 30	0.0015 a	0.009 b	0.0055 ab	0.0024 a	0.032 a
Ni 45	0.0024 a	0.008 b	0.0061 a	0.0025 a	0.030 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.0027 a	0.0135 a	0.0055 a	0.0027 a	0.0318 a
Pb 15	0.0030 a	0.0139 a	0.0059 a	0.0023 a	0.0306 a
Pb 30	0.0007 b	0.0056 b	0.0048 a	0.0028 a	0.0344 a
Pb 45	0.0015 b	0.0061 b	0.0050 a	0.0031 a	0.0330 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00265 ab	0.024 a	0.003760 bc	0.00227 ab	0.035 a
Ni0 Pb15	0.00460 a	0.021 a	0.0075 a	0.00305 ab	0.031 a
Ni0 Pb30	0.00063 b	0.006 b	0.00520 abc	0.00318 ab	0.040 a
Ni0 Pb45	0.00074 b	0.008 b	0.00501 abc	0.00459 a	0.035 a
Ni15 Pb0	0.003375 a	0.009 b	0.00524 abc	0.00248 ab	0.031 a
Ni15 Pb15	0.00249 ab	0.007 b	0.00468 abc	0.00247 ab	0.030 a
Ni15 Pb30	0.00071 b	0.004 b	0.003312 c	0.00277ab	0.036 a
Ni15 Pb45	0.000343 b	0.007 b	0.003795 bc	0.00267 ab	0.036 a
Ni30 Pb0	0.00029 b	0.0022 b	0.00634 ab	0.00332 ab	0.035 a
Ni30 Pb15	0.00236 ab	0.019 a	0.00571 abc	0.000902 b	0.030 a
Ni30 Pb30	0.00057 b	0.008 b	0.00511 abc	0.002635 ab	0.030 a
Ni30 Pb45	0.00282 ab	0.007 b	0.00471 abc	0.002805 ab	0.030 a
Ni45 Pb0	0.004385 a	0.019 a	0.00652 ab	0.00274 ab	0.026 a
Ni45 Pb15	0.00241 ab	0.005 b	0.00571 abc	0.00266 ab	0.031 a
Ni45 Pb30	0.000702 b	0.005 b	0.00551 abc	0.00253 ab	0.031 a
Ni45 Pb45	0.00209 ab	0.0023b	0.00650 ab	0.002245 ab	0.031 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.17. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of L. leucocephala shoot.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.0028 a	0.00173 a	0.00027 a	0.027 a	0.00031 a	0.50 ab
Ni 15	0.0026 a	0.00144 a	0.00025 a	0.028 a	0.00029 a	0.55 a
Ni 30	0.0024 a	0.01790 a	0.00022 a	0.023 a	0.00013 b	0.44 b
Ni 45	0.0024 a	0.00131 a	0.00024 a	0.021 a	0.00019 b	0.46 ab
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.0025 a	0.0012 b	0.00023 a	0.024 a	0.00017 b	0.453 a
Pb 15	0.0026 a	0.0011 b	0.00023 a	0.022 a	0.00022 ab	0.477 a
Pb 30	0.0025 a	0.0024 a	0..00026 a	0.027 a	0.00025 a	0.482 a
Pb 45	0.0027 a	0.0016 ab	0.00027 a	0.027 a	0.00027 a	0.533 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00282 a	0.00029 b	0.000238 a	0.025 a	0.000175 bc	0.44 a
Ni0 Pb15	0.00283 a	0.0008 b	0.000254 a	0.027 a	0.00028 abc	0.44 a
Ni0 Pb30	0.00305 a	0.0029 a	0.000295 a	0.031 a	0.00035 ab	0.49 a
Ni0 Pb45	0.00321 a	0.0031 a	0.000293 a	0.026 a	0.00452 a	0.62 a
Ni15 Pb0	0.00279 a	0.0009 b	0.000243 a	0.026 a	0.000253 bc	0.49 a
Ni15 Pb15	0.00266 a	0.00082 b	0.000238 a	0.026 a	0.0003 abc	0.59 a
Ni15 Pb30	0.00216 a	0.0031 a	0.000230 a	0.030 a	0.000303 abc	0.47 a
Ni15 Pb45	0.00275 a	0.0009 b	0.000298 a	0.030 a	0.0003 abc	0.63 a
Ni30 Pb0	0.00210 a	0.0027 a	0.000182 a	0.026 a	0.000125 c	0.44 a
Ni30 Pb15	0.00261 a	0.00191 ab	0.000228 a	0.020 a	0.00013 c	0.44 a
Ni30 Pb30	0.00219 a	0.0009 b	0.000250 a	0.020 a	0.000135 c	0.44 a
Ni30 Pb45	0.00259 a	0.0018 ab	0.000235 a	0.026 a	0.00012 c	0.44 a
Ni45 Pb0	0.00216 a	0.0009 b	0.000249 a	0.020 a	0.000135 c	0.44 a
Ni45 Pb15	0.00226 a	0.00078 b	0.000193 a	0.014 a	0.00016 c	0.44 a
Ni45 Pb30	0.00276 a	0.0026 a	0.000248 a	0.025 a	0.00023 bc	0.53 a
Ni45 Pb45	0.00262 a	0.0009 b	0.000254 a	0.026 a	0.000214 bc	0.45 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.18. Effects of nickel, lead and their interactions mean of some macro- nutrient of L. leucocephala root.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00229 c	0.00216 bc	0.65 ab	0.00408 a	0.64 bc	0.00673 a
Ni 15	0.00238 c	0.00240 ab	0.44 b	0.00327 b	0.45 c	0.00649 a
Ni 30	0.00326 b	0.00247 a	0.71 a	0.00401 a	0.73 b	0.00661 a
Ni 45	0.00374 a	0.00194 c	0.52 ab	0.00307 b	1.05 a	0.00691 a
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00242 c	0.00176 b	0.597 ab	0.00428 a	0.79 a	0.00644 a
Pb 15	0.00308 b	0.00268 a	0.610 a	0.00361 b	0.73 a	0.00657 a
Pb 30	0.00358 a	0.00261 a	0.377 b	0.00341 bc	0.60 a	0.00674 a
Pb 45	0.00258 c	0.00192 b	0.735 a	0.00312 c	0.75 a	0.00699 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00190 fg	0.00149 ghi	0.57 b	0.00290 bcd	0.59 bcde	0.00579 c
Ni0 Pb15	0.00215 efg	0.00175 fghi	1.13 a	0.00372 b	1.19 a	0.00662 abc
Ni0 Pb30	0.00284 cde	0.00294 c	0.41 b	0.00360 bc	0.89 e	0.00762 a
Ni0 Pb45	0.00226 defg	0.00247 cd	0.51 b	0.00610 a	0.68 abcd	0.00691 abc
Ni15 Pb0	0.00329 c	0.00221 def	0.51 b	0.00570 a	0.72 abcd	0.00613 bc
Ni15 Pb15	0.00247 cdef	0.00168 fghi	0.29 b	0.00358 bc	0.14 e	0.00625 abc
Ni15 Pb30	0.00154 g	0.00385 b	0.39 b	0.00140 f	0.70 abcd	0.00674 abc
Ni15 Pb45	0.00223 defg	0.00188 e-h	0.55 b	0.00239 de	0.24 de	0.00684 abc
Ni30 Pb0	0.00244 cdef	0.00134 hi	0.66 b	0.00577 a	0.78 abcd	0.00676 abc
Ni30 Pb15	0.00491 b	0.00556 a	0.58 b	0.00578 a	0.90 abc	0.00716 abc
Ni30 Pb30	0.00313 cd	0.00126 i	0.32 b	0.00276 cd	0.40 cde	0.00583 c
Ni30 Pb45	0.00255 cdef	0.00173 fghi	1.28 a	0.00170 ef	0.85 abc	0.00668 abc
Ni45 Pb0	0.00206 efg	0.00202 d-g	0.65 b	0.00275 cd	1.04 ab	0.00709 abc
Ni45 Pb15	0.00280 cdef	0.00174 fghi	0.43 b	0.00135 f	0.71 abcd	0.00624 abc
Ni45 Pb30	0.00681 a	0.00239 de	0.39 b	0.00589 a	1.23 a	0.00678 abc
Ni45 Pb45	0.00328 c	0.00160 ghi	0.61 b	0.00230 de	1.24 a	0.00754 ab

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.19. Effects of nickel, lead and their interactions mean of some micro- nutrient of L. leucocephala root.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0017 a	0.0313 b	0.0034 a	0.00252 a	0.0308 a
Ni 15	0.0024 a	0.0457 a	0.0030 a	0.0025 a	0.0307 a
Ni 30	0.0021 a	0.0413 a	0.0027 a	0.0026 a	0.0304 a
Ni 45	0.0026 a	0.0404 a	0.0031 a	0.00246 a	0.0331 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.0032 a	0.0461 a	0.0030 a	0.0026 a	0.0321 a
Pb 15	0.0015 b	0.0313 b	0.0029 a	0.0024 a	0.0319 a
Pb 30	0.0018 b	0.0422 a	0.0028 a	0.0025 a	0.0303 a
Pb 45	0.0022 b	0.0392 a	0.0035 a	0.0026 a	0.0307 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00277 abcd	0.0247 ef	0.00336 ab	0.00251 a	0.0314 a
Ni0 Pb15	0.000399 e	0.0318 ef	0.00277 ab	0.00233 a	0.0303 a
Ni0 Pb30	0.000778 de	0.0375 def	0.00287 ab	0.00269 a	0.0302 a
Ni0 Pb45	0.00292 abc	0.0314 ef	0.0044 a	0.00257 a	0.0314 a
Ni15 Pb0	0.00432 a	0.0683 a	0.00342 ab	0.00252 a	0.0305 a
Ni15 Pb15	0.00219 bcde	0.0235 f	0.00238 b	0.00211 a	0.0311 a
Ni15 Pb30	0.00239 abcde	0.0567 abc	0.00306 ab	0.00232 a	0.0307 a
Ni15 Pb45	0.000522 e	0.0344 ef	0.00297 ab	0.00294 a	0.0307 a
Ni30 Pb0	0.002315 abcde	0.0511 bcd	0.00217 b	0.00217 a	0.0305 a
Ni30 Pb15	0.000495 e	0.0399 def	0.00311 ab	0.00277 a	0.0307 a
Ni30 Pb30	0.00321 ab	0.0419 cde	0.00279 ab	0.00291 a	0.0302 a
Ni30 Pb45	0.00226 abcde	0.0324 ef	0.00288 ab	0.00273 a	0.0303 a
Ni45 Pb0	0.00351 ab	0.0402 def	0.00295 ab	0.0339 a	0.0359 a
Ni45 Pb15	0.003030 ab	0.0301 ef	0.00318 ab	0.00235 a	0.0357 a
Ni45 Pb30	0.00091 cde	0.0326 ef	0.00254 ab	0.00215 a	0.0303 a
Ni45 Pb45	0.003095 ab	0.0587 ab	0.0036 ab	0.00198 a	0.0305 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.20 . Effects of nickel, lead and their interactions mean of some non - essential heavy metal of L. leucocephala root.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00338 a	0.00116 ab	0.000243 a	0.0128 b	0.00062 a	0.553 a
Ni 15	0.00313 a	0.00165 a	0.000199 a	0.0222 a	0.00024 b	0.565 a
Ni 30	0.00312 a	0.00080 ab	0.000185 a	0.0184 ab	0.00022 b	0.528 a
Ni 45	0.002812 a	0.000357 b	0.000184 a	0.0161 ab	0.00026 b	0.545 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.003044 a	0.00162 a	0.000206 a	0.0232 a	0.00033 b	0.558 a
Pb 15	0.00324 a	0.00061 b	0.000208 a	0.0132 b	0.00054 a	0.532 a
Pb 30	0.00306 a	0.00114 ab	0.000203 a	0.0178 ab	0.00022 b	0.563 a
Pb 45	0.00310 a	0.000594 b	0.000195 a	0.0153 b	0.00026 b	0.536 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.0037 a	0.00087 b	0.000254 a	0.0250 ab	0.00060 b	0.5350 a
Ni0 Pb15	0.0035 a	0.00061 b	0.000238 a	0.00806 bc	0.0014 a	0.5422 a
Ni0 Pb30	0.0031 a	0.00247 b	0.000238 a	0.00907 bc	0.000235 c	0.6115 a
Ni0 Pb45	0.0032 a	0.00068 b	0.000244 a	0.00904 bc	0.000254 c	0.5227 a
Ni15 Pb0	0.0030 a	0.00462 a	0.000254 a	0.0266 a	0.000284 c	0.5592 a
Ni15 Pb15	0.0032 a	0.00058 b	0.000204 a	0.0165 abc	0.00023 c	0.5494 a
Ni15 Pb30	0.0031 a	0.00081 b	0.000193 a	0.0245 ab	0.000203 c	0.5384 a
Ni15 Pb45	0.0032 a	0.00059 b	0.000145 a	0.0211 abc	0.000228 c	0.6113 a
Ni30 Pb0	0.0031 a	0.00072 b	0.000145 a	0.0214 abc	0.000195 c	0.5441 a
Ni30 Pb15	0.0032 a	0.00090 b	0.000199 a	0.00819 bc	0.000297 c	0.5313 a
Ni30 Pb30	0.0033 a	0.00081 b	0.000204 a	0.0196 abc	0.000190 c	0.5509 a
Ni30 Pb45	0.00299 a	0.00079 b	0.000193 a	0.0246 ab	0.000210 c	0.4857 a
Ni45 Pb0	0.00238 a	0.00030 b	0.000170 a	0.0196 abc	0.000217 c	0.5953 a
Ni45 Pb15	0.0030 a	0.00034 b	0.000190 a	0.0202 abc	0.000 225 c	0.5066 a
Ni45 Pb30	0.00284 a	0.00049 b	0.000177 a	0.0182 abc	0.000242 c	0.5513 a
Ni45 Pb45	0.00299 a	0.00031 b	0.000199 a	0.00628 c	0.00023 c	0.5255 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.2.2.4.3 Macro, micro and some of non- essential elements for shoots and roots of *R. pseudoacacia* species

Tables 4.21, 4.22, 4.23, 4.24, 4.25 and 4.26 show the effects of Ni and Pb and their interactions were significant on some mineral elements of the shoots and roots of *R. pseudoacacia* species. It shows that some of the elements were increased with increasing the concentration of Ni and Pb from 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment. The macro elements (N, Mg and SO_4) in *R. pseudoacacia* shoots increased with increasing the Ni concentration to 30 and 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment, whereas the P elements decreased with increasing the Ni concentration. The highest values of N, Mg and SO_4 (0.00316, 0.0653 and 0.02032) % respectively were recorded in the 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (0.00228, 0.05833 and 0.01540) % were recorded in the 15 $mg.kg^{-1}$ and the control treatments. The N macro element decreased with increasing the Pb concentration to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to other treatments, while the P element decreased with 15 $mg.kg^{-1}$ Ni concentration. On the other hand, the difference between the control treatment with other treatments were not significant regarding the K, Mg, Ca and SO_4 respectively. The interactions between Ni and Pb concentrations had significant effects on Mg and SO_4 which increased with increasing Ni and Pb concentration to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, while the N and P elements decreased with increasing the Ni and Pb concentration to 30 or 45 $mg.kg^{-1}$ (See Table 4.21).

The micro elements (Cu, Fe and Mn) increased with adding $NiCl_2$ as compared to the control treatment. The highest values of the (Cu, Fe and Mn) micro elements (0.0866, 0.108, and 0.0181) % were recorded in the 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment, while the lowest values (0.0434, 0.029 and 0.0076) % were recorded in the control treatment. The micro elements Cu, Fe and Mn concentrations increased with adding $PbCl_2$ as compared to the control treatment. The highest value of the Cu, Fe and Mn micro elements (0.077, 0.101 and 0.0192) % respectively were recorded in the 15 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment which records the lowest values (0.067, 0.044 and 0.0079) %. The interactions between Ni and Pb had significant effects on Cu, Fe and Mn increased with increasing Ni and Pb concentration in the 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment. Neither Ni nor Pb nor their interactions had a significant effect on Zn and Ni shoot content of *R. pseudoacacia* species (Table 4.22).

The elements (Rb) increased with adding $NiCl_2$ as compared to the control treatment, whereas the As element decreased with some Ni concentrations. The highest value of the (Rb) heavy metal elements (0.00186 %) was recorded in the 30 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment, while the lowest values (0.000723 %) was recorded in the control treatment. The heavy metal elements (As) decreased with adding 15 and 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. The highest value (0.00073 and 0.00077) % were recorded in the control and 30 $mg.kg^{-1}$ treatments, while the lowest value of the As heavy metal element (0.00044 %) was recorded in the 15 $mg.kg^{-1}$ $PbCl_2$. Wheras the $PbCl_2$ did not affect on Pb, Rb, Cd, Ag and Ba mineral elements. The interactions between Ni and Pb concentrations had significant effects on Rb and Cd which were increased with adding 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the As element was decreased with increasing the Ni concentration, the result cleared in table (4.23).

The macro elements (Mg and Ca) of *R. pseudoacacia* roots increased with adding $NiCl_2$ as compared to the control treatment, whereas the P element decreased with increasing the Ni concentration to 30 and 45 $mg.kg^{-1}$. The highest values of Mg and Ca macro elements (0.03443 and 5.191) % respectively were recorded in the 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment of *R. pseudoacacia* roots. However, the lowest values (0.01827 and 3.919) % were recorded in the control treatment. Each of Ca and SO_4 were increased with increasing the Pb concentration especially to 15 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment, while the Mg element decreased with increasing the Pb concentration. The highest value of the Ca and SO_4 mineral elements (5.060 and 0.00119) % were recorded in the 15 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment. While the lowest values (3.816 and 0.00092) % were recorded in the control treatment. Each of N, P and K did not affect significantly by the $PbCl_2$ concentration. The interactions between Ni and Pb concentrations had significant effects on P, Mg and Ca which increased with increasing Ni and Pb concentration in the 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment; An exception the N, K and SO_4 which no significant of *R. pseudoacacia* roots (Table 4.24).

The micro elements (Fe) increased with adding $NiCl_2$ especially 30 $mg.kg^{-1}$ $NiCl_2$ concentrations (0.324 %) as compared to the control treatment of *R. pseudoacacia* roots, while the Ni micro element decreased with adding Ni concentration by the 15 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment (0.0306 %). Each of Cu, Mn and Zn does not affected significantly by adding $NiCl_2$ compared to the control treatment. The micro elements (Mn) increased with increasing the Pb concentration from $PbCl_2$ as

compared to the control treatment. The highest value of Mn micro element (0.0238 %) was recorded to 45 $mg.kg^{-1}$ $PbCl_2$, while the lowest value (0.0131 %) was recorded in the control treatment. Each of Cu, Fe, Zn and Ni did not affected by $PbCl_2$ concentration. The interactions between Ni and Pb concentrations had significant effects on Fe, Zn and Ni increased with increasing Ni and Pb concentration for 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment (Table 4. 25).

Each of Pb, Cd, Ag and Ba elements were not affected significantly by adding $NiCl_2$ or $PbCl_2$ or their interactions. The concentration of heavy metal element (As) increased with adding $NiCl_2$ by 15 $mg.kg^{-1}$ $NiCl_2$ concentrations (0.000674 %) as compared to other treatments. The Pb concentration effect was significant on the heavy metal elements (Rb and As). The heavy metal nutreints (Rb) was increased significantly by adding 15 $mg.kg^{-1}$ $PbCl_2$ concentrations to (0.00261 %) as compared to the control (0.00123 %), whereas As decreased with increasing Pb concentration to 45 $mg.kg^{-1}$ as compared to the control treatment. The interactions between Ni and Pb concentrations had significant effects on Rb and As which increased with increasing Ni and Pb concentration to 15 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, while the Pb, Cd, Ag and Ba elements which were non significant (Table 4. 26).

Table 4.21. Effects of nickel, lead and their interactions mean of some macro- nutrient of R. pseudoacacia shoot.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00277 b	0.00218 a	1.792 a	0.05833 b	6.948 a	0.01540 b
Ni 15	0.00228 c	0.00221 a	1.993 a	0.04100 c	7.367 a	0.01957 a
Ni 30	0.00316 a	0.00182 b	1.518 a	0.06536 a	7.171 a	0.01965 a
Ni 45	0.00304 ab	0.00164 b	1.511 a	0.04100 c	7.013 a	0.02032 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00302 a	0.00196 a	1.74 ab	0.05461 a	6.95 a	0.01819 ab
Pb 15	0.00318 a	0.00169 b	2.15 a	0.05424 a	7.63 a	0.02043 a
Pb 30	0.00302 a	0.00204 a	1.32 b	0.05656 a	6.85 a	0.01738 b
Pb 45	0.00204 b	0.00216 a	1.60 ab	0.05721 a	7.07 a	0.01894 ab
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00284 cd	0.00228 a	2.14 a	0.06091 bc	7.09 ab	0.01649 cde
Ni0 Pb15	0.00370 ab	0.00209 ab	2.41 a	0.07330 b	7.41 ab	0.01545 de
Ni0 Pb30	0.00296 cd	0.00205 abc	1.28 a	0.06400 bc	6.80 ab	0.01636 cde
Ni0 Pb45	0.00158 e	0.00231 a	1.33 a	0.03513 ef	6.50 ab	0.01331 e
Ni15 Pb0	0.00341 bc	0.00232 a	2.15 a	0.05662 cd	7.16 ab	0.01951 abcd
Ni15 Pb15	0.00259 d	0.00148 d	2.10 a	0.05505 cd	7.42 ab	0.02104 abc
Ni15 Pb30	0.00142 e	0.00247 a	1.27 a	0.02736 f	7.06 ab	0.01527 de
Ni15 Pb45	0.00172 e	0.00255 a	2.45 a	0.02497 f	7.84 a	0.02246 ab
Ni30 Pb0	0.00339 bc	0.00153 cd	1.26 a	0.07204 b	7.72 a	0.01501 de
Ni30 Pb15	0.00339 bc	0.00159 bcd	2.10 a	0.04232 e	7.94 a	0.02167 abc
Ni30 Pb30	0.00427 a	0.00214 ab	1.39 a	0.07413 b	6.38 ab	0.02061 abcd
Ni30 Pb45	0.00158 e	0.00203 abcd	1.32 a	0.07294 b	6.65 ab	0.02128 abc
Ni45 Pb0	0.00243 d	0.00172 bcd	1.41 a	0.02887 f	5.83 b	0.02173 abc
Ni45 Pb15	0.00303 bcd	0.00160 bcd	1.99 a	0.04626 de	7.75 a	0.02356 a
Ni45 Pb30	0.00342 bc	0.00151 cd	1.36 a	0.06074 bc	7.17 ab	0.01727bcde
Ni45 Pb45	0.00329 bc	0.00174 bcd	1.29 a	0.09579 a	7.30 ab	0.01872 abcde

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.22. Effects of nickel, lead and their interactions mean of some micro- nutrient of R. pseudoacacia shoot.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0434 b	0.029 b	0.0076 b	0.00537 a	0.032 a
Ni 15	0.0812 a	0.111 a	0.0181 a	0.00606 a	0.039 a
Ni 30	0.0856 a	0.060 b	0.0135 ab	0.00556 a	0.040 a
Ni 45	0.0801 a	0.108 a	0.0125 ab	0.00528 a	0.031 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.067 b	0.044 b	0.0079 b	0.0063 a	0.0363 a
Pb 15	0.077 a	0.101 a	0.0192 a	0.0056 a	0.0360 a
Pb 30	0.075 ab	0.063 ab	0.0124 ab	0.0054 a	0.0313 a
Pb 45	0.072 ab	0.099 a	0.0121 ab	0.00501 a	0.0375 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.021 d	0.026 b	0.0063 c	0.0063 a	0.0305 a
Ni0 Pb15	0.057 c	0.032 b	0.0081 bc	0.0065 a	0.0305 a
Ni0 Pb30	0.058 c	0.027 b	0.0082 bc	0.0052 a	0.0304 a
Ni0 Pb45	0.038 d	0.030 b	0.0077 c	0.0036 a	0.0356 a
Ni15 Pb0	0.087 ab	0.071 b	0.0092 bc	0.0061 a	0.0491 a
Ni15 Pb15	0.075 abc	0.249 a	0.031 a	0.0056 a	0.0353 a
Ni15 Pb30	0.082 ab	0.066 b	0.0085bc	0.0062 a	0.0346 a
Ni15 Pb45	0.081 ab	0.060 b	0.024 ab	0.0064 a	0.0353 a
Ni30 Pb0	0.075 abc	0.048 b	0.0089 bc	0.0064 a	0.0352 a
Ni30 Pb15	0.096 a	0.075 b	0.030 a	0.0064 a	0.4310 a
Ni30 Pb30	0.091 a	0.075 b	0.0080 bc	0.0060 a	0.0301 a
Ni30 Pb45	0.081 ab	0.042 b	0.0073 c	0.0035 a	0.0491 a
Ni45 Pb0	0.083 ab	0.033 b	0.0073 c	0.0064 a	0.0303 a
Ni45 Pb15	0.081 ab	0.048 b	0.0084 bc	0.0040 a	0.0353 a
Ni45 Pb30	0.068 bc	0.085 b	0.025 a	0.0041 a	0.0302 a
Ni45 Pb45	0.089 ab	0.265 a	0.0094 bc	0.0066 a	0.0302 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.23. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of R. pseudoacacia shoot.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.0031 a	0.000723 c	0.000261 a	0.0259 a	0.00082 a	0.589 a
Ni 15	0.0026 a	0.00161 ab	0.000295 a	0.0309 a	0.00059 b	0.623 a
Ni 30	0.0022 a	0.00186 a	0.000833 a	0.0284 a	0.00058 b	0.585 a
Ni 45	0.0032 a	0.000984 bc	0.000832 a	0.0277 a	0.00046 c	0.605 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.0027 a	0.00119 a	0.000276 a	0.0262 a	0.00073 a	0.622 a
Pb 15	0.0034 a	0.00161 a	0.000855 a	0.0308 a	0.00044 b	0.607 a
Pb 30	0.0022 a	0.00113 a	0.000269 a	0.0280 a	0.00077 a	0.594 a
Pb 45	0.0029 a	0.00126 a	0.000820 a	0.0278 a	0.00049 b	0.579 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00245 ab	0.000750 b	0.000264 b	0.0223 a	0.00100 ab	0.595 a
Ni0 Pb15	0.00251 ab	0.000545 b	0.000264 b	0.0274 a	0.00069 cd	0.586 a
Ni0 Pb30	0.00251 ab	0.000714 b	0.000249 b	0.0266 a	0.00104 a	0.587 a
Ni0 Pb45	0.00497 a	0.000885 b	0.000269 b	0.0273 a	0.00054 def	0.586 a
Ni15 Pb0	0.00307 ab	0.000898 b	0.000295 b	0.0286 a	0.00028 fg	0.707 a
Ni15 Pb15	0.00316 ab	0.00249 a	0.000323 b	0.0349 a	0.00043 efg	0.598 a
Ni15 Pb30	0.00099 b	0.00245 a	0.000308 b	0.0318 a	0.00075 abc	0.651 a
Ni15 Pb45	0.00311 ab	0.00256 a	0.000254 b	0.0283 a	0.00090 abc	0.537 a
Ni30 Pb0	0.00257 ab	0.000602 b	0.000254 b	0.0269 a	0.00061 de	0.548 a
Ni30 Pb15	0.00254 ab	0.00304 a	0.000324 b	0.0327 a	0.00041 efg	0.655 a
Ni30 Pb30	0.00260 ab	0.000882 b	0.000259 b	0.0264 a	0.00101 ab	0.597 a
Ni30 Pb45	0.00097 b	0.000975 b	0.00249 a	0.0277 a	0.00030 fg	0.541 a
Ni45 Pb0	0.00262 ab	0.000545 b	0.000290 b	0.0273 a	0.00103 a	0.638 a
Ni45 Pb15	0.00525 a	0.000354 b	0.00251 a	0.0282 a	0.00024 g	0.588 a
Ni45 Pb30	0.00258 ab	0.000469 b	0.000264 b	0.0273 a	0.00030 fg	0.542 a
Ni45 Pb45	0.00248 ab	0.000714 b	0.000264 b	0.0280 a	0.00026 g	0.652 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.24. Effects of nickel, lead and their interactions mean of some macro- nutrient of R. pseudoacacia root.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00312 a	0.00859 b	1.141 a	0.01827 c	3.919 b	0.00106 a
Ni 15	0.00306 a	0.00942 a	1.279 a	0.01964 c	4.027 b	0.00112 a
Ni 30	0.00304 a	0.00734 c	1.105 a	0.03443 a	4.536 ab	0.00099 a
Ni 45	0.00286 a	0.00784 c	1.237 a	0.02553 b	5.191 a	0.00114 a
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00308 a	0.00842 a	0.991 a	0.02754 a	3.816 b	0.00092 b
Pb 15	0.00303 a	0.00792 a	1.273 a	0.02906 a	5.060 a	0.00119 a
Pb 30	0.00290 a	0.00818 a	1.228 a	0.01887 b	4.523 ab	0.00112 a
Pb 45	0.00308 a	0.00866 a	1.269 a	0.02240 b	4.274 ab	0.00107 ab
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00327 a	0.00866 def	0.797 a	0.01432 d	3.237 c	0.00103 abc
Ni0 Pb15	0.00322 a	0.00769 efg	1.411 a	0.02445 c	4.640 bc	0.00094 c
Ni0 Pb30	0.00298 a	0.00926 bcde	1.212 a	0.01850 cd	3.962 bc	0.00118 abc
Ni0 Pb45	0.00303 a	0.00874 def	1.143 a	0.01581 cd	3.840 bc	0.00111 abc
Ni15 Pb0	0.00303 a	0.00899 cdef	1.297 a	0.02399 cd	4.159 bc	0.00087 c
Ni15 Pb15	0.00313 a	0.01038 abc	1.121 a	0.01892 cd	3.918 bc	0.00132 ab
Ni15 Pb30	0.00302 a	0.01073 ab	1.273 a	0.01679 cd	3.673 bc	0.00103 abc
Ni15 Pb45	0.00307 a	0.00756 fg	1.424 a	0.01884 cd	4.358 bc	0.00118 abc
Ni30 Pb0	0.00299 a	0.00949 abcd	0.675 a	0.04687 a	3.928 bc	0.00094 c
Ni30 Pb15	0.00306 a	0.00664 gh	1.217 a	0.03715 b	4.849 bc	0.00117 abc
Ni30 Pb30	0.00286 a	0.00571 h	1.319 a	0.01980 cd	5.051 bc	0.00082 c
Ni30 Pb45	0.00324 a	0.00749 fg	1.207 a	0.03392 b	4.315 bc	0.00102 abc
Ni45 Pb0	0.00304 a	0.00652 gh	1.196 a	0.02499 c	3.939 bc	0.00085 c
Ni45 Pb15	0.00269 a	0.00696 gh	1.343 a	0.03573 b	6.835 a	0.00134 ab
Ni45 Pb30	0.00272 a	0.00703 gh	1.107 a	0.02040 cd	5.406 ab	0.00137 a
Ni45 Pb45	0.00297 a	0.01086 a	1.302 a	0.02102 cd	4.583 bc	0.00098 bc

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.25. Effects of nickel, lead and their interactions mean of some micro- nutrient of R. pseudoacacia root.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0237 ab	0.174 b	0.0143 a	0.00443 a	0.0764 a
Ni 15	0.0149 b	0.245 ab	0.0170 a	0.00535 a	0.0306 b
Ni 30	0.0207 ab	0.324 a	0.0199 a	0.00523 a	0.0366 ab
Ni 45	0.0297 a	0.244 ab	0.0202 a	0.00422 a	0.0447 ab
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.0224 a	0.225 a	0.0131 b	0.00479 ab	0.0425 a
Pb 15	0.0223 a	0.286 a	0.0179 ab	0.00361 b	0.0380 a
Pb 30	0.0276 a	0.230 a	0.1680 ab	0.00576 a	0.0338 a
Pb 45	0.0169 a	0.245 a	0.0238 a	0.00505 ab	0.0739 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.0209 ab	0.090 b	0.00972 a	0.00264 b	0.035 b
Ni0 Pb15	0.0203 ab	0.269 ab	0.0140 a	0.00319 ab	0.035 b
Ni0 Pb30	0.0443 a	0.247 ab	0.00907 a	0.00603 ab	0.030 b
Ni0 Pb45	0.00914 b	0.089 b	0.0245 a	0.00588 ab	0.205 a
Ni15 Pb0	0.0339 ab	0.218 ab	0.0245 a	0.00732 a	0.030 b
Ni15 Pb15	0.00836 b	0.258 ab	0.00901 a	0.00281 b	0.031 b
Ni15 Pb30	0.00902 b	0.197 ab	0.00812 a	0.00577 ab	0.031 b
Ni15 Pb45	0.00863 b	0.307 ab	0.0265 a	0.00548 ab	0.030 b
Ni30 Pb0	0.00823 b	0.384 a	0.00883 a	0.00489 ab	0.055 b
Ni30 Pb15	0.0263 ab	0.317 ab	0.0252 a	0.00370 ab	0.031 b
Ni30 Pb30	0.0279 ab	0.275 ab	0.0262 a	0.00679 ab	0.029 b
Ni30 Pb45	0.0204 ab	0.321 ab	0.0196 a	0.00553 ab	0.030 b
Ni45 Pb0	0.0265 ab	0.209 ab	0.00925 a	0.00435 ab	0.49 b
Ni45 Pb15	0.0341 ab	0.302 ab	0.0233 a	0.00476 ab	0.049 b
Ni45 Pb30	0.0292 ab	0.203 ab	0.0236 a	0.00446 ab	0.044 b
Ni45 Pb45	0.0292 ab	0.263 ab	0.0247 a	0.00331 ab	0.031 b

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.26. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of R. pseudoacacia root.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00279 a	0.002099 a	0.000266 a	0.0276 a	0.000447 b	0.563 a
Ni 15	0.00274 a	0.002184 a	0.000256 a	0.0268 a	0.000674 a	0.614 a
Ni 30	0.00256 a	0.001569 a	0.000386 a	0.0302 a	0.000347 b	0.640 a
Ni 45	0.00304 a	0.001614 a	0.000311 a	0.0288 a	0.000400 b	0.573 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00292 a	0.00123 b	0.000359 a	0.0239 a	0.000574 a	0.5801 a
Pb 15	0.00263 a	0.00261 a	0.000295 a	0.0322 a	0.000416 ab	0.5998 a
Pb 30	0.00262 a	0.00148 b	0.000313 a	0.0313 a	0.000508 ab	0.5968 a
Pb 45	0.00296 a	0.00214 ab	0.000252 a	0.0260 a	0.000369 b	0.6126 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00288 a	0.00077 c	0.000264 a	0.0244 a	0.000370 c	0.517 a
Ni0 Pb15	0.00276 a	0.00243 abc	0.000295 a	0.0314 a	0.000310 c	0.545 a
Ni0 Pb30	0.00259 a	0.00247 abc	0.000254 a	0.0268 a	0.000820 ab	0.534 a
Ni0 Pb45	0.00294 a	0.00274 abc	0.000254 a	0.0278 a	0.000289 c	0.655 a
Ni15 Pb0	0.00262 a	0.00255 abc	0.000259 a	0.0268 a	0.001040 a	0.602 a
Ni15 Pb15	0.00276 a	0.00301 ab	0.000264 a	0.0282 a	0.000555 bc	0.657 a
Ni15 Pb30	0.00289 a	0.00073 c	0.000249 a	0.0256 a	0.000540 bc	0.601 a
Ni15 Pb45	0.00270 a	0.00245 abc	0.000254 a	0.0264 a	0.000562 bc	0.598 a
Ni30 Pb0	0.00258 a	0.00078 c	0.000520 a	0.0228 a	0.000541 bc	0.602 a
Ni30 Pb15	0.00273 a	0.00088 bc	0.000280 a	0.0259 a	0.000349 c	0.653 a
Ni30 Pb30	0.00219 a	0.00218 abc	0.000496 a	0.0499 a	0.000234 c	0.652 a
Ni30 Pb45	0.00276 a	0.00244 abc	0.000249 a	0.0222 a	0.000264 c	0.653 a
Ni45 Pb0	0.00363 a	0.00081 c	0.000397 a	0.0215 a	0.000347 c	0.601 a
Ni45 Pb15	0.00245 a	0.00415 a	0.000342 a	0.0432 a	0.000451 c	0.545 a
Ni45 Pb30	0.00266 a	0.00056 c	0.000254 a	0.0227 a	0.000440 c	0.601 a
Ni45 Pb45	0.00343 a	0.00094 bc	0.000254 a	0.0276 a	0.000364 c	0.545 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.2.2.4.4 Some physical and chemical properties of the soils of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species after the end of the experiment

The results are show in the tables 4.27, 4.28, 4.29, 4.30, 4.31, 4.32, 4.33, 4.34, 4.35, 4.36, 4.37 and 4.38 the effects of Ni and Pb and their interactions were significant on the mineral elements and chemical and physical characteristics of the soils after planting for three plant species such as *G. triacanthos, L. leucocephala* and *R. pseudoacacia* seedlings. They show that some of the chemical and physical characterestics of the soils increased with increasing the concentration of Ni and Pb from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment.

Chemical and physical characterestics of the *G. triacanthos* soil such as EC, O.M and $CaCO_3$ were increased with increasing the concentration of Ni for 30 and 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment, except pH and HCO_3 which decreased with increasing the concentration of Ni to 45 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatment. Whereas the CO_3 caused not detected of *G. triacanthos*. The highest value of the EC, O.M and $CaCO_3$ from the soil of *G. triacanthos* seedlings (0.68 ds/m, 0.563 % and 46.8 %) were recorded from 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. EC soil characterestics increased with increasing $PbCl_2$ concentration as compared to the control treatment, whereas pH, O.M, HCO_3 and $CaCO_3$ decreased with increasing the concentration $PbCl_2$ as compared to the control treatment. The highest value of the EC from the soil of *G. triacanthos* seedlings (0.63 ds/m) was recorded from 45 $mg.kg^{-1}$ $PbCl_2$ concentrations, while the lowest values (0.35 ds/m) was recorded in the control treatment. The interactions between Ni and Pb concentrations had significant effects on some of the soil chemical and physical characteristics such as EC and O.M which increased with increasing Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the pH, HCO_3 and $CaCO_3$ decreased with increasing the Ni and Pb concentrations, as it appears in table 4. 27.

The macro elements N, P, K and Mg decreased significantly with adding $NiCl_2$ as compared to the control treatment, except the SO_4 increased with increasing the Ni concentration to 45 $mg.kg^{-1}$ of *G. triacanthos* soil. The Ca element did not affect by Ni concentration. The highest values of N, P, K and Mg (0.00279, 0.00385, 2.166 and 0.00365) % were recorded in the control treatments and 15 $mg.kg^{-1}$, while the lowest values (0.00202, 0.00245, 0.0988 and 0.00227) % were recorded in thecontrol and 15 or 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment. The highest value of the SO_4 mineral elements (0.00142 %) was recorded in the 45 $mg.kg^{-1}$, while the lowest value of the the SO_4

(0.00066) % was recorded in the control treatment. The macro elements N, P, Mg and Ca increased to adding 30 or 45 mg.kg^{-1} $PbCl_2$ as compared 15 and control treatments, except the SO_4 which was decreased with increasing the Pb concentration to 45 mg.kg^{-1} (0.00069 %) as compared to control treatment. The highest values of N, P, Mg and Ca (0.00294, 0.00365, 0.02906 and 13.150) % were recorded in the 30 and 15 mg.kg^{-1} $PbCl_2$ concentrations, while the lowest values (0.00193, 0.00264, 0.01887 and 11.028) % were recorded in the 15 or 30 mg.kg^{-1} and the control treatment. Also, the K element which was non significant. The interactions between Ni and Pb concentrations had significant effects on some the macro elements such as N, P, Mg, Ca and SO_4 which increased with increasing Ni and Pb concentration in the 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the K was decreased with increasing the Ni and Pb concentrations 45 mg.kg^{-1}, (Table 4.28).

The micro elements Cu, Fe and Zn increased with increasing the Ni concentrations for 30 and 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control treatment. The highest value of Cu, Fe and Zn (0.00760, 0.563 and 0.00755) % were recorded in the 30 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest values (0.00551, 0.493 and 0.00589) % were recorded in the control treatment. The micro elements Cu and Zn increased with adding the $PbCl_2$, where the highest values (0.006682 and 0.00763) % were recorded in the 30 and 15 mg.kg^{-1} $PbCl_2$ concentrations and the lowest values (0.00443 and 0.00623) % were recorded in the control treatment. Whereas the Pb did not affect significantly each Fe, Mn and Ni elements. The interactions between Ni and Pb concentrations had significant effects on some micro elements such as Cu, Fe, Zn and Ni which increased with increasing Ni and Pb concentration in the 15 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment. An exception is the Mn elemnt which decreased with increasing to 30 mg.kg^{-1} concentrations, as it appear in table (4.29).

The heavy metal elements Pb, Rb and Ag increased with adding the Ni, where the highest values (0.00377, 0.00519 and 0.0525) % were recorded in the 30 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest values of Pb, Rb and Ag (0.00187, 0.00331 and 0.0406) % were recorded in the control treatment. The Cd and Ba elements did not significant effect by Ni concentration. Contrariwise the elements As decreased with adding $NiCl_2$ significantly to 0.000451% for 15 mg.kg^{-1}, whereas its concentration increased significantly with increasing $PbCl_2$ concentration to 45 mg.kg^{-1} (0.000122 %) as compared to the control treatment (0.000640 %), and the $PbCl_2$ had not any significant effects on other elements. The interactions between Ni and Pb concentrations had significant effects on some the heavy metal elements such as Ag and As which increased with increasing Ni and Pb concentration to the 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$

concentrations as compared to the control treatment, while the Pb, Rb, Cd and Ba elements which were non significant (Table 4.30).

Table 4.27. Effects of nickel, lead and their interactions mean of the physical and chemical characteristics of the soil of G. triacanthos.

Treatments	EC (ds/m)	pH	O.M	CO_3	HCO_3	$CaCO_3$
			(%)			
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.33 d	8.09 a	0.517 c	N.D	0.0376 a	44.8 b
Ni 15	0.45 c	7.93 b	0.513 d	N.D	0.0260 b	25.3 d
Ni 30	0.58 b	7.64 d	0.555 b	N.D	0.0215 c	34.0 c
Ni 45	0.68 a	7.65 c	0.563 a	N.D	0.0188 d	46.8 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.35 d	8.150 a	0.553 b	N.D	0.035 a	43.2 a
Pb 15	0.50 c	7.800 c	0.558 a	N.D	0.025 b	39.0 b
Pb 30	0.55 b	7.813 b	0.540 c	N.D	0.021 d	37.1 c
Pb 45	0.63 a	7.548 d	0.498 d	N.D	0.022 c	31.4 d
Interaction between Ni & Pb con.						
Ni0 Pb0	0.30 f	8.20 a	0.55 c	N.D	0.043 a	48.0 a
Ni0 Pb15	0.30 f	8.20 a	0.55 c	N.D	0.043 a	48.0 a
Ni0 Pb30	0.30 f	8.20 a	0.55 c	N.D	0.033 c	44.0 c
Ni0 Pb45	0.40 e	7.79 d	0.42 f	N.D	0.0324 d	39.0 f
Ni15 Pb0	0.30 f	8.00 c	0.56 b	N.D	0.0322 e	36.0 h
Ni15 Pb15	0.40 e	8.00 c	0.57 a	N.D	0.0238 g	25.0 i
Ni15 Pb30	0.50 d	8.10 b	0.48 d	N.D	0.0237 h	20.0 j
Ni15 Pb45	0.60 c	7.60 e	0.44 e	N.D	0.0244 f	20.0 j
Ni30 Pb0	0.40 e	8.20 a	0.55 c	N.D	0.0411 b	42.0 e
Ni30 Pb15	0.60 c	7.52 f	0.55 c	N.D	0.0177 l	36.0 h
Ni30 Pb30	0.60 c	7.46 i	0.56 b	N.D	0.0127 o	38.0 g
Ni30 Pb45	0.70 b	7.38 k	0.56 b	N.D	0.0144 n	20.0 j
Ni45 Pb0	0.40 e	8.20 a	0.55 c	N.D	0.0227 i	47.0 b
Ni45 Pb15	0.70 b	7.48 h	0.56 b	N.D	0.0162 m	47.0 b
Ni45 Pb30	0.80 a	7.49 g	0.57 a	N.D	0.0180 k	46.5 c
Ni45 Pb45	0.80 a	7.42 j	0.57 a	N.D	0.0183 j	46.5 c

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.28. Effects of nickel, lead and their interactions mean of some macro- nutrient of the soil of G. triacanthos.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00279 a	0.00320 b	2.166 a	0.00365 a	11.999 ab	0.00078 c
Ni 15	0.00229 b	0.00385 a	0.988 b	0.00316 b	12.046 ab	0.00066 d
Ni 30	0.00233 b	0.00245 c	1.320 b	0.00352 a	11.797 b	0.00110 b
Ni 45	0.00202 c	0.00253 c	1.089 b	0.00227 c	12.501 a	0.00142 a
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00230 b	0.00308 b	1.540 ab	0.00245 c	11.028 c	0.00105 a
Pb 15	0.00193 c	0.00264 c	1.314 ab	0.00301 b	13.150 a	0.00113 a
Pb 30	0.00294 a	0.00365 a	1.059 b	0.00366 a	12.133 b	0.00110 a
Pb 45	0.00226 b	0.00266 c	1.656 a	0.00348 a	12.0324 b	0.00069 b
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00200 efg	0.00285 c	3.042 a	0.00256 efg	10.722 cd	0.00074 fg
Ni0 Pb15	0.00181 fg	0.00492 ab	2.867 a	0.00306 de	13.611 a	0.00108 d
Ni0 Pb30	0.00423 a	0.00189 d	1.042 c	0.00604 a	10.584 cd	0.00065 gh
Ni0 Pb45	0.00313 b	0.00314 c	1.712 bc	0.00290 ef	13.079 ab	0.00064 gh
Ni15 Pb0	0.00237 de	0.00434 b	0.798 c	0.00250 efg	11.619 c	0.00077 efg
Ni15 Pb15	0.00180 fg	0.00318 c	1.091 c	0.00216 fgh	13.792 a	0.00074 fg
Ni15 Pb30	0.00286 bc	0.00504 a	0.896 c	0.00422 c	11.945 bc	0.00046 h
Ni15 Pb45	0.00213 ef	0.00284 c	1.168 c	0.00376 cd	10.828 cd	0.00067 g
Ni30 Pb0	0.00290 bc	0.00312 c	1.042 c	0.00253 efg	9.992 d	0.00095 de
Ni30 Pb15	0.00218 def	0.00096 e	0.852 c	0.00493 b	13.447 a	0.00191 b
Ni30 Pb30	0.00210 ef	0.00285 c	0.735 c	0.00146 h	13.047 ab	0.00093 def
Ni30 Pb45	0.00214 ef	0.00287 c	2.678 ab	0.00518 b	10.704 cd	0.00062 gh
Ni45 Pb0	0.00193 efg	0.00199 d	1.279 c	0.00221 fgh	11.778 bc	0.00173 c
Ni45 Pb15	0.00193 efg	0.00149 de	0.445 c	0.00188 gh	11.749 bc	0.00078 efg
Ni45 Pb30	0.00258 cd	0.00482 ab	1.565 bc	0.00292 ef	12.958 ab	0.00235 a
Ni45 Pb45	0.00164 g	0.00182 d	1.065 c	0.00209 gh	13.519 a	0.00082 efg

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.29. Effects of nickel, lead and their interactions mean of some micro- nutrient of the soil of G. triacanthos.

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Treatments	Cu	Fe	Mn	Zn	Ni
	(%)				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.00551 b	0.493 b	0.135 a	0.00589 b	0.0725 a
Ni 15	0.00485 b	0.510 ab	0.109 a	0.00710 a	0.0289 a
Ni 30	0.00760 a	0.563 a	0.068 a	0.00755 a	0.0397 a
Ni 45	0.0588 b	0.534 ab	0.069 a	0.00737 a	0.0853 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.00443 b	0.506 a	0.131 a	0.00623 b	0.0327 a
Pb 15	0.00628 a	0.518 a	0.069 a	0.00763 a	0.0796 a
Pb 30	0.00682 a	0.565 a	0.113 a	0.00699 ab	0.0834 a
Pb 45	0.00588 a	0.511 a	0.069 a	0.00707 ab	0.0306 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00332 f	0.414 b	0.348 a	0.00472 d	0.0295 b
Ni0 Pb15	0.00412 ef	0.489 ab	0.055 b	0.00596 cd	0.199 a
Ni0 Pb30	0.00592 bcde	0.586 a	0.067 b	0.00591 cd	0.0285 b
Ni0 Pb45	0.00702 abcd	0.489 ab	0.075 b	0.00699 abcd	0.0326 b
Ni15 Pb0	0.00398 ef	0.488 ab	0.052 b	0.00622 abcd	0.0236 b
Ni15 Pb15	0.00501 def	0.485 ab	0.067 b	0.00738 abc	0.0293 b
Ni15 Pb30	0.00683 abcd	0.576 a	0.262 a	0.00727 abc	0.0324 b
Ni15 Pb45	0.00358 ef	0.492 ab	0.056 b	0.00754 abc	0.0304 b
Ni30 Pb0	0.00559 cdef	0.552 ab	0.055 b	0.00736 abc	0.0387 b
Ni30 Pb15	0.00814 ab	0.534 ab	0.083 b	0.00851 ab	0.0445 b
Ni30 Pb30	0.00789 abc	0.604 a	0.056 b	0.00679 abcd	0.0451 b
Ni30 Pb45	0.00879 a	0.561 a	0.078 b	0.00757 abc	0.0305 b
Ni45 Pb0	0.00483 def	0.567 a	0.069 b	0.00664 abcd	0.0392 b
Ni45 Pb15	0.00789 abc	0.566 a	0.074 b	0.00869 a	0.0453 b
Ni45 Pb30	0.00667 abcd	0.493 ab	0.067 b	0.00799 abc	0.228 a
Ni45 Pb45	0.00415 ef	0.509 ab	0.070 b	0.00619 bcd	0.0291 b

Table 4.30. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of the soil of G. triacanthos.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	(%)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00187 b	0.00331 b	0.000223 a	0.0406 b	0.00111 a	0.495 a
Ni 15	0.00237 b	0.00378 ab	0.000287 a	0.0486 ab	0.000451 b	0.519 a
Ni 30	0.00377 a	0.00519 a	0.000322 a	0.0525 a	0.000647 ab	0.490 a
Ni 45	0.00263 ab	0.00404 ab	0.000343 a	0.0508 a	0.000677 ab	0.480 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00275 a	0.00377 a	0.000293 a	0.0502 a	0.000640 b	0.492 a
Pb 15	0.00258 a	0.00385 a	0.000256 a	0.0433 a	0.00044 b	0.524 a
Pb 30	0.00316 a	0.00392 a	0.000362 a	0.0471 a	0.000584 b	0.502 a
Pb 45	0.00215 a	0.00478 a	0.000265 a	0.0518 a	0.00122 a	0.467 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00187 ab	0.00283 a	0.000197 a	0.0406 bcd	0.000875 b	0.501 a
Ni0 Pb15	0.00249 ab	0.00371 a	0.000198 a	0.0357 d	0.000323 b	0.500 a
Ni0 Pb30	0.00219 ab	0.00281 a	0.000268 a	0.0456 abcd	0.000400 b	0.486 a
Ni0 Pb45	0.000941 b	0.00443 a	0.000232 a	0.0406 bcd	0.00284 a	0.491 a
Ni15 Pb0	0.00226 ab	0.00313 a	0.000231 a	0.0454 abcd	0.000276 b	0.530 a
Ni15 Pb15	0.00251 ab	0.00328 a	0.000238 a	0.0506 abcd	0.000226 b	0.591 a
Ni15 Pb30	0.00245 ab	0.00415 a	0.000342 a	0.0387 cd	0.000578 b	0.544 a
Ni15 Pb45	0.00227 ab	0.00458 a	0.000336 a	0.0595 abc	0.000726 b	0.414 a
Ni30 Pb0	0.00384 ab	0.00479 a	0.000263 a	0.0505 abcd	0.000658 b	0.419 a
Ni30 Pb15	0.00453 a	0.00484 a	0.000243 a	0.0407 bcd	0.000585 b	0.525 a
Ni30 Pb30	0.00415 a	0.00520 a	0.000532 a	0.0569 abcd	0.000633 b	0.537 a
Ni30 Pb45	0.00255 ab	0.00592 a	0.000251 a	0.0616 ab	0.000712 b	0.479 a
Ni45 Pb0	0.00302 ab	0.00434 a	0.000480 a	0.0644 a	0.000752 b	0.517 a
Ni45 Pb15	0.000801 b	0.00413 a	0.000347 a	0.0459 abcd	0.000627 b	0.480 a
Ni45 Pb30	0.00386 ab	0.00351 a	0.000306 a	0.0472 abcd	0.000727 b	0.439 a
Ni45 Pb45	0.00284 ab	0.00417 a	0.000241 a	0.0455 abcd	0.000603 b	0.484 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Chemical and physical characterestics of the *L. leucocephala* soil changed significantly with $NiCl_2$ and $PbCl_2$ application, where each of the EC and HCO_3 increased significantly to 0.63 ds/m and 0.039 % for 30 and 15 $mg.kg^{-1}$ $NiCl_2$ treatments as compared to the control treatment which the highest values of pH, O.M and $CaCO_3$ (8.06, 0.58 % and 47.42 %) were recorded in the control treatment. Whereas the CO_3 caused not detected of *L. leucocephala* soil. Each of EC and O.M were increased significantly with increasing the concentrations of $PbCl_2$ as compared to the control treatment, while pH, HCO_3 and $CaCO_3$ decreased significantly with increasing the concentration of $PbCl_2$ to 45 $mg.kg^{-1}$ as compared to the control treatment. The highest value of the EC and O.M (0.55 ds/m and 0.56 %) were recorded for the 45 $mg.kg^{-1}$ $PbCl_2$ concentrations, while the lowest values (0.40 ds/m and 0.518 %) were recorded in the control treatment. The highest values of pH, HCO_3 and $CaCO_3$ (8.13, 0.0383 % and 46.9 %) for 15 and control, while the lowest values (7.68, 0.0241 % and 46.5 %) for 45 $mg.kg^{-1}$. The interactions between Ni and Pb concentrations had significant effects on the soil chemical and physical characteristics EC and O.M were they increased with increasing Ni and Pb concentration in the 15 or 30 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the pH, HCO_3 and $CaCO_3$ were decreased with increasing the Ni and Pb concentrations, as it shown in table (4.31).

The mineral elements of *L. leucocephala* soils. N, P, K and Ca were decreased by adding $NiCl_2$ as compared to the control treatment, except the Mg and SO_4 element was increased with increasing the Ni concentration. The highest values of N, P, K and Ca (0.00246, 0.00287, 1.219 and 12.05) % were recorded in the control treatment of *L. leucocephala* soils, while the lowest values (0.00172, 0.00105, 0.816 and 10.63) % were recorded to 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. The highest values of the Mg and SO_4 (0.00559 and 0.00093) % were recorded in the 30 or 45 $mg.kg^{-1}$ concentrations, while the lowest values (0.003221 and 0.00054) % were recorded in the control and 45 $mgkg^{-1}$. The macro elements N and P increased with increasing the Pb concentration to 30 and 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment, except the Mg and SO_4 element were decreased with 45 and 30 $mg.kg^{-1}$ Pb concentration. The highest values of N and P (0.00240 and 0.00268) % were recorded in the 45 and 30 $mg.kg^{-1}$ $PbCl_2$ concentrations, while the lowest values (0.00181 and 0.00214) % were recorded in the control and 15 $PbCl_2$ treatments. The highest values of the Mg and SO_4 0.00506 and 0.00080 % were recorded in the 30 and 15 $mg.kg^{-1}$ treatment as compared to the other treatments. While the K and Ca element which were non significant. The interactions between Ni and Pb concentrations had significant effects on Mg which was increased with increasing Ni and Pb concentration to 30 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ as compared to the control treatment, however the N, P, Mg and Ca were increased with increasing Ni and Pb concentration

in the 15, 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the K and SO_4 which were decreased with increasing Ni and Pb concentration (Table 4.32).

The micro elements Fe, Mn and Zn decreased with adding $NiCl_2$ as compared to the control treatment, the highest values of Fe, Mn and Zn (0.548, 0.119 and 0.0078) % were recorded in the control treatment, the lowest values (0.417, 0.057 and 0.0059) % at 30 and 45 mg.kg^{-1}. The Cu and Ni which were non significant. The micro element (Cu and Zn) decreased with increasing the Pb concentration to 30 and 45 mg.kg^{-1} $PbCl_2$, where the highest value of Cu (0.0063 and 0.0069) % were recorded in the control treatment. The Fe and Ni elements which were non significant. Moreover, 45 mg.kg^{-1} $PbCl_2$ increased Mn significantly to (0.118 %). The Fe and Ni did not significant effect by Pb concentration. The interactions between Ni and Pb concentrations had significant effects on Mn which increased with increasing Ni and Pb concentration for 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment. An exception are Cu, Fe and Zn elements which decreased with increasing the Ni and Pb concentration at 15 and 30 mg.kg^{-1}, while the Ni element which was non significant (Table 4.33).

The element Rb was decreased with increasing the Ni concentrations from 15 to 45 mg.kg^{-1} $NiCl_2$ concentrations as compared to the control. The highest value of the Rb heavy metal element (0.0108 %) was recorded in the control treatment, while the lowest value (0.00428 %)was recorded in the 45 mg.kg^{-1} $NiCl_2$ concentrations. Adding $NiCl_2$ did not affect significantly each Pb, Cd, Ag, As and Ba content compared to the control treatment. The Pb concentration was non- significant on the heavy metal elements of *L. leucocephala* soils compared to other treatments. The interactions between Ni and Pb concentrations had significant effects on Rb, Cd and As which were increased with increasing Ni and Pb concentration to 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, while the Pb, Ag and Ba did not significant effect by Ni and Pb concentration (Table 4.34).

Table 4.31. Effects of nickel, lead and their interactions mean of the physical and chemical characteristics of the soil of L. leucocephala.

Treatments	EC (ds/m)	pH	O.M	CO_3	HCO_3	$CaCO_3$
			(%)			
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.38 d	8.06 a	0.58 a	N.D	0.037 b	47.42 a
Ni 15	0.55 b	7.87 b	0.48 d	N.D	0.039 a	46.38 c
Ni 30	0.63 a	7.77 c	0.49 c	N.D	0.034 c	46.13 d
Ni 45	0.40 c	7.52 d	0.57 b	N.D	0.023 d	46.63 b
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.40 c	8.13 a	0.518 d	N.D	0.0383 b	46.8 b
Pb 15	0.45 b	7.74 b	0.518 c	N.D	0.0385 a	46.9 a
Pb 30	0.55 a	7.69 c	0.528 b	N.D	0.0339 c	46.4 d
Pb 45	0.55 a	7.68 d	0.560 a	N.D	0.0241 d	46.5 c
Interaction between Ni & Pb con.						
Ni0 Pb0	0.30 e	8.2 a	0.55 e	N.D	0.04 f	48.0 a
Ni0 Pb15	0.30 e	8.2 a	0.55 e	N.D	0.04 f	48.0 a
Ni0 Pb30	0.40 d	7.99 d	0.59 b	N.D	0.04 f	47.2 b
Ni0 Pb45	0.50 c	7.88 f	0.64 a	N.D	0.02 j	46.5 d
Ni15 Pb0	0.30 e	8.10 b	0.51 f	N.D	0.04 d	47.0 c
Ni15 Pb15	0.50 c	7.98 e	0.48 g	N.D	0.04 c	47.0 c
Ni15 Pb30	0.70 a	7.69 i	0.46 h	N.D	0.04 a	46.0 e
Ni15 Pb45	0.70 a	7.72 g	0.45 i	N.D	0.02 g	45.5 f
Ni30 Pb0	0.50 c	8.00 c	0.46 h	N.D	0.04 b	46.0 e
Ni30 Pb15	0.60 b	7.67 j	0.48 g	N.D	0.04 b	46.0 e
Ni30 Pb30	0.70 a	7.71 h	0.48 g	N.D	0.02 g	45.5 f
Ni30 Pb45	0.70 a	7.72 g	0.56 d	N.D	0.02 g	47.0 c
Ni45 Pb0	0.50 c	8.20 a	0.55 e	N.D	0.02 k	46.0 e
Ni45 Pb15	0.40 d	7.1 l	0.56 d	N.D	0.02 i	46.5 d
Ni45 Pb30	0.40 d	7.40 k	0.58 c	N.D	0.02 h	47.0 c
Ni45 Pb45	0.30 e	7.40 k	0.59 b	N.D	0.02 g	47.0 c

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.32. Effects of nickel, lead and their interactions mean of some macro- nutrient of the soil of L. leucocephala.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00246 a	0.00287 a	1.219 a	0.00322 c	12.05 a	0.00081 b
Ni 15	0.00176 c	0.00286 a	0.919 b	0.00331 c	11.23 b	0.00070 c
Ni 30	0.00226 b	0.00264 a	0.816 b	0.00435 b	10.71 c	0.00093 a
Ni 45	0.00172 c	0.00105 b	0.991 b	0.00559 a	10.63 c	0.00054 d
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00181 c	0.00218 bc	0.929 a	0.00402 b	11.127 a	0.00076 a
Pb 15	0.00186 c	0.00214 c	1.032 a	0.00407 b	11.124 a	0.00080 a
Pb 30	0.00213 b	0.00268 a	0.962 a	0.00506 a	11.219 a	0.00064 b
Pb 45	0.00240 a	0.00242 b	1.0238 a	0.00331 c	11.152 a	0.00077 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00172 defg	0.00202 d	1.291 ab	0.00462 bc	11.153 cd	0.00091 abcd
Ni0 Pb15	0.00188 cdef	0.00224 d	1.342 a	0.00153 f	13.994 a	0.00075 cdef
Ni0 Pb30	0.00196 cde	0.00421 a	0.806 cde	0.00498 bc	10.919 de	0.00063 fg
Ni0 Pb45	0.00426 a	0.00300 c	1.442 a	0.00176 f	12.143 bc	0.00094 ab
Ni15 Pb0	0.00166 efg	0.00377 ab	0.7801 de	0.00298 e	11.071 d	0.00068 efg
Ni15 Pb15	0.00151 fg	0.00339 bc	1.202 abc	0.00283 e	11.057 d	0.00066 efg
Ni15 Pb30	0.00224 bc	0.00225 d	1.179 abcd	0.00425 cd	12.626 b	0.00072 def
Ni15 Pb45	0.00164 efg	0.00203 d	0.513 e	0.00315 e	10.148 def	0.00074 cdef
Ni30 Pb0	0.00248 b	0.00147 ef	0.765 de	0.00360 de	11.126 cd	0.00090 abcd
Ni30 Pb15	0.00192 cdef	0.00175 de	0.883 bcde	0.00530 b	9.449 f	0.00092 abc
Ni30 Pb30	0.00252 b	0.00340 bc	0.706 e	0.00493 bc	11.179 cd	0.00082 bcde
Ni30 Pb45	0.00212 bcd	0.00391 ab	0.912 bcde	0.00358 de	11.089 cd	0.000108 a
Ni45 Pb0	0.00139 g	0.00145 ef	0.879 bcde	0.00493 bc	11.156 cd	0.00053 gh
Ni45 Pb15	0.00214 bcd	0.00119 fg	0.699 e	0.00662 a	9.995 ef	0.00088 bcd
Ni45 Pb30	0.00179 defg	0.00084 g	1.155 abcd	0.00606 a	10.155 def	0.00039 hi
Ni45 Pb45	0.00157 efg	0.00073 g	1.229 ab	0.00473 bc	11.228 cd	0.00033 i

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.33. Effects of nickel, lead and their interactions mean of some micro- nutrient of the soil of L. leucocephala.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.0053 a	0.548 a	0.119 a	0.0078 a	0.0346 a
Ni 15	0.0056 a	0.569 a	0.064 b	0.0064 b	0.0351 a
Ni 30	0.0060 a	0.417 b	0.062 b	0.0059 b	0.0384 a
Ni 45	0.0052 a	0.447 b	0.057 b	0.0061 b	0.0379 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.0063 a	0.523 ab	0.062 b	0.0069 ab	0.0372 a
Pb 15	0.0064 a	0.435 b	0.051 b	0.0062 bc	0.0347 a
Pb 30	0.0048 b	0.566 a	0.071 b	0.0061 c	0.0365 a
Pb 45	0.0046 b	0.458 b	0.118 a	0.0072 a	0.0376 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00698 ab	0.482 a	0.084 b	0.00729 abc	0.039 a
Ni0 Pb15	0.00819 a	0.637 a	0.0509 b	0.00849 a	0.0329 a
Ni0 Pb30	0.00214 f	0.569 a	0.0753 b	0.00778 ab	0.0342 a
Ni0 Pb45	0.00404 de	0.506 a	0.269 a	0.00782 ab	0.0329 a
Ni15 Pb0	0.00598 bc	0.563 a	0.0594 b	0.00639 bcd	0.0383 a
Ni15 Pb15	0.00829 a	0.565 a	0.0464 b	0.00702 abcd	0.0326 a
Ni15 Pb30	0.00451 cde	0.578 a	0.085 b	0.00628 bcd	0.03359 a
Ni15 Pb45	0.00214 ef	0.572 a	0.0631 b	0.00611 cde	0.0335 a
Ni30 Pb0	0.00693 ab	0.562 a	0.0577 b	0.00729 abc	0.0384 a
Ni30 Pb15	0.00408 de	0.273 b	0.0505 b	0.00336 f	0.0329 a
Ni30 Pb30	0.00713 ab	0.576 a	0.0725 b	0.00559 de	0.044 a
Ni30 Pb45	0.00593 bc	0.259 b	0.0685 b	0.00723 abcd	0.0384 a
Ni45 Pb0	0.00539 bcde	0.484 a	0.0508 b	0.00655 bcd	0.0337 a
Ni45 Pb15	0.00502 cde	0.264 b	0.0554 b	0.00594 cde	0.040 a
Ni45 Pb30	0.00539 bcde	0.542 a	0.0530 b	0.00462 ef	0.0325 a
Ni45 Pb45	0.00477 cde	0.497 a	0.0699 b	0.00749 abc	0.046 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.34. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of the soil of L. leucocephala.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00254 a	0.0108 a	0.000296 a	0.0504 a	0.00073 ab	0.522 a
Ni 15	0.00260 a	0.00353 b	0.000303 a	0.0506 a	0.00060 b	0.448 a
Ni 30	0.00247 a	0.00373 b	0.000297 a	0.0475 a	0.00063 ab	0.527 a
Ni 45	0.00289 a	0.00428 b	0.000776 a	0.0467 a	0.0014 a	0.503 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00294 a	0.00428 a	0.000769 a	0.0455 a	0.00065 ab	0.524 a
Pb 15	0.00278 a	0.00395 a	0.000305 a	0.0506 a	0.00074 ab	0.457 a
Pb 30	0.00271 a	0.00976 a	0.000292 a	0.0478 a	0.00054 b	0.519 a
Pb 45	0.00207 a	0.00432 a	0.000305 a	0.0514 a	0.00140 a	0.500 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.0028 a	0.0043 b	0.000310 b	0.0454 ab	0.000585 b	0.481 ab
Ni0 Pb15	0.0026 a	0.0060 b	0.000291 b	0.0485 ab	0.000871 b	0.538 ab
Ni0 Pb30	0.0024 a	0.0287 a	0.000296 b	0.0549 ab	0.000680 b	0.597 ab
Ni0 Pb45	0.0023 a	0.0041 b	0.000286 b	0.0528 ab	0.000772 b	0.473 ab
Ni15 Pb0	0.0029 a	0.0029 b	0.000248 b	0.0447 ab	0.000567 b	0.491 ab
Ni15 Pb15	0.0028 a	0.0040 b	0.000362 b	0.0609 a	0.000882 b	0.409 b
Ni15 Pb30	0.0024 a	0.0031 b	0.000307 b	0.0505 ab	0.000461 b	0.419 b
Ni15 Pb45	0.0024 a	0.0042 b	0.000298 b	0.0461 ab	0.000492 b	0.473 ab
Ni30 Pb0	0.0029 a	0.0062 b	0.000281 b	0.0389 b	0.000902 b	0.640 a
Ni30 Pb15	0.0029 a	0.00153 b	0.000286 b	0.0527 ab	0.000622 b	0.412 b
Ni30 Pb30	0.0033 a	0.0032 b	0.000281 b	0.0465 ab	0.000652 b	0.586 ab
Ni30 Pb45	0.00079 a	0.0041 b	0.000342 b	0.0518 ab	0.000357 b	0.469 ab
Ni45 Pb0	0.0032 a	0.0038 b	0.00224 a	0.0528 ab	0.000557 b	0.482 ab
Ni45 Pb15	0.0028 a	0.0042 b	0.000281 b	0.0402 b	0.000592 b	0.469 ab
Ni45 Pb30	0.0028 a	0.0040 b	0.000286 b	0.0391 b	0.000382 b	0.476 ab
Ni45 Pb45	0.0028 a	0.0049 b	0.000296 b	0.0548 ab	0.00399 a	0.585 ab

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Chemical and physical characterestics of the *R. pseudoacacia* soil including EC, pH, O.M and HCO_3 were increased by adding $NiCl_2$ as compared to the control treatment, except the $CaCO_3$ which decreased with adding $NiCl_2$ as compared to the control treatment. Whereas the CO_3 not detected of *R. pseudoacacia.* The highest value of the EC, pH, O.M and HCO_3 for the soil of *R. pseudoacacia* seedlings (0.425 ds/m, 8.05, 0.56 % and 0.04858 %) were recorded for 15 or 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. While the lowest values (0.325 ds/m, 7.72, 0.52 % and 0.04323 %) were recorded in the control treatment. The EC and HCO_3 were increased with increasing $PbCl_2$ cocentrations from 15 to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment, whereas the pH, O.M and $CaCO_3$ were decreased with increasing the concentration from15 to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. The highest value of the EC and HCO_3 (0.45 ds/m and 0.0485 %) were recorded for the 45 $mg.kg^{-1}$ $PbCl_2$ concentrations, while the lowest values (0.30 ds/m and 0.0446 %) were recorded in the control treatment. The interactions between Ni and Pb concentrations had significant effects on soil EC, O.M and HCO_3 were increased with increasing Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the pH and $CaCO_3$ were decreased with increasing the Ni and Pb concentrations, as it appear from the table (4.35).

The macro, micro and some non - eesentioal heavy metal elements of *R. pseudoacacia* soils. Whereas each of the macro elements (N, P, K and Mg) were decreased with adding $NiCl_2$ as compared to the control treatment, except the SO_4 was increased with increasing the $NiCl_2$ concentration. The highest values of N, P, K and Mg macro elements (0.00279, 0.00385, 2.166 and 0.00365) % were recorded in the control treatment and 15 $mg.kg^{-1}$. While the lowest values (0.00202, 0.00245, 0.988 and 0.00227) % respectively were recorded in the 15 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. The Ca element which was non significant. The macro elements (N, P, Mg and Ca) were increased with adding 30 or 15 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment, except SO_4 which were decreased with increasing the $PbCl_2$ concentration. The highest value of the mineral elements N, P, Mg and Ca (0.00294, 0.00365, 0.00366 and 13.150) % respectively were recorded in the 15 or 30 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment (0.00193, 0.00264, 0.00245 and 11.028) %. The highest value of SO_4 (0.00105 %) was recorded in the control treatment, while the lowest value (0.00069 %) was recorded in the 45 $mg.kg^{-1}$ $PbCl_2$ concentrations. In addition the K element which was non significant. The interactions between Ni and Pb concentrations had significant effects on N, P, Mg, Ca and SO_4 were increased with increasing Ni and Pb concentration in the 15 and 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control

treatment, except the K was decreased with increasing the Ni and Pb concentration, as it clear in table (4.36).

The micro elements (Cu, Fe and Zn) were increased significantly to (0.00760, 0.0563 and 0.00755) % with 30 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment and 30 $mg.kg^{-1}$. While the lowest values (0.00485, 0.493 and 0.00589) % respectively were recorded in the 15 $mg.kg^{-1}$ and the control treatments. The Mn and Ni which were non significant. The micro elements Cu and Zn were increased significantly with adding $PbCl_2$ concentration for the Cu elements and by 15 $mg.kg^{-1}$ $PbCl_2$ for the Zn element as compared to the control treatment. The highest values of Cu and Zn micro elements (0.00682 and 0.00763) % respectively were recorded in the 30 and 15 $mg.kg^{-1}$ $PbCl_2$ concentrations treatments. While the lowest values (0.00443 and 0.00623) % were recorded in the control treatment. Each of Fe, Mn and Ni did not significant effect by Pb concentration. The interactions between Ni and Pb concentrations had significant effects on Cu, Fe, Zn and Ni were increased with adding Ni and Pb concentration in the 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, except the Mn which was decreased with increasing the Ni concentration, as it appear in the table (4.37).

The non – essential heavy metals (Pb, Rb and Ag) were increased significantly to (0.00377, 0.00519 and 0.525) % with increasing the Ni concentrations to 30 $mg.kg^{-1}$ $NiCl_2$ as compared to the control treatments (0.00187, 0.00331 and 0.0406) %, while Cd, As and Ba which were non significant. The heavy metal element (As) was increased with increasing the Pb concentration to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. The highest value of the (As) heavy metal element (0.00682 %) was recorded in the 45 $mg.kg^{-1}$ concentrations as compared to the control treatment (0.000509 %) . Whereas adding $PbCl_2$ did not affect significantly on each of Pb, Rb, Cd, Ag and Ba elements respectively. The interactions between Ni and Pb concentrations had significant effect on Ag which was increased with increasing Ni and Pb concentration to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment, while Pb, Rb, Cd, As and Ba which were non significant effect by Ni and Pb concentration, the result of table (4.38).

Table 4.35. Effects of nickel, lead and their interactions mean of the physical and chemical characteristics of the soil of R. pseudoacacia.

Treatments	EC (ds/m)	pH	O.M	CO_3	HCO_3	$CaCO_3$
			(%)			
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.325 c	7.76 c	0.52 c	N.D	0.04323 d	48.00 a
Ni 15	0.425 a	8.05 a	0.51 d	N.D	0.04733 c	46.90 d
Ni 30	0.400 b	7.77 b	0.55 b	N.D	0.04855 b	47.31 b
Ni 45	0.425 a	7.72 d	0.56 a	N.D	0.04858 a	47.20 c
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.300 d	8.13 a	0.543 a	N.D	0.0446 d	47.63 a
Pb 15	0.400 c	7.99 b	0.540 b	N.D	0.0464 c	47.48 b
Pb 30	0.430 b	7.69 c	0.533 c	N.D	0.0483 b	47.07 d
Pb 45	0.450 a	7.50 d	0.532 c	N.D	0.0485 a	47.24 c
Interaction between Ni & Pb con.						
Ni0 Pb0	0.300 c	8.20 a	0.55 c	N.D	0.04270 o	48.0 a
Ni0 Pb15	0.300 c	8.10 b	0.55 c	N.D	0.04270 o	48.0 a
Ni0 Pb30	0.300 c	7.38 i	0.50 g	N.D	0.04360 n	48.0 a
Ni0 Pb45	0.400 b	7.37 i	0.48 h	N.D	0.04390 m	48.0 a
Ni15 Pb0	0.300 c	8.20 a	0.54 d	N.D	0.04420 l	48.0 a
Ni15 Pb15	0.500 a	8.10 b	0.50 g	N.D	0.04540 k	47.0 g
Ni15 Pb30	0.500 a	8.00 c	0.51 f	N.D	0.04970 f	46.1 k
Ni15 Pb45	0.400 b	7.91 d	0.51 f	N.D	0.05000 c	46.5 j
Ni30 Pb0	0.300 c	8.10 b	0.53 e	N.D	0.04570 j	47.5 c
Ni30 Pb15	0.400 b	7.83 e	0.55 c	N.D	0.04870 g	47.8 b
Ni30 Pb30	0.400 b	7.75 f	0.56 b	N.D	0.04980 e	46.96 i
Ni30 Pb45	0.500 a	7.43 h	0.56 b	N.D	0.05001 b	46.97 h
Ni45 Pb0	0.300 c	8.00 c	0.55 c	N.D	0.04580 i	47.0 g
Ni45 Pb15	0.400 b	7.93 d	0.56 b	N.D	0.04860 h	47.1 f
Ni45 Pb30	0.500 a	7.64 g	0.56 b	N.D	0.04990 d	47.2 e
Ni45 Pb45	0.500 a	7.32 j	0.58 a	N.D	0.05002 a	47.5 d

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.36. Effects of nickel, lead and their interactions mean of some macro- nutrient of the soil of R. pseudoacacia.

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Treatments	N	P	K	Mg	Ca	SO_4
	(%)					
Nickel con. ($mg.kg^{-1}$ soil)						
Ni 0	0.00279 a	0.00320 b	2.166 a	0.00365 a	11.999 ab	0.00078 c
Ni 15	0.00229 b	0.00385 a	0.988 b	0.00316 b	12.046 ab	0.00066 d
Ni 30	0.00233 b	0.00245 c	1.32 b	0.00352 a	11.797 b	0.00110 b
Ni 45	0.00202 c	0.00253 c	1.089 b	0.00227 c	12.501 a	0.00142 a
Lead con. ($mg.kg^{-1}$ soil)						
Pb 0	0.00230 b	0.00308 b	1.540 ab	0.00245 c	11.028 c	0.00105 a
Pb 15	0.00193 c	0.00264 c	1.314 ab	0.00301 b	13.150 a	0.00113 a
Pb 30	0.00294 a	0.00365 a	1.059 b	0.00366 a	12.133 b	0.00110 a
Pb 45	0.00226 b	0.00266 c	1.656 a	0.00348 a	12.0324 b	0.00069 b
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00200 efg	0.00285 c	3.042 a	0.00256 efg	10.722 cd	0.00074 fg
Ni0 Pb15	0.00181 fg	0.00492 ab	2.867 a	0.00306 de	13.611 a	0.00108 d
Ni0 Pb30	0.00423 a	0.00189 d	1.042 c	0.00604 a	10.584 cd	0.00065 gh
Ni0 Pb45	0.00313 b	0.00314 c	1.712 bc	0.00290 ef	13.079 ab	0.00064 gh
Ni15 Pb0	0.00237 de	0.00434 b	0.798 c	0.00250 efg	11.619 c	0.00077 efg
Ni15 Pb15	0.00180 fg	0.00318 c	1.091 c	0.00216 fgh	13.792 a	0.00074 fg
Ni15 Pb30	0.00286 bc	0.00504 a	0.896 c	0.00422 c	11.945 bc	0.00046 h
Ni15 Pb45	0.00213 ef	0.00284 c	1.168 c	0.00376 cd	10.828 cd	0.00067 g
Ni30 Pb0	0.00290 bc	0.00312 c	1.042 c	0.00253 efg	9.992 d	0.00095 de
Ni30 Pb15	0.00218 def	0.0096 e	0.852 c	0.00493 b	13.447 a	0.00191 b
Ni30 Pb30	0.00210 ef	0.00285 c	0.735 c	0.00146 h	13.047 ab	0.00093 def
Ni30 Pb45	0.00214 ef	0.00287 c	2.678 ab	0.00518 b	10.704 cd	0.00062 gh
Ni45 Pb0	0.00193 efg	0.00199 d	1.279 c	0.00221 fgh	11.778 bc	0.00173 c
Ni45 Pb15	0.00193 efg	0.00149 de	0.445 c	0.00188 gh	11.749 bc	0.00078 efg
Ni45 Pb30	0.00258 cd	0.00482 ab	1.565 bc	0.00292 ef	12.958 ab	0.00235 a
Ni45 Pb45	0.00164 g	0.00182 d	1.065 c	0.00209 gh	13.519 a	0.00082 efg

Table 4.37. Effects of nickel, lead and their interactions mean of some micro- nutrient of the soil of R. pseudoacacia.

Treatments	Cu	Fe	Mn	Zn	Ni
	%				
Nickel con. (mg.kg^{-1} soil)					
Ni 0	0.00550 b	0.493 b	0.068 a	0.00589 b	0.0725 a
Ni 15	0.00485 b	0.510 ab	0.109 a	0.00710 a	0.0289 a
Ni 30	0.00760 a	0.563 a	0.068 a	0.00755 a	0.0397 a
Ni 45	0.0588 b	0.534 ab	0.069 a	0.00737 a	0.0853 a
Lead con. (mg.kg^{-1} soil)					
Pb 0	0.00443 b	0.506 a	0.131 a	0.00623 b	0.0327 a
Pb 15	0.00628 a	0.518 a	0.0695 a	0.00763 a	0.0796 a
Pb 30	0.00682 a	0.565 a	0.113 a	0.00699 ab	0.0834 a
Pb 45	0.00588 a	0.511 a	0.0698 a	0.00707 ab	0.0306 a
Interaction between Ni & Pb con.					
Ni0 Pb0	0.00332 f	0.414 b	0.348 a	0.00472 d	0.0295 b
Ni0 Pb15	0.00412 ef	0.489 ab	0.055 b	0.00596 cd	0.199 a
Ni0 Pb30	0.00592 bcde	0.586 a	0.067 b	0.00591 cd	0.0285 b
Ni0 Pb45	0.00702 abcd	0.489 ab	0.075 b	0.00699 abcd	0.0326 b
Ni15 Pb0	0.00398 ef	0.488 ab	0.052 b	0.00622 abcd	0.0236 b
Ni15 Pb15	0.00501 def	0.485 ab	0.067 b	0.00738 abc	0.0293 b
Ni15 Pb30	0.00683 abcd	0.576 a	0.262 a	0.00727 abc	0.0324 b
Ni15 Pb45	0.00358 ef	0.492 ab	0.056 b	0.00754 abc	0.0304 b
Ni30 Pb0	0.00559 cdef	0.552 ab	0.055 b	0.00736 abc	0.0387 b
Ni30 Pb15	0.00814 ab	0.534 ab	0.083 b	0.00851 ab	0.0445 b
Ni30 Pb30	0.00789 abc	0.604 a	0.056 b	0.00679 abcd	0.0451 b
Ni30 Pb45	0.00879 a	0.561 a	0.078 b	0.00757 abc	0.0305 b
Ni45 Pb0	0.00483 def	0.567 a	0.069 b	0.00664 abcd	0.0392 b
Ni45 Pb15	0.00789 abc	0.566 a	0.074 b	0.00869 a	0.0453 b
Ni45 Pb30	0.00667 abcd	0.493 ab	0.067 b	0.00799 abc	0.228 a
Ni45 Pb45	0.00415 ef	0.509 ab	0.070 b	0.00619 bcd	0.0291 b

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.38. Effects of nickel, lead and their interactions mean of some non - essential heavy metal of the soil of R. pseudoacacia.

Treatments	Pb	Rb	Cd	Ag	As	Ba
	%					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.00187 b	0.00331 b	0.000223 a	0.0406 b	0.000636 ab	0.483 a
Ni 15	0.00237 b	0.00378 ab	0.000287 a	0.0486 ab	0.000528 b	0.566 a
Ni 30	0.00377 a	0.00519 a	0.000322 a	0.0525 a	0.000652 a	0.550 a
Ni 45	0.00263 ab	0.00404 ab	0.000343 a	0.0508 a	0.000522 b	0.492 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.00275 a	0.00377 a	0.000293 a	0.0502 a	0.000509 b	0.539 a
Pb 15	0.00258 a	0.00385 a	0.000256 a	0.0433 a	0.000551 b	0.479 a
Pb 30	0.00316 a	0.00392 a	0.000362 a	0.0471 a	0.000595 ab	0.531 a
Pb 45	0.00215 a	0.00478 a	0.000265 a	0.0518 a	0.000682 a	0.542 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.00187 ab	0.00283 a	0.000197 a	0.0406 bcd	0.000537 a-e	0.540 a
Ni0 Pb15	0.00249 ab	0.00371 a	0.000198 a	0.0357 d	0.000690 a-d	0.452 a
Ni0 Pb30	0.00219 ab	0.00281 a	0.000268 a	0.0456 a-d	0.000685 a-d	0.499 a
Ni0 Pb45	0.000941 b	0.00443 a	0.000232 a	0.0406 bcd	0.000631 a-d	0.441 a
Ni15 Pb0	0.00226 ab	0.00313 a	0.000231 a	0.0454 a-d	0.000422 de	0.538 a
Ni15 Pb15	0.00251 ab	0.00328 a	0.000238 a	0.0506 a-d	0.000606 a-d	0.462 a
Ni15 Pb30	0.00245 ab	0.00415 a	0.000342 a	0.0387 cd	0.000439 cde	0.642 a
Ni15 Pb45	0.00227 ab	0.00458 a	0.000336 a	0.0595 abc	0.000647 a-d	0.623 a
Ni30 Pb0	0.00384 ab	0.00479 a	0.000263 a	0.0505 a-d	0.000507 b-e	0.584 a
Ni30 Pb15	0.00453 a	0.00484 a	0.000243 a	0.0407 bcd	0.000627 a-d	0.496 a
Ni30 Pb30	0.00415 a	0.00520 a	0.000532 a	0.0569 a-d	0.000777 a	0.493 a
Ni30 Pb45	0.00255 ab	0.00592 a	0.000251 a	0.0616 ab	0.000697 abc	0.628 a
Ni45 Pb0	0.00302 ab	0.00434 a	0.000480 a	0.0644 a	0.000570 a-d	0.495 a
Ni45 Pb15	0.000801 b	0.00413 a	0.000347 a	0.0459 a-d	0.000282 e	0.505 a
Ni45 Pb30	0.00386 ab	0.00351 a	0.000306 a	0.0472 a-d	0.000482 cde	0.492 a
Ni45 Pb45	0.00284 ab	0.00417 a	0.000241 a	0.0455 a-d	0.000755 ab	0.476 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.3. Effects of nickel, lead and their interactions on some qualitative and quantitative determination of some phytochemicals total phenolic, flavonoid, glycoside, alkaloid, steroid, terpene, tannin and saponin of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves

4.3.1 Qualitative determination of the phytochemical constituents of the studied plant species

Table (4.39) presents the results of the secondary metabolite including total phenole, flavonoid, glycoside, alkaloid, steroid, terpene, tannin and saponin obtained by used water, methanol and ethyl acetate extracts of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves. The methanol and ethyl-acetate extracts revealed the presence of all the tested phytochemicals, except for the saponins that presence only by water extract. Whereas the metahanol and ethyl acetate extracts show a positive result for phenols, flavonoids, glycosides, alkaloids, steroids, terpenes and tannins. The presence of phytochemicals such as phenols, flavonoids, glycosides and alkaloids of all extract solvents water, methanol and ethyl acetate showed positive results of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves. The results of qualitative phytochemical analysis of different plant species revealed the existence of some bioactive constituents and their corresponding concentrations are presented in increasing order of magnitude: saponins > phenols > flavonoids > glycosides > alkaloids > terpenes > steroids > tannins were presence of a wide range of phytochemicals thus indicating the *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves provide the anticipated promising biological response. While polarity of the solvent is the key concern in extracting secondary plant metabolites. This study, primarily aimed to carry out a preliminary phytochemical screening to detect the major classes of bioactive compounds presented in *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves and three solvents water (aqueous), methanol, and ethyl acetate were used to determine the best plant species and solvents can be used for extraction. The results obtained in the present study revealed the concentration of the bioactive compounds of different plant dry leaves extracts. The methanol and ethyl - acetate extracts for thee plant dry leaves contain a higher content of bioactive compounds, which can be used for further researches on these plants.

Table 4.39. The qualitative screening phytochemical for G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) dry leaves by three extract solvents (water, methanol and ethyl acetate).

Phytochemical constituents	Name of the test	Observation	Water extract			Methanol extract			Ethyl-Acetate extract		
			G.	L.	R.	G.	L.	R.	G.	L.	R.
Phenols	Folin–Ciocalteu method	Blue colour	+++	+++	+++	++++	++++	++++	++	++	++
Flavonoids	Dowd method	Red color	++	+	++	+++	++	+++	+	±	+
Glycosides	Phenol-Sulphuric Acid solution	Yellow color	±	+++	+	+	++++	+	±	++	±
Alkaloids	Wagner's test	Brown colored	±	+	±	++	++	++	+	+	±
Steroids	Liebermann-Burchard test	Darkgreen color	–	–	–	+	++	+	++	+	±
Terpenoids	Salkowski test	Reddish brown	–	–	–	++	++	+	++	++	±
Tannins	Braemer test	Darkgreen color	–	–	–	+	++	+	±	++	±
Saponins	Foam test	Formation of foam	++++	+++	+	–	–	–	–	–	–

Note: ++++ (Very High) +++ (High), ++ (Moderate) + (low) ± (negligible) and - (Nil).

4.3.2 Quantitative determination of the phytochemical constituents of three plant species

The results shown in the tables 4.40, 4.41, 4.42, 4.43, 4.44, 4.45, 4.46 and 4.47 indicate that the effect of Ni, Pb and their interactions were significant on quantitative phytochemical studies including total phenolic, flavonoid, glycoside, alkaloid, steroid, terpene, tannin and saponin for *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves, extracted by methanol, water and ethylacetate.

For each of total phenolic (TPC), flavonoids (TFC), glycosides (TGC), alkaloids (TAC) content using methanol as an extraction solvent gave the highest values of these compounds followed by water then ethylacetate for the three studied species.

For each of total steroid (TSTC), terpenes (TTEC), tannins (TTC) content, only methanol and ethylacetate were used as extracting solvents, whereas total saponine (TSC) was extracted only by water.

The highest value of the total phenolic content (TPC) was extracted; first by methanol solvent (44.76 and 26.39) mg/g for *G. triacanthos* and *R. pseudoacacia* dry leaves, second by water solvent (9.54 and 5.96) mg/g for *G. triacanthos* and *R. pseudoacacia*, third by ethyl-acetate solvent (6.27 and 2.35) mg/g for *G. triacanthos and R. pseudoacacia* respectively were recorded from 30 or 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest value of the TPC was extracted for methanol solvent (34.08 and 24.57) mg/g for *G. triacanthos* and *R. pseudoacacia* dry leaves, for water solvent (5.48 and 4.33) mg/g for *G. triacanthos* and *R. pseudoacacia*, and for ethyl-acetate solvent (2.56 and 1.84) mg/g only for *G. triacanthos* and *R. pseudoacacia* respectively were recorded in the control treatment, while the TPC decreased by three solvent extracts of *L. leucocephala* as compared to the control treatment. Some of the TPC which extracted by three solvent for the three plant species were increased with increasing the Pb element concentrations from 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the control treatment. The interactions between the Ni and Pb concentrations also had significant effects and increased with increasing Ni or pb concentrations from 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the TPC of different plant species by different solvent extracts, except the TPC of *L. leucocephala* was decreased by water solvent extract, as it seen in the table (4.40).

Higher total flavonoid content (TFC) were extracted from methanol and water for all plant species (*G. triacanthos, L. leucocephala* and *R. pseudoacacia*) dry leaves. The highest value of the TFC was extracted; first by methanol solvent (14.89, 8.59 and 14.78 mg/g) for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves, second by water solvent (3.83, 2.02 and 3.16) mg/g for *G. triacanthos*, *L.*

leucocephala and *R. pseudoacacia*, third by ethylacetate solvent (2.30, 0.77 and 1.96) mg/g for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves respectively were recorded for the control and 15, 30 and 45 $mg.kg^{-1}$, while the lowest value of the TFC was extractions; First by methanol solvent (9.38, 7.94 and 11.09) mg/g for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* dry leaves, second by water solvent (2.62, 1.50 and 1.86) mg/g for *G. triacanthos, L. leucocephala* and *R. pseudoacacia*, third by ethylacetate (0.33, 0.59 and 0.92) mg/g for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* respectively were recorded to 15, 30 and 45 $mg.kg^{-1}$ treatments. Some of the TFC by the three solvent extractions for the three plant species increased with increasing the Pb element concentrations from 30 and 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the other treatments. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or pb concentrations from 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the TFC of the three plant species by different solvent extracts, except the TFC was decreased by three solvent extracts of *G. triacanthos*, as clear in table (4.41).

Total glycoside content (TGC) by three of the solvent extractions (methanol, water and ethylacetate) for one of the plant species (*L. leucocephala* dry leaves) increased with increasing the Ni element concentration. The highest value of the TGC was extractions; First by the methanol solvent (1.44, 47.46 and 1.38) mg/g for three studied species, second by water solvent (35.80 mg/g) only for *L. leucocephala* , third by ethyl-acetate solvent (12.27 mg/g) only for *L. leucocephala* dry leaves respectively were recorded for 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to other treatments, while the lowest value of the TGC was extractions; First by methanol solvent (0.48, 36.28 and 0.46 mg/g) for three studied species, second by water solvent (23.57 mg/g) for *L. leucocephala*, third by ethylacetate solvent (7.89 mg/g) only for *L. leucocephala* dry leaves respectively were recorded for 15 $mg.kg^{-1}$ $NiCl_2$ concentration, while the TGC was decreased with increasing Ni concentration for *G. triacanthos* and *R. pseudoacacia* by water and ethyl acetate solvent extraction. Some of the TGC by the different solvent extractions for the different plant species were increased with increasing the Pb element concentrations from 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or pb concentrations from 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the TGC of the different plant species by different solvent extracts, as presented in table (4.42).

Adding $NiCl_2$ increased significantly total alkaloids content (TAC) in *G. triacanthos* and *L. leucocephala* where it extracted by methanol, where it reached to 2.20 and 4.88 mg/g for 45 and 30 $mg.kg^{-}$

^{1} as compared to 15 mg.kg^{-1}, whereas it decreased significantly to 0.30 mg/g dry weight in the 45 mg.kg^{-1} for *R. pseudoacacia* as compared to the control treatment. When adding $NiCl_2$ increased significantly total alkaloids to 0.30 and 0.011 mg/g in the 45 or 15 mg.kg^{-1} for *L. leucocephala* and *R. pseudoacacia* by water as compared to the control treatment and 15 mg.kg^{-1}. Adding $NiCl_2$ to *G. triacanthos* that extracted with water decreased significantly total alkaloids with increasing $NiCl_2$ to 0.046 mg/g for 45 mg.kg^{-1} treatment as compared to the control treatment. Total alkaloids increased with increasing concentrations of $NiCl_2$ to 0.609 and 0.022 mg/g for 15 and 45 mg.kg^{-1} of *L. leucocephala* and *R. pseudoacacia* by ethyl acetate, whereas it decreased to 0.093 mg/g in the 45 mg.kg^{-1} for *G. triacanthos* as compared to control treatment. Regarding the effects of $PbCl_2$ in the studied species that extracted with methanol lead to increasing the total alkaloid in *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species to 2.022, 4.88 and 0.49 mg/g for 30 and 45 mg.kg^{-1}, whereas with water and ethylacetatte using $PbCl_2$ increased total alkaloid significantly to 0.073 and 0.151 mg/g for *G. triacanthos*, whereas it decreased significantl to 0.206 and 0.407 for *L. leucocephala* and to 0.0047 and 0.010 mg/g for *R. pseudoacacia* when 15 or 45 mg.kg^{-1}. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or Pb concentrations from 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on some TAC of the different plant species by different solvent extracts, as shown in table (4.43).

Using $NiCl_2$ increased significantly total steroid content (TSTC) in *L. leucocephala* and *R. pseudoacacia* dry leaves to 0.077, 0.026, 0.057 and 0.034 mg/g dry matter extracted with methanol and ethylacetate solvents for 30 and 45 mg.kg^{-1} as compared to other treatment. Whereas adding $NiCl_2$ decreased significantly *G. triacanthos* species, content of total steroid to 0.029 and 0.0435 mg/g for methanol and ethylacetate extracts compared to the control treatment (0.44 and 0.0672 mg/g) respectively. The concentration 45 mg.kg^{-1} $PbCl_2$ increased *G. triacanthos* content of total steroid significantly to 0.047 and 0.071 mg/g for methanol and ethyacetate extracts. Only the treatment 30 mg.kg^{-1} $PbCl_2$ increased total steroid significantly to 0.077 mg/g in *L. leucocephala* dry leaves with methanol extraction, whereas using ethylacetate with 15 mg.kg^{-1} $PbCl_2$ decreased total steroid significantly to 0.0374 mg/g for *L. leucocephala* as compared to control treatments. Whereas with methanol and ethylacetate solvents using $PbCl_2$ decreased total steroid significantly to 0.0225 and 0.0233 mg/g for *R. pseudoacacia* when 15 mg.kg^{-1}. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or Pb concentrations from 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on the total steroid content with ethylacetate solvent extract for the three studied species, whereas with methanol

solvent extract was decreased with increasing high concentrations of the different plant species, as see in the table (4.44).

Regarding total terpenes content (TTEC) adding $NiCl_2$ by 15 or 30 or 45 $mg.kg^{-1}$ increased total terpenes significantly to 4.44, 3.68, 1.39, 4.16, 3.39 and 1.02 mg/g dry wight as compared to control and 15 treatment for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species extracted with methanol and ethylacetate solvents. Adding $PbCl_2$ increased total terpenes content significantly to 3.69, 3.73, 3.79, 3.44 and 1.08 mg/g dry weight for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species extracted by methanol and ethylacetate solvents. Interactions between Ni and Pb treatments records significant differences between them, when adding $NiCl_2$ and/ or $PbCl_2$ increased total terpenes significantly for all treatments compared to the control for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* using methanol or ethylacetate solvents, it is clearified in table (4.45).

Using $NiCl_2$ increased significantly total tannin content (TTC) extracted by methanol and ethylacetate for *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species where highest values were 0.055, 2.01, 0.092 and 1.02 recorded for 30 or 45 $mg.kg^{-1}$. $NiCl_2$ significance decreased in tannins when $NiCl_2$ was added where methanol was used as solvent extraction for *R. pseudoacacia* and ethylacetate for *L. leucocephala* both treatments the control gave the highest tannins (0.28 and 3.44) mg/g respectively. $PbCl_2$ also increased *L. leucocephala* and *R. pseudoacacia* content of tannins significantly in some concentrations compared to the control treatment, except *G. triacanthos* species which their content of tannins decreased with adding $PbCl_2$. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or Pb concentrations from 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the total tannin content of the different plant species by different solvent extracts, as it displayed in the table (4.46).

Total saponin content (TSC) extracted only by water for the studied plant species it decreased with increasing the Ni element concentrations as compared to the control treatment. The highest value of the TSC was (54.14, 32.10 and 1.64) mg/g for *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves were recorded in the control treatment, while the lowest value of the TSC was (24.14, 19.68 and 0.23) mg/g for *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* dry leaves were recorded from 30 or 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. The TSC increased with increased the Pb element concentrations from 45 and 30 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment only for *L. leucocephala* and *R. pseudoacacia* species where highest values (30.93 and 1.40 mg/g) were recorded for the 45 and 30

mg.kg^{-1} concentrations, and for *G. triacanthos* adding $PbCl_2$ decreased the TSC significantly compared to the control treatment (46.64) mg/g. The interactions between the Ni and Pb concentrations had significant effects and increased with increasing Ni or Pb concentrations from 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on the TSC for *L. leucocephala* and *R. pseudoacacia* dry leaves by one of the solvent extracts (water) as compared to other treatments, except for *G. triacanthos* decreased with increasing high concentrations, as seen in table (4.47).

Table 4.40. Effects of nickel, lead and their interactions mean of total phenolic content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Phenolic mg/g dry weight								
	Methanol extract			Water extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. (mg.kg^{-1} soil)									
Ni 0	34.08 c	45.27 a	24.74 c	5.48 b	12.08 a	4.33 c	2.56 d	6.62 a	1.84 c
Ni 15	33.77 c	44.48 b	24.57 c	5.49 b	11.78 b	4.46 c	2.80 c	6.78 a	1.95 b
Ni 30	41.38 b	40.65 c	26.39 a	8.90 a	10.80 c	5.54 b	3.78 b	1.01 c	2.35 a
Ni 45	44.76 a	28.64 d	25.08 b	9.54 a	7.62 c	5.96 a	6.27 a	1.82 b	1.80 c
Lead con. (mg.kg^{-1} soil)									
Pb 0	41.04 ab	40.14 b	26.00 a	7.89 a	10.56 b	5.40 a	4.25 a	4.03 a	1.84 c
Pb 15	30.76 c	33.43 d	22.39 b	6.73 b	8.92 d	4.08 b	4.12 ab	4.10 a	1.62 d
Pb 30	41.35 a	38.16 c	26.33 a	7.99 a	10.27 c	5.49 a	3.01 c	3.97 a	1.95 b
Pb 45	40.84 b	47.32 a	26.05 a	6.80 b	12.53 a	5.32 a	4.02 b	4.14 a	2.54 a
Interaction between Ni & Pb con.									
Ni0 Pb0	38.95 e	51.89 a	28.35 bc	6.15 cd	13.81 ab	5.06 d	3.01 fg	7.48 b	1.965 e
Ni0 Pb15	36.11 h	48.05 d	26.23 e	5.86 cd	12.87 c	4.43 e	2.89 g	7.03 c	1.885 ef
Ni0 Pb30	30.32 jk	40.30 f	22.14 g	4.95 cd	10.81 e	3.89 f	2.16 h	5.96 d	1.723 fg
Ni0 Pb45	30.95 j	40.85 f	22.25 g	4.96 cd	10.84 e	3.94 ef	2.17 h	6.03 d	1.783 f
Ni15 Pb0	26.98 l	35.30 g	19.31 h	4.09 d	9.19 f	3.29 g	2.22 h	5.48 e	1.571 gh
Ni15 Pb15	30.14 k	40.05 f	22.03 g	5.06 cd	10.74 e	4.15 ef	2.52 h	6.17 d	1.712 fg
Ni15 Pb30	39.85 d	52.29 a	28.96 b	6.64 c	13.93 a	5.41 d	3.30 f	8.10 a	2.312 cd
Ni15 Pb45	38.12 f	50.29 b	27.98 c	6.18 cd	13.27 bc	5.0 d	3.17 fg	7.39 b	2.215 d
Ni30 Pb0	59.10 b	51.05 b	28.09 c	10.41 ab	13.31 bc	7.92 b	2.98 fg	0.64 j	2.40 c
Ni30 Pb15	36.15 h	18.33 j	26.23 e	10.08 ab	4.89 i	4.92 d	4.05 e	1.15 i	1.178 i
Ni30 Pb30	33.10 i	44.05 e	24.08 f	9.06 b	11.94 d	4.22 ef	2.39 h	0.64 j	2.728 b
Ni30 Pb45	37.16 g	49.20 c	27.15 d	6.04 cd	13.04 c	5.11 d	5.68 c	1.61 h	3.082 a
Ni45 Pb0	39.14 e	22.32 i	28.25 c	10.91 ab	5.94 h	5.33 d	8.81 a	2.51 f	1.406 h
Ni45 Pb15	20.63 m	27.31 h	15.05 i	5.92 cd	7.17 g	2.82 g	7.0 b	2.06 g	1.717 fg
Ni45 Pb30	62.14 a	15.99 k	30.16 a	11.29 a	4.40 i	8.44 a	4.20 e	1.20 i	1.025 i
Ni45 Pb45	57.14 c	48.94 c	26.84 de	10.03 ab	12.97 c	7.25 c	5.07 d	1.52 hi	3.062 a

*Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.41. Effects of nickel, lead and their interactions mean of total flavonoid content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Flavonoid mg/g dry weight								
	Methanol extract			Water extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. (mg.kg^{-1} soil)									
Ni 0	14.89 a	8.13 bc	11.39 c	3.79 a	1.54 c	1.96 b	2.30 a	0.64 b	0.94 c
Ni 15	14.59 a	7.94 c	11.09 d	3.83 a	1.50 d	1.86 b	2.27 b	0.62 b	0.92 c
Ni 30	13.12 b	8.59 a	13.64 b	3.47 b	1.86 b	3.06 a	0.33 d	0.77 a	1.25 b
Ni 45	9.38 c	8.33 ab	14.78 a	2.61 c	2.02 a	3.16 a	0.59 c	0.59 c	1.96 a
Lead con. (mg.kg^{-1} soil)									
Pb 0	12.92 b	8.37 a	13.52 ab	3.44 b	1.83 b	2.65 a	1.37 b	0.62 b	1.31 a
Pb 15	10.95 c	7.34 b	10.35 c	3.03 c	1.38 c	2.13 b	1.36 b	0.53 c	1.34 a
Pb 30	12.56 b	8.62 a	13.65 a	3.10 c	1.87 a	2.71 a	1.35 b	0.63 b	1.01 b
Pb 45	15.55 a	8.66 a	13.37 b	4.12 a	1.84 b	2.57 a	1.41 a	0.84 a	1.40 a
Interaction between Ni & Pb con.									
Ni0 Pb0	17.06 ab	9.23 b	12.94 d	4.34 a	1.72 de	2.42 d	2.61 b	0.707 d	1.055 ef
Ni0 Pb15	15.85 c	8.88 bc	12.19 e	4.32 a	1.62 f	2.02 defg	2.42 d	0.696 d	1.035 ef
Ni0 Pb30	13.31 e	7.15 e	10.10 g	3.14 b	1.40 h	1.69 fgh	2.09 e	0.548 ef	0.824 fg
Ni0 Pb45	13.35 e	7.28 e	10.34 g	3.35 b	1.40 h	1.70 fgh	2.10 e	0.599 e	0.827 fg
Ni15 Pb0	11.44 f	6.25 f	8.92 h	3.11 b	1.22 i	1.50 h	1.81 f	0.500 fg	0.717 g
Ni15 Pb15	13.21 e	7.10 e	10.13 g	3.61 b	1.34 h	1.64 gh	2.05 e	0.532 f	0.818 fg
Ni15 Pb30	17.31 a	9.17 b	13.06 d	4.32 a	1.79 c	2.21 de	2.68 a	0.729 d	1.102 de
Ni15 Pb45	16.41 bc	9.27 b	12.24 e	4.29 a	1.67 ef	2.11 def	2.54 c	0.714 d	1.053 ef
Ni30 Pb0	16.07 c	8.91 bc	19.20 b	4.25 a	2.64 b	3.32 bc	0.23 l	0.797 c	1.061 ef
Ni30 Pb15	5.75 i	8.31 cd	12.06 e	1.70 de	1.61 f	2.90 c	0.34 k	0.346 h	1.323 cd
Ni30 Pb30	14.38 d	8.08 d	11.08 f	3.49 b	1.50 g	3.02 bc	0.22 l	0.909 b	0.727 g
Ni30 Pb45	16.26 c	9.06 b	12.20 e	4.44 a	1.69 de	3.02 bc	0.51 i	1.031 a	1.899 b
Ni45 Pb0	7.12 h	9.10 b	13.02 d	2.07 cd	1.74 cd	3.35 b	0.82 g	0.535 f	2.424 a
Ni45 Pb15	8.99 g	5.07 g	7.02 i	2.50 c	0.93 j	1.94 efg	0.64 h	0.473 g	2.199 a
Ni45 Pb30	5.22 i	10.09 a	20.37 a	1.43 e	2.81 a	3.92 a	0.40 j	0.319 h	1.399 c
Ni45 Pb45	16.18 c	9.04 b	18.70 c	4.43 a	2.61 b	3.44 b	0.51 i	1.023 a	1.809 b

*Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.42. Effects of nickel, lead and their interactions mean of total glycoside content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Glycoside mg/g dry weight								
	Methanol extract			Water extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. ($mg.kg^{-1}$ soil)									
Ni 0	1.39 b	42.03 c	1.33 b	1.24 b	27.33 c	1.26 b	0.73 b	9.47 c	0.79 b
Ni 15	0.48 d	36.28 d	0.46 d	1.34 a	23.57 d	1.42 a	0.83 a	7.89 d	0.87 a
Ni 30	1.44 a	55.03 a	1.38 a	0.80 c	35.80 a	0.85 c	0.68 c	12.27 a	0.71 c
Ni 45	0.81 c	47.46 b	0.78 c	0.49 d	30.89 b	0.50 d	0.46 d	10.73 b	0.48 d
Lead con. ($mg.kg^{-1}$ soil)									
Pb 0	1.39 a	38.42 d	1.35 a	1.22 a	24.99 d	1.28 a	0.68 b	8.55 d	0.711 b
Pb 15	1.12 b	39.93 c	1.07 b	1.15 b	26.02 c	1.20 b	0.66 b	8.91 c	0.706 b
Pb 30	0.70 d	60.63 a	0.66 d	1.07 c	39.45 a	1.09 c	0.63 c	13.64 a	0.655 c
Pb 45	0.91 c	41.82 b	0.88 c	0.43 d	27.13 b	0.46 d	0.74 a	9.25 b	0.773 a
Interaction between Ni & Pb con.									
Ni0 Pb0	2.44 a	38.25 i	2.34 a	1.21 e	25.08 h	1.23 d	0.75 e	8.36 h	0.81 e
Ni0 Pb15	1.61 b	42.27 g	1.52 b	1.32 d	27.40 g	1.35 c	0.84 d	9.63 g	0.91 d
Ni0 Pb30	0.53 i	57.46 c	0.51 h	1.81 b	37.43 c	1.85 b	1.14 c	13.06 c	1.22 c
Ni0 Pb45	0.99 g	30.13 l	0.95 f	0.61 k	19.39 k	0.623 j	0.21 j	6.83 i	0.22 i
Ni15 Pb0	0.36 j	28.21 m	0.32 i	1.93 a	18.29 l	2.04 a	1.24 b	6.22 j	1.32 b
Ni15 Pb15	0.31 jk	27.10 n	0.30 i	1.73 c	17.39 m	1.82 b	1.15 c	5.67 k	1.19 c
Ni15 Pb30	0.34 jk	47.42 f	0.33 i	1.16 f	31.12 f	1.21 d	0.73 e	10.32 f	0.75 f
Ni15 Pb45	0.91 h	42.40 g	0.89 g	0.54 l	27.49 g	0.60 j	0.20 j	9.35 g	0.21 i
Ni30 Pb0	1.53 c	50.16 e	1.50 b	0.94 h	32.25 e	1.02 f	0.27 hi	11.21 e	0.28 h
Ni30 Pb15	1.40 d	55.31 d	1.32 c	0.84 i	36.18 d	0.91 g	0.30 gh	12.30 d	0.31 h
Ni30 Pb30	1.62 b	75.36 a	1.54 b	1.03 g	49.25 a	1.07 e	0.41 f	17.08 a	0.41 g
Ni30 Pb45	1.19 f	39.28 h	1.14 e	0.39 m	25.34 h	0.41 k	1.72 a	8.48 h	1.81 a
Ni45 Pb0	1.25 e	37.08 j	1.22 d	0.81 i	24.14 i	0.84 h	0.44 f	8.41 h	0.43 g
Ni45 Pb15	1.16 f	35.03 k	1.11 e	0.72 j	23.12 j	0.73 i	0.35 g	8.06 h	0.41 g
Ni45 Pb30	0.29 k	62.27 b	0.24 j	0.25 n	40.00 b	0.21 l	0.23 ij	14.09 b	0.24 i
Ni45 Pb45	0.54 i	55.48 d	0.55 h	0.19 o	36.29 d	0.20 l	0.81 d	12.34 d	0.84 e

*Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.43. Effects of nickel, lead and their interactions mean of total alkaloid content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Alkaloid mg/g dry weight								
	Methanol extract			Water extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. (mg.kg^{-1} soil)									
Ni 0	1.66 c	4.57 c	0.46 a	0.070 a	0.21 d	0.0010 c	0.142 a	0.464 c	0.0020 c
Ni 15	1.63 d	4.54 c	0.44 a	0.069 a	0.22 c	0.00098 d	0.140 a	0.458 c	0.0020 c
Ni 30	2.01 b	4.88 a	0.42 b	0.062 b	0.28 b	0.011 a	0.129 b	0.538 b	0.022 a
Ni 45	2.20 a	4.67 b	0.30 c	0.046 c	0.30 a	0.010 b	0.093 c	0.609 a	0.021 b
Lead con. (mg.kg^{-1} soil)									
Pb 0	2.00 ab	4.79 c	0.40 b	0.063 b	0.272 a	0.0062 a	0.128 b	0.555 a	0.013 a
Pb 15	1.489 c	4.15 d	0.34 c	0.053 d	0.206 b	0.0047 d	0.106 d	0.407 b	0.010 c
Pb 30	2.022 a	4.88 a	0.39 b	0.059 c	0.258 a	0.0060 b	0.119 c	0.552 a	0.012 b
Pb 45	1.994 b	4.83 b	0.49 a	0.073 a	0.271 a	0.0059 c	0.151 a	0.556 a	0.012 b
Interaction between Ni & Pb con.									
Ni0 Pb0	1.88 de	5.13 d	0.50 bc	0.81 a	0.25 bc	0.0013 i	0.163 a	0.53 cd	0.0023 g
Ni0 Pb15	1.74 g	4.83 f	0.49 c	0.075 b	0.24 bcd	0.0011 k	0.152 c	0.49 e	0.0021 gh
Ni0 Pb30	1.51 i	4.15 hi	0.41 e	0.064 c	0.13 g	0.0009 l	0.120 f	0.41 f	0.0018 hi
Ni0 Pb45	1.51 i	4.17 h	0.42 e	0.062 c	0.21 def	0.0009 l	0.131 e	0.42 f	0.0018 hi
Ni15 Pb0	1.32 k	3.61 j	0.34 f	0.053 d	0.18 f	0.0008 m	0.112 g	0.36 g	0.0016 i
Ni15 Pb15	1.45 j	4.08 i	0.41 e	0.063 c	0.20 ef	0.0009 l	0.125 ef	0.41 f	0.0018 hi
Ni15 Pb30	1.93 d	5.33 b	0.52 ab	0.081 a	0.26 b	0.0011 ij	0.164 a	0.54 c	0.0023 g
Ni15 Pb45	1.84 ef	5.15 d	0.51 abc	0.080 a	0.25 bc	0.0011 jk	0.160 ab	0.52 cde	0.0023 g
Ni30 Pb0	2.89 b	5.19 cd	0.53 a	0.081 a	0.39 a	0.0113 c	0.162 a	0.79 b	0.0230 b
Ni30 Pb15	1.74 g	4.84 f	0.19 i	0.029 g	0.24 bcd	0.0106 f	0.060 j	0.41 f	0.0216 d
Ni30 Pb30	1.60 h	4.45 g	0.45 d	0.064 c	0.22 cde	0.0098 g	0.140 d	0.44 f	0.0194 e
Ni30 Pb45	1.82 f	5.03 e	0.51 abc	0.074 b	0.24 bc	0.0109 d	0.154 bc	0.51 de	0.0222 c
Ni45 Pb0	1.92 d	5.25 c	0.24 h	0.036 f	0.26 b	0.0115 b	0.073 i	0.53 cd	0.0232 b
Ni45 Pb15	1.03 l	2.83 k	0.28 g	0.044 e	0.14 g	0.0061 h	0.090 h	0.29 h	0.0125 f
Ni45 Pb30	3.05 a	5.61 a	0.17 j	0.026 g	0.41 a	0.0122 a	0.052 k	0.84 a	0.0244 a
Ni45 Pb45	2.82 c	4.98 e	0.50 abc	0.077 ab	0.38 a	0.0108 e	0.157 abc	0.78 b	0.0221 c

*Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.44. Effects of nickel, lead and their interactions mean of totall steroid content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Steroid mg/g dry weight					
	Methanol extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.044 a	0.034 b	0.0253 b	0.0672 a	0.0314 c	0.026 c
Ni 15	0.045 a	0.033 b	0.0246 c	0.0672 a	0.0301 d	0.025 d
Ni 30	0.040 b	0.077 a	0.0260 a	0.0613 b	0.0570 a	0.031 b
Ni 45	0.029 c	0.044 b	0.0248 c	0.0435 c	0.0543 b	0.034 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.040 b	0.041 b	0.0263 a	0.060 b	0.0457 a	0.0312 a
Pb 15	0.032 d	0.030 b	0.0225 c	0.050 d	0.0374 b	0.0233 b
Pb 30	0.038 c	0.077 a	0.0261 ab	0.058 c	0.0449 a	0.0309 a
Pb 45	0.047 a	0.040 b	0.0259 b	0.071 a	0.0448 a	0.0309 a
Interaction between Ni & Pb con.						
Ni0 Pb0	0.0515 a	0.039 b	0.0285 b	0.0735 cd	0.0359 f	0.0299 d
Ni0 Pb15	0.0435 c	0.036 b	0.0270 cd	0.0714 d	0.0319 h	0.0279 ef
Ni0 Pb30	0.0404 d	0.030 b	0.0230 e	0.0619 f	0.0290 i	0.0229 h
Ni0 Pb45	0.0405 d	0.031 b	0.0230 e	0.0620 f	0.0289 i	0.0239 g
Ni15 Pb0	0.0360 e	0.027 b	0.0205 g	0.0540 g	0.0250 j	0.0210 j
Ni15 Pb15	0.0395 d	0.029 b	0.0220 f	0.0600 f	0.0285 i	0.0225 h
Ni15 Pb30	0.0525 a	0.039 b	0.0285 b	0.0795 a	0.0325 gh	0.0295 d
Ni15 Pb45	0.0505 ab	0.038 b	0.0275 c	0.0755 bc	0.0345 fg	0.0285 e
Ni30 Pb0	0.0505 ab	0.059 b	0.0275 c	0.0765 b	0.0605 bc	0.0445 b
Ni30 Pb15	0.0185 h	0.036 b	0.0265 d	0.0275 j	0.0565 d	0.0275 f
Ni30 Pb30	0.0435 c	0.177 a	0.0235 e	0.0665 e	0.0525 e	0.0245 g
Ni30 Pb45	0.0485 b	0.037 b	0.0265 d	0.0745 bc	0.0585 cd	0.0275 f
Ni45 Pb0	0.0225 g	0.039 b	0.0285 b	0.0345 i	0.0615 b	0.0295 d
Ni45 Pb15	0.0275 f	0.021 b	0.0145 h	0.0415 h	0.0325 gh	0.0155 j
Ni45 Pb30	0.0155 i	0.062 b	0.0295 a	0.0245 k	0.0655 a	0.0465 a
Ni45 Pb45	0.0485 b	0.057 b	0.0265 d	0.0735 cd	0.0575 d	0.0435 c

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.45. Effects of nickel, lead and their interactions mean of total terpenes content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Terpene mg/g dry weight					
	Methanol extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R*
Nickel con. (mg.kg^{-1} soil)						
Ni 0	2.92 c	3.51 b	0.97 c	3.12 c	3.24 b	0.78 c
Ni 15	2.87 d	3.68 a	0.87 d	3.72 b	3.39 a	0.76 d
Ni 30	4.44 a	3.14 c	0.99 b	4.16 a	2.89 c	1.02 a
Ni 45	3.22 b	2.60 d	1.39 a	2.57 d	2.39 d	0.98 b
Lead con. (mg.kg^{-1} soil)						
Pb 0	3.41 b	3.25 c	1.22 a	2.54 d	2.99 c	0.97 b
Pb 15	3.69 a	3.73 a	0.92 d	3.72 b	3.44 a	0.63 d
Pb 30	3.08 d	2.64 d	1.12 b	3.50 c	2.44 d	1.08 a
Pb 45	3.28 c	3.31 b	0.96 c	3.79 a	3.05 b	0.86 c
Interaction between Ni & Pb con.						
Ni0 Pb0	2.16 n	2.72 h	0.65 l	2.17 n	2.51 i	0.61 l
Ni0 Pb15	4.23 d	4.85 c	0.71 k	4.71 c	4.51 c	0.62 k
Ni0 Pb30	2.34 m	2.70 h	0.80 i	2.62 l	2.49 j	1.26 d
Ni0 Pb45	2.95 i	3.76 d	1.72 a	2.97 i	3.46 e	0.62 k
Ni15 Pb0	3.94 e	4.97 b	1.59 d	3.91 e	4.57 b	1.35 b
Ni15 Pb15	3.20 h	5.66 a	0.61 m	3.59 h	5.21 a	0.51 m
Ni15 Pb30	2.79 k	1.90 l	0.66 l	2.68 k	1.75 n	0.35 o
Ni15 Pb45	1.56 p	2.20 k	0.62 m	4.69 d	2.02 m	0.81 h
Ni30 Pb0	2.91 j	2.76 g	1.36 e	3.67 g	2.53 h	0.73 i
Ni30 Pb15	3.83 f	2.60 i	0.76 j	2.59 m	2.39 k	0.92 g
Ni30 Pb30	5.06 b	3.43 f	1.33 f	4.85 b	3.16 g	1.75 a
Ni30 Pb45	5.95 a	3.77 d	0.51 n	5.50 a	3.48 d	0.67 j
Ni45 Pb0	4.62 c	2.55 j	1.28 g	1.48 p	2.35 l	1.19 e
Ni45 Pb15	3.48 g	1.80 m	1.62 c	2.92 j	1.66 o	0.45 n
Ni45 Pb30	2.13 o	2.54 j	1.69 b	3.85 f	2.34 l	0.96 f
Ni45 Pb45	2.67 l	3.49 e	0.98 h	2.021 o	3.22 f	1.34 c

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.46. Effects of nickel, lead and their interactions mean of total tannin content of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	Tannin mg/g dry weight					
	Methanol extract			Ethylacetate extract		
	G.	*L.*	*R.*	*G.*	*L.*	*R.*
Nickel con. (mg.kg^{-1} soil)						
Ni 0	0.041 c	1.90 c	0.28 a	0.051 c	3.44 a	0.78 c
Ni 15	0.041 c	1.88 d	0.27 b	0.051 c	3.40 b	0.77 d
Ni 30	0.051 b	2.01 a	0.25 c	0.092 a	3.10 c	0.94 b
Ni 45	0.055 a	1.92 b	0.18 d	0.088 b	2.19 d	1.02 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	0.050 a	2.00 b	0.25 b	0.074 a	3.06 b	0.935 b
Pb 15	0.037 b	1.72 d	0.21 d	0.061 c	2.56 d	0.705 d
Pb 30	0.051 a	2.02 a	0.24 c	0.074 a	2.91 c	0.943 a
Pb 45	0.050 a	1.99 c	0.29 a	0.073 b	3.60 a	0.931 c
Interaction between Ni & Pb con.						
Ni0 Pb0	0.048 cd	2.17 d	0.319 b	0.059 f	3.93 b	0.891 f
Ni0 Pb15	0.041 f	2.01 i	0.296 g	0.055 h	3.65 g	0.828 i
Ni0 Pb30	0.038 g	1.71 m	0.251 i	0.046 i	3.09 j	0.701 l
Ni0 Pb45	0.038 g	1.71 l	0.252 i	0.046 i	3.10 i	0.705 k
Ni15 Pb0	0.033 h	1.50 o	0.215 k	0.041 j	2.71 l	0.616 n
Ni15 Pb15	0.037 g	1.68 n	0.247 j	0.046 i	3.04 k	0.691 m
Ni15 Pb30	0.049 c	2.21 b	0.323 a	0.060 f	3.99 a	0.907 d
Ni15 Pb45	0.047 cd	2.12 f	0.311 d	0.057 g	3.84 d	0.871 g
Ni30 Pb0	0.072 b	2.14 e	0.314 c	0.098 b	3.87 c	1.341 b
Ni30 Pb15	0.045 e	2.01 j	0.116 n	0.092 d	1.43 o	0.825 i
Ni30 Pb30	0.041 f	2.81 k	0.272 h	0.085 e	3.35 h	0.755 j
Ni30 Pb45	0.046 de	2.07 g	0.304 e	0.095 c	3.74 e	0.845 h
Ni45 Pb0	0.048 cd	2.18 c	0.141 m	0.098 b	1.73 n	0.895 e
Ni45 Pb15	0.026 i	1.17 p	0.171 l	0.054 h	2.11 m	0.479 o
Ni45 Pb30	0.076 a	2.31 a	0.099 o	0.106 a	1.23 p	1.411 a
Ni45 Pb45	0.071 b	2.05 h	0.301 f	0.094 c	3.71 f	1.305 c

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.47. Effects of nickel, lead and their interactions mean of total saponin content of water extract of G. triacanthos (G.), L. leucocephala (L.) and R. pseudoacacia (R.) leaves.

Treatments	**Saponin mg/g dry weight**		
	G.	***L.***	***R.***
Nickel con. (mg.kg^{-1} soil)			
Ni 0	54.14 a	32.10 a	1.64 a
Ni 15	26.24 c	26.95 b	0.70 b
Ni 30	34.45 b	19.68 d	0.23 c
Ni 45	24.14 d	26.01 c	0.70 b
Lead con. (mg.kg^{-1} soil)			
Pb 0	46.64 a	29.29 b	0.47 c
Pb 15	33.50 b	28.83 c	1.17 b
Pb 30	27.89 d	15.70 d	1.40 a
Pb 45	30.93 c	30.93 a	0.23 d
Interaction between Ni & Pb con.			
Ni0 Pb0	73.13 a	40.31 c	0.000 d
Ni0 Pb15	64.69 b	42.19 b	1.875 b
Ni0 Pb30	38.44 e	10.31 o	3.745 a
Ni0 Pb45	40.31 d	35.62 e	0.938 c
Ni15 Pb0	42.19 c	38.44 d	0.938 c
Ni15 Pb15	11.22 n	31.88 f	1.875 b
Ni15 Pb30	17.81 m	20.63 i	0.000 d
Ni15 Pb45	33.75 h	16.88 l	0.000 d
Ni30 Pb0	32.81 i	15.94 m	0.000 d
Ni30 Pb15	37.49 f	25.31 g	0.938 c
Ni30 Pb30	35.63 g	17.81 k	0.000 d
Ni30 Pb45	31.89 j	19.69 j	0.000 d
Ni45 Pb0	38.43 e	22.48 h	0.938 c
Ni45 Pb15	20.63 k	15.94 m	0.000 d
Ni45 Pb30	19.69 l	14.06 n	1.875 b
Ni45 Pb45	17.81 m	51.56 a	0.000 d

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4.4 Effects of nickel, lead and their interactions on some anatomical studies

4.4.1 Anatomical characteristics of leaf, petiole, stem and root of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species

G. triacanthos anatomical characteristic of the leaf including lamina and midrib were their thickness increased with increasing Ni concentrations. Lamina and midrib of the leaf consist of cuticle, upper epidermis, mesophyll, vascular bundle and lower epidermis. The highest values of the lamina and midrib thickness (5.6, 24.1, 192.3, 70.4 and 23.7 micron) and (1.8, 7.4, 83, 43.8 and 6.2 micron) at 40x were recorded for 30 or 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (3.9, 17.1, 151, 55.5 and 20.6 micron) and (1.4, 5, 64.8, 28.8 and 4.0 micron) were recorded in the control treatment. Most parameters thickness of the lamina and midrib of *G. triacanthos* leaf increased with increasing the Pb concentrations to 15, 30 and 45 $mg.kg^{-1}$ $PbCl_2$ as compared to the other treatment, except for the cuticle thickness of the lamina which was non significant. Some of the interactions between concentrations of Ni and Pb also had significant effects and increased thickness with using Ni and Pb on all the anatomical studies of *G. triacanthos* leaf, (Table 4.48) and (Figures 4.4 and 4.5).

Anatomical characteristic of the petiole (periderm, cortex, fiber, accessory bundle, phloem and xylem) thickness increased with increasing Ni concentrations of *G. triacanthos* species. The highest values of periderm, cortex, fiber, accessory bundle, phloem and xylem thickness (7, 22, 10.3, 29.4, 11.2 and 15.8 micron) at 10x were recorded for the $NiCl_2$ application, while the lowest values (6, 15.2, 7, 18.8, 7.9 and 14.9 micron) at 10x were recorded in the control treatment. The petiole parameters thickness increased with using $PbCl_2$ as compared to the control treatment and 30 $mg.kg^{-1}$, except the phloem was decreased with increasing Pb concentration to 15 $mg.kg^{-1}$. The interactions between Ni and Pb also had significant effects by using $NiCl_2$ and $PbCl_2$ on all the anatomical consistent of *G. triacanthos* petiole, as shown in table (4.49) and figure (4.6).

Anatomical characteristic of the stem (periderm, cortex, fiber, phloem and xylem) thickness increased with increasing Ni concentrations of *G. triacanthos* stem. The highest values of periderm, cortex, fiber, phloem and xylem thickness (13.7, 21.8, 7.1, 9.95 and 117.1 micron) at 4x were recorded from 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentration, while the lowest values (7.4, 13.9, 4.2, 7.9 and 61.6 micron) were recorded in the control treatment. 30 $mg.kg^{-1}$ $PbCl_2$ concentration increased all stem component thickness treatment as compared to the control and 15 $mg.kg^{-1}$. Some of the interactions between concentrations of Ni and Pb

had significant effects and increased stem component significant thickness, whereas others decreased them compared to the control treatment, (See Table 4.50 and Figure 4.7).

Anatomical characteristic of the root consist of periderm, cortex, phloem and xylem where their thickness increased with Ni application. The highest values of periderm, cortex, phloem and xylem thickness (10.4, 41.4, 28.9 and 5.9 micron) at 4x were recorded for 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (8.7, 20.9, 12.9 and 5.3 micron) were recorded in the control treatment. The cortex and phloem thickness increased with adding Pb concentration as compared to the control treatment and 15 $mg.kg^{-1}$, except the periderm and xylem which were decreased with adding the Pb to 15 and 30 $mg.kg^{-1}$ as compared to the control treatment. The interactions between Ni and Pb concentrations had significant effects and cortex, phloem and xylem increased with increasing Ni or pb concentrations from 15 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations, except the periderm which was decreased with adding the Pb concentration as compared to 15 $mg.kg^{-1}$ of *G. triacanthos* roots, as shown in Table 4.50 and Figure 4.8.

Table 4.48. Effects of nickel, lead and their interactions mean of some anatomical characteristics of G. triacanthos leaf.

Treatments	Lamina (Thickness micron, 40x)					Midrib (Thickness micron, 10x)				
	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis
Nickel con. ($mg.kg^{-1}$ soil)										
Ni 0	3.9 b	17.1 d	151.0 c	55.5 c	20.6 b	1.4 b	5.0 c	64.8 d	28.8 c	4.0 c
Ni 15	5.2 a	22.3 b	182.7 b	59.7 b	22.9 a	1.5 ab	5.4 c	70.0 c	38.1 b	5.2 b
Ni 30	5.6 a	20.5 c	175.1 b	61.2 b	23.7 a	1.8 a	6.1 b	74.8 b	43.8 a	5.2 b
Ni 45	5.2 a	24.1 a	192.3 a	70.4 a	23.0 a	1.7 a	7.4 a	83.0 a	38.3 b	6.2 a
Lead con. ($mg.kg^{-1}$ soil)										
Pb 0	5.2 a	20.7 b	185.2 a	56.4 c	20.9 b	1.7 a	6.0 b	76.4 a	39.3 a	5.4 ab
Pb 15	4.8 a	23.6 a	176.0 b	61.3 b	22.1 ab	1.8 a	6.3 b	71.2 b	34.4 b	5.0 b
Pb 30	4.7 a	18.1 c	165.7 c	63.5 ab	24.1 a	1.2 b	4.8 c	69.6 b	34.9 b	4.3 c
Pb 45	5.1 a	21.8 b	174.2 bc	65.5 a	23.1 ab	1.8 a	6.9 a	75.5 a	40.4 a	5.8 a
Interaction between Ni & Pb con.										
Ni0 Pb0	3.9 ef	15.1 f	132.6 i	53.7 de	19.0 d	1.4 cde	3.3 h	80.2 c-e	34.9 ef	3.4 f
Ni0 Pb15	3.3 f	18.3 c-e	153.5 gh	79.2 b	20.8 bcd	1.4 de	4.7 fg	51.3 i	17.3 i	4.5 ef
Ni0 Pb30	3.1 f	17.2 def	144.0 hi	44.2 fg	20.4 bcd	1.3 de	5.1 efg	47.3 i	23.4 h	3.4 f
Ni0 Pb45	5.2 cde	17.9 c-f	173.7 def	44.8 fg	22.4 ad	1.4 cde	7.0 bcd	80.5 bcd	39.5 cd	4.6 e
Ni15 Pb0	3.9 ef	20.9 bc	223.2 a	83.1 ab	19.1 d	1.4 cde	3.9 gh	58.9 h	36.2 def	5.6 b-e
Ni15 Pb15	5.1 cde	26.1 a	204.0 bc	58.2 d	21.6 bcd	1.5 cde	6.1 cde	73.8 efg	37.6 cde	4.5 ef
Ni15 Pb30	7.1 b	20.7 bc	138.7 hi	44.2 fg	23.5 a-d	1.3 de	4.4 fgh	74.3 defg	37.5 cde	4.8 de
Ni15 Pb45	4.6 def	21.5 b	165.1 efg	53.2 e	27.5 a	1.7 b-e	7.2 bc	73.1 fg	41.0 c	5.9 bcd
Ni30 Pb0	8.5 a	25.4 a	175.9 def	47.6 f	24.8 abc	2.1 abc	10.3 a	78.2 b-f	59.0 a	6.2 bc
Ni30 Pb15	5.0 cde	21.6 b	181.2 de	36.2 h	22.7 a-d	2.4 a	6.3 cde	78.2 b-f	44.3 b	5.0 cde
Ni30 Pb30	4.6 def	15.5 ef	156.3 f-h	80.0 b	27.1 a	1.0 e	4.1 gh	72.3 fg	38.0 cde	4.4 ef
Ni30 Pb45	4.1 ef	19.5b-d	187.1 cd	81.2 ab	20.0 cd	1.6 b-e	3.7 gh	70.5 g	33.8 f	5.3 cde
Ni45 Pb0	4.6 def	20.7 bc	209.2 ab	41.3 g	20.8 bcd	1.7 bcd	6.2 cd	88.2 a	27.1 g	6.6 ab
Ni45 Pb15	5.7 cd	28.3 a	165.5 efg	71.7 c	23.1 a-d	1.8 bcd	8.1 b	81.3 bc	38.1 cde	5.9 bcd
Ni45 Pb30	4.0 ef	18.9b-d	223.7 a	85.6 a	25.5 ab	1.1 de	5.6 def	84.7 ab	40.8 c	4.7 de
Ni45 Pb45	6.4 bc	28.1 a	170.9d-g	82.9 ab	22.6 a-d	2.3 ab	9.7 a	77.8 cdef	47.3 b	7.4 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.49. Effects of nickel, lead and their interactions mean of some anatomical characteristics of G. triacanthos petiole at 10x.

Treatments	Periderm	Cortex	Fiber	Accessory bundle	Phloem	Xylem
	Thickness (Micron)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	6.0 b	15.2 d	7.0 c	18.8 d	7.9 c	14.9 b
Ni 15	6.2 b	22.0 a	10.1 ab	23.5 c	8.7 bc	16.8 a
Ni 30	6.9 a	19.7 b	10.3 a	29.4 a	11.2 a	14.9 b
Ni 45	7.0 a	17.6 c	9.3 b	27.4 b	9.6 b	15.8 ab
Lead con. (mg.kg^{-1} soil)						
Pb 0	5.7 b	16.6 c	7.5 c	23.5 b	10.3 a	15.4 b
Pb 15	6.1 b	17.8 b	9.7 ab	27.5 a	8.3 b	16.4 ab
Pb 30	7.1 a	22.2 a	9.2 b	24.2 b	9.1 ab	13.6 c
Pb 45	7.2 a	18.0 b	10.3 a	24.1 b	9.5 a	17.0 a
Interaction between Ni & Pb con.						
Ni0 Pb0	5.5 ef	13.7 hij	6.6 ghi	10.0 f	7.7 ef	14.6 cd
Ni0 Pb15	5.7 def	17.2 d-g	6.2 hi	34.2 a	4.8 g	12.6 de
Ni0 Pb30	6.2cde	18.3 def	7.5 fgh	14.2 e	10.8 bc	15.4 c
Ni0 Pb45	6.6 cde	11.6 j	7.8 fgh	16.9 e	8.3 def	17.1 c
Ni15 Pb0	6.1 cde	19.2 cd	9.6 de	23.1 cd	10.3 bcd	19.9 b
Ni15 Pb15	5.7 def	19.2 cd	10.7 cd	21.7 d	9.1 b-f	12.3 de
Ni15 Pb30	7.07 bcd	36.6 a	8.0 efg	20.9 d	7.0 f	10.7 e
Ni15 Pb45	6.0 cde	13.0 ij	12.0 bc	28.5 b	8.5 cdef	24.2 a
Ni30 Pb0	4.4 f	15.9 fgh	8.2 efg	33.4 a	14.6 a	11.4 e
Ni30 Pb15	7.06 bcd	13.4 ij	7.6 fgh	26.6 bc	9.9 bcde	17.3 c
Ni30 Pb30	7.05 bcd	18.9 cde	13.1 ab	34.7 a	9.3 bcde	16.0 c
Ni30 Pb45	9.1 a	30.6 b	12.3 b	23.1 cd	10.9 b	15.0 cd
Ni45 Pb0	7.8 bcde	17.4 defg	5.8 i	27.5 b	8.8 bcdef	15.6 c
Ni45 Pb15	6.1 cde	21.2 c	14.5 a	27.3 bc	9.4 bcde	23.5 a
Ni45 Pb30	8.0 ab	15.1 ghi	8.1 efg	27.0 bc	10.1 bcd	12.6 de
Ni45 Pb45	7.13 bc	16.7 efg	9.0 ef	28.0 b	10.1 bcd	11.7 e

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.50. Effects of nickel, lead and their interactions mean of some anatomical characteristics of G. triacanthos stem and root at 4x

Treatments	Stem (Thickness micron)					Root (Thickness micron)			
	Periderm	Cortex	Fiber	Phloem	Xylem	Periderm	Cortex	Phloem	Xylem
Nickel con. (mg.kg^{-1} soil)									
Ni 0	7.4 c	13.9 c	4.2 c	7.9 b	61.6 c	8.7 c	20.9 d	12.9 b	5.3 b
Ni 15	7.7 c	18.3 b	6.0 b	9.66 a	62.0 c	10.4 a	29.6 c	13.9 b	5.7 ab
Ni 30	8.9 b	17.6 b	6.0 b	9.95 a	94.9 b	9.5 b	38.6 b	13.0 b	5.5 ab
Ni 45	13.7 a	21.8 a	7.1 a	9.64 a	117.1 a	10.4 a	41.4 a	28.9 a	5.9 a
Lead con. (mg.kg^{-1} soil)									
Pb 0	9.9 b	13.2 c	5.5 b	9.09 b	94.4 a	11.2 a	29.7 b	17.9 b	6.6 a
Pb 15	8.2 c	17.6 b	5.5 b	8.65 b	67.7 c	8.3 c	35.3 a	15.5 c	5.0 c
Pb 30	11.2 a	23.3 a	6.9 a	9.81 a	96.5 a	9.0 c	30.4 b	15.6 c	5.0 c
Pb 45	8.4 c	17.4 b	5.5 b	9.69 a	77.1 b	10.4 b	34.9 a	19.7 a	5.8 b
Interaction between Ni & Pb con.									
Ni0 Pb0	11.7 b	5.4 j	3.0 e	7.12 f	55.9 d	11.9 b	27.5 fg	9.0 i	6.9 ab
Ni0 Pb15	4.6 h	13.5 i	3.9 e	8.05 ef	67.5 cd	6.8 ij	12.1 i	10.5 hi	5.7 bcd
Ni0 Pb30	6.1 gh	19.9 bcd	6.2 bcd	8.08 ef	64.5 cd	8.2 ghi	15.9 hi	10.4 hi	3.0 f
Ni0 Pb45	7.1 fg	16.8 fgh	3.8 e	8.75 de	58.6 d	7.8 ghi	28.0 efg	21.8 d	5.2 cde
Ni15 Pb0	8.4 d-g	14.9 hi	7.1 b	9.86 bcd	61.8 d	15.5 a	18.1 h	15.8 ef	5.8 bcd
Ni15 Pb15	7.9 efg	22.1 b	7.0 b	8.84 cde	41.6 e	7.2 hij	30.5 def	17.7 e	4.1 e
Ni15 Pb30	8.1 efg	17.9 d-g	5.7 cd	9.91 a-d	67.0 cd	8.2 ghi	26.5 fg	9.6 i	6.9 ab
Ni15 Pb45	6.3 gh	18.2 c-f	4.2 e	10.02 abc	77.7 c	10.5 b-e	43.3 b	12.6 gh	5.9 bcd
Ni30 Pb0	8.9 c-f	15.4 ghi	5.9 bcd	9.28 bcd	114.7 b	6.0 j	38.7 bc	24.2 cd	6.0 bcd
Ni30 Pb15	9.9 b-e	15.1 hi	5.4 d	10.54 ab	60.0 d	8.9 efg	56.7 a	8.3 i	5.1 cde
Ni30 Pb30	6.0 gh	19.1 c-f	5.9 bcd	10.14 ab	100.0 b	8.8 fgh	23.5 g	5.4 j	5.2 cde
Ni30 Pb45	10.9 bc	20.6 bc	6.9 bc	9.84 bcd	105.1 b	14.3 a	35.3 cd	14.0 fg	5.9 bcd
Ni45 Pb0	10.7 bcd	17.2 e-h	6.0 bcd	10.09 ab	145.1 a	11.4 bc	34.7 cd	22.7 d	7.6 a
Ni45 Pb15	10.2 b-e	19.5 cde	5.6 cd	7.19 f	101.8 b	10.2 c-f	42.0 b	25.6 c	5.0 cde
Ni45 Pb30	24.5 a	36.6 a	9.8 a	11.13 a	154.5 a	10.8 bcd	55.8 a	36.9 a	4.8 de
Ni45 Pb45	9.5 b-e	14.1 i	7.0 bc	10.15 ab	67.5 cd	9.2 d-g	33.0 de	30.4 b	6.1 bc

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

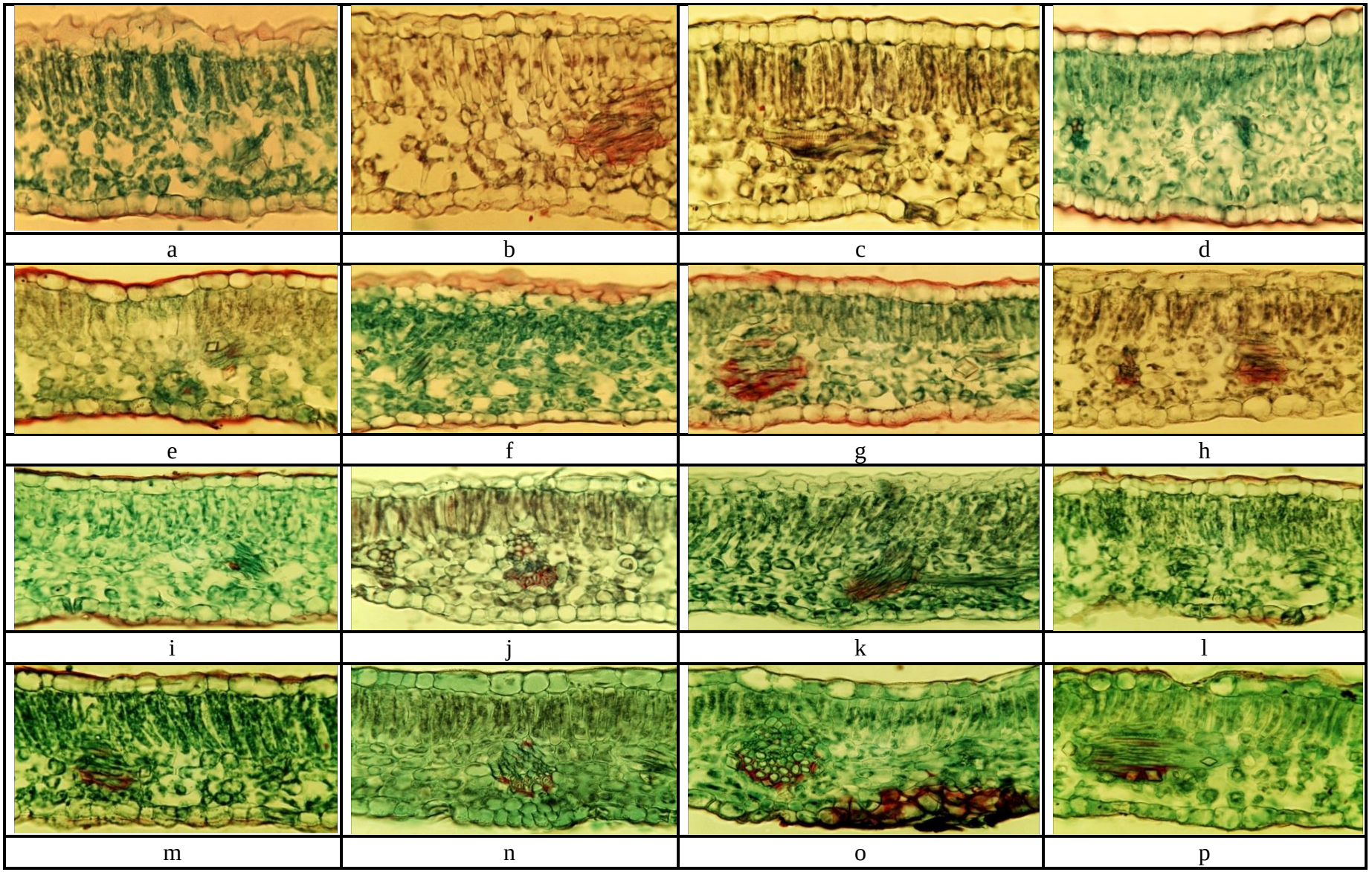


Fig 4.2. *Leaves (lamina) anatomy of G. triacanthos at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

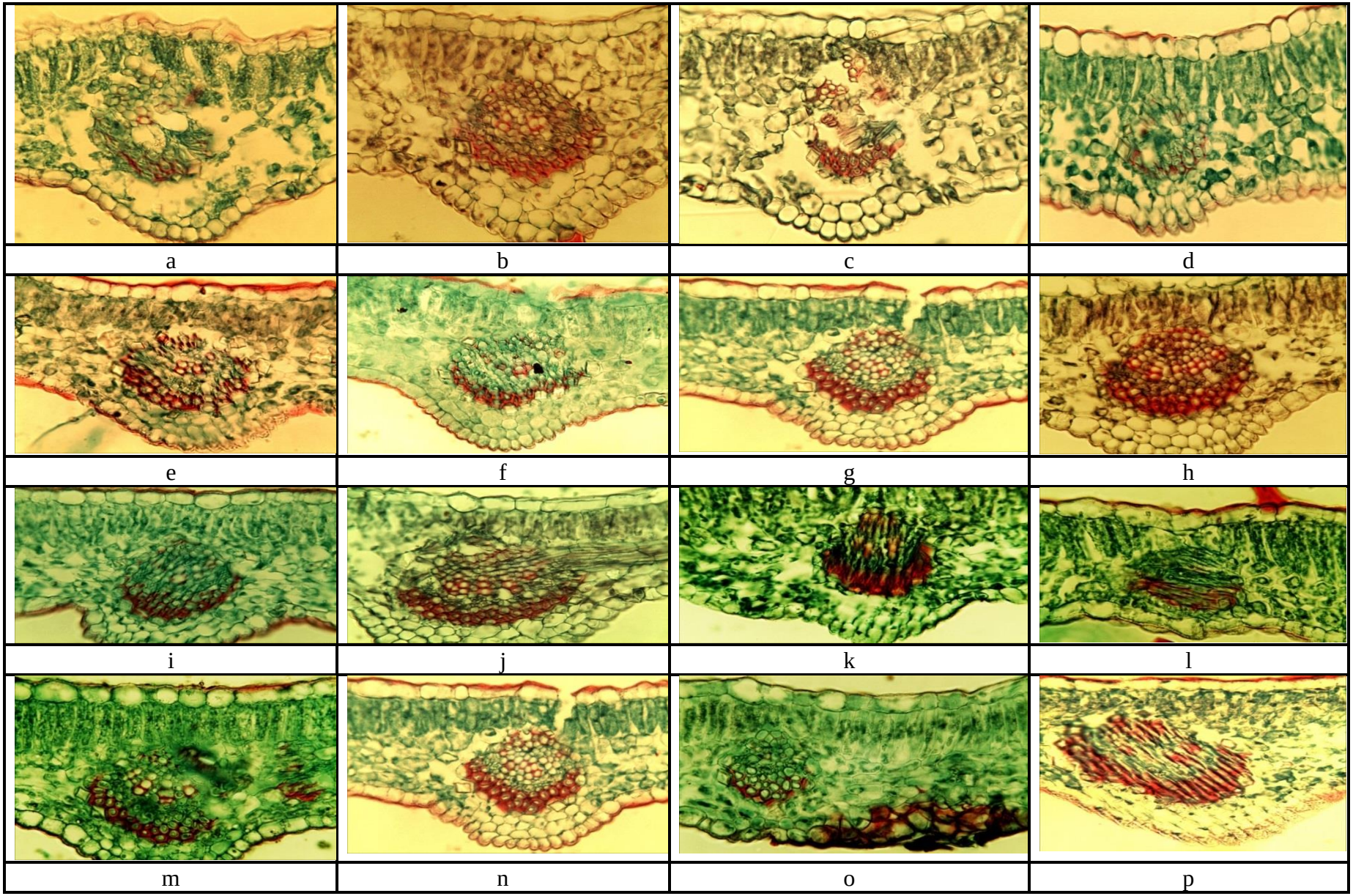


Fig 4.3. *Leaves (midrib) anatomy of G. triacanthos at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

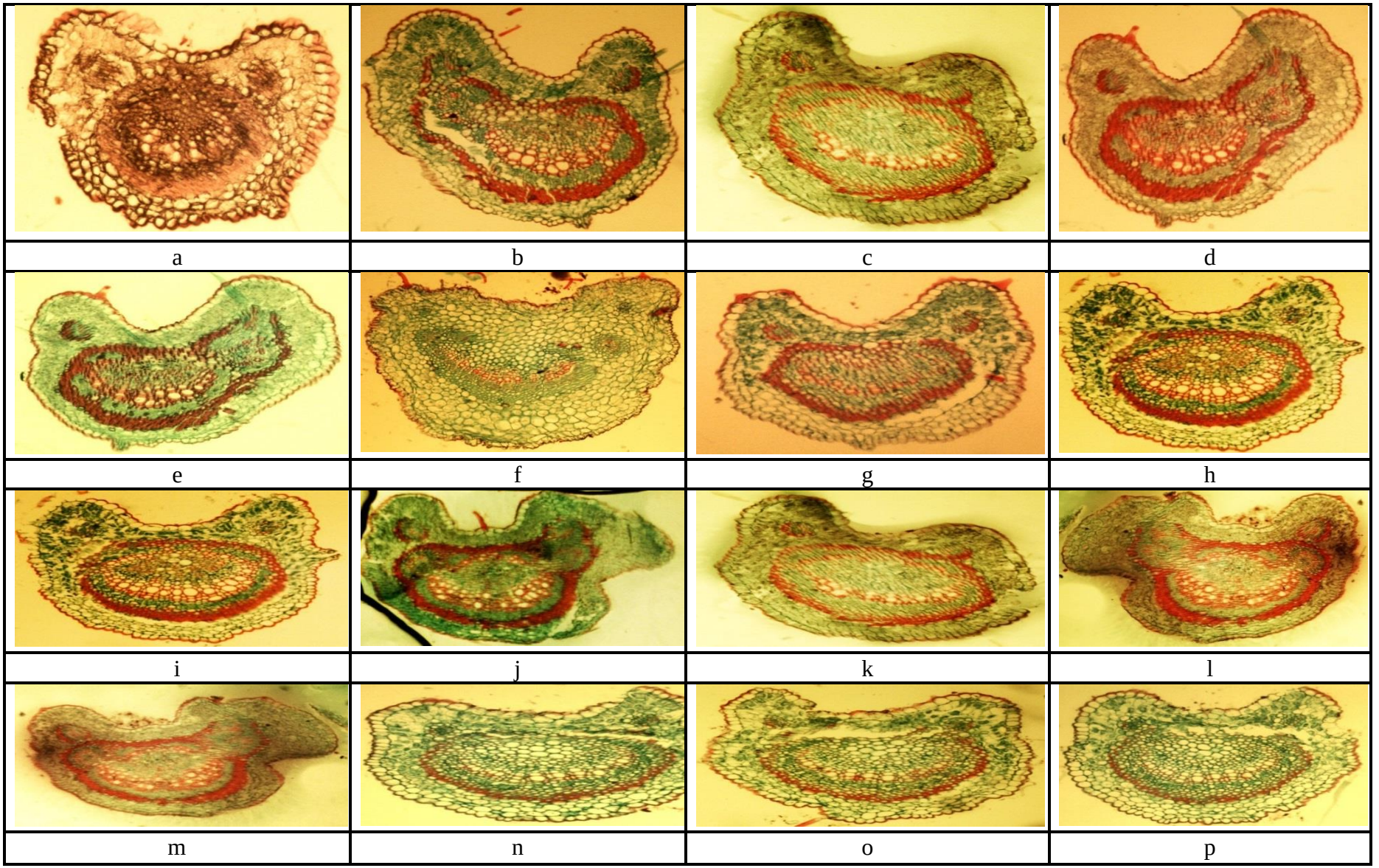


Fig 4.4. *Petiole anatomy of G. triacanthos at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

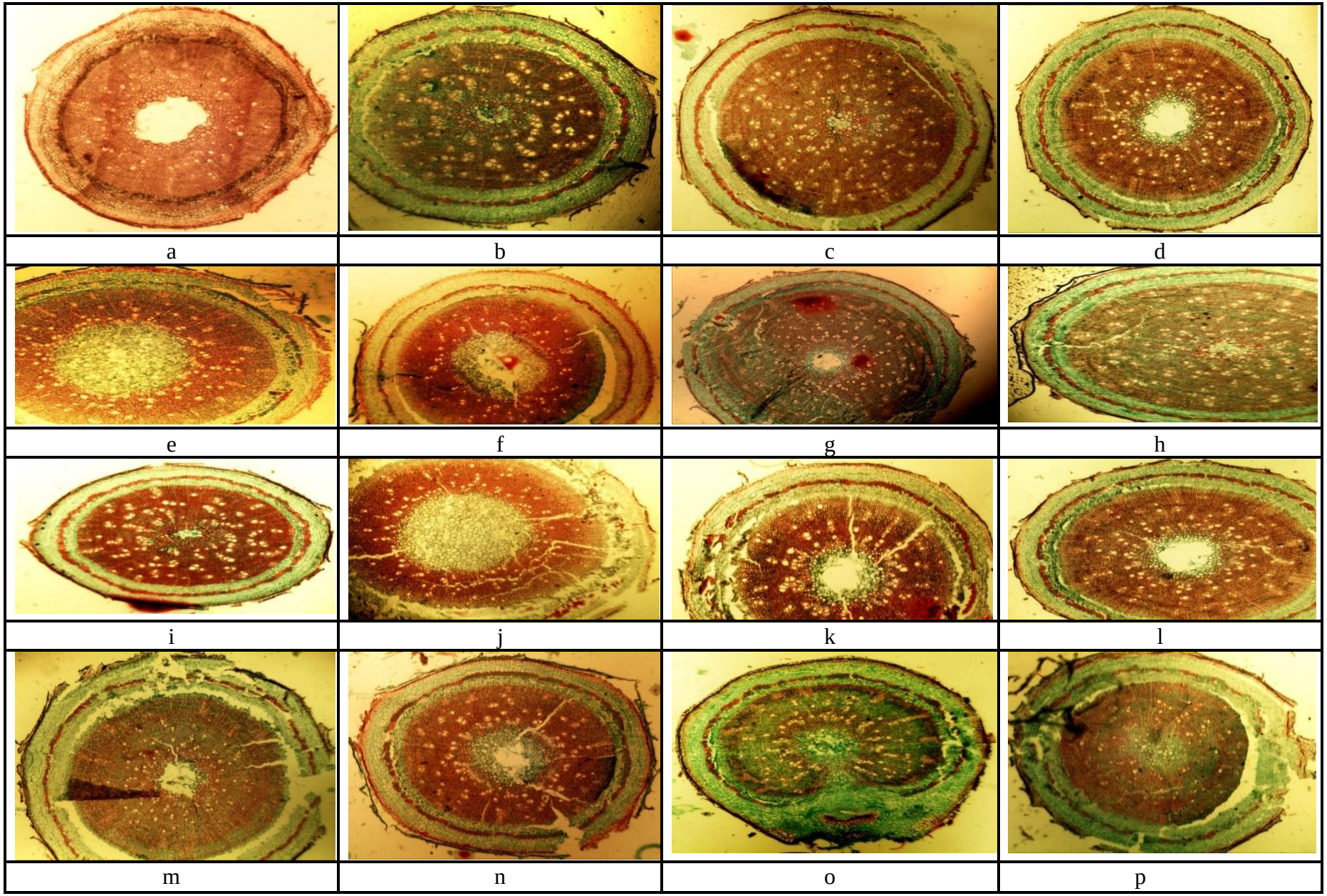


Fig 4.5*. Stem anatomy of G. triacanthos at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

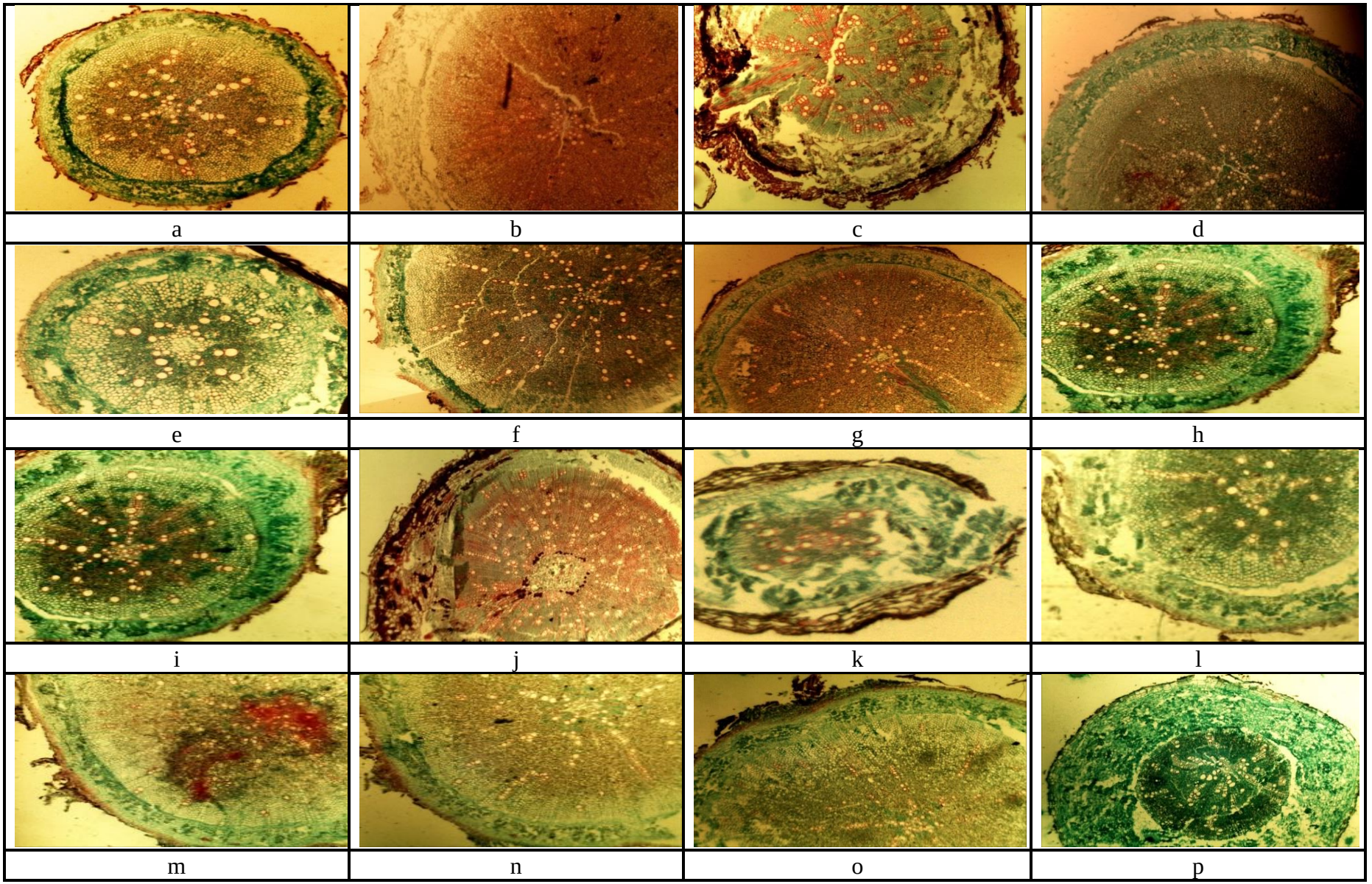


Fig 4.6. *Root anatomy of G. triacanthos at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

L. leucocephala leaf anatomical characteristics consist of lamina and midrib which lamina and midrib of the leaf consist of cuticle, upper epidermis, mesophyll, vascular bundle and lower epidermis. The highest thickness values (4.3, n.s, 179.6, 61.8 and n.s micron) and (1.8, 5.2, 87.6, 43.3 and 5.9 micron) at 40x were recorded for 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (2.7, n.s, 139.5, 54.3 and n.s micron) and (1.1, 4.3, 62.5, 31.3 and 3.3) micron at 40x respectively were recorded in the control treatment. The cuticle thickness of the lamina increased with increasing the Pb concentrations to 45 $mg.kg^{-1}$ $PbCl_2$ as compared to 30 $mg.kg^{-1}$, whereas each of upper epidermis and vascular bundle of the lamina decreased with increasing the Pb concentrations to 30 $mg.kg^{-1}$, whereas mesophyll and lower epidermis which were non significant. The cuticle, upper epidermis, mesophyll and vascular bundle of the midrib thickness decreased with increasing the Pb concentrations to 45 and 30 $mg.kg^{-1}$ as compared to the control treatment and 15 $mg.kg^{-1}$, whereas the lower epidermis of midrib which was non significant. The interactions between concentrations of Ni and Pb had significant effects and the upper and lower epidermis of the lamina and mesophyll thickness of the midrib which were decreased with increasing Ni or Pb concentrations for 30 or 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations, whereas all the other parameters thickness of the lamina and midrib were increased with increasing Ni or Pb concentrations for 15, 30 and 45 $mg.kg^{-1}$, as shown in Table (4.51) and Figures (4.11 and 4.12).

Anatomical characteristics of the petiole consist of periderm, cortex, fiber, accessory bundle, phloem and xylem were their thickness increased with increasing Ni concentrations. The highest values of periderm, cortex, fiber, accessory bundle, phloem and xylem thickness (11.7, 18.0, 10.8, 52.5, 16.2 and 40.5 micron) at 10x were recorded for 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ treatments, while the lowest values (7.2, 14.6, 8.9, 45.6, 12.3 and 29.3 micron) at 10x were recorded in the control treatment. All parameters thickness increased with increasing the Pb concentrations to 30 or 45 $mg.kg^{-1}$ $PbCl_2$ as compared to other treatment, except periderm and xylem were decreased significantly with increasing the Pb concentrations. The interactions between Ni and Pb concentrations had significant effects and increased thickness with increasing Ni or Pb concentrations to 15, 30 and 45 and 30 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on all the anatomical studies of *L. leucocephala* petiole, as it appears in Table (4.52) and Figure (4.13).

Anatomical characteristic of the stem consist of periderm, cortex, fiber, phloem and xylem which increased with increasing Ni concentrations. The highest values of periderm, cortex, fiber, phloem and xylem thickness (18.2, 9.3, 9.2, 15 and 104.8 micron) at 4x were recorded for 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (15.3, 6.6, 6.5, 9.3 and 67.1 micron) at 4x were recorded in the control treatment. The cortex and xylem thickness increased with using Pb application as compared to 15

and 30 mg.kg^{-1} treatment, whereas the periderm, fiber and phloem thickness decreased with increasing the Pb concentrations as compared to control and 15 mg.kg^{-1}. The interactions between concentrations of Ni and Pb also had significant effects and increased with increasing Ni or Pb concentrations for 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on all the anatomical studies of *L. leucocephala* stem, (See Table 4.53 and Figure 4.14).

Anatomical characteristic of the root consist of periderm, cortex, phloem and xylem. The highest values of periderm, cortex, phloem and xylem thickness (20, 69.5, 20.3 and 8.8 micron) at 4x were recorded for 30 or 45 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest values (15.3, 47.7, 16.5 and 7.5 micron) at 4x were recorded in the control treatment. The periderm and cortex thickness decreased with Pb concentrations as compared to the control treatment, except the phloem increased with increasing Pb concentration to 45 mg.kg^{-1} as compared to 30 mg.kg^{-1}, whereas the xylem which was non significant. The interactions between concentrations of Ni and Pb had significant effects and the cortex and phloem thickness increased with increasing Ni or Pb concentrations to 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations, except the xylem which decreased with increasing Ni or Pb concentrations to 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations as compared to the control treatment of *L. leucocephala* roots , (See Table 4.53 and Figure 4.15.

Table 4.51. Effects of nickel, lead and their interactions mean of some anatomical characteristics of L. leucocephala leaf.

Treatments	Lamina (Thickness micron, 40x)					Midrib (Thickness micron, 10x)				
	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis
Nickel con. ($mg.kg^{-1}$ soil)										
Ni 0	2.7 c	16.5 a	139.5 c	54.3 c	16.7 a	1.1 b	4.3 b	62.5 d	31.3 c	3.3 c
Ni 15	3.2 bc	16.8 a	161.0 b	57.1 b	16.8 a	1.6 a	4.7 ab	70.9 c	39.0 b	4.7 b
Ni 30	3.3 b	16.9 a	166.3 b	60.5 a	17.0 a	1.8 a	4.6 ab	87.6 a	39.2 b	5.1 b
Ni 45	4.3 a	18.2 a	179.6 a	61.8 a	17.2 a	1.8 a	5.2 a	78.4 b	43.3 a	5.9 a
Lead con. ($mg.kg^{-1}$ soil)										
Pb 0	3.4 b	19.9 a	162.4 a	73.8 a	18.1 a	1.7 a	5.1 ab	87.4 a	46.1 a	5.1 a
Pb 15	3.3 bc	17.6 b	161.6 a	64.0 b	16.3 a	2.0 a	4.4 bc	67.2 b	34.1 c	4.7 a
Pb 30	2.9 c	15.1 c	161.5 a	45.2 d	16.2 a	1.4 b	4.1 c	65.4 c	34.9 c	4.7 a
Pb 45	3.9 a	15.8 c	160.9 a	50.6 c	17.1 a	1.3 b	5.3 a	79.3 b	37.7 b	4.6 a
Interaction between Ni & Pb con.										
Ni0 Pb0	2.7 de	18.3 abc	115.4 e	71.1 c	14.8 c	1.2 cd	4.9 ab	71.8 f	26.5 d	3.8 def
Ni0 Pb15	1.6 f	13.8 d	142.2 d	67.6 c	15.5 c	1.3 cd	4.7 ab	56.2 g	30.1 d	3.2 ef
Ni0 Pb30	2.6 def	15.8 bcd	125.1 e	39.0 fg	18.3 bc	1.1 cd	2.7 c	42.1 h	27.0 d	2.6 f
Ni0 Pb45	4.0 abc	18.1 abc	175.2 bc	39.3 fg	18.0 bc	1.0 d	4.9 ab	79.9 e	41.6 c	3.8 def
Ni15 Pb0	3.1 bcd	21.5 a	166.8 c	78.3 ab	16.4 bc	1.3 cd	5.1 ab	100.5 a	52.8 b	5.6 bc
Ni15 Pb15	2.7 de	18.4 abc	177.6 abc	72.7 bc	17.6 bc	2.4 a	5.8 a	41.7 h	19.2 e	3.6 def
Ni15 Pb30	2.9 cde	14.7 cd	180.8 ab	43.3 ef	17.9 bc	1.5 cd	3.8 bc	86.6 bcd	58.2 a	5.9 bc
Ni15 Pb45	4.0 abc	12.6 de	119.1 e	34.1 g	15.4 c	1.4 cd	4.3 abc	54.7 g	25.8 d	3.7 def
Ni30 Pb0	3.1 bcd	19.7 a	177.9 abc	78.2 ab	17.4 bc	2.1 ab	5.4 ab	88.5 bc	44.7 c	5.0 bcd
Ni30 Pb15	4.1 ab	19.7 a	146.2 d	44.8 ef	14.1 c	2.5 a	2.9 c	81.0 de	43.1 c	4.4 cde
Ni30 Pb30	2.0 ef	19.9 a	165.2 c	42.1 ef	15.1 c	1.3 cd	4.4 abc	98.1 a	29.8 d	4.9 bcd
Ni30 Pb45	4.1 ab	13.5 de	175. 8 bc	82.0 a	21.4 ab	1.4 cd	5.9 a	82.9 cde	39.2 c	6.1 ab
Ni45 Pb0	4.7 a	20.0 a	189.3 a	67.7 c	23.6 a	2.3 a	5.1 ab	88.8	60.3 a	5.9 b
Ni45 Pb15	4.9 a	18.6 abc	180.6 ab	71.0 c	18.1 bc	1.7 bc	4.0 abc	89.9 bc	44.2 c	7.3 a
Ni45 Pb30	4.0 abc	10.0 e	175.1 bc	56.5 d	13.5 c	1.7 bc	5.6 ab	35.0 i	24.5 d	5.3 bc
Ni45 Pb45	3.5 bcd	19.2 ab	173.5 bc	46.8 e	13.6 c	1.5 cd	6.0 a	99.9 a	44.1 c	5.0 bcd

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.52. Effects of nickel, lead and their interactions mean of some anatomical characteristics of L. leucocephala petiole at 10x.

Treatments	Periderm	Cortex	Fiber	Accessory bundle	Phloem	Xylem
	Thickness (Micron)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	7.2 c	14.6 b	8.9 c	45.6 b	12.3 c	29.3 c
Ni 15	7.7 c	18.0 a	9.5 bc	50.7 a	15.1 b	35.4 b
Ni 30	10.4 b	17.1 a	10.1 ab	52.5 a	16.5 a	40.5 a
Ni 45	11.7 a	17.1 a	10.8 a	52.0 a	16.2 a	37.8 b
Lead con. (mg.kg^{-1} soil)						
Pb 0	10.0 a	15.9 b	10.1 ab	44.9 d	15.8 a	38.6 a
Pb 15	8.2 c	16.9 ab	9.6 bc	47.5 c	13.9 b	38.0 a
Pb 30	9.6 ab	16.2 b	9.0 c	55.5 a	16.4 a	33.7 b
Pb 45	9.2 b	17.9 a	10.5 a	53.0 b	14.0 b	32.6 b
Interaction between Ni & Pb con.						
Ni0 Pb0	5.5 g	11.1 g	6.6 g	30.3 f	11.9 hi	35.3 de
Ni0 Pb15	4.9 g	18.5 cd	9.6 cde	39.9 e	11.2 i	20.7 g
Ni0 Pb30	8.7 ef	10.8 g	8.5 ef	59.8 a	14.0 fghi	37.2 cde
Ni0 Pb45	9.8 de	17.9 d	10.7 c	52.6 cd	13.0 ghi	23.9 fg
Ni15 Pb0	10.3 b-e	11.3 g	10.9 c	43.4 e	12.0 hi	38.7 cd
Ni15 Pb15	8.1 f	20.5 bc	10.1 cd	51.5 cd	12.0 hi	42.5 bc
Ni15 Pb30	7.6 f	19.0 bcd	7.1 fg	54.9 abc	16.6 cde	26.3 f
Ni15 Pb45	4.7 g	21.2 b	9.9 cde	53.1 bcd	19.8 ab	34.2 de
Ni30 Pb0	12.8 a	14.4 ef	7.1 fg	51.3 cd	18.7 bc	46.1 ab
Ni30 Pb15	7.5 f	15.3 e	8.9 de	55.3 abc	17.1 cd	49.8 a
Ni30 Pb30	10.1 cde	19.5bcd	10.2 cd	48.8 d	20.9 a	31.9 e
Ni30 Pb45	11.1 a-d	19.3 bcd	14.2 b	54.7 abc	9.4 j	34.0 de
Ni45 Pb0	11.6 abc	29.6 a	16.0 a	54.6 abc	20.8 a	34.3 de
Ni45 Pb15	12.2 a	13.0 fg	9.6 cde	43.3 e	15.3 def	39.0 cd
Ni45 Pb30	11.8 ab	15.6 e	10.2 cd	58.4 ab	14.7 efg	39.5 cd
Ni45 Pb45	11.1 a-d	13.1 fg	7.2 fg	51.6 cd	13.9 fgh	38.3 cd

* Means followed by the same letters with in columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.53 Effects of nickel, lead and their interactions mean of some anatomical characteristics of L. leucocephala stem and root at 4x.

Treatments	Stem (Thickness micron)					Root (Thickness micron)			
	Periderm	Cortex	Fiber	Phloem	Xylem	Periderm	Cortex	Phloem	Xylem
Nickel con. (mg.kg^{-1} soil)									
Ni 0	15.3 b	6.6 c	6.5 c	9.3 c	67.1 c	15.3 d	47.7 c	16.5 c	7.5 c
Ni 15	18.1 a	7.6 b	6.8 c	10.8 b	76.3 b	16.8 c	48.9 c	19.7 ab	7.7 bc
Ni 30	16.3 b	9.3 a	9.2 a	10.5 b	104.8 a	18.6 b	60.1 b	20.5 a	8.8 a
Ni 45	18.2 a	9.0 a	7.5 b	15.0 a	78.2 b	20.0 a	69.5 a	18.7 b	8.3 ab
Lead con. (mg.kg^{-1} soil)									
Pb 0	19.1 a	7.8 b	7.6 b	12.0 b	82.3 b	20.2 a	61.9 a	20.6 a	7.9 ab
Pb 15	16.1 b	7.2 c	8.6 a	13.7 a	82.0 b	16.6 c	55.2 b	17.6 b	7.6 b
Pb 30	17.0 b	10.1 a	7.5 b	10.9 c	73.0 c	16.2 c	47.3 c	15.7 c	8.4 a
Pb 45	15.8 b	7.4 bc	6.3 c	9.1 d	89.0 a	17.8 b	61.8 a	21.5 a	8.5 a
Interaction between Ni & Pb con.									
Ni0 Pb0	17.9 bc	6.0 ef	9.5 abc	9.6 ef	44.3 h	17.9 bcd	51.0 efg	18.0 c-f	7.8 cde
Ni0 Pb15	9.4 e	4.7 g	7.0 e	9.6 ef	71.5 e	12.4 i	40.1 i	17.5 c-g	6.5 def
Ni0 Pb30	11.7 de	9.8 bc	5.1 fg	10.7 ef	75.8 de	13.9 hi	55.0 de	13.9 hi	7.7 cde
Ni0 Pb45	22.2 a	6.0 ef	4.5 g	7.5 gh	76.9 de	17.2 def	44.9 hi	16.6 e-i	8.0 cd
Ni15 Pb0	22.1 a	9.1 c	8.8 bc	8.9 fgh	80.0 d	15.4 fgh	59.2 d	20.5 c	6.2 ef
Ni15 Pb15	17.0 bc	9.0 c	8.4 cd	13.9 b	51.7 g	17.6 cde	52.3 ef	19.5 cde	8.9 bc
Ni15 Pb30	18.3 bc	6.8 de	4.9 fg	11.0 cde	62.5 f	18.6 bcd	50.0 e-h	18.8 cde	7.3 c-f
Ni15 Pb45	15.0 cd	5.4 fg	5.3 fg	9.3 efg	110.8 b	15.8 efg	34.0 j	20.1 cd	8.3 bc
Ni30 Pb0	17.2 bc	6.0 ef	7.2 de	12.3 bc	99.8 c	17.7 cd	84.0 b	28.9 a	11.7 a
Ni30 Pb15	17.6 bc	5.3 fg	9.9 ab	13.0 b	130.7 a	19.2 bc	46.3 f-i	14.5 ghi	8.4 bc
Ni30 Pb30	17.7 bc	15.2 a	10.5 a	9.1 efg	80.3 d	17.9 bcd	40.2 i	13.3 i	8.8 bc
Ni30 Pb45	12.5 de	10.6 b	9.2 abc	7.2 h	108.3 b	19.7 b	70.0 c	25.2 b	6.4 def
Ni45 Pb0	19.0 ab	9.8 bc	5.0 fg	16.8 a	105.0 bc	29.9 a	53.7 ef	15.0 f-i	5.9 f
Ni45 Pb15	20.1 ab	9.9 bc	9.2 abc	18.1 a	74.3 de	17.1 def	82.0 b	18.8 cde	6.6 def
Ni45 Pb30	20.4 ab	8.7 c	9.5 abc	12.7 bc	73.4 de	14.5 gh	44.2 hi	17.0 d-h	9.8 b
Ni45 Pb45	13.3 d	7.4 d	6.2 ef	12.3 bcd	60.0 f	18.6 bcd	98.1 a	24.1 b	11.2 a

* Means followed by the same letters with in columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

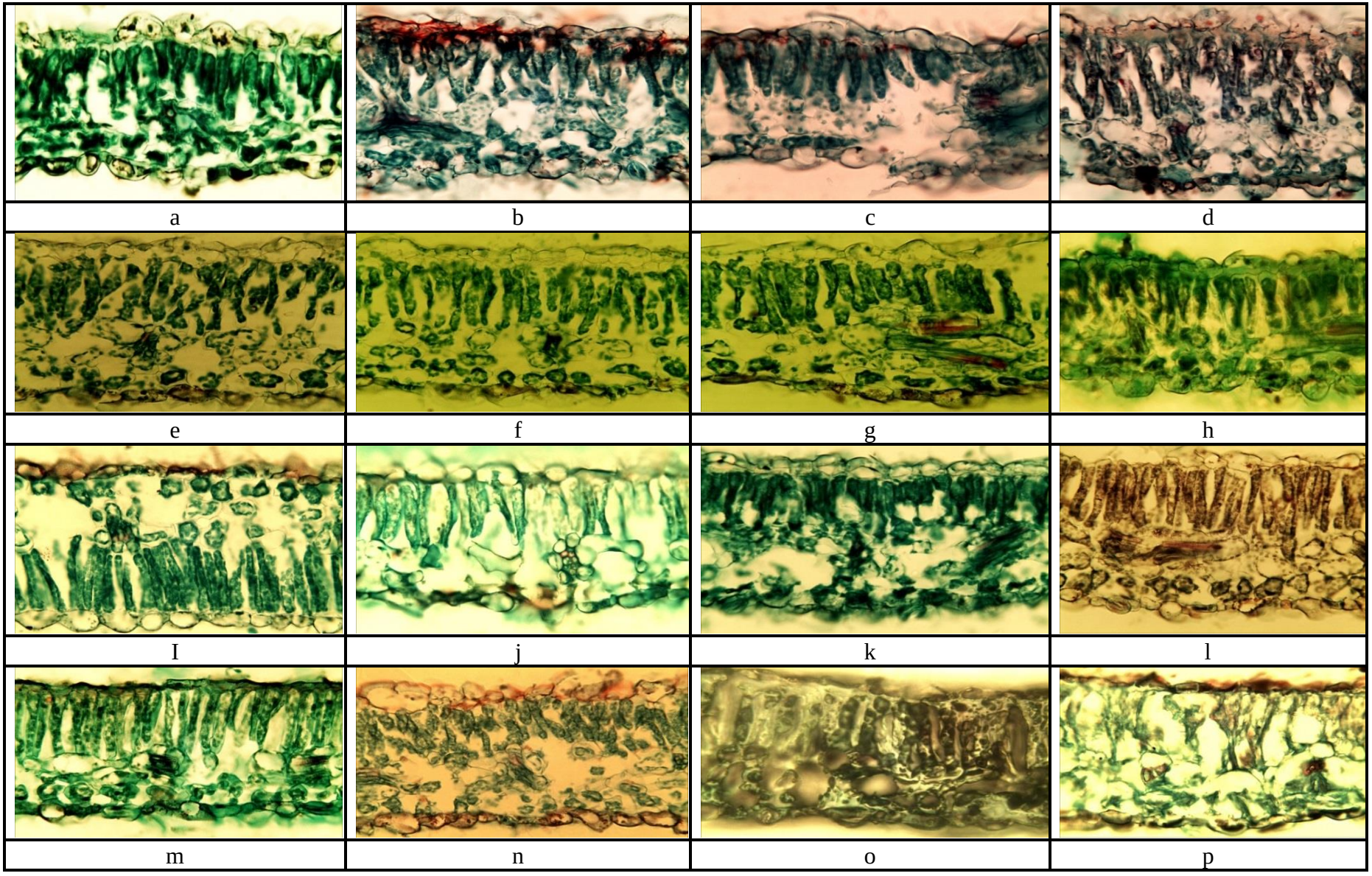


Fig 4.7. *Leaves (lamina) anatomy of L. leucocephala at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

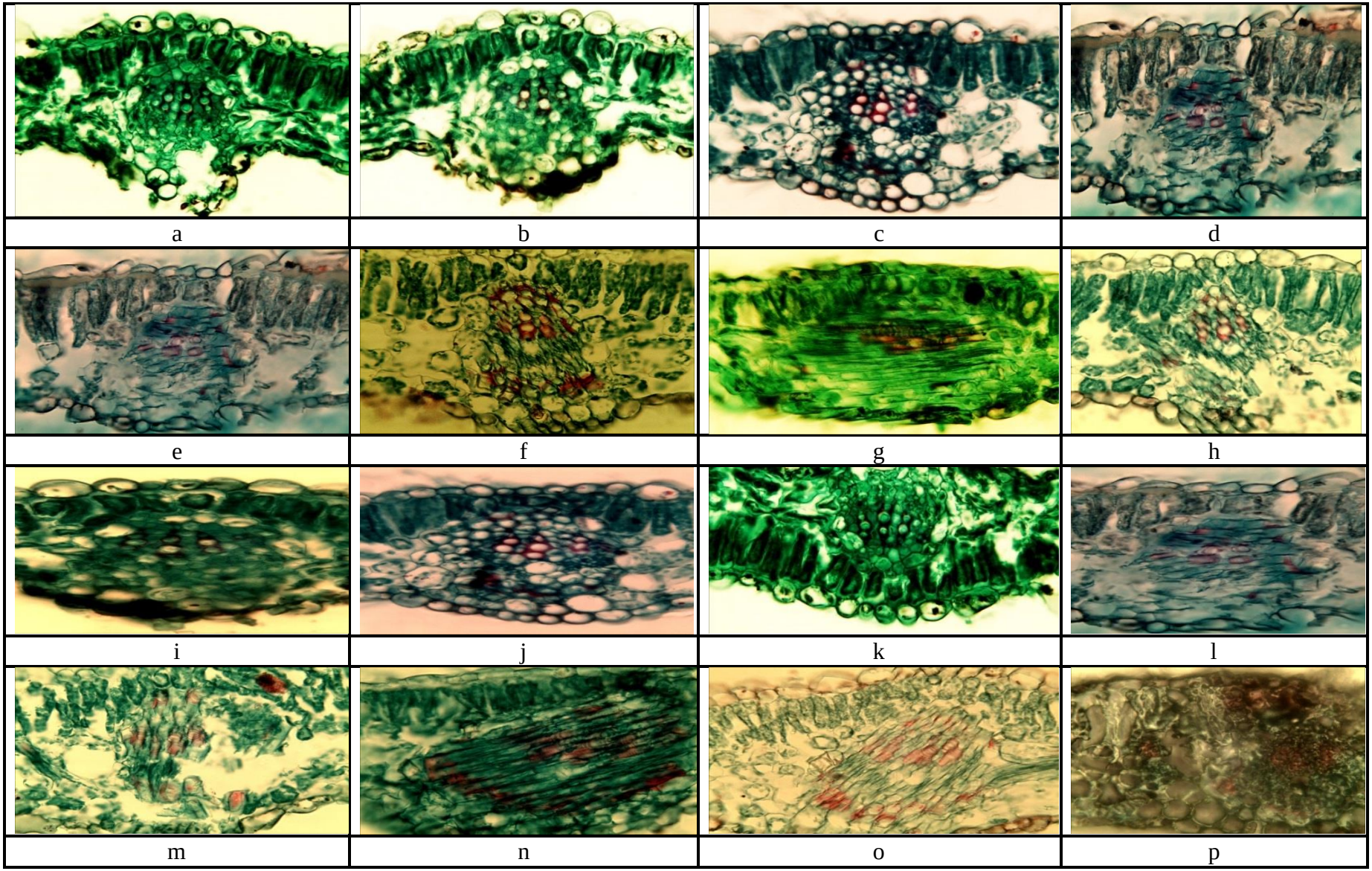


Fig 4.8*. Leaves (midrib) anatomy of L. leucocephala at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

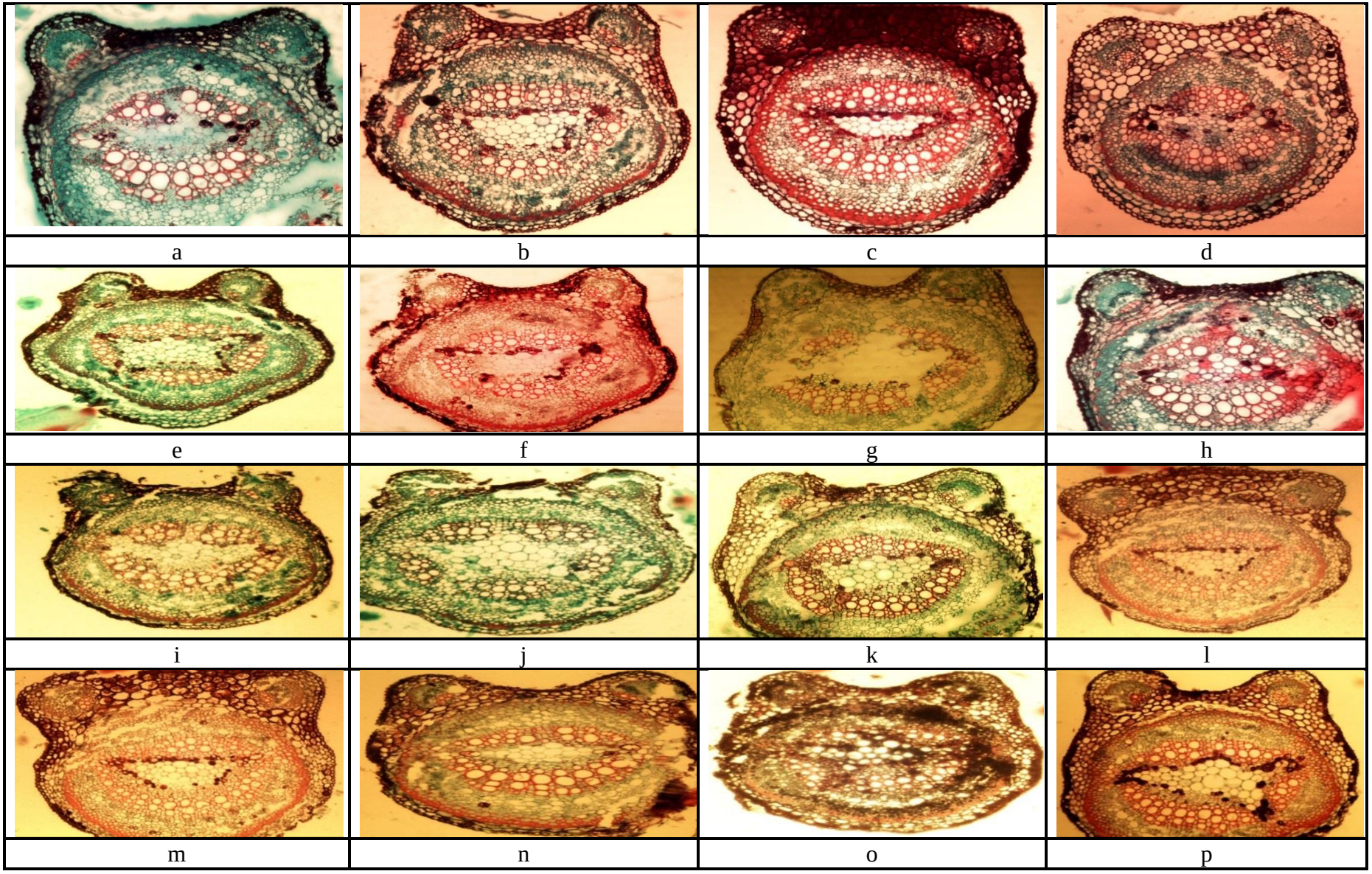


Fig 4.9. *Petiole anatomy of L. leucocephala at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

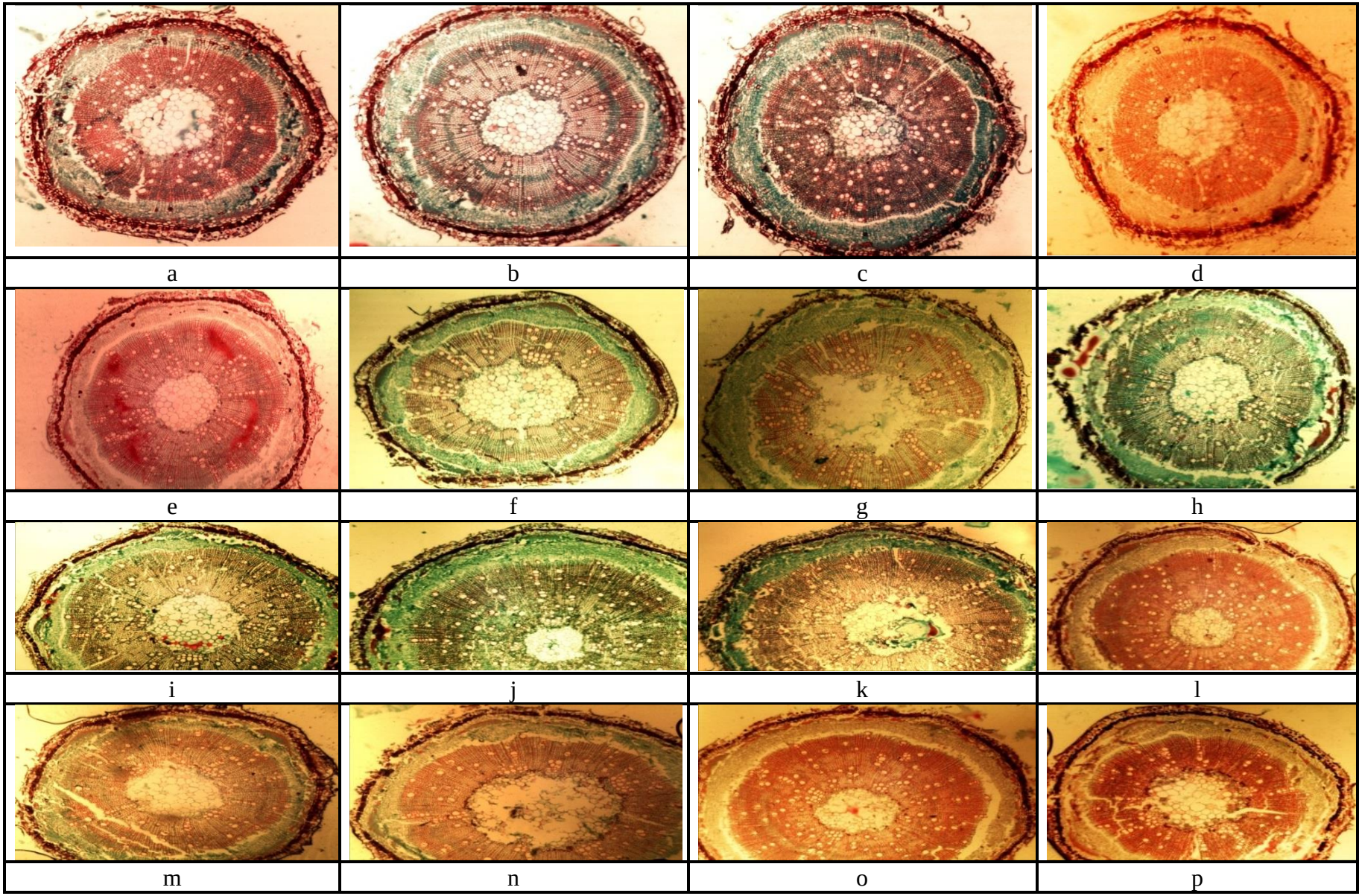


Fig 4.10*. Stem anatomy of L. leucocephala at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

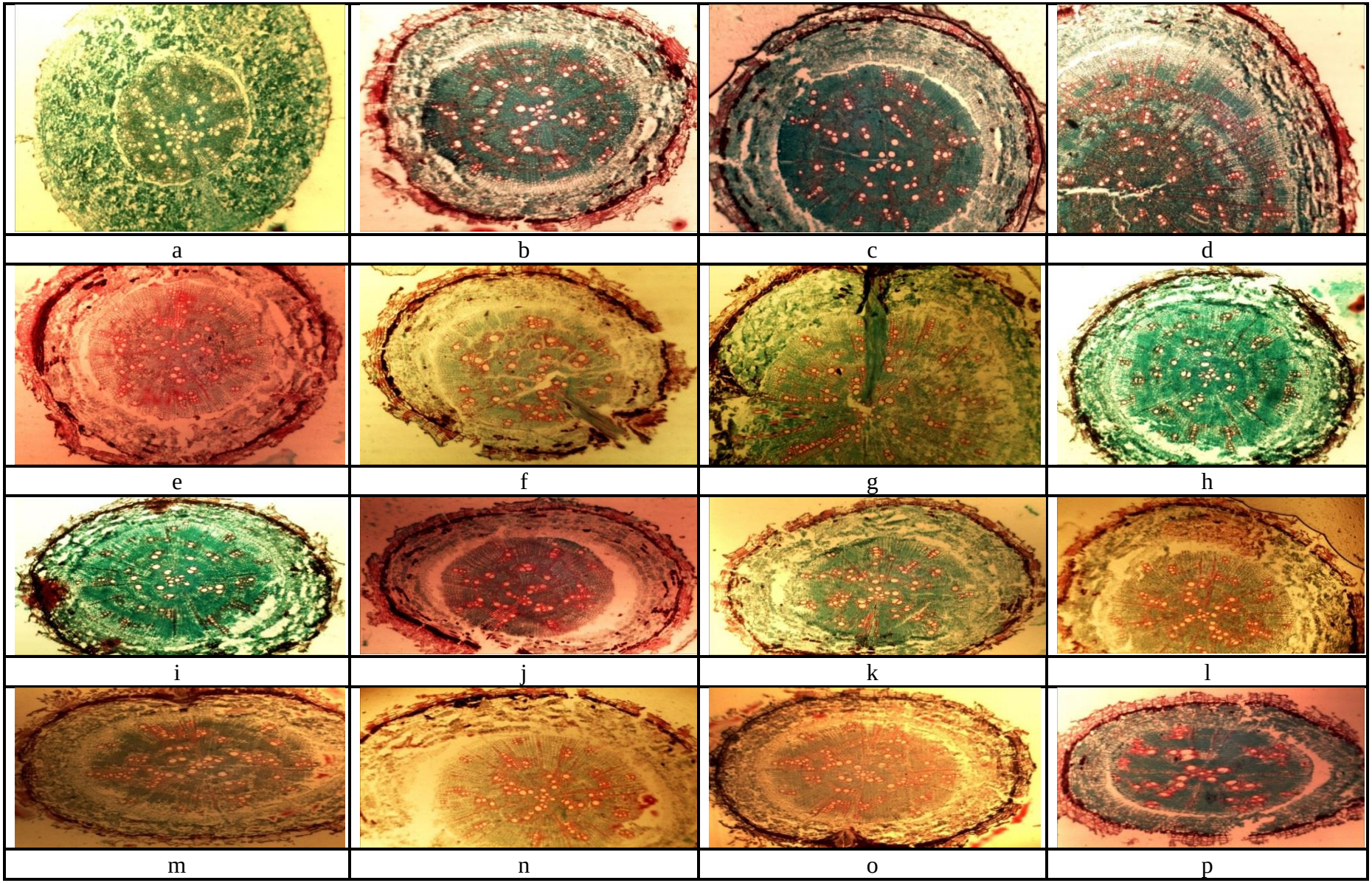


Fig 4.11. *Root anatomy of L. leucocephala at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

R. pseudoacacia anatomical characteristics of the leaf consists of lamina and midrib. Lamina and midrib of the leaf consists of cuticle, upper epidermis, mesophyll, vascular bundle and lower epidermis. The highest value of lamina and midrib thickness (4.2, 26, 198, n.s, and 15.7 micron) and (2.1, 7.6, 121.4, 85 and 6.0 micron) at 40x respectively were recorded for 30 and 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest value of lamina and midrib (3.2, 17.9, 140, n.s and 13.3 micron) and (1.6, 6, 94.8, 49.4 and 4.6 micron) were recorded in the control treatment. The lamina (cuticle and upper epidermis) and midrib (vascular bundle) thickness were increased with increasing the Pb concentrations to 45 and 30 $mg.kg^{-1}$ $PbCl_2$ as compared to the other treatment, except the upper epidermis and mesophyll of the midrib which decreased with increasing the Pb concentrations to 45 $mg.kg^{-1}$ as compared to the control treatment and 30 $mg.kg^{-1}$, whereas the mesophyll, vascular bundle and lower epidermis of the lamina and the cuticle and lower epidermis of the midrib which were non significant. The interactions between concentrations of Ni and Pb had significant effects and increased with increasing Ni or Pb concentrations 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on all anatomical characterestics of *R. pseudoacacia* leaf, (Table 4.54) and (Figures 4.18 and 4.19).

Anatomical characteristics of the petiole consist of periderm, cortex, fiber, accessory bundle, phloem and xylem were thickness increased with increasing Ni concentrations. The highest value of periderm, cortex, fiber, accessory bundle, phloem and xylem thickness (7.5, 17.5, 10.0, 36.4, 19.1 and 26.1 micron) at 10x were recorded for 30 or 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (5.3, 11.5, 7.2, 22.1, 10.6 and 18.6 micron) were recorded for the control treatment. Each of periderm, fiber and accessory bundle thickness were increased with increasing the Pb concentration to 30 or 45 $mg.kg^{-1}$ $PbCl_2$ as compared to 15 $mg.kg^{-1}$ treatment, except the cortex which was decreased with increasing the Pb concentrations for 45 $mg.kg^{-1}$ as compared to the control treatment, whereas the phloem and xylem which were non significant. The interactions between concentrations of Ni and Pb also had significant effects wherethey increased with increasing Ni or Pb concentrations for 15 or 30 or 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on all the anatomical studies, (Table 4.55) and (Figure 4.20).

Anatomical characteristics of the stem (periderm, cortex, fiber, phloem and xylem) thickness increased with increasing Ni concentrations. The highest value of periderm, cortex, fiber, phloem and xylem (15.3, 13.4, n.s, 17.2 and n.s micron) at 4x were recorded from 45 $mg.kg^{-1}$ $NiCl_2$ concentrations, while the lowest values (8.2, 11.1, n.s, 12.3 and n.s micron) at 4x were recorded in the control treatment. The periderm, cortex, fiber, phloem and xylem thickness increased with increasing the Pb concentrations for 30 or 45

mg.kg^{-1} $PbCl_2$ as compared to the control treatment and 15 mg.kg^{-1}. The interactions between concentrations of Ni and Pb also had significant effects and increased thickness with increasing Ni or Pb concentrations from 30 and 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on all the anatomical parameters of *R. pseudoacacia* stems, (Table 4.56) and (Figure 4.21).

Anatomical characteristics of the root (periderm, cortex, phloem and xylem) thickness were increased with increasing Ni concentrations. The highest values of periderm, cortex, phloem and xylem thickness (12.4, n.s, 22.9 and 8.8 micron) at 4x were recorded for 30 or 45 mg.kg^{-1} $NiCl_2$ concentrations, while the lowest values (7.6, n.s, 9.3 and 6.9 micron) were recorded in the control treatment. The periderm, cortex, phloem and xylem thickness increased with increasing the Pb concentrations to 45 mg.kg^{-1} $PbCl_2$ as compared to 15 and 30 mg.kg^{-1} treatment. The interactions between concentrations of Ni and Pb also had significant effects and increased thickness with increasing Ni or Pb concentrations from 15 or 30 or 45 mg.kg^{-1} $NiCl_2$ or $PbCl_2$ concentrations on all the anatomical studies of *R. pseudoacacia* roots, (See Table 4.56 and Figure 4.22).

Table 4.54. Effects of nickel, lead and their interactions mean of some anatomical characteristics of R. pseudoacacia leaf.

Treatments	Lamina (Thickness micron, 40x)					Midrib (Thickness micron, 10x)				
	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis	Cuticle	Upper Epidermis	Mesophyll	Vascular bundle	Lower Epidermis
Nickel con. (mg.kg^{-1} soil)										
Ni 0	3.2 c	17.9 d	140.8 c	37.0 a	13.3 b	1.6 b	6.0 b	94.8 c	49.4 c	4.6 c
Ni 15	3.7 b	20.6 c	161.2 b	39.8 a	13.7 b	1.8 ab	6.6 b	95.3 c	64.5 b	4.7 c
Ni 30	3.9 ab	26.0 a	198.0 a	67.0 a	13.7 b	1.9 ab	6.2 b	121.4 a	61.1 b	6.0 a
Ni 45	4.2 a	23.6 b	169.3 b	40.2 a	15.7 a	2.1 a	7.6 a	112.3 b	85.0 a	5.4 b
Lead con. (mg.kg^{-1} soil)										
Pb 0	3.5 b	23.9 a	160.9 a	40.0 a	15.1 a	1.0 a	7.4 a	100.3 b	52.1 d	5.3 a
Pb 15	3.5 b	18.6 c	173.8 a	39.0 a	13.4 a	2.1 a	6.9 ab	113.7 a	72.4 b	5.2 a
Pb 30	4.0 a	21.2 b	165.5 a	66.7 a	13.7 a	1.7 a	6.1 b	116.2 a	76.8 a	5.1 a
Pb 45	4.0 a	24.6 a	169.2 a	38.2 a	14.1 a	1.8 a	6.0 b	93.5 b	58.6 c	5.1 a
Interaction between Ni & Pb con.										
Ni0 Pb0	3.5 d	15.1 hi	129.4 fg	24.2 b	15.11 abc	2.0 abc	5.6 def	88.6 efg	45.4 e	4.7 d-g
Ni0 Pb15	3.7 cd	14.8 i	148.1 ef	34.0 b	14.2 abcd	1.8 bc	4.5 f	110.4 bcd	63.0 d	3.0 h
Ni0 Pb30	3.7 cd	22.1 c-f	100.8 g	41.8 b	10.6 d	1.6 c	7.4 b-e	96.8 d-g	45.2 e	5.8 bcd
Ni0 Pb45	1.8 e	19.8 efg	184.7 cde	48.0 b	13.2 bcd	1.2 c	6.3 c-f	84.2 g	44.0 e	5.1 def
Ni15 Pb0	3.7 cd	22.0 cde	147.6 ef	44.4 b	14.5 abcd	2.2 abc	7.2 bcde	95.8 d-g	50.7 e	3.8 gh
Ni15 Pb15	3.3 d	16.3 ghi	219.8 ab	39.6 b	11.8 cd	1.7 bc	7.8 bcd	95.4 d-g	62.9 d	4.9 def
Ni15 Pb30	3.8 cd	22.0 c-f	126.1 fg	42.4 b	14.5 a-d	1.5 c	6.3 cde	104.5 cde	81.3 c	4.4 fg
Ni15 Pb45	3.9 bcd	21.1 def	151.4 ef	32.7 b	13.9 bcd	1.9 bc	4.9 f	85.6 fg	63.0 d	5.6 b-e
Ni30 Pb0	3.9 bcd	28.8 ab	216.2 abc	46.2 b	12.9 bcd	1.5 c	8.7 ab	95.8 d-g	47.5 e	7.4 a
Ni30 Pb15	3.2 d	18.9 fgh	159.5 de	42.6 b	10.9 d	1.88 bc	5.2 ef	247.8 a	62.2 d	6.3 abc
Ni30 Pb30	3.8 bcd	24.3 cd	244.0 a	137.2 a	16.8 ab	1.7 bc	5.5 ef	141.4 a	89.1 b	5.6 b-f
Ni30 Pb45	4.7 abc	31.9 a	172.5 de	41.9 b	14.2 abcd	2.7 ab	5.4 ef	100.6 d-g	45.5 e	4.5 efg
Ni45 Pb0	2.9 d	28.7 ab	150.4 ef	45.1 b	18.0 a	1.8 bc	7.9 bc	121.1 bc	64.7 d	5.3 c-f
Ni45 Pb15	3.7 cd	24.2 cd	167.7 de	40.0 b	16.5 ab	3.0 a	10.0 a	101.2 d-g	101.6 a	6.5 ab
Ni45 Pb30	4.8 ab	16.2 ghi	191.1 bcd	45.6 b	13.1 bcd	2.2 abc	5.3 ef	123.0 b	91.6 b	4.5 efg
Ni45 Pb45	5.6 a	25.5 bc	168.2 de	30.3 b	15.13 abc	1.6 bc	7.3 b-e	103.7 def	82.0 c	5.4 b-f

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.55. Effects of nickel, lead and their interactions mean of some anatomical characteristics of R. pseudoacacia petiole at 10 x.

Treatments	Periderm	Cortex	Fiber	Accessory bundle	Phloem	Xylem
	Thickness (Micron)					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	5.3 b	11.5 c	7.2 c	22.1 c	10.6 b	18.6 c
Ni 15	5.5 b	15.0 b	9.0 ab	29.1 b	17.3 a	22.1 a
Ni 30	5.3 b	15.3 b	10.0 a	29.3 b	18.9 a	26.7 a
Ni 45	7.5 a	17.5 a	7.7 bc	36.4 a	19.1 a	26. 1 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	5.5 bc	21.6 a	7.5 b	27.2 b	16.4 ab	23.0 a
Pb 15	5.1 c	13.5 c	7.1 b	21.1 c	14.5 b	22.6 a
Pb 30	6.8 a	15.3 b	9.5 a	34.1 a	18.1 a	24.4 a
Pb 45	6.3 ab	8.8 d	9.8 a	34.6 a	16.9 ab	24.1 a
Interaction between Ni & Pb con.						
Ni0 Pb0	4.8 d-g	14.3 de	7.6 d	24.6 e	9.8 d	19.1 cde
Ni0 Pb15	4.1 efg	13.0 efg	6.3 d	13.4 f	9.4 d	17.4 de
Ni0 Pb30	5.8 a-e	11.9 efg	6.4 d	39.0 b	13.3 cd	22.2 a-e
Ni0 Pb45	6.5 a-e	6.7 i	8.4 cd	11.5 f	9.7 d	15.6 e
Ni15 Pb0	2.6 g	29.8 a	7.8 d	25.3 e	7.8 d	20.0 b-e
Ni15 Pb15	5.3 b-f	11.0 fg	7.3 d	16.1 f	13.3 cd	18.0 cde
Ni15 Pb30	7.0 a-d	11.6 efg	9.1 bcd	26.9 e	22.8 ab	23.6 a-d
Ni15 Pb45	7.2 a-d	7.5 hi	11.7 abc	48.0 a	25.4 ab	29.1 a
Ni30 Pb0	6.5 a-e	16.3 cd	7.0 d	24.1 e	19.0 bc	25.3 abc
Ni30 Pb15	3.2 fg	11.5 efg	7.4 d	22.5 e	9.1 d	27.3 ab
Ni30 Pb30	6.5 a-e	23.5 b	13.7 a	37.5 bc	24.6 ab	29.8 a
Ni30 Pb45	5.1 c-f	9.9 gh	12.1 ab	33.1 cd	22.9 ab	24.4 a-d
Ni45 Pb0	8.2 a	25.8 b	7.6 d	34.8 bcd	29.1 a	27.5 ab
Ni45 Pb15	7.7 abc	18.7 c	7.6 d	32.3 d	26.0 ab	27.5 ab
Ni45 Pb30	7.9 ab	14.3 def	8.6 cd	32.9 cd	11.9 cd	22.0 a-e
Ni45 Pb45	6.4 a-e	11.2 efg	7.0 d	45.7 a	9.6 d	27.5 ab

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.56. Effects of nickel, lead and their interactions mean of some anatomical characteristics of R. pseudoacacia stem and root at 4x.

Treatments	Stem (Thickness micron)					Root (Thickness micron)			
	Periderm	Cortex	Fiber	Phloem	Xylem	Periderm	Cortex	Phloem	Xylem
Nickel con. (mg.kg^{-1} soil)									
Ni 0	8.2 c	11.1 b	6.5 a	12.3 b	39.9 a	7.6 b	39.2 a	9.3 c	6.9 c
Ni 15	10.8 b	11.2 b	6.8 a	12.7 b	40.1 a	7.8 b	42.3 a	17.3 b	7.7 bc
Ni 30	12.1 b	11.5 b	7.2 a	16.2 a	42.6 a	7.7 b	40.8 a	22.9 a	8.2 ab
Ni 45	15.3 a	13.4 a	7.0 a	17.2 a	42.5 a	12.4 a	42.3 a	19.4 b	8.8 a
Lead con. (mg.kg^{-1} soil)									
Pb 0	10.0 c	9.6 d	5.8 c	12.4 b	40.6 b	9.1 b	45.0 a	17.4 a	8.3 a
Pb 15	12.3 b	11.2 c	6.5 bc	12.1 b	40.0 b	8.4 b	36.6 b	12.7 b	6.7 b
Pb 30	10.1 c	13.8 a	8.0 a	16.6 a	38.7 b	7.0 c	37.2 b	19.1 a	8.0 a
Pb 45	14.1 a	12.5 b	7.2 ab	17.3 a	45.8 a	11.1 a	45.9 a	19.7 a	8.4 a
Interaction between Ni & Pb con.									
Ni0 Pb0	6.0 f	10.2 fg	4.7 d	12.5 bcd	43.7 ab	9.1 cde	44.8 abc	8.1 g	6.2 de
Ni0 Pb15	10.6 e	12.0 def	6.2 bcd	7.5 d	35.8 c	4.7 h	33.2 f	12.5 efg	5.1 e
Ni0 Pb30	4.1 f	11.0 efg	8.6 ab	15.8 b	36.0 c	5.3 gh	34.0 f	9.4 fg	8.0 bcd
Ni0 Pb45	12.2 de	11.0 efg	6.4 bcd	13.3 bc	44.2 a	11.5 bc	45.0 abc	7.6 g	8.2 bcd
Ni15 Pb0	12.4 cde	9.4 fg	6.9 a-d	9.9 cd	38.3 bc	5.9 fgh	41.6 a-e	16.8 cde	8.0 bcd
Ni15 Pb15	11.5 de	8.6 g	6.6 a-d	11.9 bcd	46.6 a	8.9 de	37.4 def	10.7 fg	7.2 cd
Ni15 Pb30	4.1 f	15.0 bc	7.1 abc	13.0 bc	28.2 d	5.9 fgh	43.3 a-d	19.9 cd	7.7 bcd
Ni15 Pb45	15.4 c	11.6 ef	6.6 a-d	15.8 b	47.4 a	10.6 bcd	46.7 ab	21.6 c	7.7 bcd
Ni30 Pb0	11.0 de	5.5 h	6.5 a-d	12.2 bcd	45.9 a	6.8 e-h	48.7 a	22.3 c	9.0 abc
Ni30 Pb15	3.8 f	14.4 bcd	4.8 cd	15.6 b	32.9 cd	8.1 def	35.8 ef	14.9 def	6.6 de
Ni30 Pb30	18.3 b	9.5 fg	8.6 ab	13.3 bc	45.5 a	8.4 def	33.2 f	18.1 cde	7.0 d
Ni30 Pb45	15.3 c	16.6 b	8.8 a	23.2 a	46.1 a	7.6 efg	45.0 abc	36.4 a	10.2 a
Ni45 Pb0	10.7 e	13.1 cde	5.1 cd	15.1 bc	34.6 c	14.5 a	44.7 abc	22.2 c	10.1 a
Ni45 Pb15	23.1 a	9.8 fg	8.5 ab	13.0 bc	44.2 a	11.8 b	40.1 b-f	12.9 efg	7.9 bcd
Ni45 Pb30	13.8 cd	19.7 a	7.6 ab	23.8 a	45.3 a	8.5 def	38.3 c-f	29.2 b	9.4 ab
Ni45 Pb45	13.5 cd	10.8 efg	6.9 a-d	16.9 b	45.4 a	14.8 a	46.4 ab	13.4 efg	7.6 bcd

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

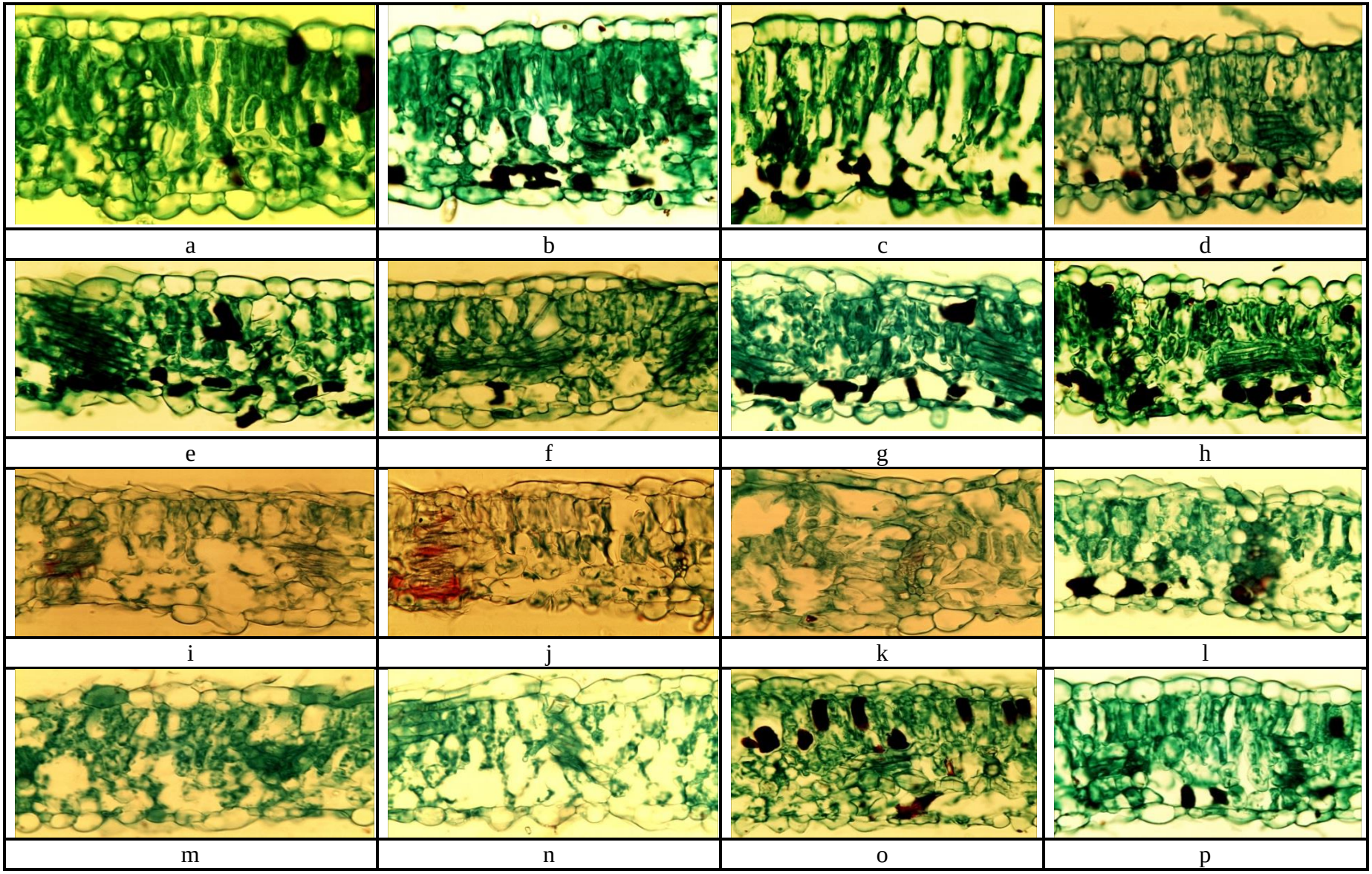


Fig 4.12. *Leaves (lamina) anatomy of R. pseudoacacia at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

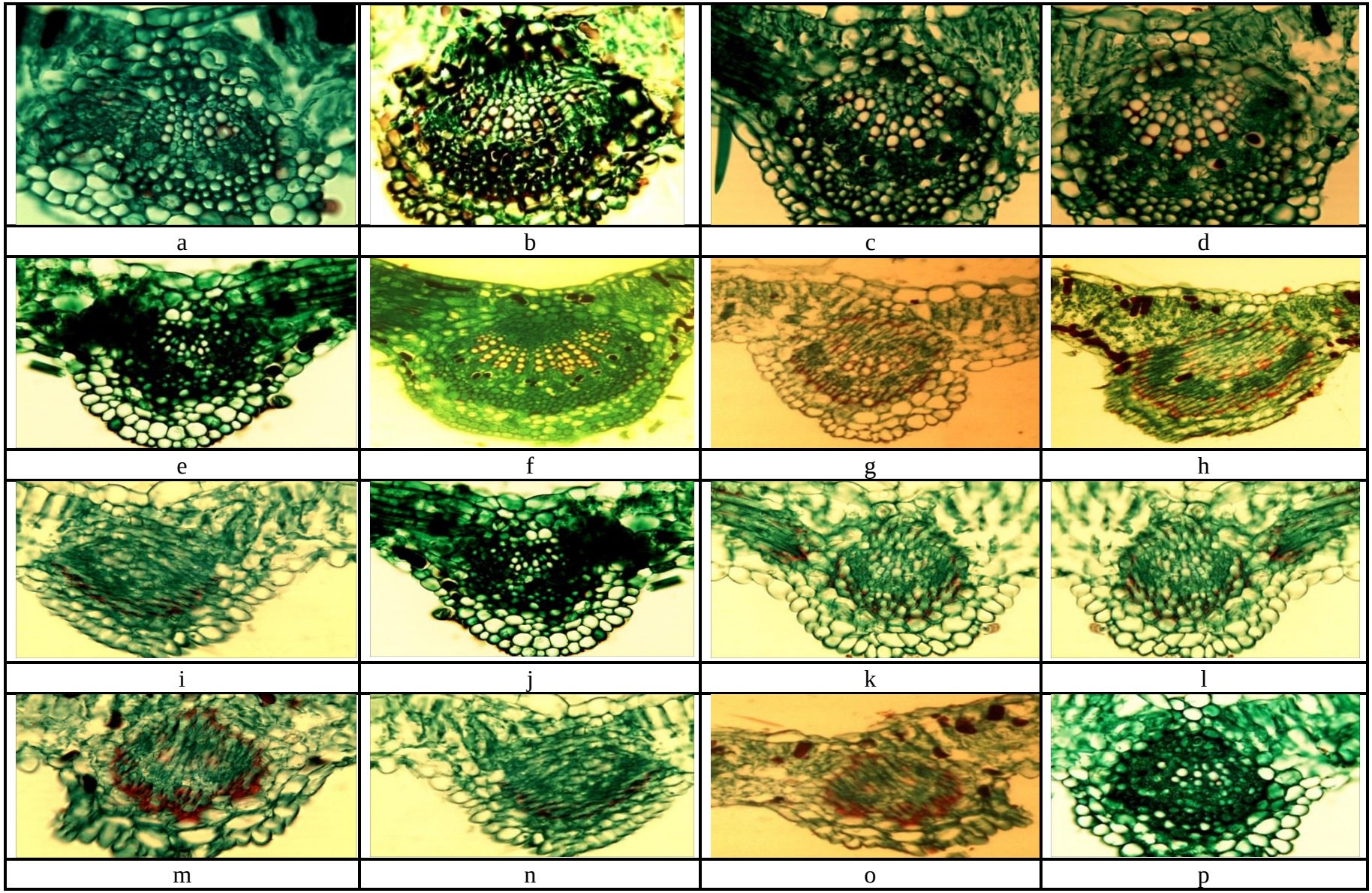


Fig 4.13. *Leaves (midrib) anatomy of R.pseudoacacia at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

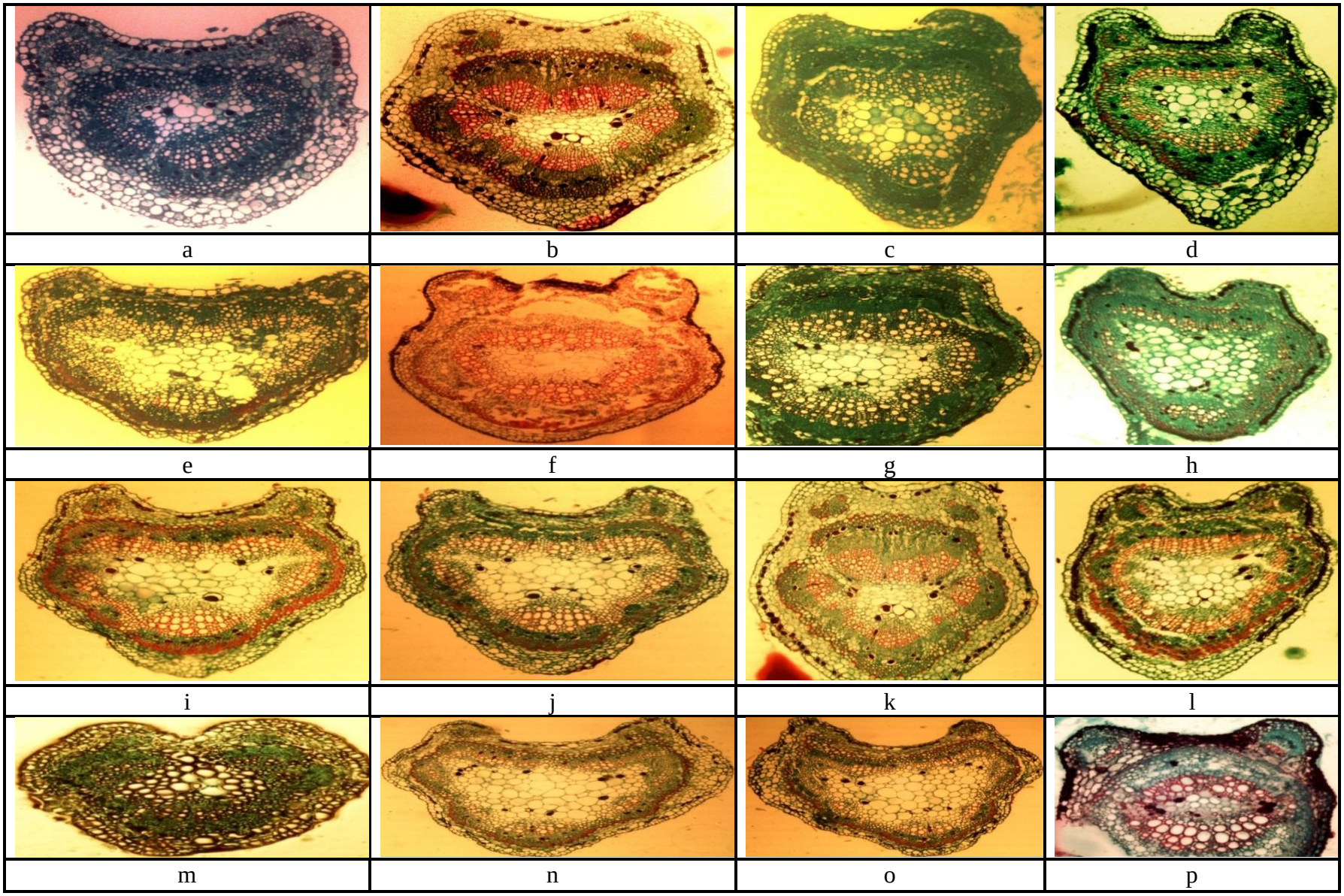


Fig 4.14. *Petiole anatomy of R. pseudoacacia at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

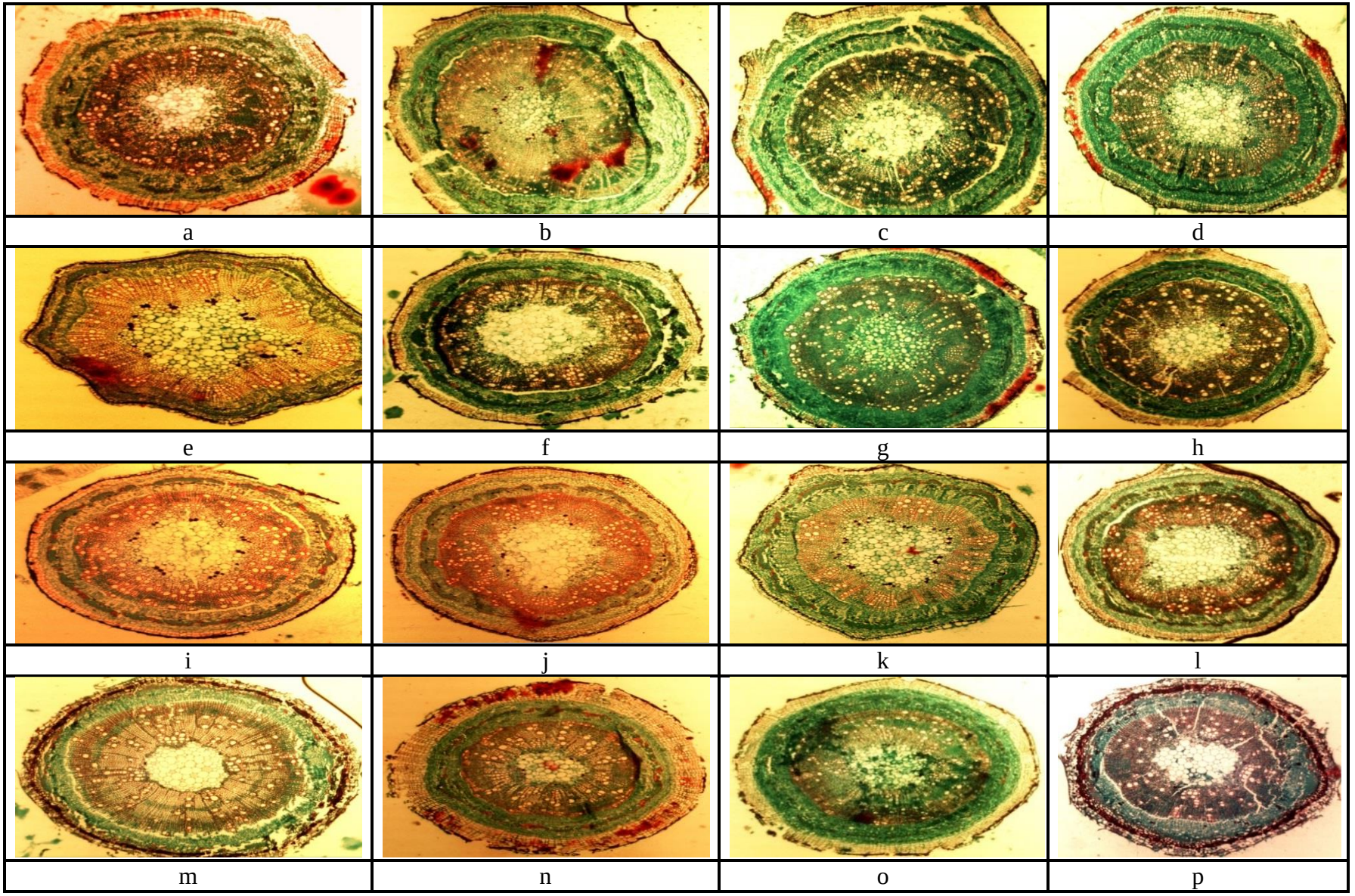


Fig 4.15*. Stem anatomy of R. pseudoacacia at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

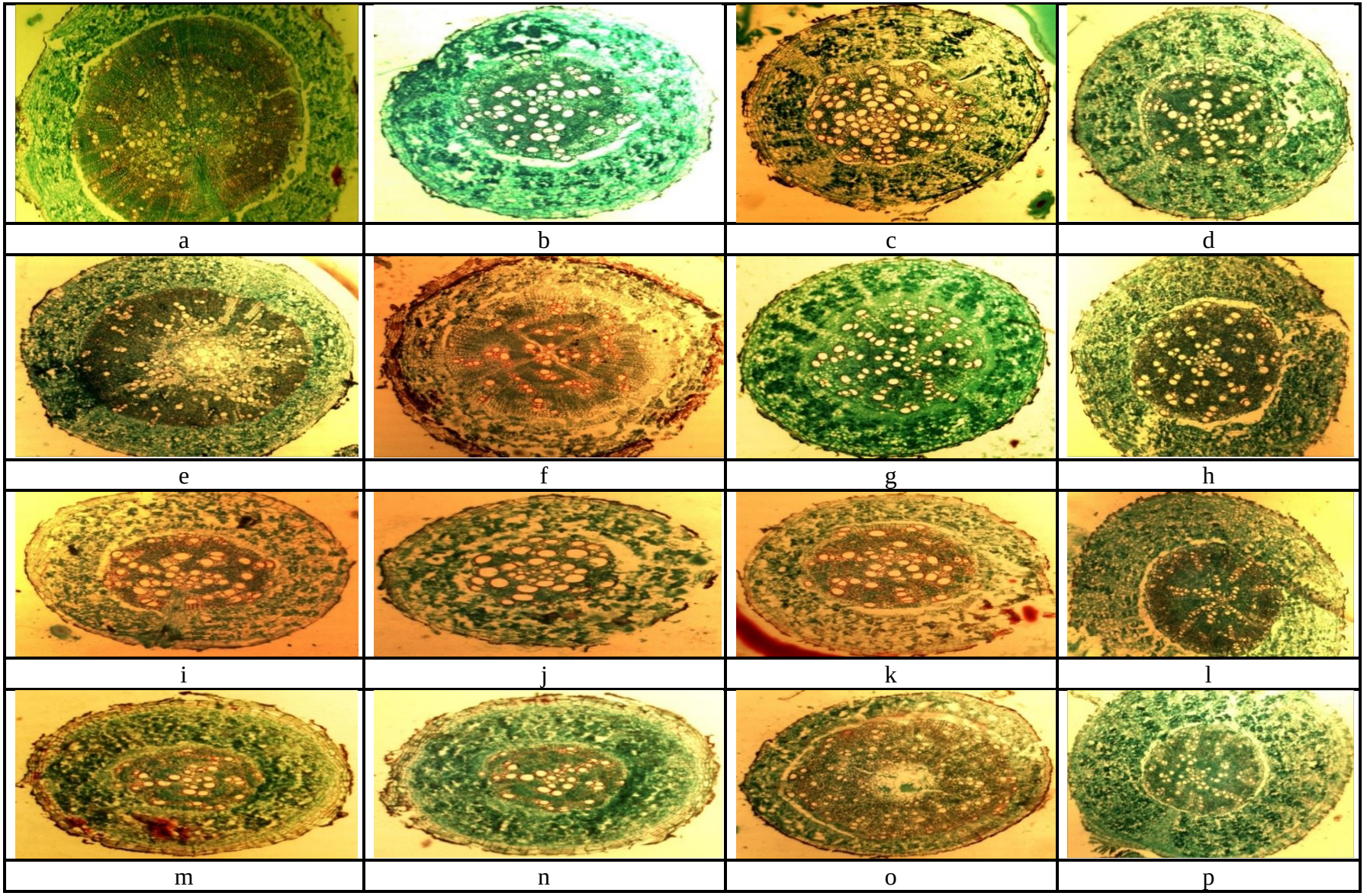


Fig 4.16. *Root anatomy of R. pseudoacacia at 4x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg^{-1} soil.*

4.4.2 Stomata characterestics (number, length and width) of stomata in upper (adaxial) and lower (abaxial) surfaces of *G. triacanthos, L. leucocephala* and *R. pseudoacacia*

Results in the tables 4.57, 4.58 and 4.59 show that the effect of Ni and Pb were significant on some of stomata characterestics such as number, length and width of stomata in upper and lower surface for each of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* leaves.

G. triacanthos genus there were no stomata on the upper leaves surfaces. Length stomata of lower surface of *G. triacanthos* decreased significantly with increasing Ni concentrations for 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment. The highest value of stomata length in the lower surface of *G. triacanthos* species was (36.8 micron) at 40x to the control treatment, while the lowest value of stomata length in the lower surface was (34.3 micron) at 40x for 45 $mg.kg^{-1}$ treatment, whereas the number and width of stomata which were non significant. The number, length and width of stomata in the lower surface of *G. triacanthos* species affect non significantly with Pb concentrations. The interactions between concentrations of Ni and Pb had significant effects and decreased with increasing Ni and Pb concentrations especially for 30 or 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the number and length of stomata in the lower surface of *G. triacanthos*, except the width of somata which was non significant (Table 4.57) and (Figures 4.2 and 4.3).

The number of stomata in the upper and lower surfaces and length of stomata on the upper surface of *L. leucocephala* were significantly decreased with increasing Ni concentrations to 45 $mg.kg^{-1}$ $NiCl_2$ concentrations. The highest value of the number of stomata in the upper and lower surfaces and length of stomata on the upper surface of *L. leucocephala* (5.2, 11.1 and 29.7 micron) at 40x for the control treatment, while the lowest values (3.6, 8.8 and 26.1 micron) for the 45 and 30 $mg.kg^{-1}$ $NiCl_2$ concentrations, whereas width of stomata in the upper and lower surfaces and length of stomata in the lower surface which were non significant. The number of stomata in the upper and lower surfaces, width of stomata in the upper surface of *L. leucocephala* decreased with increasing the Pb concentrations for 30 or 45 $mg.kg^{-1}$ $PbCl_2$ concentrations as compared to the control treatment, whereas length of stomat in the lower surface increased with $PbCl_2$ concentration to 30 $mg.kg^{-1}$. Also length of stomata on the upper surface and with of stomata in the lower surface which were non significant. The interactions between concentrations of Ni and Pb had significant effects and decreased with increasing Ni and Pb concentrations for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the number of stomata in the upper and lower surfaces

and width in the upper surface, whereas length of stomata in the upper and lower surfaces and width of stomata in the lower surface which were non significant of *L. leucocephala* (Table 4.58) and (Figures 4.9 and 4.10).

The number of stomata in the upper and lower surfaces, length of stomata in the upper and lower surfaces and width of stomata in the lower surface of *R. pseudoacacia* significantly decreased with increasing Ni concentrations for 45 $mg.kg^{-1}$ $NiCl_2$. The highest value of stomata number in the upper and lower surfaces, length of stomata in the upper and lower surfaces and width of stomata in the lower surface of *R. pseudoacacia* (5.9, 9.5, 19.6, 25.6 and 15.4 micron) at 40x were recorded in the control treatment, while the lowest values (4.4, 7.6, 16.0, 18.1 and 11.8 micron) were recorded for 45 $mg.kg^{-1}$ $NiCl_2$ concentrations as compared to the control treatment, whereas width of stomata on the upper surface which was non significant. The number and width of stomata in the upper surface of *R. pseudoacacia* increased with increasing the $PbCl_2$ concentrations to 15 $mg.kg^{-1}$ as compared to the control treatment, also length of stomata in the upper and lower surfaces, number and width of stomata in the lower surface which were non significant. The interactions between concentrations of Ni and Pb had significant effects and decreased with increasing Ni and Pb concentrations to 30 or 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations on the number, length and width of stomata in the upper and lower surfaces of *R. pseudoacacia* (See Table 4.59 and Figures 4.16 and 4.17).

Table 4.57. Effects of nickel, lead and their interactions mean of abaxial stomata characteristics of G. triacanthos species at 40x.

Treatments	Number	Length	Width
		Micron	
Nickel con. (mg.kg^{-1} soil)			
Ni 0	10.83 a	36.8 a	20.4 a
Ni 15	10.33 a	36.8 a	20.3 a
Ni 30	10.17 a	35.0 ab	20.3 a
Ni 45	10.08 a	34.3 b	20.2 a
Lead con.(mg.kg^{-1} soil)			
Pb 0	10.8 a	37.1 a	20.2 a
Pb 15	10.0 a	35.4 a	19.6 a
Pb 30	10.3 a	35.4 a	20.2 a
Pb 45	10.3 a	35.1 a	21.2 a
Interaction between Ni & Pb con.			
Ni0 Pb0	11.7 ab	41.09 a	20.6 a
Ni0 Pb15	11.7 ab	36.2 bc	18.9 a
Ni0 Pb30	11.0 ab	35.0 bc	21.6 a
Ni0 Pb45	9.0 cd	34.9 bc	20.7 a
Ni15 Pb0	10.7 abcd	36.0 bc	18.7 a
Ni15 Pb15	10.3 abcd	37.0 abc	21.6 a
Ni15 Pb30	11.0 abc	38.5 ab	19.5 a
Ni15 Pb45	9.3 bcd	35.5 bc	21.5 a
Ni30 Pb0	10.0 abcd	35.3 bc	21.8 a
Ni30 Pb15	8.3 d	33.7 bc	17.5 a
Ni30 Pb30	10.3 abcd	35.7 bc	19.9 a
Ni30 Pb45	12.0 a	35.4 bc	21.8 a
Ni45 Pb0	10.7 abcd	35.7 bc	19.8 a
Ni45 Pb15	9.7 abcd	34.6 bc	20.3 a
Ni45 Pb30	9.0 cd	32.5 c	19.9 a
Ni45 Pb45	11.0 abc	34.4 bc	20.8 a

* There were no stomata at the adaxial leaves surfaces.

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.58. Effects of nickel, lead and their interactions mean of some stomata characteristics of L. leucocephala species at 40x.

Treatments	Adaxial stomata			Abaxial stomata		
	Number	Length	Width	Number	Length	Width
	Micron					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	5.2 a	29.7 a	15.5 a	11.1 a	33.2 a	18.9 a
Ni 15	4.2 b	26.5 b	15.0 a	10.0 b	31.8 a	18.1 a
Ni 30	3.6 c	26.1 b	13.8 a	9.4 bc	30.0 a	17.5 a
Ni 45	3.6 c	26.3 b	14.6 a	8.8 c	31.3 a	16.9 a
Lead con. (mg.kg^{-1} soil)						
Pb 0	4.4 a	26.0 a	16.9 a	10.7 a	30.0 b	17.3 ab
Pb 15	4.0 ab	28.3 a	13.8 b	9.4 bc	30.2 b	16.4 b
Pb 30	3.7 b	26.3 a	13.4 b	9.0 c	33.6 a	18.9 a
Pb 45	4.4 a	27.9 a	14.8 b	10.2 ab	32.4 ab	18.9 a
Interaction between Ni & Pb con.						
Ni0 Pb0	6.3 a	28.1 ab	16.5 abc	11.7 ab	33.2 ab	19.4 a
Ni0 Pb15	5.0 bc	29.1 ab	14.8 abc	11.0 a-d	30.9 ab	17.1 a
Ni0 Pb30	4.3 cd	27.8 ab	13.1 c	9.3 c-f	34.7 a	19.8 a
Ni0 Pb45	5.0 bc	33.9 a	17.7 ab	12.3 a	34.0 ab	19.2 a
Ni15 Pb0	5.7 ab	25.7 b	18.3 a	10.3 b-e	27.6 b	16.7 a
Ni15 Pb15	2.0 f	26.9 b	13.3 c	10.3 b-e	31.9 ab	16.4 a
Ni15 Pb30	4.3 cd	26.6 b	13.8 bc	8.7 efg	33.4 ab	19.2 a
Ni15 Pb45	4.7 cd	26.9 b	14.7 abc	10.7 a-e	34.2 ab	20.1 a
Ni30 Pb0	3.0 ef	26.4 b	16.0 abc	11.3 abc	29.4 ab	16.7 a
Ni30 Pb15	4.7 bcd	28.3 ab	13.0 c	7.3 g	27.4 b	15.9 a
Ni30 Pb30	2.3 f	25.0 b	13.4 c	9.3 c-f	32.9 ab	19.0 a
Ni30 Pb45	4.3 cd	24.7 b	12.9 c	9.7 c-f	30.2 ab	18.6 a
Ni45 Pb0	2.7 ef	24.0 b	16.9 abc	9.3 c-f	29.8 ab	16.2 a
Ni45 Pb15	4.3 cd	28.8 ab	14.3 abc	9.0 d-g	30.6 ab	16.2 a
Ni45 Pb30	3.7 de	26.0 b	13.3 c	8.8 efg	33.4 ab	17.6 a
Ni45 Pb45	3.7 de	26.2 b	13.4 bc	8.0 fg	31.3 ab	17.7 a

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

Table 4.59. Effects of nickel, lead and their interactions mean of some stomata characteristics of R. pseudoacacia species at 40x.

Treatments	Adaxial stomata			Abaxial stomata		
	Number	Length	Width	Number	Length	Width
	Micron					
Nickel con. (mg.kg^{-1} soil)						
Ni 0	5.9 a	19.6 a	12.0 a	9.5 a	25.6 a	15.4 a
Ni 15	5.6 a	18.6 ab	11.9 a	8.4 b	22.9 b	12.7 b
Ni 30	5.5 a	18.2 b	10.8 a	8.2 bc	18.5 c	11.9 b
Ni 45	4.7 b	16.0 c	10.9 a	7.6 c	18.1 c	11.8 b
Lead con. (mg.kg^{-1} soil)						
Pb 0	4.9 b	18.2 a	10.6 b	8.3 a	22.0 a	12.5 a
Pb 15	6.3 a	17.4 a	12.8 a	8.8 a	21.6 a	13.0 a
Pb 30	5.4 b	18.1 a	10.6 b	8.0 a	20.7 a	12.5 a
Pb 45	5.1 b	18.6 a	11.7 ab	8.6 a	20.7 a	13.4 a
Interaction between Ni & Pb con.						
Ni0 Pb0	6.3 a	17.1 def	12.3 abc	7.7 cde	25.6 a	15.2 abc
Ni0 Pb15	6.7 a	17.8 def	13.4 ab	9.3 bc	25.6 a	16.6 a
Ni0 Pb30	6.3 a	22.0 a	11.0 abc	8.0 bcde	25.5 a	14.2 a-d
Ni0 Pb45	4.3 cd	21.3 ab	11.5 abc	13.0 a	25.6 a	15.5 ab
Ni15 Pb0	5.3 abc	21.0 abc	10.8 abc	8.3 bcd	22.4 bc	15.1 abc
Ni15 Pb15	6.7 a	17.8 def	14.4 a	9.7 b	24.5 abc	9.5 d
Ni15 Pb30	4.7 bcd	17.3 def	10.2 bc	9.3 bc	21.3 cd	12.8 a-d
Ni15 Pb45	5.7 abc	18.3 cde	12.2 abc	6.3 e	24.5 ab	13.5 a-d
Ni30 Pb0	4.3 cd	19.7 abcd	9.1 c	9.3 bc	19.4 de	11.0 bcd
Ni30 Pb15	6.3 a	16.7 def	11.6 abc	8.7 bcd	20.8 cd	12.3 a-d
Ni30 Pb30	5.3 abc	17.7 def	10.8 abc	7.7 cde	17.6 ef	11.2 bcd
Ni30 Pb45	6.0 ab	18.5 cde	11.6 abc	7.0 de	16.0 f	13.1 a-d
Ni45 Pb0	3.7 d	14.8 f	10.3 cd	8.0 bcde	20.6 cd	10.4 cd
Ni45 Pb15	5.3 abc	17.3 def	11.9 abc	7.3 de	16.6 ef	13.1 a-d
Ni45 Pb30	5.3 abc	15.3 f	10.2 bc	7.0 de	18.5 def	11.8 a-d
Ni45 Pb45	4.3 cd	16.4 ef	11.3 abc	8.0 bcde	16.7 ef	11.4 bcd

* Means followed by the same letters within columns are significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

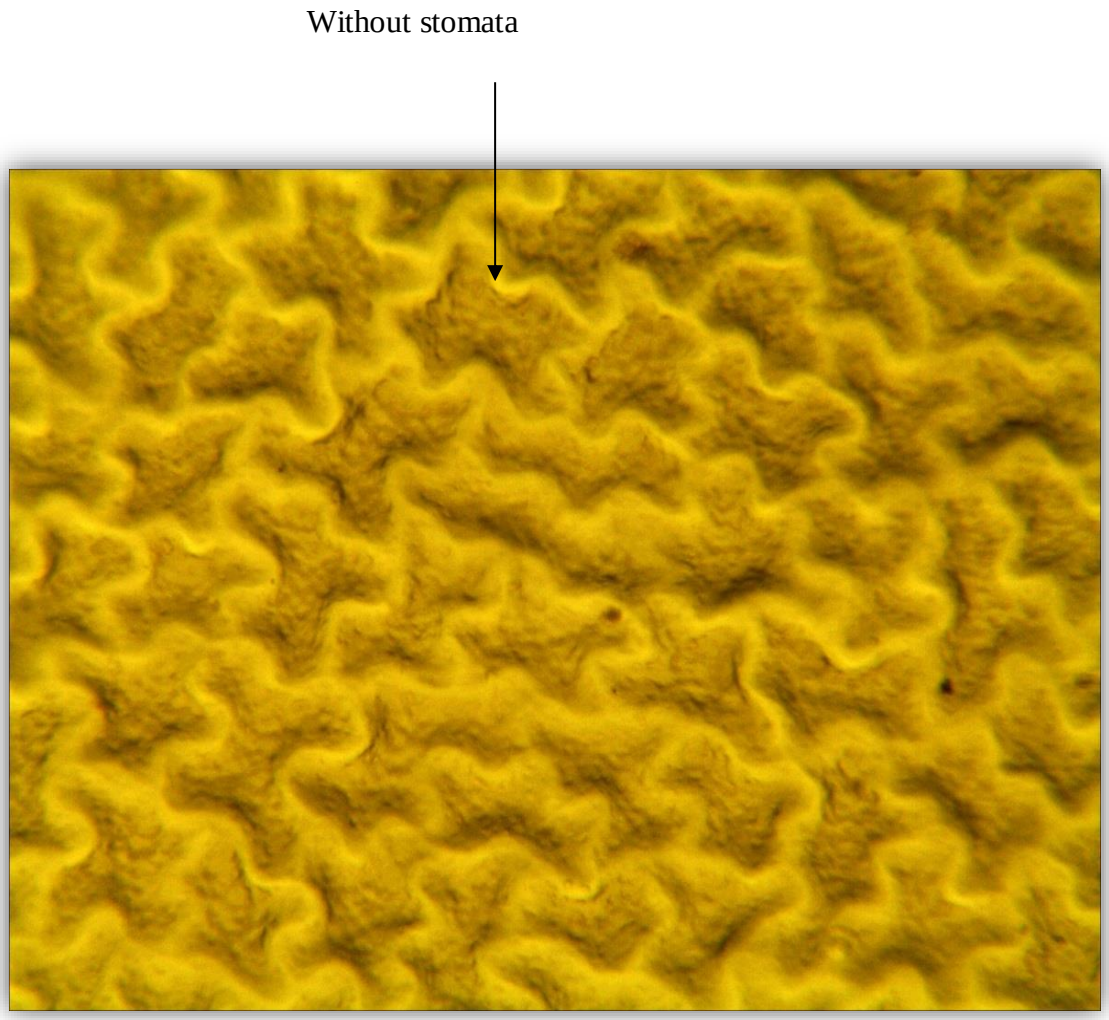


Fig 4.17. *Upper (adaxial) leaf surface of G. triacanthos for the control treatment at 40x, (Ni0Pb0 mg.kg^{-1} soil).*

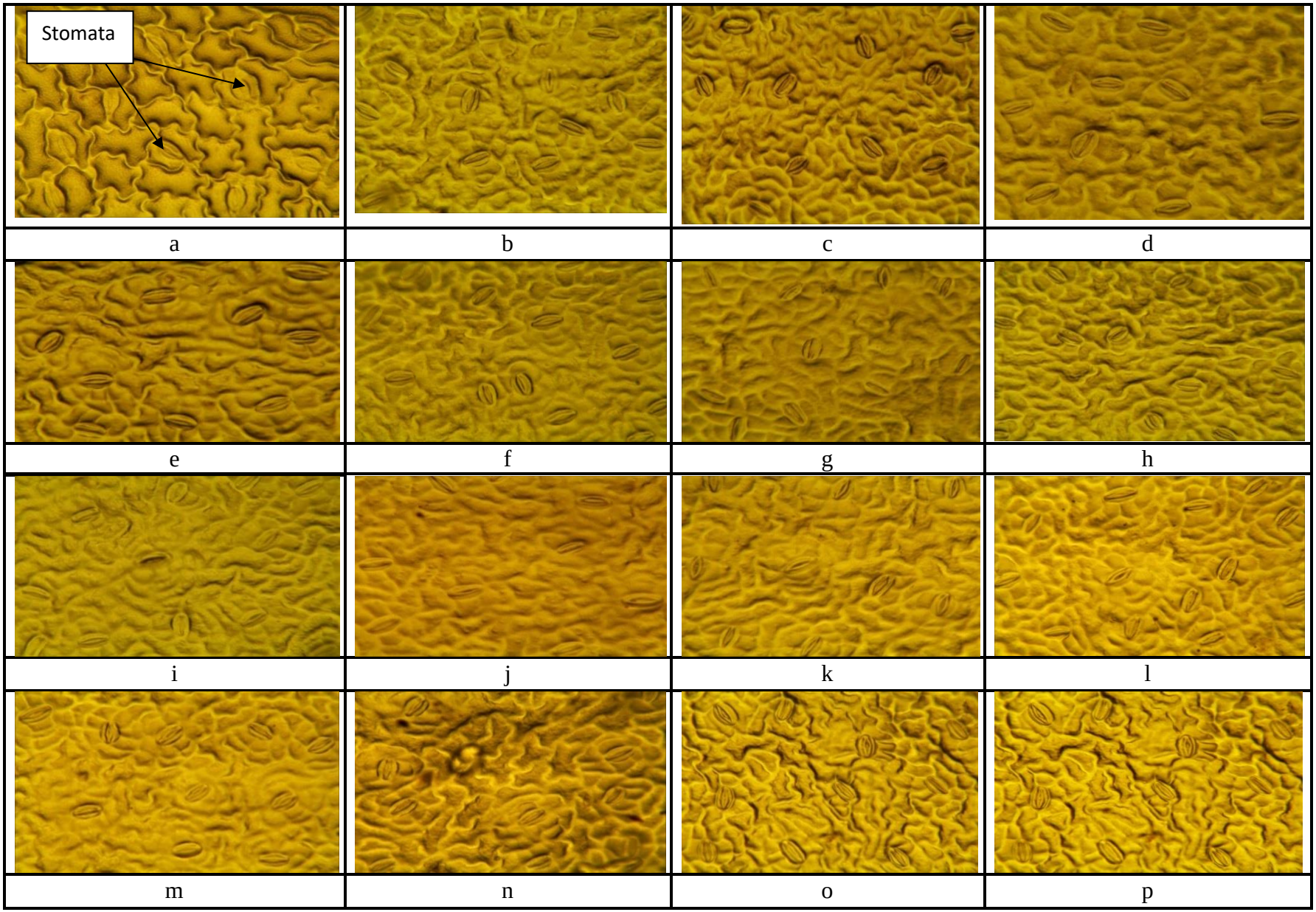


Fig 4.18. *Stomata of lower (abaxial) leaves surfaces of G. triacanthos at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

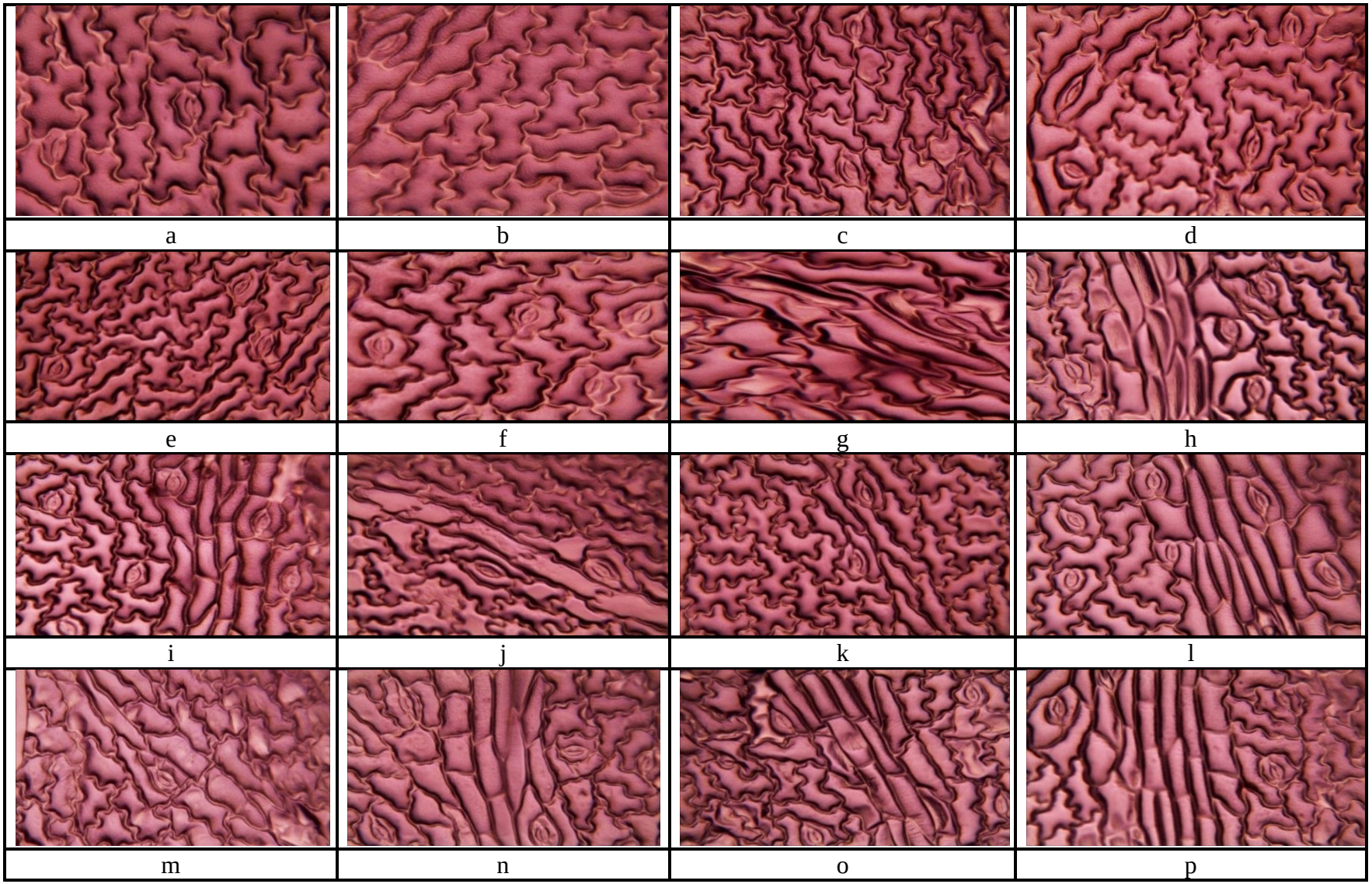


Fig 4.19. *Stomata of upper (adaxial) leaves surfaces of L. leucocephala at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

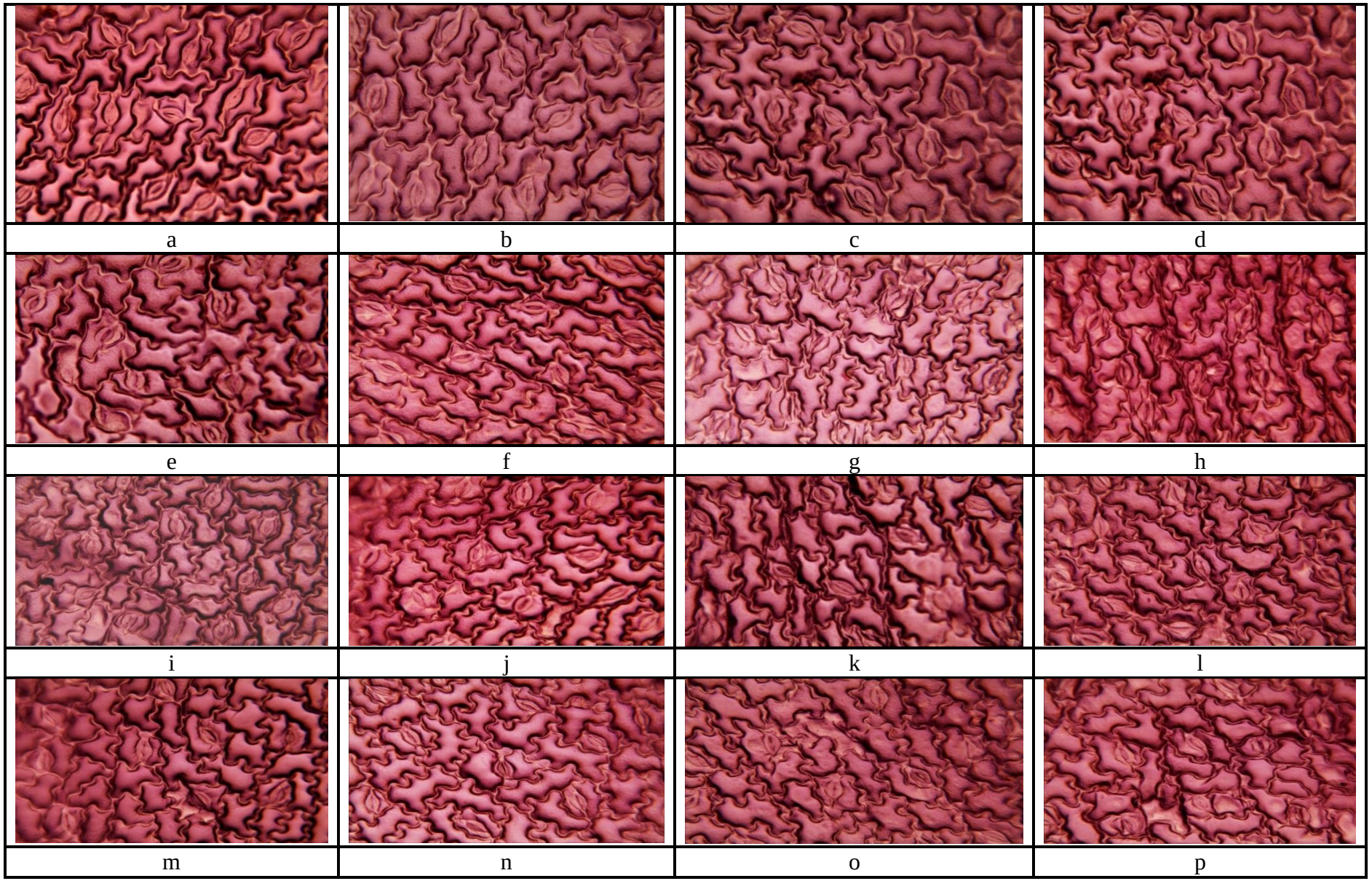


Fig 4.20. *Stomata of lower (abaxial) leaves surfaces of L. leucocephala at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-^{1} soil.*

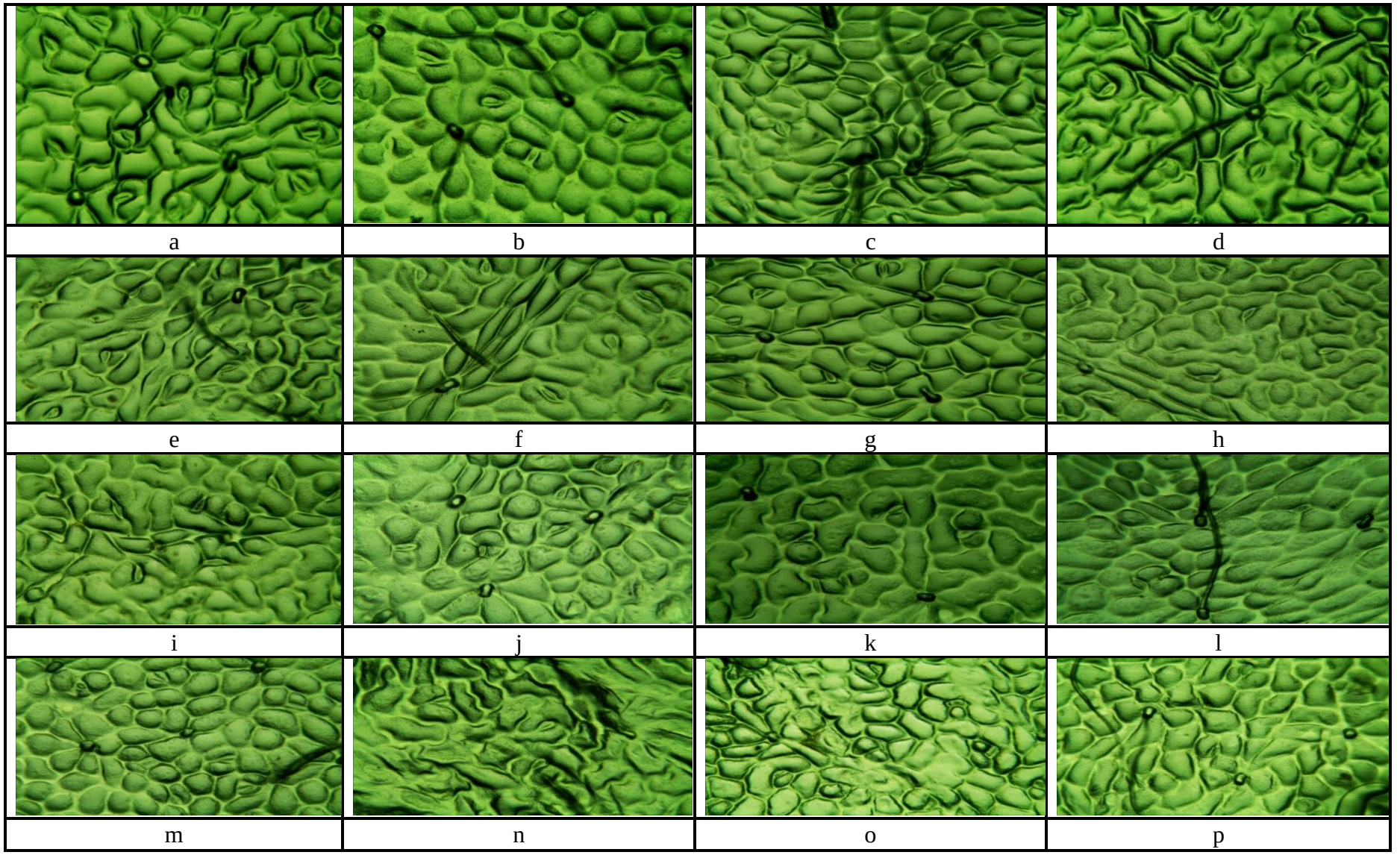


Fig 4.21. *Stomata of upper (adaxial) leaves surfaces of R. pseudoacacia at 40x; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg^{-1} soil.*

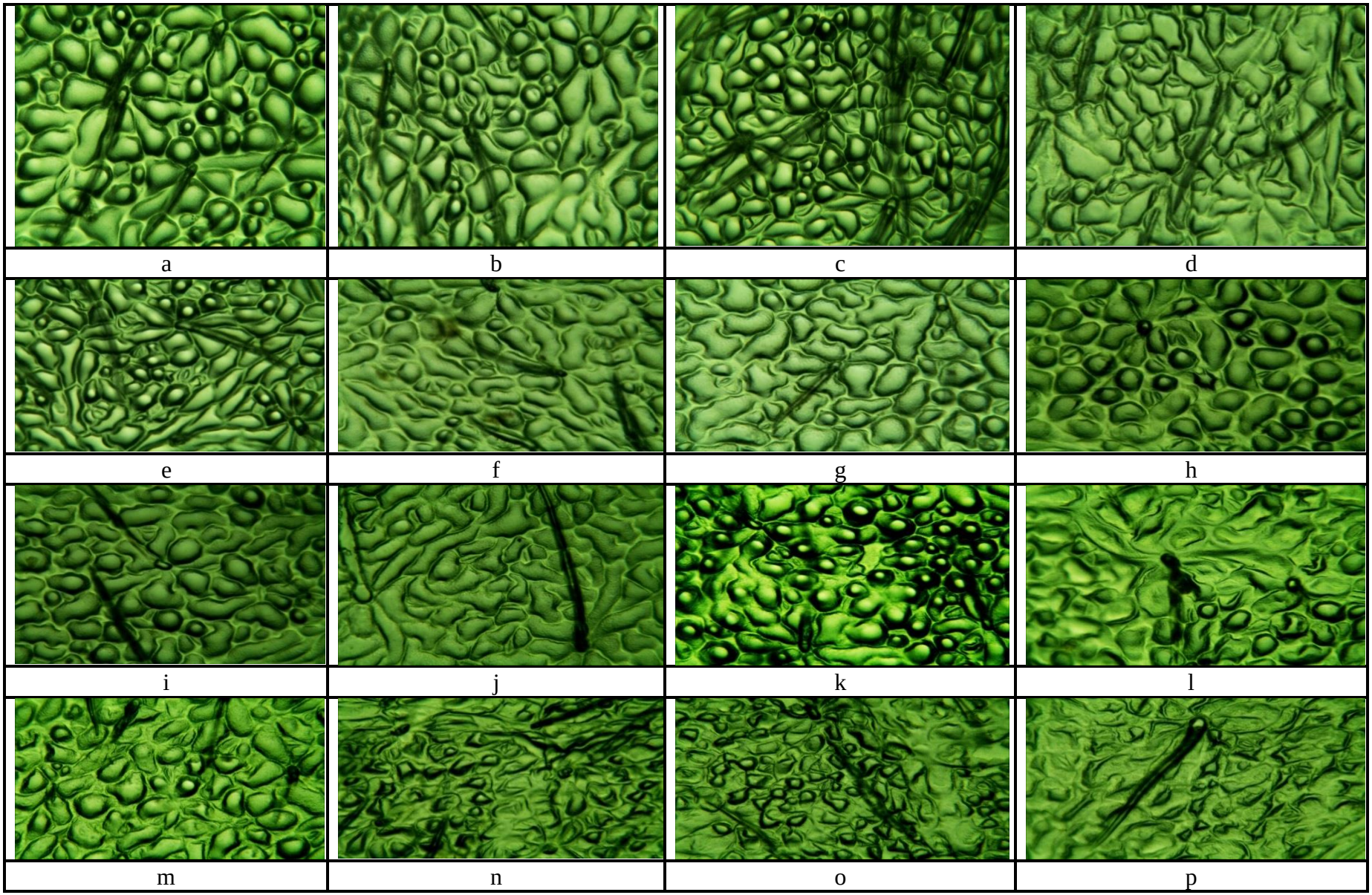


Fig 4.22. *Stomata of lower (abaxial) leaves surfaces of R. pseudoacacia at 40x ; (a. Ni0Pb0, b. Ni0Pb15, c. Ni0Pb30, d. Ni0Pb45, e. Ni15Pb0, f. Ni15Pb15, g. Ni15Pb30, h. Ni15Pb45, i. Ni30Pb0, j.Ni30Pb15, k. Ni30Pb30, l. Ni30Pb45, m. Ni45Pb0, n. Ni45Pb15, o. Ni45Pb30, p. Ni45Pb45) mg.kg-[1] soil.*

Chapter Five

Discussions

5.1 Effects of nickel, lead and their interactions on seed performance and some vegetative growth of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia*

Low seeds germination for the studied species were clear which may be due to germination condition especially the high temperature, the average temperature during the germination period was April – May (Table 3.1) that is more than the optimal temperature for most seeds germination. Regarding the results of these species after seeds soaking for 24 hours, it was seen that the seeds coat of *G. triacanthos*, was more rigid than *R. pseudoacacia* seeds, which is more rigid than *L. leucocephala* seeds. This property is from the results of Tables 4.1, 4.2 and 4.3 where it was seen that the effect of Ni and Pb on *G. triacanthos* germination performance was less than other species, which may due to the accumulation of the metals in the coat of seeds, while the endosperm appeared to be lacking of Ni and Pb, and the coats of seed can decrease the amount of Ni and Pb entering the seeds (Le´on *et al.*, 2005). Decreasing in germination velocity is clear where Ni is used for the three species because Ni is considered as one of the essential mineral elements for plants which is required in small amounts for healthy growth and development, Kun *et al.* (2021), Joseph *et al.* (2018); Abdel-Aziz *et al.* (2014). In many plant species, increasing concentrations of Ni inhibit and delay seed germination and seedling growth, generally due to the suppression of amylase and protease activity (Sergin and Kozhevnikova, 2006). Figure 3.2 it is shows that the growth of each of *L. leucocephala* and *R. pseudoacacia* species was more than that of *G. triacanthos* species, and this may due to genetic factors and the existence of root nodules, which the host plants deliver energy as photosynthate, on the other hand receives nitrogen for its growth and development (Yusuf *et al.*, 2011). The results from table (4.1) indicate that Ni or Pb or their interactions had significant effects on some seeds performance and vegetative growth parameters. The percentage of seed germination was non significant, but velocity of germination was significantly decreased with increasing concentrations of the Ni, Pb and their interactions as compared to the control treatment. Morphologyical parameters such as plant leaves number, plant leaf area, root length and root diameter were significantly decreased with increasing Ni concentrations as compared to the control. Moreover, increasing levels of nickel chloride decreased seed germination and the growth of plant and cause toxicity because nickel is one of the essential micronutrients for germination of seeds and subsequent plant growth at low

concentrations; whereas it becomes toxic with high concentrations in the plant and soil (Pavlova *et al*., 2018). However the efficacy of elements tolerance depends on the species of the plant Singh (2020) and Anwar *et al.* (2022). Also, the plant height and root diameter decreased with Pb application, except leaf area which was increased with Pb concentrations as compared to the control treatment. Whereas, increasing levels of Pb element inhibits and delays seedlings growth, it inhibits photosynthesis process, declines in minerals nutrition, growth stunting, and other symptoms, and may lead to a cell death at high level concentrations. All these disorders distressed normal physiological activities of the plant (Fazal, 2015). The interactions between Ni and Pb concentrations on the leaf area and root diameter were significant and decreased with increasing $NiCl_2$ and $PbCl_2$ concentrations, except plant hight and plant leaves number were increased with increasing $NiCl_2$ and $PbCl_2$ concentrations. The results show that Ni and Pb were less toxic metals for *G. triacanthos* species. They also show that the low concentrations of nickel chloride are required for the normal growth of plants. Whereas, increasing in nickel chloride levels will reduce seed germination and subsequent plant growth as a result to toxicity (Ahmad *et al*., 2009). However the tolerance efficiency will depends on the species of the plant. From the Figure (4.1), it is clear that *G. triacanthos* has no root nodules, where *Gledistsia* genus is phyllogenetically located at the base of the fabaceae family where there is no nodules among the species of this genus (Faria *et al.*, 2002), however the root form some hypertrophic outgrowth, where there are bacteria found inside structures which are very like to infection threads in legumes, and nitrogenase enzyme is present in this material (Faria *et al.*, 2002). Same results were gained by many researchers. Existing of roots nodules reflects on the activity of nitrate reductase enzyme, where it behaved inversely and the highest activity were recorded in G. *triacanthos* species followed by *L. leucocephala* and *R. pseudoacacia* species.

L. leucocephala species, results of table (4.2) indicated that decrease in the percent of seed germination may due to harmful effects of Ni and Pb on the mineral nutrients content, sugar transport, water balance, distract membranes function and ion balance of cytoplasm particularly K^+ ion (Kumar *et al*., 2019). This study appears that the velocity of germination significantly increased with Ni application (Zayed *et al.*, 2012). The results indicate that the effect of Ni was toward increasing the vegetative growth, compared to the Pb application, and this may due to Ni is consider as an essential element for the growth and development of plants, whereas Pb is not, because Ni at low concentrations is one of essential micronutrients for seeds germination and plant growth, yet, its toxicity appears with high concentration in the soil and plants, although tolerance efficiency depends on the plant species (Fazal, 2015). Excessive amount of toxic elements caused decrease in germination of seeds and growth of plants, whereas Pb is one

of the heavy metals not essential in cell metabolism processes, but its absorption is very easy; thus so they accumulated in different parts of plants. Pb element inhibits seed germination, also delays seedlings growth, inhibits photosynthesis process, declines in minerals nutrition, growth stunting, and other symptoms, and may lead to a cell death at high level concentrations. The root cells are responsible for the transport of lead from the external medium to the interior of the cell using the cation channels of the plasma membrane, in particular the Ca^{+2} channels (Seregin and Kozhevnikova, 2008). All these disorders distressed normal physiological activities of the plant (Fazal, 2015; Sharma and Dubey, 2005). The plants already developed many tolerance mechanisms that are triggered as a response to the exposure to Pb element, this element usually affects plant root systems which respond rapidly to either by callus synthesis and deposition, ending Pb entering by creating a barriers, or uptakes large amount of Pb then restoration in the vacuole which supplemented many changes in root branching and growth, and finally by translocation the Pb to the hyper-accumulators plant shoots. The inhibitory effect of the studied heavy metals Pb on seed germination could be the result of ionic toxicity on emryo viability and/or due to an osmotic effect of tested solutions which impairs water uptake by seeds, the most important factor for germination process. Many other possible causes of germination inhibition under heavy metal stress have been reported in previous studies: alteration of selection permeability properties of cell membrane, accelerated breakdown of stocked nutrients in the endosperm (Shafiq *et al.*, 2008). The plant species *L. leucocephala* was more tolerant to the increase of Ni and Pb concentrations levels. Some species can grow at higher metal concentrations, which are highly toxic to other species. The results show that Ni and Pb were less toxic metals for *L. leucocephala* plant species. The same result was described by Shweti *et al.* (2018) that Ni at low concentration is considered essential micronutrient for seed germination and the growth for wheat (0.5, 1, 2, 4, 6 and 8 mM Ni as $NiCl_2.6H_2O$). However, toxicity will appears with high concentration in the soil and plants. In addition Pavlova *et al*. (2018) studied the effect of Ni on *Alyssum markgrafii* and *A. mural* seeds and where the ability to germination decreased with increasing the Ni concentrations, whereas hypocotyl elongation were stimulated at low Ni concentrations, but the root elongation was inhibited for both species, which firm that the plant roots were more sensitive than hypocotyl to Ni element. Because it appears toxicity with high concentrations in the soil and plants; However, tolerance efficiency depends on the plants species. The same results were obtained by Shweti *et al.* (2018) who established that low concentrations of Ni are considered as stimulator for wheat seed emerging, whereas, high concentrations were toxic, where it decreased significantly other plant characteristics, which may due to activation of the stores to the rest of the root nodules, prompting root

nodules dry weight (Sahu *et al.,* 2020). Where Pb treatment causes a significant reduction in the seed germination, length of shoot and many other characteristics when compared to the control treatment (Mehboob *et al.,* 2018, Muhammad *et al.,*2008, Seregin and Kozhevnikova, 2006; Le´on *et al.,* 2005).

The results in table 4.3 demonstrat that little amount of nickel chloride is required for stimulating *R. pseudoacacia* seed performance and seedling growth, whereas increasing levels of nickel chloride decreased seed germination and the growth of plant cause toxicity, because nickel is one of the essential micronutrients for germination of seeds and subsequent plant growth at low concentrations, yet it becomes toxic with high concentrations in the plant and soil. However the efficacy of elements tolerance depends on the species of the plant (Kabira *et al.,* 2018, Sharifi-Rad, 2017, Jaime *et al*., 2012; Singh *et al.,* 2010). Inhibition of root growth is clear in plants treated with Pb which agrees with Fazal, (2015) and Sharma and Dubey (2005). Differences were observed between all the studied plant species regarding most of the vegetative growth parameters because heavy metals uptake and accumulation vary even from species to species within a genus (Kun *et al*., 2021), and the contaminants distribution in a plant is controled by the plant physiological character, and the distribution depends on the mobility of the contaminant in the plant tissues, on the type of plant, and the conditions of growth (Štofejová *et al*, 2021). Results show that high concentrations of nickel were more toxic than lead, because of the higher differences between Ni treatments than that of Pb element. The results were agree with that of Ahmad *et al*. (2009), who established that Ni concentrations at low levels promoted significantly sunflower seed germination and improved the growth of seedling, fresh and dry weights and germinating of seeds, while higher concentrations caused a significant inhibition in seeds germination. Inversely Pb application causes a significant decrease in plant root length, because those plants grow under the stress of heavy metals suffering a shortage in the energy amount required for a good growth of the plants grown in stressed environment (Tai *et al.,* 2022; Kang *et al.,* 2018). Heavy metals might cause an inhibition in root growth that alters water balance and nutrient absorption, thereby affecting their transportation to the aboveground plant parts and thus negatively affecting shoot growth and ultimately decreasing biomass accumulation (Singh *et al.,* 2016). The reduction of biomass could be the result of a direct effect of Pb on photosynthesis or on the physiological processes of the plant (Sharma and Dubey, 2005). Metals and chemicals in higher concentration the plant germination, growth and production are mainly associated with the physiological, biochemical and genetic of the plant system (Sethy and Ghosh, 2013). Nodule growth may be linked to total plant growth and depend upon environmental factors (e.g. nitrate supply, salt stress, drought stress) which directly effects plant growth (Voisin *et al.,* 2003).

As it is clear from Figure 4.1 all the nodules for *L. leucocephala* and *R. pseudoacacia* species were distributed on secondary roots. The rhizobia existed inside nodules fixed the atmospheric N into ammonia which was considered a nitrogen source for the legumes. Increasing nodules number and dry matter and some other parameters for *L. leucocephala* and *R. pseudoacacia* species may be due to the existence of some rhizobia including that strains resistant to heavy metals and can stimulate the growth of plants under the stress of heavy metal (De Angelis *et al.,* 2022). Generally, it was noticed that effects of Pb was less compared to Ni because lead not an essential element and is considered relatively unavailable for living organisms due to the fact that immobilization in soil and the transportation from roots to plants is limited (Kacálková *et al.,* 2014). The results show that all three plant under HMs stress showed different decline in plant growth characterestics compared with the control. this may be due to the high content of heavy metals in the soil, which limited the uptake of nutrients by the plants thus leads to a decrease in plant growth.

5.2 Effects of nickel, lead and their interactions on some physiological characterestics of *G. triacanthos*, *L. leucocephala* and *R. pseudoacacia* species

5.2.1 Photosynthetic pigments

Results from table (4.4) shows that the effect of Ni, Pb and their interactions was significant on some photosynthetic pigments (chlorophyll a, b and total carotenoids) of the studied species. The chl a and b increased with increasing Ni and Pb concentrations. Total carotenoids decreased with increasing the elements concentration. The high Ni concentration has been found to change the photosynthetic pigments such as Chl a, Chl b and TC. Carotenoids are plant pigments that function as non-enzymatic antioxidants. They are the main pigments known to be involved in protecting plant organs from stresses. At high concentrations, Ni directly damages photosynthetic apparatus of leaf in several ways. It has been recognized that magnesium is an essential constituent of heme group of chlorophyll; moreover, other nutrients like iron and manganese are needed for metabolic activities of chlorophyll. Therefor, their lower absorption rates can reduce synthesis of chlorophyll at high Ni concentration. In addition, Ni at high level breakdowns remaining chlorophyll in chloroplast that causes chlorosis of leaves and ultimately necrosis of plant parts (Batool, 2018). Pb ions significantly block -amino laevulinate dehydrogenase, the primary enzyme for the manufacture of chlorophyll (Singh *et al*., 2012). The chlprophyll content of the three plants after stress of higher concentration of HMs showed different degrees of decreased or increased compacted to contral which may be due the fact that the heavy metals stress leads to the disruption of the chloroplast

structure and disruption of the electron transport chain, the Ni and Pb synergestic to Zn, since higher level of Zn can displace the Mg cofactor in chloropyll thereby disrupting photosynthesis limiting growth and causing plants to fade to green. The pollution with heavy metals has many deleterious effects; the most important of them is the effect on chemical composition of plant tissues. Physiological phenomena affected by Ni toxicity include changes in the concentration of photosynthetic pigments resulting in reduction in net photosynthetic rate, stomatal conductance, transpiration rate, and water-use efficiency. Excessive Ni contents have also been shown to alter the concentration of physiologically important organic molecules such as soluble sugars, free amino acids, and soluble proteins. Heavy metal has deleterious effects on the content and functionality of the photosynthetic pigments. This can be caused by the inhibition of pigment synthesis or direct oxidative damage to the pigments. They comprise impairments of chlorophyll synthesis resulting in chlorotic leaves, changed ratios of chlorophyll a and b and photosynthetic activity, inhibition of photosynthesis induces oxidative stress, which can contribute to the degradation of photosynthetic structures and induction of senescence, and to stress acclimation of plants through signaltransduction processes. Heavy metals inhibit chlorophyll and carotenoid biosynthesis and retard the incorporation of these pigments in photosystems. The decrease in pigment photosynthesis as a consequence of reduced absorption of essential mineral nutrientsis an indirect reason for plant chlorosis (Li *et al.,* 2018; SaiKachout *et al.,* 2015).

Soils should have an impact on chlorophyll since several elements, such as nitrogen (N), magnesium (Mg), iron (Fe) and phosphorous (P) indirectly, are needed for chlorophyll production. Precipitation may have an effect on the photochemical activity of chloroplasts because water is the medium used by plants to transport nutrients. Mineral salts must be dissolved in water in order for plants to absorb them. Consequently, there is a direct connection between the production of chlorophyll and water. The absence of water in leaves affects the production of chlorophyll, encourages its breakdown, and hastens the yellowing of leaves. Additionally, there is indirect evidence that soils and climate, particularly temperature, work together to influence chlorophyll (Chl), suggesting that Chl could be a useful feature for predicting how plants will react to climate change (Li *et al.,* 2018). From the results appear that *G. triacanthos* and *L. leucocephala* were less affected compared to *R. pseudoacacia* for Ni and Pb elements, where the values of Chl a and TC were as follows: (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*), for Chl b as follows (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*). Same results were obtained from each of Kycko *et al.,*(2019), Batool (2018); Amini and Amirjani, (2013). These results confirmed by Singh *et al.,* (2012), who found that the effect of Pb and Ni on Chl a, Chl b and TC content of Black gram

(*Vigna mungo* L.) seedlings were evaluated under 10, 50 and 100 µM concentration. These concentrations significantly affected chlorophyll and carotenoid content of Black gram as compared to control. Pb and Ni at 10 µM concentration resulted in less significant effect on chlorophyll, a, b and total carotenoids. The TC was less affected compared to Chl a and Chl b, while higher concentrations (50 and 100 µM) significantly reduced chlorophyll and total carotenoid contents of the seedlings.

5.2.2 Enzymatic, non – enzymatic antioxidants and protein content

The results presented from tables (4.5 – 4.8) show that the effect of Ni, Pb and their interactions were significant on some of the enzymatic and non enzymatic antioxidants. The POD, NR, AA and TPr increased with increasing concentrations of Ni and Pb from 45 $mg.kg^{-1}$ of the studied species, except the NR was decreased from 45 $mg.kg^{-1}$ of *L. leucocephala* and *R. pseudoacacia.* While the CAT, Pr and CHO decreased with increasing concentrations of Ni and Pb from 45 $mg.kg^{-1}$ of the studied species, except the CHO increased from 45 $mg.kg^{-1}$ of *L. leucocephala.* The Pb and Ni are considered as a dangerous pollutant to plants, because they have an effect on many biological processes like photosynthesis, carbohydrate synthesis and cell wall by decrease of exchange between inner and outer (Taha *et al.,* 2008). Antioxidant responses to avoid damages caused by ROS to cellular components, as well as to maintain growth, metabolism, development, and overall productivity, the balance between production and elimination of ROS at the intracellular level must be tightly regulated and/or efficiently metabolized. This equilibrium between the production and detoxification of ROS is sustained by enzymatic and non-enzymatic antioxidants. Excess ROS is harmful to the plant; thereby, to restore the cellular redox balance, both enzymatic and non-enzymatic systems are activated to detoxify the toxic levels of ROS. The observed increase in enzymatic activities and decrease in oxidative damage are closely related. The expression of many antioxidant enzymes is positively correlated with higher tolerance levels against abiotic stresses (Gull *et al*., 2019; Caverzan *et al.,* 2016). Plant produce excesses ROS under complex heavy metals which can rapidly damage biomolecular structure (DNA, RNA and protein) and membrances through lipid peroxidation, leading to metabolic disorders and cell death in plants.

$$O_2^{\cdot -} \xrightarrow{SOD} H_2O_2 \xrightarrow[CAT]{POD} H_2O + O_2$$

CAT play important role in H_2O_2 decomposing the decrease in CAT activity of plants after HMs stress is compensated by the increase POD activity to scavenge H_2O_2 . plant produce higher levels of AA under stress condition. Ascorbic acid act as powerful antioxidant in plant, helps in neutralize harmful molecules

of ROS that produce under stress condition, thus AA protect plant cell from damageenzzymes.many plant required AA as acofactor for proper fanction, these enzyme involved in cell division cell elongation and hormone synthesis. The majority of biological functions performed by proteins in cells (and occasionally other biomolecules as well) are carried out by these biological macromolecules. Generally speaking, plant proteins provide a variety of enzymatic, structural, and functional functions (such as photosynthesis, biosynthesis, transport, and immunology). They also act as storage mediums to meet the growth and nutritional demands of developing seedlings. All plant tissues contain proteins, which are categorized as simple proteins and further broken down into four categories. These four plant protein types; albumins, globulins, prolamins, and glutelins are mostly linked to seed storage proteins. Proteins and carbohydrates are among the various nutrients that are kept in plants' storage organs. Typically, these proteins are referred to as storage proteins (Rasheed *et al.,* 2020). Their main purpose is to be converted into amino acids, which serve as the fundamental units of emerging proteins in the subsequent generation of growing plants (Khan *et al.,* 2017). Protein content may increase in response to heavy metal stress. This accumulation is believed to be notably due to the synthesis of defense proteins involved in the maintaining the redox state of the cell such as ascorbate, or metal sequestration/detoxication (methalothionines, glutathione, phytochelatins), Pandey and Sharma, (2002). In this study it was indicated that each of the antioxidants value for the studied species was as follows: NR and AA (*G. triacanthos* > *L. leucocephala* >*R. pseudoacacia*), POD and CHO (*R. pseudoacacia* > *G. triacanthos* > *L. leucocephala*), CAT (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala*), Pr (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*) and TPr of *G. triacanthos* > *L. leucocephala* (shoot > root), except *R. pseudoacacia* (root > shoot). These results were also confirmed by Mame *et al.*, 2021, Hussain *et al.*, 2020, Gull *et al*., 2019, Andresen *et al.*, 2018, Bielen *et al.*, 2013, Singh *et al.*, 2012, Yan *et al.*, 2008, Dey *et al*, 2007; Tzvetkova and Dimitar, 1996).

5.2.3 Mineral elements contents

The movement of metals ions from the soil solution via root to shoot is called translocation. Translocation is primarily controlled by two processes. First is root pressure and second one is leaf transpiration. Following translocation to leaves metal can reabsorbed from leaf cells. This rate of long distance metal transport is different in the plants. The metal is then sorbed at the metal surface and moves into the root cells through the cellular membrane using apoplastic (passive diffusion) and symplastic (active diffusion) pathways (Sabreena *et al,* 2022; Verma *et al.,* 2013). Lead is a metal of very low

mobility; most of its portion is retained in soil at the root level. Pb uptake by plants is passive and conducted by the hairy root system and can penetrate leaves by their waxy cuticles. Once transported into the plant, it can cause damages to membranes, enzymes and various protein components. Small proportions of Pb may inhibit respiration and photosynthesis due to the disturbance of electron transfer chain reaction (Doganlar and atmaca, 2011). Higher level of lead and nickel may disturb cell membrane integrity affecting cell signaling and nutrients transport, overall lead can disrupt cell organelles and cellular processes. The Pb and Ni are considered as a dangerous pollutant for plants, because they have an effect on many biological processes like photosynthesis, carbohydrate synthesis and cell wall by decrease of exchange between inner and outer (Amari *et al.,* 2017). Results from the tables (4.9 – 4.38) show that Ni or Pb or their interactions had significant effects on some mineral elements in the shoot, root and soil of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species. Some of the macro, micro and heavy metal elements increased with increasing concentrations of the Ni and Pb elements from the shoot, root and soil of the three tree species. According this study, the highest accumulation of the plant organs, soil and species was as follows: shoot > root > soil and *G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*. Some of the mineral elements increased with increasing Ni and Pb concentrations from 15, 30 and 45 $mg.kg^{-1}$ of the shoot, root and soil of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species as follows; the mineral elements of *G. triacanthos* increased with increasing Ni, Pb and their interactions of the shoot, root and soil: (P, Mg, SO_4, Fe, Mn, Zn, Cd; N, P, K, Mg, Ca, SO_4, Fe, Zn, As and SO_4, Cu, Fe, Zn, Pb, Rb, Ag), (P, Mg, Zn, Cd, As; P, Mg, SO_4 and N, Mg, Ca, Cu, Zn) and (N, P, Mg, SO_4, Cu, Zn, Pb, Cd, As; N, P, Ca, SO_4, Cu, Fe, Mn, As, Rb and Ca, Cu, Fe, Mn, Zn, Ni, Ag, As). The mineral elements of *L.leucocephala* increased with increasing Ni, Pb and their interactions of the shoot, root and soil: (N, Mg, SO_4; N, P, Ca, Fe, Ag and Mg), (SO_4 , As; N, P and N, P, Mg, Mn) and (Ca, Rb, As; N, P, K, Mg, Ca, SO_4, Cu, Fe, Rb, Ag, As and Mg, Mn, Pb, Cd, As) and the mineral elements of *R. pseudoacacia* increased with increasing Ni, Pb and their interactions of the shoot, root and soil: (N, SO_4, Cu, Fe; Mg, Ca, Fe, As and SO_4, Cu, Zn, Pb, Rb, Ag), (Cu, Fe, Mn; Ca, SO_4, Mn and N, Mg, Cu, Zn, Ba) and (Mg, Cu, Fe, Mn, Rb, Cd; P, Mg, Ca, Fe, Zn, Ni, Rb and N, Ca, SO_4, Cu, Fe, Zn, Ni, Ag). Heavy metals and plants have complex relationships. Some of heavy metals are essential nutrients in trace concentrations for healthy growth as plants require the nutrients for essential physiological functions. Both high and low heavy metal concentrations in soil can have a negative impact on crop growth because these metals interfere with how plants use their energy sources, such as respiration and photosynthesis, and they also cause the organelles that make up their cells to age and eventually die. Because heavy metals build up in

plant tissues and induce a variety of morphological, physiological, and biochemical reactions, plants react differently to pollution depending on the species (Gratani *et al.,* 2021; Rahul *et al.,* 2016). The concentration of these metals in the environment (air, soil, or water), bioavailability, cation exchange capacity, the time since last vegetation, the climate, and numerous other variables. The distribution of metals in various plant tissues is largely influenced by the mechanism of metal translocation in plants. The degree of accumulation and dispersion of heavy metals in the upper vegetative portions are determined by a number of variables, including biochemical, anatomical, and physiological ones. Instead of using the low-metal-content soil, constant uptake and translocation can raise the metal concentrations in plant tissues. The primary cause of such results appears to be the root activity, which appears to facilitate the translocation of metals. The leaves of green plants, in particular, take a lot of heavy metals from the soil and roots. The accumulation of heavy metals in plants depends on several factors such as plant species, metal forms, soil parts, and properties such as solubility (Al-Heety *et al.,* 2021; Bislimi *et al.,* 2021). The intraction among different elements such as (Ni and Pb) and essential nutrients likes N, P, Mg, Fe, Cu , Zn and some non-essential elements in higher plants can exhibite anatagonistics or synergestic effect , in the context of Ni and Pb, they might antagonistically affect the availability or uptake of N, P, S, Mg, Fe, Cu ann vary depending on the spesific plant species the concentration of Ni and Pb the concentration and shape of essential nutrients. Zn because higher level of Ni and Pb could be compete with these essential nutrients for uptake by plant roots, potentially leading to nutrient imbalance and decrease plant health vice versa for synergestic. It is important to note that the effect of antagonistic or synergism c Soil characteristics strongly influence the solubility of metals. Under acid and oxidizing environments, most of the HMs are readily mobile and are strongly retained under alkaline and reducing conditions. Plants use root cells to limit and restrict the uptake and movement of HMs into the plant tissues. Such a process involves various defense mechanisms (root sorption, metal precipitation and exclusion). Because heavy metals are immobilized in the vacuoles of the root cells when plants are exposed to them, they become less toxic, which may be a natural toxicity response of the plant and there is a high accumulation in the roots of the plants. In the rhizosphere, a variety of root exudates serve as HM ligands to create HM complexes, which limit the bioavailability and lethality of HMs. The accessibility of HMs from the soil to the roots is further limited by the exclusion barriers that exist between the root and shoot system. Following chelation, the HM ligand complexes are moved from the cytosol to non-toxic storage areas like the vacuole, leaf petioles, leaf sheaths, and trichomes (Sabreena *et al*, 2022; Verma *et al.,* 2013). For maximum nutrient utilization efficiency, antagonistic (negative) nutrient interactions should be reduced

and synergistic (positive) nutrient interactions should be increased when creating fertilizers with the proper nutritional composition (René *et al.*, 2017). The most deteriorating is the destruction of the plasmalemma which ineffect disturbs the permeability for water and nutrients and lead to impaired plant growth. The main proceses responsible for Pb accumulation in root tissues is the deposition of Pb as pyrophosphate on cell wall. Particulary the pectic acid is most active in Pb sorption thus the Pb influenced the elasticity and pasticity of cell wall. The stimulating effect of Pb once uptake by plant root may be asecondary effect of the disturbance of transmembrane transportions, the same reasons of anatagonistic of Pb with Zn. The interferance of Pb with Ca is of metabolic important Pb can mimic the physiological behavior of Ca, thus can inhibit some enzyme. The differences between the species response to nickel and lead may be due to the uptake of heavy metals from the soil solution depending on species, form and concentration of metal, the soil. In this study, it is assumed that each of *G. triacanthos* > *L. leucocephala* > *R. pseudoacacia* plants may be better adapted and are tolerant to heavy metal stress conditions and more suitable for remediation of the soil because they have more ability to accumulation of hazard elements. Same results were confirmed by (Aloud *et al.*, 2022, Bislimi *et al.*, 2021, Szwalec *et al.*, 2018, Amari *et al.*, 2017, Palowsk *et al.*, 2016; Stankovic *et al.*, 2009).

The results from tables (4.27, 4.31 and 4.35) indicate that *G. triacanthos, L. leucocephala*, and *R. pseudoacacia* were response to nickel and lead which 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. The phytoremediation process, which also affects the physical qualities of the soils, can be strongly influenced by the soil conditions. Depending on the species, form, and concentration of the metals, the acidity of the soil or nutrient solution, and the composition of the organic matter, the changes in how different species react to nickel and lead may be caused by the uptake of heavy metals from the soil solution. For example, increased pH from 5.5 – 6.5 decrease the availability of Pb and Ni. However, it has been shown that at pH values between 5.5 and 7.5, phosphate or carbonate precipitates limit the solubility of lead (Pb) in the soil, leaving only a minimal quantity of Pb available to plants (Amari *et al.*,2017). A soil for phytoremediation should have physical and chemical characteristics that maximize plant growth rates by creating hospitable conditions for the establishment of new plants. The soil's physical characteristics, including texture, structural status, aeration, water conductivity, compaction, saturated hydraulic conductivity, and penetration resistance, as well as the soil's microenvironment (temperature, moisture content, and heat exchange), are crucial to the remediation process. In many instances, it is advised to enhance the soils' physical qualities by adding ingredients in

order to maintain high remediation rates (Solomou *et al.*, 2022). The same results were confirmed by (Sharma *et al.*, 2022, Solomou *et al.*, 2022; Seregin *et al.*, 2006).

5.3 Effects of nickel, lead and their interactions on some phytochemical contents

The results from tables (4.40 – 4.47) found that effect of Ni or Pb or their interactions had a significan effect on all phytochemicals parameters total phenolic, flavonoid, glycoside, alkaloid, steroid, terpene, tannin and saponin. It showed that all the qualitative and quantitative phytochemicals increased with increasing Ni and Pb concentrations. However, a number of important factors, including genotype, size and maturity, soil conditions, fertilizer, irrigation, location, climate, and season, affect the variability of phytochemicals in plants, in order to increase and improve the phytochemical content of plants, several elements might be used (Usman *et al.*, 2022, Shrestha *et al.*, 2015; Godwill *et al.*, 2013). This variation in Ni concentration among plant species may be related to the differences of organic anions that secreted by plant root that modified the pH in the rhizospher may considerably decrease Ni adsorption by soil and then increase it is bioavailability. Plant under Ni or Pb stress, the absorption of nutrient, root development and metabolosms are strongly related inaddition the photosynthesis , respiration and transpiration inhibite. The quantitative phytochemicals of the studied plant dry leaves included these secondary metabolites TPC, TFC, TGC, TAC, TSTC, TTEC, TTC and TSC. These phytochemicals were extracted with three solvents, viz., methanol, water and ethyl - acetate. Qualitative and quantitave phytochemicals extracted were affected by solvent polarity, the ratio of solvent and plant materials, material particle size, extraction method and temperature. The plant developmental stage, plant components, and the extraction and separation solvents have a direct impact on the phytochemicals (Wakeel *et al.*, 2019). Different minerals play different roles in the metabolism of medicinal plants. Numerous effects on plant metabolism result from the severity or shortage of these factors. The concentration of these minerals in the soil, whether they are activators or inhibitors, is crucial to the secondary plant metabolism. Additionally, minerals are essential for these medicinally significant plants to reproduce. However, the metabolic activities of such valued medicinal plants are negatively impacted by soils with differing mineral element compositions (Mishra,*et al.*, 2012). Same results were obtained from each of (Sobati-Nasab *et al.*, 2021, Mansur *et al.*, 2020, Ogoko, 2018, Usman *et al.*, 2018; Madhu *et al.*, 2017). In this study has evaluated highest value of qualitative and quantitave phytochemicals for three medicinal plants by three solvent extractions as followes:

- TPC by methanol, water and ethyl acetate (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*).

- TFC by methanol, water and ethyl acetate (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala).*

- TGC by methanol, water and ethyl acetate (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia).*

- TAC by methanol, ethyl acetate and water (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia).*

- TSTC by methanol and ethyl acetate (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia).*

- TTEC by methanol and ethyl acetate (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia).*

- TTC by methanol and ethyl acetate (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos).*

- TSC by water (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia).*

According this study, it was determined that the best solvent extraction method for all parameters was methanol, except total saponin content (water) and the amazing species i.e *L. leucocephala* and *G. triacanthos* for all parameters.

5.4 Effects of nickel, lead and their interactions on some anatomical studies of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* species

Results from the tables (4.48 – 4.59) show that effect of Ni or Pb or their interactions were significant on all anatomical parameters for leaf, petiole, stem, root, adaxial and abaxial stomata number, length and width. There are some anatomical changes which can caused by heavy metals such as Ni or Pb applied alone or in combination caused an increase in thickness of leaves (lamina and midrib), petioles, stems and roots of all seedling organs. These change may be due to decrease in cell division that resulted in an increase in cell wall thickness and/or a disturbance in the activity and content of phyto-hormones like auxin in the roots exposed to heavy metals may be the cause of a loss in root growth. High absorption and accumulation of heavy metals in roots of different plants caused in different anatomical changes, in plants affected by an increased Ni stress. The metal ions enter roots, they are stored in roots or translocate to shoots through particularly xylem, but directly, absorb heavy metal from water in addition to translocation from root, in some plants involves binding toxic metals at cell walls of roots and leaves, or storing them in a vacuoles or complex them to certain organic acids or proteins and others plants make stable metal complexes in the root cells to prevent metal translocation from the roots to above-ground tissues (Hamza *et al.,* 2020; Al-Saadi *et al.,* 2013). The Pb and Ni related impact on the plant may result from direct toxicity of metal accumulated in tissues, Amari *et al.* (2017). In the present study, the changes in the root,

stem and leaves anatomical structure of three plants were consistent with translocation of heavy metals to the cell wall of various tissues systems inadition implosion xylem conduct needed to be reinforced and having liquded cell wall because heavy metal deposite in cell wall binded to lignin. Stomata's density and aperture are lowered by heavy metals. Stomata close as a result of the early effects of metal toxicity on roots and stems, or as a direct result of toxic metals interacting directly with guard cells. An excess of both essential and non-essential metals induces ion stress in plants and causes multiple direct or indirect effects, which concern practically all physiological functions. In the absence of transpiration, e.g., when stomata are closed, water movement is driven by the active pumping of solutes in roots. At hight amount of Ni, and Pb induce a wide range of toxic effects to plants at morphological, physiological, and biochemical levels. Minerals have a diversified role in medicinal plant metabolism. Each and every aspect of plant biochemistry, physiology, anatomy, etc. is affected due to mineral nutrient composition of soils (Amari *et al.*, 2017, Chaudhari *et al.*, 2016; Rucinska-Sobkowiak, 2016). The same results were obtained from each of (Gao *et al.*, 2019, Amari *et al.*, 2017, Rucinska-Sobkowiak, 2016; Gupta *et al.*, 2013). These result agree with Khudhur and Omer (2015) who mentioned that stomata number on lower surface is more than stomata number on upper surface. They also noted that stomata characteristics like number, length and width are affected by genetic constitution, season, leaf position and leaf surface (upper or lower). In addition the number and size of stomata are influenced by genotype and environment factors such as: water availability, light intensity, temperature, and CO_2 concentration. The higher the intensity of light, the frequency of stomata on both leaf surfaces will also increase, although the increase in frequency is not significant. Plants that grow in dry environments with high light intensity tend to have a lot of stomata, but their size is small compared to plants that grow in wet and protected environments (Maylani *et al.*, 2020). The same results were obtained from Amari *et al.* (2017).

According to this study as shown on the anatomical properties that the highest thickenss or abnormality or irregularity occurred in all the shapes of the three studied plants as follows:

- **Leaf (Lamina):** Cuticle (*G. triacanthos > R. pseudoacacia > L. leucocephala*), Upper Epidermis (*G. triacanthos > R. pseudoacacia > L. leucocephala*), Mesophyll (*G. triacanthos > R. pseudoacacia > L. leucocephala*), Vascular bundle (*G. triacanthos > L. leucocephala > R. pseudoacacia*) and Lower Epedermis (*G. triacanthos > L. leucocephala > R. pseudoacacia*).
- **Leaf (Midrib):** Cuticle (*R. pseudoacacia > G. triacanthos > L. leucocephala*), Upper Epidermis (*R. pseudoacacia > G. triacanthos > L. leucocephala*), Mesophyll (*R. pseudoacacia > G.*

triacanthos > *L. leucocephala*), Vascular bundle (*R. pseudoacacia* > *L. leucocephala* > *G. triacanthos*) and Lower Epedermis (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala*) .

- **Petiole:** Periderm (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*), Cortex (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*), Fiber (*L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*), Accessory bundle (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*), Phloem (*R. pseudoacacia* > *L. leucocephala* > *G. triacanthos*) and Xylem (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*).
- **Stem:** Periderm (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*), Cortex (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala*), Fiber (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*), Phloem (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala*) and Xylem (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*).
- **Root:** Periderm (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*), Cortex (*L. leucocephala* > *R. pseudoacacia* > *G. triacanthos*), Phloem (*G. triacanthos* > *R. pseudoacacia* > *L. leucocephala*) and Xylem (*R. pseudoacacia* > *L. leucocephala* > *G. triacanthos*).
- **Adaxial stomata:** Number (*R. pseudoacacia* > *L. leucocephala*), Length (*L. leucocephala* > *R. pseudoacacia*) and Width (*L. leucocephala* > *R. pseudoacacia*).
- **Abaxial stomata:** Number (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*), Length (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*) and Width (*G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*).

CONCLUSIONS

AND

RECOMMENDATIONS

Conclusions

The current study concludes that:

1. Soil contamination with Ni and Pb is a serious problem where they which affects the seed performance which significantly decreased for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations for *G. triacanthos, L. leucocephala and R. pseudoacacia* species, except velocity of germination of *L. leucocephala* which increased with increasing concentration to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$.
2. The application of Ni and Pb elements decreased most of the morphological characterestics by 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ concentrations for the three plant species, except plant height, leaf area, root dry matter and number of nodules of *L. leucocephala* and root diameter and nodules dry matter of *R. pseudoacacia* were they increased with increasing the concentration to 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$.
3. Ni and Pb affects on photosyntetic pigments Chl a and Chl b were increased at 45 $mg.kg^{-1}$, except TC was decreased at 45 $mg.kg^{-1}$ for the three species.
4. The adverse effects of Ni and Pb induced antioxidant defense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant to heavy metal stress. Enzymatic and non - enzymatic antioxidants such as the activities of POD, AA and TPr were increased for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of the studied species. Whereas the NR was decreased for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ for *L. leucocephala* and *R. pseudoacacia* species, except the NR was increased for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ for *G. triacanthos* species. The activities of CAT, Pr, CHO were decreased at 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of the studied species, except the CHO was increased from 45 $mg.kg^{-1}$ of *L. leucocephala* species.
5. Some of macro, micro and non - essential heavy metals of the shoot, root and the soil were decreased at 15, 30 and 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ of the studied species.
6. Soil contaminated with Ni and Pb lead to increasing all the phytochemical parameters such as total (phenolic, flavonoid, glycoside, alkaloid, steroid, terpene, tannin and saponin) with three solvent extractions (methanol, water and ethylacetate) for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$ for all the studied species.
7. The plant grow in soil polluted with Ni and Pb affects on all the anatomical characterestics i.e leaf, petiole, stem, root and stomata of *G. triacanthos, L. leucocephala* and *R. pseudoacacia* where their thickness were increased especially for 45 $mg.kg^{-1}$ $NiCl_2$ or $PbCl_2$.

8. The use of phytoremediation of soils from heavy metals depositions is of great interest with a low cost. The most important proceeding that can be executed are the organization of green belts around cities. In respect to this study results, it suggested that *L. leucocephala* had the best heavy metal tolerance reactions, so it is recommended to cultivating this species in that areas contaminated with heavy metals especially nickel and lead round Iraq and Kurdistan regions.

Recommendations

From the results of this study we can recommend the following:

1. Further studies are required using other concentration of Ni and Pb which may give other results and finding the toxicity of these elements.
2. Using other heavy metals such as Cd, Cr, Ag, As and Hg to study their effects on *G. triacanthos, L. leucocephala* and *R. pseudoacacia.*
3. Conducting the experiment hydroponically for an exact study of the nutrient status of the plant under the study factors.
4. Estimating some antioxidant enzyme on heavy metal stressed seeds of *G. triacanthos, L. leucocephala* and *R. pseudoacacia*.
5. In the present study non enzymatic protectants have been studied. Studies on others like glycinebetaine, glutathione, trehalose, organic acides, etc are suggested.
6. Further studies are required using other solvent extracts such as ethanol, chloroform, hexane and acetone for qualitative and quantitative phytochemical analysis which may give accurate results.
7. Lipid peroxidation id the most reliable indicator for classifying genotypes are tolerant or sensitive under stressful conditions, so measuring of malondialdehyde (MDA) is required.
8. Estimating hormones on stressed *G. triacanthos, L. leucocephala and R. pseudoacacia s*pecies.
9. Estimating H_2O_2 to determine the level of oxidative stress in the studied plants.

REFERENCES

References

- Abdalla, M. Y. A and Mahmoud A. M. A. (2008). Effect of long-term stress with stress heavy metals combinations on growth and chemical composition of some ornamental shrubs. *J. Agri. Sci.,* 33 (12): 8695 – 8707.
- Abdel-Aziz, H. A. and Galal Y. G. M. (2014). Influence of different levels of phosphorus and potassium on growth of *Leucaena leucocephala* seedlings. *J. Soil Sci. and Agri. Eng.* 5 (2): 135-14.
- Aebi, H. E. (1974). *Catalase In : Methods of Enzymatic Analysis. Weinheim/NewYork. 673-680.*
- Ahmad, M. S. A, Mumtaz H, Muhammad A, Rashid A and Mhuhammad Y. A. (2009). Effect of nickel on seed germination of some elite sunflower (*Helianthus annuus* L.) cultivars. *Pak. J. Bot.* 41(4): 1871-1882.
- Ahmad, S, Inayat S. F, Rizwanul H, Samdun N. Kh and Malik Z. A. (2010). Standardization and estimation of nitrate reductase activity in the leaves of *Ammi majus L.* (Bishops weed) in relation to sulphur deficiency and seed yield. *Austral. J. Cro. Sci.* 4 (7): 515-522.
- Ahmadloo, F, Tabari M, Yousefzadeh H, Kooch Y and Rahmani A. (2012). Effects of soil nutrient on seedling performance of *Arizona cypress* and medite cypress. *Ann. Bio. Res.* 3(3): 1369-1380.
- Al-Barzinji, I. M, Khudhur S. A and Abdulrahman N. M. (2015). Effects of some dates, pre-treatment sowing, soil texture and foliar spraying of zinc on seedling of *Dalbergia sissoo* (Roxb.). *ARO- Sci. J. Koya Uni.* III (1): 09.
- Al-Hayani, I. H. H. (2015). Effect of glutathione and hydrogen peroxide and their interaction on some qualitative and quantitative characteristics of *Vigna radiata* L. plant (PhD thesis), *Colleg. Edu. Pure Scien. Ibn Al-Haitham, Uni. Bagh.* 96-224.
- AL-Heety, L. F, Hasan O. M and Al-Heety E. A. M. S. (2021). Heavy metal pollution and ecological risk assessment in soils adjacent to electrical generators in Ramadi City, *Iraq. Iraqi J. Sci.* 62(4): 1077–1087.
- Ali, S, Hayat K, Iqbal A, Xie L. (2020). Implications of abscisic acid in the drought stress tolerance of plants. *Agronom.*10(9): 1323.
- Al-Mohammadi, SH and Al-Mohammadi F. (2002). Statistics and experimental design. Dar osama for publishing and distribution. *Amman, Jordan.* 375 .

- Aloud, S.S, Alotaibi K.D, Almutairi K.F and Albarakah F.N. (2022). *Assessment of Heavy Metals Accumulation in Soil and Native Plants in an Industrial Environment, Saudi Arabia. Sustainability*. *14: 5993. https://www.mdpi.com*.
- Al-Saadi, M. S. A, Al-Asaadi W. M and Al-Waheeb A.N.H. (2013). The effect of some heavy metals accumulation on physiological and anatomical characteristic of some *Potamogeton L*. Plant. *J. Ecolo. Environ*. Sci.4(1): 100-108. http://www.bioinfopublication.org.
- Al-Sahaf, F. H. (1989). *Applied Plant Nutrition. Ministry of Higher Education and Scientific Research, University of Baghdad. Al-Hikma House. Iraq. (In Arabic).*
- Álvarez, S. P, Cabezas-Montero D, Debora-Duarte B. N, Tapia M. A. M, Sida-Arreola, J. P, Sánchez E and Héctor-Ardisana E. F. (2019). Used response to antioxidative enzymes in rice under stress due to lead and nickel. *Rev. Mex. Cienc. Agrí.* 10 (1): 570.
- Amari, T and Abdelly T. G. C. (2017). Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. South Afric. *J. Bot.* 111: 99–110.
- Amini, F and Amirjani M. R. (2013). Effect of Ni and Pb on chlorophyll content and metals accumulation in *Medicago sativa*. *Isfahan Univ. Techn. J.* 2 (6) :11-20. https://agris.fao.org/agris-search.
- Andresen, E, Edgar P and Hendrik K. (2018). Trace metal metabolism in plants. *J. Exper. Bot.* 69 (5): 909 – 954.
- Anwar, KH, Aqsa N, Yamin B, Farwa I, Noshin I, Ghulam Y and Muhammad K. (2022). Lead harms seed germination and growth of *Albizia lebbeck L*. benth and *Prosopis juliflora* (sw.). *Pak. J. Bot*. 54(1): 121-124.
- Azmat, R, Haider S, Nasreen H, Aziz F and Riaz M. (2009). A viable alternative mechanism in adapting the plants to heavy metal environment. *Pak. J. Bot.* 41(6): 2729-2738.
- Baran, U and Ekmekci Y. (2021). Physiological, photochemical and antioxidant responses of wild and cultivated carthamus species exposed to nickel toxicity and evaluation of their usage potential in phytoremediation. *Environ. Sci. Pollut. Res. Int.* 29(3): 4446-4460.
- Bates, L. S, Waldron R. P and Tears I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.
- Batool, S. (2018). Effect of nickel toxicity on growth, photosynthetic pigments and dry matter yield of *Cicer arietinum* L. varieties. *Asian. J. Agri. Biol*. 6(2):143-148..

- Bezini, E, Abdelguerfi A, Nedjimi B, Touati M, Adli B and Yabrir B. (2019). Effect of some heavy metals on seed germination of *Medicago arborea L*. (Fabaceae). *Agri. conspec. sci.* 84 (4): 357-364.
- Bhat, B A, Ahmad M, Amin T, Ahmad A and Shah W A. (2015). Evaluation of phytochemical screening, anticancer and antimicrobial activities of *Robinia Pseudoacacia*. Americ. *J. Pharm. Health Res*. 3(2): 2321-3647. www.ajphr.com.
- Bielen, An, Remans T, Vangronsveld J and Cuypers A. (2013). The influence of metal stress on the availability and redox state of ascorbate, and possible interference with its cellular functions. *Int. J. Mol. Sci.* 14(3): 6382-6413. www.mdpi.com.
- Bislimi, K, Sahiti H, Halili J, Bici M and Mazreku I. (2021). Effect of mining activity in accumulation of heavy metals in soil and plant (*Urtica dioica L*). *J. Eco. Eng*. 22(1): 1–7.
- Brownlee, C. (2001). The long and short of stomatal density signals. *Tren. Plant Sci*. 6 (10): 441-442.
- Casson, S and Gray J. E. (2008). Influence of environmental factors on stomatal development. *New phytologist*. 178 (1): 9-23.
- Caverzan, A, Alice C and Sandra P. B. (2016). Antioxidant responses of wheat plants under stress. *Genet. Molec. Biol*. 39(1): 1-6.
- Chapman, H. D. and Pratt P. F. (1978). Methods of Analysis for Soils, Plants and Waters. Univ. *California Div. Agric. Sci. Oakland*. https://www.scirp.org.
- Chaudhari, J, Patel K and Patel V. (2016). Exploring the toxic effects of Pb & Ni on stem anatomy of *Pisum Sativum* L. *Int. J. Chem. Environ. Bio Sci*. 4(1): 1-32.
- Dana, ED, García-de-Lomas J, Jiménez-Cantizano FA and Verloove F. (2022) *Gleditsia triacanthos L.* honeylocust, Leguminosae: first record as an invader of riparian woodland in Southern Spain. *Bio. Invasions Record*. 11(4): 819–829.
- De Angelis, A, Gasco L, Parisi G, Danieli P.P. A. (2022). Multipurpose leguminous plant for the mediterranean countries: *Leucaena leucocephala* as an alternative protein source. *J. Animals*. 11(8): 2230.
- De Macedo, F.G, Bresolin J. D, Santos E. F, Furlan F, Lopes da Silva WT, Polacco JC and Lavres J. (2016). Nickel availability in soil as influenced by liming and its role in soybean nitrogen metabolism. *Front. Plant Sci.* 7:1358. www.frontiersin.org.

- Deivasigamani, R. (2018). Phytochemical analysis of *Leucaena leucocephala* on various extracts. *J Phyto*.7(6): 480-482. www.phytopharmajournal.com.
- Deswal, M and Laura J. S. (2018). Effect of heavy metals cadmium, nickel and lead on the seed germination and early seedling growth of pisum sativum. *Res. J. Life Bioinfor. Pharm. Chem. Sci.* 4(2): 368.www.rjlbpcs.com.
- Dey, S.K, Jayashree D, Sanjukta P and Debasmita P. (2007).Changes in the antioxidative enzymes activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Braz. J. Plant Physio*.19(1): 53-60.
- Doganlar, Z. B and Atmaca M.(2011). Influence of airborne pollution on Cd, Zn, Pb, Cu and Al accumulation and physiological parameters of plant leaves in Antakya (Turkey). *Water, air and soil pollu.*214: 509-523.
- Easlon, H.M and Bloom, A. J. (2014). Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Appli. plant sci*. 2(7): 153.
- El-Amier, Y, Elhindi Kh, El-Hendawy S, Al-Rashed S and Abd-El Gawad A. (2019). Antioxidant system and biomolecules alteration in *Pisum sativum* under heavy metal stress and possible alleviation by 5-aminolevulinic acid. *Molecules,* 24 (22): 4194.
- El-bsheer, S. El. A. (2018). Development of methods for the determination of vitamin C content in some dry fruits and leaves, laloub and mesquite (*Balanites aegyptiaca* and *Prosopis juliflora*). *Sudan Uni. Sci. Techn. Colleg. Gradu. Stud*. (PhD Thesis). http://repository.sustech.edu.
- Ertekin, E. N, Ertekin İ, Bilgen M, Üniversites A Fakültesi Z, Bölümü T. B, Türkiye A, Üniversitesi H. M. K and Fakültesi Z. (2020). Effects of some heavy metals on germination and seedling growth of sorghum. *J. Agri Nat* 23 (6): 1608-1615.
- Fahr, M; Laplaze L; Bendaou N; Hocher V; El Mzibri M; Bogusz D and Smouni A. (2013). Effect of lead on rootg rowth. Fun. Plant Eco. 4: 175. www.frontiersin.org.
- Faizy, H. S, Sahi B. G, Mahdi H. S and Hamid H. M. S. (2022). The impact of heavy metals (cadmium & nickel) on seed germination, photothynsises pigments and vegetative growth of fenugreek (*Trigonell afoenum-Graecum*). *Plant J. Uni. Duho*. 25(1): 88-100.
- Faria, S. M, Olivares F. L and Xavier R. P. (2002). *Nodule-Structure in the Roots of Gleditsia Spp. a Non-Nodulating Legume Genus. A chapter in book: Nitrogen Fixation: From Molecules to Crop Productivity: 337-337. https://www.researchgate. net.*

- Fazal, H. (2015). A Mini review on lead (Pb) toxicity in plants. *J. Bio and Life Sci*, 6(2): 2157-6076. www.macrothink.org.
- Gao T. P, Wang H. M, Changming Li, Mingbo Z, Wang X, Liu Y, Yang Y, Liu Y, Yao G, Chang G, Xu D and Fang X. (2019). Effects of complex heavy metal stress on the hydrodynamic parameters, stem anatomy and photosynthetic properties of three desert species in the jinchang mining area. *J. Res. Sq*. 1(3): 855904.
- Giuliani, C, Lazzaro L, Lippi M. M, Calamassi R and Foggi B. (2015). Temperature-related effects on the germination capacity of black locust (*Robinia pseudoacacia L.*, Fabaceae) seeds. *Folia Geobot*, 50:275–282.
- Godwill, En. A, Paul N, Chidubem N. J, Innocent O. T, Chinenye E. B. (2013). Comparative qualitative analysis of the phytochemical load of water, methanol, ethyl acetate and hexane extracts of six selected medicinal plants. *Intern. J. Pharm. and Phytochem. Res*. 5(3);164-167. www.ijppr.com.
- Gratani, L, Vasheka O and Bigaran F. (2021). Metal accumulation capability by *Platanus acerifolia* (Aiton), *Ailantus altissima* (Mill.), *Robinia pseudoacacia* and *Quercus ilex*, largely distributed in the city of rome. *Ameri. J. Plant Sci*., 12(1): 163-185. https://www.scirp.org.
- Gull, A, Ajaz A. L and Noor U. I. W. (2019). *Abiotic and Biotic Stress in Plants. https://www.intechopen.com/books.*
- Gupta, P. K. (2004). *Soil, Plant, Water and Fertilizer Analysis. Fourth Edition. Agrobios, India. https://content.kopykitab.com/ebooks*.
- Hallmann, E. (2020). Quantitative and qualitative identification of bioactive compounds in edible flowers of black and bristly locust and their antioxidant activity. *J. Biomole*. 10 (12):1603, www.mdpi.com.
- Hamad, SH.O and. Anwer, L. S. (2021). Effect of pre-treatments and sowing depths on germination and early growth of *Leucaena leucocephala* seeds. *Zanco J. Pur. Appli*. Sci. 33 (1): 53-61.
- Hamza, Sh M, Malik Al-Saadi S A. A and Hussain Al-Abbawy D. A. (2020). A study of physical and anatomical characteristics of the hwavy metal accumulation of *Juncus rigdus* deefontains, 1798 (family,juncaaceae) in basrah province, southeren of Iraq. *Bull. Iraq nat. Hist. Mus.* 16 (1): 63- 81.

- Haschke, M. (2014). *Laboratory Micro-X-Ray Fluorescence Spectroscopy: Instrumentation and Applications. Kindle Edition. https://www.amazon.com/ ebook 55.*
- Haseeb, M, Iqbal S, Hafeez MB, Saddiq MS, Zahra N, Raza A, lbrahim M U, Iqbal J, Kamran M, Ali Q, Javed T, Ali H. M. and Siddiqui M. H. (2022). Phytoremediation of nickel by quinoa: Morphological and physiological response. *J. Plon*.17(1): 0262309.
- Heuzé, V, Tran G, Sauvant D and Lebas F. (2018). Honey locust (*Gleditsia triacanthos)*. A programme by inrae, Cirad, Afz and FAO. 11: 34. https://www.feedipedia.org.
- Huiying, Li and Hongji Luo, Deying Li, Tao Hu and Jinmin Fu. (2012). Antioxidant enzyme activity and gene expression in response to lead stress in *Perennial ryegrass. J. Amer. Soc. Hort. Sci.* 137(2):80–85.
- Hussain, S, Abdul Kh, Mehmood Ali N, Mohsin T, Hafiz Athar H, Sadam H, Tariq Sh. and Tariq M.(2020). *Metal Toxicity and Nitrogen Metabolism in Plants. Springer Nature Singapore. https://www.researchgate.net.*
- Islam, Sh. Md, Sana S, Ehsanul H. Md, Mushiur R. S. M, Samad A, Al- Noman Ab, Alam R, Rana S, Islam M. R, Mondol D, Shariful I. Md, Torikul I. Md and Mazumder K. (2021). Methanol, ethyl acetate and n-hexane extracts of *Tragia involucrata* L. leaves exhibit anxiolytic, sedative and analgesic activity in Swiss *albino mice*. *Heli. J.* 7(1): 05814.
- Ismail, S, Khan F and Iqbal M. Z. (2013). Phytoremediation assessing tolerance of tree species against heavy metal (Pb and Cd) toxicity. *Pak. J. Bot*. 45(6): 2181-2186.
- Jaime, A, Teixeira da S, Naeem M, Idrees M. (2012). *Beneficial and Toxic Effects of Nickel in Relation to Medicinal and Aromatic Plants. Medi. Arom. Plant Sci. Biotechn. 6 (1): 94-104.*
- Joseph, J, Reddy J and Sayantan D. (2018). Effect of nickel uptake on selected growth parameters of *Amaranthus viridis L. J. Appli. Natur. Scie.*10 (3): 1011 – 1017.
- Kabir, M, Iqbal M.Z, Shafiq M and Farooqi Z.R. (2010). Effects of lead on seedling growth of *Thespesia populnea L. Plan. Soil Environ*. 56; (4): 194 –199.
- Kabira, M, Iqbal M. Z, Shafiq M, Farooqid Z. R. (2018). The effects of lead and cadmium individually and in combinations on germination and seedling growth of *Leucaena leucocephala*. *Ameri. Scienti. Res. J. Eng. Techn. Sci* 43(1): 33-43. http://asrjetsjournal.org.
- Kacálková, L, Tlustoš P and Száková J. (2014). Chromium, nickel, cadmium, and lead accumulation in maize, sunflower, willow, and poplar. *Pol. J. Environ. Stud*. 23(3):753-761.

- Kadiry, L. (2009). Effect of lead accumulation on growth and mineral composition of eggplant seedlings *(Solarium melongena.). New Zeal. J. Cro. Horti. Scie*. 37(3): 189-199.
- Kafle, A, Timilsina A, Gautam A, Adhikari K, Bhattarai A and Aryal N. (2022). Phytoremediation: mechanisms, plant selection and enhancement by natural and synthetic agents. *Environ. Advanc.* 8: 100203. www.sciencedirect.com
- Kaloo, M. A, Bhat B. A and Rafiqi G. (2018). Preliminary phytochemical screening of extracts of *Robinia pseudoacacia*. *Int. J. Pharm. Pharmacol*. 2 (2): 2581-3080.
- Kang, X, Yu X, Zhang Y, Cui Y, Tu W, Wang Q, Li Y, Hu L, Gu Y, Zhao K, Xiang Q, Chen Q, Ma M, Zou L, Zhang X and Kang J. (2018). Inoculation of sinorhizobium saheli leads to reduced metal uptake for *Leucaena leucocephala* grown in mine tailings and metal-polluted soils. *Front. Microbiol*. 9 :1853. www.frontiersin.org.
- Kartoori, S, Sumalatha G. M, Shuba A. C, Komala N. T and Biradar P. N. K. (2018). Role of enzymatic antioxidants defense system in seeds. *Int. J. Curr. Microbiol. App. Sci*. 7: 584-594. http://www.ijcmas.com.
- Khalid, S, Shahid M, Niazi N Kh, Murtaza B and Bibi I. (2016). A com- parison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Expl. Elsevier*. 182 : 247 - 268.
- Khalid, S, Shahid M, Niazi N. Kh, Murtaza B, Bibi I, Dumat C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Explo*. 182: 247-268.
- Khan, R. H, Siddiqi M. Kh and Salahuddin P. (2017). Protein Structure and Function. https://austinpublishinggroup.com/ebooks/basic-biochemistry.
- Khudhur, S. A and Omer T. J. (2015). Effect of NAA and IAA on stem cuttings of *Dalbergia sissoo* (Roxb). *J. Bio. Life Sci*. 6(2): 208-220.
- Khudhur, S. A. (2019). Effects of different times of cutting soaking and concentrations of IAA on morphological features of *Robinia pseudoacacia* stem cuttings. *J. Advan. Lab. Res. Bio*. 10 (4): 111-122.
- Kumar, V, Singh J and Kumar P. (2019). *Heavy Metals Accumulation in Crop Plants: Sources, Response Mechanisms, Stress Tolerance and Their Effects. Agro Environ Med. Agri. Environ. Sci. Acad. Haridwar.India. Book. 33-37.*
- Kumari, S and Amarnath M. (2021). *Heavy Metal Contamination. https://www.intechopen.com.*

- Kun, Li, Han Xu, Ruiqiang Ni, Shi G, Sergiode M, Chuanrong Li, Weixing Sh, Yikun Zh and Xing Zh. (2021). Impact of *Robinia pseudoacacia* stand conversion on soil properties and bacterial community composition in Mount Tai,China. *Fores. Eco*. 8 (19): 2-12.
- Kycko, M, Romanowska El Z and Zagajewski B. (2019). Lead-induced changes in fluorescence and spectral characteristics of pea leaves. *J. Remot. Sens*. 11(16): 1885. www.mdpi.com.
- Lahare, R P, Yadav H Sh, Bisen Y K and Dashahre A K. (2021). Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus* from durg district, Chhattisgarh. *India. Sch. Bull. J*. 7(1): 1-6
- Le´on, V, Jacques R, Roger N, Roxane B, Xavier M, Saliou Bouraima-Madje BI, Josette V and Rene P. (2005). Effects of three nickel salts on germinating seeds of *grevillea exulvar, Rubiginosa, an Endemic* and *Serpentine proteaceae. Annal. Botan.* 95 (4): 609–618, www.aob.oupjournals.org.
- Li,Y, He N, Hou J, Xu L, Liu C, Zhang J, Wang Q, Zhang X and Wu X. (2018). Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front. Ecol. Evol*. 6 : 64. www.frontiersin.org.
- Lichtenthaler, H. K and Wellburn, A. R (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Socie.Trtans*.11(5): 591–592.
- Madhu, Ch, Krishna K M, Reddy K R, Omar M, Bhawani S and Kalakonda S. (2017). Comparative studies on phytochemical screening and metal analysis of hydroalcoholic extracts of *Beta Vulgaris*, *Carica Papaya*, and *Vitis vinifera. Am. J. Pharm. Tech. Res*. 7(3): 2249-3387.
- Malar, S, Vikram S. Sh, Favas P. J and Perumal V. (2014). Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths, *Eichhornia crassipes* (Mart.). *Botan. Stud*. 55(1): 54.
- Mame, S. O. O and Hussein S. M. A. (2021). Impact of ascorbic acid on the concentration of carotene and prolin in the legume (*Vicia faba*) plants grown with soil treated with Pb and Ni. *Ecol. Environ. Cons*. 27: 169-172.
- Mami, Y, Ahmadi G, Shahmoradi M and Ghorbani H R. (2011). Influence of different concentration of heavy metals on the seed germination and growth of tomato. *African J. Environ. Sci. Techn.* 5(6): 420-426. http://www.academicjournals.org.

- Mansur, N. Fh, Hanafiah M. A. K. M and Ismail M. (2020). Pb (II) Adsorption onto urea treated *Leucaena leucocephala* leaf powder: Characterization, kinetics and isotherm studies. *An Intern. Quar. Scient. J.* 19 (1): 311-318.
- Maylani, E. D, Yuniati R and Wardhana W. (2020). The effect of leaf surface character on the ability of water hyacinth, *Eichhornia crassipes* (Mart.) solms. to transpire water. *In Iop. Conf. Seri. Mate.Sci. Eng.* 902 (1): 012070.
- Mehboob, S, Iqbal M. Z, Shafiq M, Kabir M and Farooq Z. R. (2018). Effects of lead on seed germination and seedling of wheat (*Triticum sativum)*. *Glob. Sci. J.* 6 (8): 590. www.globalscientificjournal.com.
- Menage, P. M. A and Pridmore B. (1973). *Automated Determination of Phosphate Using Bray Extractant. 20 (1): 80-82. http://hdl.handle.net.*
- Mishra, B. K, Rastogi A and Shukla S. (2012). Regulatory role of mineral elements in the metabolism of medicinal plants. *Medic.Arom. Plant Sci. Biotechn*. 6 (1): 1-23.
- Muhammad, Sh, Iqbal M. Z and Mohammad A. (2008). Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala. J. Appl. Sci. Environ. Manage.* 12(2): 61 – 66. www.bioline.org.
- Musa, S. I, Awayewaserere K. O and Njoku K. L. (2019). Effects of dump site soil on the leaf structures of *Luffa cylindrical* (Sponge gourd) and *Amaranthus viridis* (Green Amaranth). *J. Appl. Sci. Environ. Manage.* 23 (2): 309-313.
- Mustafa, T. D. (2009). *Trees and Shrubs of Kurdistan Region and Iraq. Ministry of Agriculture-General Directorate of Horticulture, Forestry and Rangeland. Book. 105-129.*
- Najmaddin, Ch and Saeed M. M. (2020). Anatomical and palynological studies of some *Bougainvillea glabra* Cultivars. *Sys. Rev. Pharm*.11(11): 1177-1191.
- Nas, F. S and Ali M. (2018). The effect of lead on plants in terms of growing and biochemical parameters. *J. Eco. Environ. Sci*. 3 (4): 265-268.
- Nedjimi, B. (2021). Phytoremediation: A sustainable environmental technology for heavy metals decontamination. *Sn. Appl. Sci*. 3 :286.
- Nezih, M. (1985).The peroxidase enzyme activity of some vegetables and its resistance to heat. *Food Agric*. 36:877 – 880.
- Nyiramigisha, Ph, Komariah and Sajidan. (2021). Harmful impacts of heavy metal contamination in the soil and crops grown around dumpsites. *Agricul. Sci*. 9 (0): 271–282.

- Ogoko, E. C. (2018). Phytochemical and heavy metal analysis of *Gongronema latifolium*, *Talinum triangulare* and *Amaranthus hybridus*. *Acad. Res*. 4 (1): 8-15. http://arpgweb.com.
- Oladele, Eo, Yahaya T, Odeigah Pgc and Taiwo IA. (2017). The Influence of Pb and Zn contaminated soil on the germination and growth of bambara nut (*Vigna subterranea*). *J. Appl. Sci. Environ. Manage.* 21 (4): 761-768. www.bioline.org.
- Olorunmaiye, K. S, Madandola H. A, Oguntoye M. O and Apeh L. E. (2019). Germination and seedling development studies *Leucaena leucocephala* (Lam.) de Wit and *Parkia biglobosa*. *Benth. Bio. Environ. Sci. J. Trop*. 16(1): 151 – 158.
- Orwa, C. A, Mutua K. R, Jamnadass R. S and Anthony. (2010). *Agroforestree Database: A Tree Reference and Selection Guide. Eastern Africa. http://www.world agroforestry.org*.
- Palowsk, B, Małkowska E, Kurtyka R, Szymanowska-Pułka J, Gucwa-Przepióra E, Małkowski Ł, Woźnica A and Małkowski E. (2016). Bioaccumulation of heavy metals in selected organs of black locust (*Robinia pseudoacacia*) and their potential use as air contamination bioindicators. *Pol. J. Environ. Stud.* 25(5): 2081-2092.
- Pandey, A. K, Zorić L, Sun T, Karanović D, Fang P, Borišev M, Wu X, Luković J and Xu P. (2022). The anatomical basis of heavy metal responses in legumes and their impact on plant–rhizosphere interactions. *J. Plants*. 11(19): 2554.
- Pandey, N and Sharma C. P.(2002). Effect of heavy metals CO^{2+} , Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. *Plant Scie.* 163(4): 753-758.
- Pant, H, Lobo V and Singh R. (2020). Lead toxicity in plants and phytoremediation potential of aromatic plants for lead contaminated soils. *J. Medi. Arom. Plant. Sci.* 42(4), 205-219.
- Patra, A, Dutta A, Jatav S. S, Choudhary S and Chattopadhyay A. (2019). Horizon of nickel as essential to toxic element. *Intern. J. Chem. Stud*.7(2): 1185-1191.
- Pavlova, D, Donaltina V, Kalina V, Aida B, Besmira Xh. (2018). Effect of nickel on seed germination of alyssum species with potential for phytomiming in Albania. *Fresen. Environ. Bulle.* 27 (3):1345-1352.
- Pishchik, V, Mirskaya G, Chizhevskaya E, Chebotar V and Chakrabarty D. (2021). Nickel stress-tolerance in plant-bacterial associations. *Peer. J*. 9: 12230
- Pitotti, A, Elizalde B. E and Anese M. (1995). Effect of caramelization and maillard reaction products on peroxidase activity. *J. Food Biochem*. 18(6): 445-457.

- Pompeu, G. B, Gratão P. L, Vitorello V. A and Azevedo R. A. (2008). Antioxidant isoenzyme responses to nickel-induced stress in tobacco cell suspension culture. *Sci. Agric*. 65(5): 548-552.
- Rahul, J and Jain M. K. (2016). Effect of heavy metals on some selected roadside plants and its morphological study. Natu. Environ. Pollu. Techn. Intern. Quar. *Scienti. J*. 15 (4): 1133-1142.
- Rai, P and Mishra R. M. (2013). Effect of urban air pollution on epidermal traits of road side tree species, *Pongamia pinnata* (L.). *J. Environ. Sci., Toxicol. Food Techn*. 2(6): 4 - 7.
- Rajkumar, K, Shubharani R and Sivaram V. (2015). Phytochemical screening and antioxidant activity of leaves and stem bark extracts of *Garcinia imberti*- an endangered plant. *Intern. J. Pharm. Sci. Res*. 6(9): 4016-4021.
- Ranal, M. A and Santana D. G. d. (2006). How and why to measure the germination process. Brazi. *J. Bot*. 29 (1): 1-11.
- Rasheed, F, Markgren J, Hedenqvist M and Johansson E. (2020). Modeling to understand plant protein structure-function relationships-implications for seed storage proteins. *Molecul*. 25(4): 873
- Ren, R, Li Z, Zhang L, Jiang H, Zhou X and Liu Y. (2021). Enzymatic and non-enzymatic antioxidant systems impact the viability of cryopreserved *Paeonia suffruticosa* pollen. *J. plant cell, tissue and organ culture*. 144 (1): 233–246.
- René, P. J. J, Rietraa, M. H, Chistian O. D and Prem S. B. (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communi. soil sci. and plant analy*. 48(16): 1895–1920.
- Roman, A. M, Truta A. M, Viman O, Morar I. M, Spalevic V, Dan C, Sestras R. E, Holonec L and Sestras A. F. (2022). Seed germination and seedling growth of *Robinia pseudoacacia* depending on the origin of different geographic provenances. *J. Diversity*. 14 (1): (34).
- Rucinska-Sobkowiak, R. (2016). Water relations in plants subjected to heavy metal stresses. *Acta. Physiol. Plant*. 38 : 257 .
- Ryan, J, Estefan G and Rashid A. (2001). *Soil and Plant Analysis Laboratory Manual - Second Edition. Beirut, Lebanon: Intern. Cent. Agri. Res. Dry Area. https://repo.mel.cgiar.org/handle.*
- Sabreena, H. S, Bhat S. A, Kumar V, Ganai B. A. and Ameen F. (2022). Phytoremediation of heavy metals: An indispensable contrivance in green remediation technology. *Plants*. 11(9): 1255. https://www.mdpi.com.

- Sachan, P and Lal N. (2017). An overview of nickel (Ni^{2+}) essentiality, toxicity and tolerance strategies in plants. *Asia. J. Bio*. 2(4): 1-15.
- Sachdev, S, Shamim A. A, Mohammad I. A, Masayuki F and Mirza H. (2021). Abiotic stress and reactive oxygen species: Generation, signaling and defense mechanisms. *J. antiox*. 10: 277. https://www.mdpi.com.
- Sahu, Sh. S, Mohanty P and Mahalik G. (2020). Effects of heavy metals on growth and nodulation of rhizobium sp. in *Vigna radiate* (L) wilczek. *Intern. J. Modern Agricul*. 9 (4): 2305 – 7246.
- Saikachout, S, Ben M. A, Ennajah A, Leclerc J. C, Ouerghi Z and Karray B. N. (2015). Effects of metal toxicity on growth and pigment contents of annual halophyte (*A. hortensis* and *A. rosea*). *Int. J. Environ. Res*. 9(2): 613-620.
- Saio, V and Syiem D. (2015). Phytochemical analysis of some traditionally used medicinal plants of North – East India. *J. Sci. Environ*. 1: 2394-6490.
- Santos-Sánchez, N. F, Raúl-Salas C, Claudia-Villanueva C and Beatriz-Hernández C. (2019). *Antioxidant Compounds and their Antioxidant Mechanism. Book. https://www.intechopen.com.*
- Saraiva, M. P, Maia C. F, Silva B R S, Batista B. L and Lobato A. K. S. (2020). 24-Epibrassinolide induces protection against nickel excess in soybean plants. *Univ. Feder. Rur. Amaz.* (PhD thesis). http://repositorio.ufra.edu.
- Seneviratne, M, Rajakaruna N, Rizwan M, Madawala H. M. S. P, Ok Y. S and Vithanage M. (2017). Heavy metal-induced oxidative stress on seed germination and seedling development. Pub. Environ. *Geochem. Heal*. 41(4): 1813-1831.
- Septina, E, Yetti R. D and Rivai H. (2020). Phytochemical and pharmacological activities of chinese petai (*Leucaena leucocephala*). *Int. J. Pharm. Sci. Medi*. 5(12): 3.426. www.ijpsm.com.
- Seregin, I. V and Kozhevnikova A. D. (2006). Physiological role of nickel and its toxic effects on higher plants. *Russi. J. Plant Physi*. 53(2): 257–277.
- Sethy, S. K and Ghosh Sh. (2013). Effect of heavy metals on germination of seeds. *J. Natu. Sci. Bio and Medi.* 4 (2): 272–275.
- Shafiq, M., Zafar I. M and Athar M. (2008). Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. *J. App. Sci. Environ. Manag*. 12(2): 61-66.
- Shah Asif, KN. Q and Rather MA. (2018). Scarification treatments employed for breaking impervious status in seeds of Honey locust (*Gleditsia SPP.). J. Pharmaco. Phytoch.* 7(3): 968-971.

- Sharifi-Rad, M. (2017). Phytotoxic effects of heavy metals on seed germination and seedling growth of medical plant, hyssop (*Hyssopus officinalis* L.) in laboratory conditions, *J. Medi. Plan. Prod.* 6 (2): 247-253.
- Sharma, J. K, Kumar N, Singh N. P and Santal A. R. (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Front. Plant Sci*. 14:1076876.
- Sharma, P and Dubey R. S. (2005). Lead toxicity in plants. *Brazil. J. Plant Physio*. 17(1), 35-52.
- Sharma, P, Kaur A, Batish D. R, Kaur Sh and Chauhan B. (2022). Critical insights into the ecological and invasive attributes of *Leucaena leucocephala*, a tropical agroforestry species. *Front. Agro.* 4 : :890992. www.frontiersin.org.
- Shelton, H. M, Gutteridge R. C, Mullen B. F and Bray R. A. (1998). *Leucaena- Adaptation, Quality and Farming Systems. Austral. Cent. Inte rn. Agri. Res. https://www.aciar.gov.au.*
- Shrestha, P, Adhikari S, Lamichhane B and Shrestha B. G. (2015). Phytochemical screening of the medicinal plants of Nepal. *J. Environ. Sci.* 1 (6): 11-17. www.iosrjournals.org.
- Shweti, A. K and Verma J. S. (2018). Effects of nickel chloride on germination and seedling growth of different wheat (*Triticum aestivum)* cultivars. *J. Pharmaco. Phytochem*. 7(4): 2227-2234.
- Singh, A. L, Jat R. S, Chaudhari V, Bariya H and Sharma S. J. (2010). Toxicities and tolerance of mineral elements boron, cobalt, molybdenum and nickel in crop plants. *Plant Str*. 4 (2): 31-56.
- Singh, G, Agnihotri R. K, Reshma R. Sh and Ahmad M. (2012). Effect of lead and nickel toxicity on chlorophyll and proline content of Urd (*Vigna mungo* L.) seedlings. *Inter. J. Plant Physio. Bioch*. 4(6): 136-141. http://www.academicjournals.org.
- Singh, P. (2020). The effect of nickel on seed germination and seedling growth of *Raphanus sativus. J. Emerg.Techno. Innovat. Res*. 7 (8): 1328. www.jetir.org.
- Singh, S, Parul P, Rachana S, Vijay P. S and Sheo M. P. R. (2016). Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics and ionomics. *Front. Plant Sci.* 6: 1143.www.frontiersin.org.
- Sirhindi, G, Mir MA, Abd-Allah EF, Ahmad P and Gucel S. (2016). Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity and gene expression in *Glycine max* under nickel toxicity. *Front. Plant Sci*. 7: 591.

- Sobati-Nasab, Z, Alirezalu A and Noruzi P. (2021). Effect of foliar application of nickel on physiological and phytochemical characteristics of pot marigold *(Calendula officinalis*). *J. Agri. and Food Res.* 3: 100108.
- Solomou, A. D, Germani R, Proutsos N, Petropoulou M, Koutroumpilas P, Galanis C, Maroulis G and Kolimenakis A. (2022). Utilizing mediterranean plants to remove contaminants from the soil environment. *J. Agri*. 12 (2): 238.
- Ssenku, J. E, Muhammad N, Ingvar B and Hannington O. (2017). *Phytoremediation Potential of Leucaena leucocephala for Heavy Metal-Polluted and Heavy Metal-Degraded Environments. Book. 189-209. https://www.researchgate.net.*
- Stancovic, D, Igic R, Šijacic-nikolic M, Vilotic D and Pajevic S. (2009). Contents of the heavy metals nickel and lead in leaves of *Paulownia elongate* and *Paulownia fortune*. *Arch. Biol. Sci., Belgrade,* 61 (4), 827-834.
- Staszak, AM, Małecka A, Ciereszko I and Ratajczak E. (2020). Differences in stress defence mechanisms in germinating seeds of *Pinus sylvestris* exposed to various lead chemical forms. *J. Pon*. 15 (9): 0238448.
- Štofejová, L, Fazekaš J and Fazekašová D. (2021). Analysis of heavy metal content in soil and plants in the dumping ground of *Magnesite mining*. *J. Sustainability*. 13 (8): 4508.
- Szwalec, A, Lasota A, Kedzior R and Mundala P. (2018). Variation in heavy metal content in plants growing on a zinc and lead tailings dump. *Appl. Eco. Environ. Res*.16(4): 5081-5094.
- Taha, D. N, Ahmed A. K and Alaa K. H. Kh. (2008). Determination of lead, nickel and zinc in plants of North Al-Diwaniya City. *J. Kerba. Iraq*. 6 (4): 58.
- Tai, Ch K L, Ruiqiang N, Chaofan L, Lingyu X, Caihong Zh, Chuanrong L, Weixing Sh, Huiling G and Yikun Zh. (2022). The effect of *Robinia pseudoacacia* expansion on the soil particle size distribution on Moun Tai, China. *J. catena*. 6 (367): 105774.
- Thiele, I. and Palsson B. O. (2010). A protocol for generating a high-quality genome-scale metabolic reconstruction. *J. Natu. Proto*. 5(1): 93-121.
- Tognetti, P. M, Mazia N and Ibáñez G. (2019). Seed local adaptation and seedling plasticity account for *Gleditsia triacanthos* tree invasion across biomes. *Annal. Botan*. 124: 307–318. www.academic.oup.com.
- Tzvetkova, N and Dimitar K. (1996). Effect of air pollution on carbohydrate and nutrients concentrations in some decieduous tree species. *Bulg. J. Plant Physio*. 22(1–2): 53–63.

- Townsend, C .C and Guest E. (1974). Flora of Iraq. Baghdad, Iraq: Ministry of Agriculture and Agrariian Reform of the Republic of Iraq. 3: 54-661.
- Ushie, O. A, Aikhoje E. F, Aasegh T. J, Brahim A. I, Bako B. and Ettah A. O. (2022). Estimation of total alkaloids, saponins, flavonoid, tannin and phyenols in *Thayma tococcus* danielli leaves. *Worl. J. Pharm. Medical Res*. 8(4): 242-246. www.wjpmr.com.
- Usman, M, Khan W. R, Yousaf N, Akram S, Murtaza G, Kudus, K. A, Ditta A, Rosli Z, Rajpar M. N and Nazre M. (2022). Exploring the phytochemicals and anti-cancer potential of the members of fabaceae family: A comprehensive review. *Molecul*. 27: 3863.
- Usman, S. S, Hamza A, Abdussalam U. S, Muttaka A, Bello B. A and Bashir N. A. (2018). Proximate and phytochemical analysis and levels of heavy metal (Pb, Cd, Cr, Ni and Cu) of some medicinal plants collected from gombe state, Nigeria. *J. Biol. Sci.* 4 (4): 9-17. www.sciarena.com.
- Vatansever, R, Ibrahim I. O and Ertugrul F. (2010). Essential and beneficial trace elementsin plants, and their transport in roots. *Appl. Biochem. Biotechno*.
- Verma, D. K and Gupta A. P. (2013). Removal of heavy metals from whole sphere by plants working as bioindicators. *Bas. Res. J. Pharm. Sci.* 1(1): 01-07. http//www.basicresearchjournals.org.
- Voisin, A. S, Salon C, Jeudy C and Warembourgf R. (2003). Root and nodule growth in *Pisum sativum L*. in relation to photosynthesis: Analysis using c-labelling. *Ann. Bot*. 92(4): 557–563.
- Wakeel, A, Jan S. A, Ullah I, Shinwari Z. K and Xu M. (2019). Solvent polarity mediates phytochemical yield and antioxi-dant capacity of *Isatis tinctoria*. *Peer. J*. 9 (7): 7857.
- Yadav, V, Arif N, Kováč J, Singh V. P, Tripathi D. K, Chauhan D. K and Vaculík M. (2021). Structural modifications of plant organs and tissues by metals and metalloids in the environment. *J. Plant Physio. Biochem*. 159: 100-112.
- Yan, A, Wang Y, Tan S, Mohd Y. M, Ghosh S and Chen Z. (2020). Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. *Front. Plant Sci*. 11: 359. www.frontiersin.org.
- Yan, R, Gao S, Yang W, Cao M, Wang S and Chen F. (2008). Nickel toxicity induced antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. cotyledons. *Plant Soil Environ*. 54(7): 294 – 300.
- Yusuf, M, Fariduddin Q, Hayat S and Ahmad A. (2011). Nickel: An overview of uptake, essentiality and toxicity in plants. *Bull. Environ. Contam. Toxicol*. 86 (1): 1–17.

- Zainab, Q, Tanees CH. M, Xiongming D, Lori H and Tehseen A. M. (2021). Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* L. in response to extreme abiotic conditions. *J. Cotton Res*. 4 (9): 7494.
- Zayed, M. Z, Ho W. S, Fasihuddin B. A and Pang S. L. (2012). Effects of length of soaking in 100^{o}C water and ethyl methane sulphonate on germination of *Neolamarckia cadamba* and *Leucaena leucocephala* seeds. *4th Regional Conference on Natural Resources in the Tropics. Univ. Malaysia.*
- Zayed, M. Z, Sallam S. M. A and Shetta N. D. (2018). *Leucaena leucocephala* as one of the miracle timber trees. *Intern. J. Pharmac. Pharmaceuti. Sci.* 10 (1): 1-7.
- Zengin, F. K and Munzuroglu O. (2005). Effect of some heavy metals on content of chlorophyll, protein and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta. Biol. Cracov. Ser. Botan*. 47(2): 157–164.

جمهورية العراق الفدرالي
حكومة اقليم الكوردستان
وزارة التعليم العالي والبحث العلمي
جامعة كوية

الصفات الفسيولوجية والتشريحية لنمو بعض النباتات البقولية في ارض ملوثة بالنيكل والرصاص

أطروحة مقدمة

الى فاكلتي العلوم والصحة فى جامعة كوية، وهي جزء من متطلبات نيل درجة دكتوراه علوم Ph.D. فى فسلجة النبات

سركول احمد خضر

حاصلة على شهادة الماجستير في فسيولوجيا النبات عام (2013) في علوم الأحياء - جامعة كوية

بأَشراف:

پ. د. إقبال محمدغريب البرزنجي

2023

الخلاصة

تم إجراء هذه الدراسة في حقل مفتوح في مدينة كويه من 25 آذار إلى 20 آب 2021. هدف الدراسة الحالية هو دراسة تنظيف التربة الملوثة بالنيكل والرصاص وتداخلاتهما على أداء البذور والخصائص المورفولوجية والفسيولوجية والكيميائية والتشريحية لثلاثة أنواع من أشجار الغابات التابعة لعائلة البقوليات وهي *Gleditsia triacanthos* و *Leucaena leucocephala* و *Robinia pseudoacacia* . تم زراعة البذور السليمة من هذه الأنواع من البقوليات في 25 آذار 2021. تم استخدام تجربة ذات تصميم عشوائي كامل بثلاث مكررات لكل نوع لدراسة تأثير خلط التربة مع التراكيز (0 و 15 و 30 و 45 ملغم/كغم تربة) من مركبات كلوريد النيكل ($NiCl_2$) وكلوريد الرصاص ($PbCl_2$) وتداخلاتهما على كل نوع بشكل منفصل، وتم تسمية هذه التراكيز بـــ Ni0 و Ni15 و Ni30 و Ni45 للملح الأول و Pb0 و Pb15 و Pb30 و Pb45 للملح الثاني.

أظهرت النتائج أن كل من النيكل (Ni) والرصاص (Pb) لم يكن له تأثير معنوي على نسبة انبات بذور *G. triacanthos*، بينما زادت مساحة أوراق النبات بشكل ملحوظ عند استخدام التراكيز Ni15 وNi30 و P15 وP30 وP45، بينما لم يؤثر بشكل ملحوظ على خصائص النمو الأخرى للنبات مقارنة بمجموعة معاملات السيطرة. بالنسبة لنبات *L. leucocephala*، بدا أنه أكثر تحملاً لزيادة تراكيز المعادن Ni وPb. بالنسبة لنبات *R. pseudoacacia*، أدى استخدام النيكل (Ni) إلى زيادة ملحوظة في ارتفاع النبات لجميع المعاملات، ونسبة المادة الجافة في الجذور، وعدد العقد الجذرية عند تراكيز Ni30 وNi45، بينما أدى استخدام الرصاص (Pb) إلى زيادة ملحوظة في مساحة أوراق النبات. لم يتم رؤية أي عقد جذرية في نوع *G. triacanthos*. يتبين أيضا من النتائج أن التثبيط الناجم عن عناصر Ni وPb يعتمد على تركيز العنصر ونوع المعدن ونوع النبات. أشارت النتائج إلى أن مستويات منخفضة من كلوريد النيكل ضرورية لنمو النبات، في حين أن زيادة تراكيز كلوريد النيكل تثبط انبات البذور ونمو النبات وقد تسبب التسمم، ومع ذلك، تعتمد كفاءة التحمل على نوع النبات. وفي الختام، تأثرت جميع الأنواع المدروسة بعناصر Ni وPb التي تمت إضافتها إلى التربة مما ينعكس على أداء البذور والشتلات.

أظهر النوع *L. leucocephala* تحملًا أكبر للمعادن الثقيلة Ni وPb. أظهرت النتائج أن مستويات صبغات التمثيل الضوئي (كلوروفيل أ وكلوروفيل ب) زادت عند التركيز 45 ملغم/كغم في أنواع النباتات *G. triacanthos* و *L. leucocephala* و *R. pseudoacacia* ، في حين انخفض إجمالي الكاروتينويدات عند نفس التركيز. أظهرت المعادن الثقيلة تأثيرات ضارة على محتوى ووظائف صبغات التمثيل الضوئي. انخفض تركيز صبغات التمثيل الضوئي نتيجة للامتصاص المنخفض للعناصر المعدنية الأساسية ما يعد سببًا غير مباشر لإصفرار النباتات. عند مقارنة استجابة أنواع نباتات *G. triacanthos* و *L. leucocephala* و *R. pseudoacacia* للنيكل والرصاص، لوحظ أن الأنواع *G. triacanthos* و *L. leucocephala* تأثرت بأقل قدر مقارنة بـ *R. pseudoacacia*. كانت قيم الكلوروفيل أ و الكاروتينويدات الكلي تتبع النظام التالي *G. triacanthos* > *L. leucocephala* > *R. pseudoacacia*، أما بالنسبة لكلوروفيل ب، فكان الترتيب كالتالي *L. leucocephala* > *G. triacanthos* > *R. pseudoacacia*.

فيما يتعلق بتطبيقات النيكل والرصاص وتفاعلاتهما على بعض مضادات الأكسدة الأنزيمية وغير الأنزيمية، بالإضافة إلى نشاط إنزيم النيترات الاختزالي (NR) لأنواع النبات *G. triacanthos* و *L. leucocephala* و *R. pseudoacacia*،

أظهرت نتائج هذه الدراسة زيادة ملحوظة في نشاط إنزيم البيروكسيديز وانخفاض ملحوظ في نشاط إنزيم الكاتاليز ومحتوى البرولين ومحتوى الكربوهيدرات الكلي في أوراق الأنواع الثلاثة مع زيادة تركيزات النيكل والرصاص، باستثناء محتوى الكربوهيدرات الكلي الذي زاد فقط في نبات *L. leucocephala*. كما زاد محتوى حمض الأسكوربيك بشكل ملحوظ مع زيادة تركيزات النيكل والرصاص في الأنواع المدروسة. انخفض نشاط إنزيم NR بشكل ملحوظ في أنواع *L. leucocephala* و *R. pseudoacacia*، وعلى العكس من ذلك، ازداد نشاط إنزيم NR بشكل ملحوظ في النوع *G. triacanthos* . يمكننا استنتاج زيادة عامة أو انخفاض في محتوى بعض مضادات الأكسدة في الأوراق لجميع الأنواع المدروسة. ومع ذلك، هناك بعض الاختلافات الخاصة بكل نوع نباتي فيما يتعلق بالمحتويات الأخرى، وهو ما يعكس آليات مختلفة لتحمل النباتات لتأثيرات المعادن الثقيلة.

تأثيرات النيكل (Ni) والرصاص (Pb) وتداخلاتهما على بعض عناصر العناصر الاساسية الاولية والثانوية وبعض المعادن الثقيلة غير الضرورية في الأجزاء الخضرية والجذور والتربة وخصائص التربة الفيزيائية والكيميائية بعد الزراعة تمت انخفاضها في المعاملات 15 و 30 و 45 ملغم / كغم للأنواع الثلاثة المدروسة. ووفقًا لهذه الدراسة ، كان أعلى تراكم في أعضاء النباتات والأنواع على النحو التالي: الأجزاء العلوية> الجذور> التربة و *R. pseudoacacia*> *L. leucocephala*> *G. triacanthos*. تتمتع المعادن الثقيلة والنباتات بعلاقات معقدة، حيث أن بعض المعادن الثقيلة هي عناصر غذائية ضرورية بتراكيز قليلة للنمو الصحي لأن النباتات تحتاج إلى هذه العناصر الغذائية للوظائف الفسيولوجية الأساسية. يمكن أن تؤثر تراكيز المعادن الثقيلة العالية والمنخفضة في التربة سلبًا على نمو المحاصيل، حيث تتداخل هذه المعادن مع الوظائف الأيضية في النباتات ، بما في ذلك تثبيط عملية البناء الضوئي والتنفس ، وتدهور أجهزة الخلية الرئيسية ، حتى يمكن أن تؤدي إلى موت النباتات. تسبب تراكم المعادن الثقيلة في أنسجة النباتات استجابات مورفولوجية وفسيولوجية وبيوكيميائية متنوعة.

تأثيرات النيكل (Ni) والرصاص (Pb) وتداخلاتهما على نوعية وكمية المركبات النباتية (الفينول، الفلافونويد، الجليكوزيد، القلويد، الستيرويد، التيربين، التانين، والسابونين) الكلي باستخدام ثلاث طرق لاستخلاص المذيبات (الميثانول، الماء، وخلات الإيثيل) ازدادت من 45 ملغم/كغم للأنواع الثلاثة. أظهرت النتائج أن مذيب الميثانول كان الأفضل في استخراج المركبات النباتية نوعا وكما، باستثناء محتوى الصابونين الكلي الذي استخرج فقط باستخدام مذيب الماء لأوراق *G. triacanthos* و *L. leucocephala* و *R. pseudoacacia*. تأثر استخلاص المركبات النباتية نوعاً وكماً بقطبية المذيب، ونسبة المذيب إلى مواد النبات، وحجم جسيمات المواد، وطريقة الاستخلاص ودرجة الحرارة. يعتمد محتوى المركبات النباتية مباشرة على مرحلة تطور النبات وأجزائه والمذيبات المستخدمة في الاستخلاص وطريقة العزل. تلعب المعادن دورًا متنوعًا في أيض النباتات الطبية، وتسبب وفرة أو ندرة هذه المعادن آثارًا متعددة على أيض النباتات.

استخدام النيكل (Ni) والرصاص (Pb) وتفاعلاتها على بعض الخصائص التشريحية للنباتات مثل الأوراق والعناقيد والسيقان والجذور وعدد وطول وعرض الثغور زادت عند تعرض شتلات *G. triacanthos* و *L.leucocephala* و *R. pseudoacacia* لتراكيز 30 و 45 ملغرام لكل كيلوغرام من التربة. تتسبب امتصاص وتراكم المعادن الثقيلة بكميات كبيرة في الجذور لأنواع النباتات المختلفة في تغيرات تشريحية مختلفة ضها لمستويات عالية من النيكل. يؤدي وجود زائد للمعادن

الأساسية وغير الأساسية إلى جهد الأيونات في النباتات ويسبب آثارًا متعددة مباشرة أو غير مباشرة تؤثر في جميع الوظائف الفسيولوجية تقريبًا.

کۆماری فیدرِاڵی عیَراق
حکومەتی هة ریَمی کوردستان
وەزارەتی خوێندنی باڵاو توێژینەوەی زانستی
زانـکۆکۆیـــة

تایبەتمەندی کارئەندامزانی و، تویکاري لەلایەن هەندێک گەشەی ڕووەکە پاقلەمەنیەکان لە خاکیکی پیسبوو بە نیکل و قورقوشم

تێزێکە پێشکەش بە

فاکەڵتی زانست و تەندروستی لە زانکۆی کۆیە

وەکو جێبەجێکردنی بەشێک لە پێداویستیەکانی بۆ پلەی دکتۆرای فەلسەفەی زانست لە بایۆلۆجی / فیزیۆلۆژیای ڕووەک

سـەرگـوڵ ئەحمەد خضر

ماستەر لە فیزیۆلۆژیای ڕووەک – 2013

بایۆلۆجی / زانکۆی کۆیە / فاکەڵتی زانست و تەندروستی

بە سەرپەرشتی: پ. د.اقبال محمد غریب بەرزنجی

2723

پوخـــــــتـــــــه

ئەم توێژینەوەیە لە کێڵگەیەکی کراوەدا لە شاری کۆیە لە ٢٥ی ئازار تا ٢٠ی ئابی ٢٠٢١ ئەنجامدراوە، ئامانجی ئەم لێکۆڵینەوەیە چارەسەرکردنی رووەکی بۆ خاکێکی پیسکراو بە هەریەك لە کانزای Ni ، Pb و بە هاوبەشی لەسەر چەکەرەی تۆو و مۆرفۆلۆژیی وفیزیۆلۆژیی و تایبەتمەندی فیتۆکیمیایی و تویکاری بۆ *Gleditsia triacanthos* ، *Leucaena leucocephala*و *Robinia pseudoacacia* وەک سێ جۆری داری دارستان سەر بە خێزانی پاقلەمەنیەکان.

تۆوی تەندروستی ئەم جۆرانە لە ٢٥ی ئازاری ٢٠٢١ چێنراوە . تاقیکردنەوەیەکی فاکتۆریاڵ بە دیزاینێکی تەواو هەڕەمەکی لەگەڵ سێ دووبارەکردنەوە بۆ هەر جۆرێک بەکارهات بۆ لێکۆڵینەوە لە کاریگەرییەکانی تێکەڵکردنی خاک لەگەڵ جیاوازی خەستی (0، 15، 30 و 45 ملغم/کغم خاک)ی دوو کانزای قورس کلۆریدی نیکل ($NiCl_2$) و کلۆریدی قورقوشم ($PbCl_2$) و بەهاوبەش لەسەر هەر جۆرێک بە جیا کە بە Ni0 ، Ni15، Ni30و Ni45 بۆ خوێی یەکەم وPb0 ، Pb15، Pb30و Pb45 بۆ خوێی دووەم ئاماژەیان پێکرا .

ئەنجامەکان دەریانخست کە نە Ni و نە Pb کاریگەرییەکی بەرچاویان لەسەر ڕێژەی چەکەرەکردنی تۆوی *G. triacanthos* نەبووە، لە کاتێکدا، ڕووبەری گەڵاکانی ڕووەک بە شێوەیەکی بەرچاو زیادی کرد لە Ni15 و Ni30، P15، P30، و P45 لە کاتێکدا کاریگەرییەکی بەرچاویان لەسەر سیفەتە ڕووەکییەکانی تر نەبووە بە بەراورد بە مامەڵەنەکراوی. بۆ *L. leucocephala* پێدەچوو زیاتر بەرگەی زیادبوونی چڕیی کانزاکانی Ni و Pb بگرێت. بۆ *R. pseudoacacia* بەکارهێنانی Ni دەبێتە هۆی زیادبوونی بەرچاو بۆ دریژی ڕووەکەکە بۆ هەموو مامەڵەکان، و لەڕێژەی سەدی مادەی وشکی ڕەگ، گرێی ڕەگی ڕووەکەکان ژمارەیان لە هەردوو Ni30، Ni45، لە کاتێکدا بەکارهێنانی Pb دەبێتە هۆی

زیادبوونی بەرچاو لە ڕووبەری گەڵاکانی ڕووەک. هیچ گرێیەکی ڕەگ لە جۆری *G. triacanthos* بەدی نەکرا. ئەنجامەکان ئەوە پیشان دەدەن کە کاریگەرییە ڕێگریکەرەکانی توخمەکانی Ni و Pb پەیوەستە بە چڕییەکانیان و جۆری کانزاکە و جۆری ڕووەکەکە. دەرکەوت کە ئاستی نزم لە کلۆریدی نیکل پێویستە بۆ گەشەی ڕووەکەکان، لە کاتێکدا زیادکردنی خەستی ڕێگری لە ڕواندنی تۆو و گەشەی ڕووەکەکان دەکات و تەنانەت ڕەنگە ببێتە هۆی ژەهراویبوون. بەڵام کارایی بەرگەگرتن بەپێی جۆری ڕووەکەکە دەگۆڕێت. لە کۆتاییدا، هەموو ئەو جۆرانەی کە لێکۆڵینەوەیان لەسەر کراوە کاریگەرییان لەسەر بووە بەهۆی زیادکردنی Ni و Pb بۆ خاکەکە، کە کاریگەری لەسەر تۆو و نەمامەکان هەبووە. *L. leucocephala* بەرگەگرتنی زیاتری بۆ کانزا دەگمەنەکان Ni و Pb نیشان دا بە بەراورد بە دوو جۆرەکەی تر، لەگەڵ خەستی زیاتر، بە تایبەت 45 ملغم/کغم خاک، کە کاریگەری بەرچاوی زیاتری لەسەر تایبەتمەندییە پشکنینکراوەکان هەبوو.

تۆێژینەوەکە کاریگەرییەکانی پیسبوونی خاک بە نیکل و قورقوشم و هەروەها کارلێکەکانیان لەسەر بەڵگە کارئەندام زانیە جیاوازەکان تاوتوێ کردووە. ئەنجامەکان دەریانخست کە لە خەستی 45 ملغم/کغم خاک ، ئاستی کلۆرۆفیل a و کلۆرۆفیل b لە *G. triacanthos*، *L. leucocephala*، و *R. pseudoacacia* زیادی کردووە، لە کاتێکدا ئاستی کۆی گشتی کارۆتینۆید کەمیکردووە. دەرکەوت کە میتالە قورسەکان کاریگەری نەرێنییان لەسەر پوختەو و کارایی ڕەنگە ڕۆشنەپێکهاتن هەیە. کەمبوونەوەی ڕۆشنەپێکهاتن، کە لە ئەنجامی کەمبوونەوەی هەڵمژینی مادده خۆراکییە کانزاییە بنەڕەتییەکان بووە، بە شێوەیەکی ناڕاستەوخۆ بەشداری کردووە لە زەردبوونی ڕووەک. بە بەراوردکردنی ئەنجامەکان *G. triacanthos*، *L. leucocephala*، و *R. pseudoacacia* بۆ نیکل و قورقوشم، دەرکەوت کە *G. triacanthos* و *L. leucocephala* کەمتر کاریگەرییان لەسەر بووە لە چاو *R. pseudoacacia*. ڕێژەی کلۆرۆفیل a و کۆی گشتی کارۆتینۆید بەم ڕیزبەندییە بوون: *G.*

triacanthos > L. leucocephala > R. pseudoacacia، لە کاتێکدا بۆ کلۆرۆفیل b، ڕێزبەندی *L. leucocephala > G. triacanthos > R. pseudoacacia* بوو.

لە ڕووی دژە ئۆکسێنەرە ئەنزیمییەکان و نائەنزیمییەکان، هەروەها چالاکیی ئەنزیمەکانی نیترات ڕیدکتێز (NR)، توێژینەوەکە کاریگەرییەکانی نیکل، قورقوشم و کارلێکەکانیان لەسەر *G. triacanthos*، *L. leucocephala* و *R. pseudoacacia* تاقیکردەوە. ئەنجامەکان دەریانخست بە زیادبوونی بەرچاوی چالاکیی ئەنزیمەکانی پەرۆکسیدایز و کەمبوونەوەی بەرچاوی چالاکیی ئەنزیمەکانی کاتالێز و پرۆلین وکۆی ڕێژەی کاربۆهیدرایت لە گەڵاکانی هەر سێ جۆرەکەدا دەرخست لەگەڵ زیادبوونی خەستی نیکل و قورقوشم، جگە لە کۆی ڕێژەی کاربۆهیدرات کە تەنها لە جۆری *L. leucocephala* . ڕێژەی ترشی ئەسکۆربیک بە شێوەیەکی بەرچاو زیادی کردووە لەگەڵ زیادبوونی خەستی نیکل و قورقوشم لە هەموو لێکۆڵینەوەکەدا. چالاکیی ئەنزیمی NR لە *L. leucocephala* و *R. pseudoacacia* بە شێوەیەکی بەرچاو کەمیکردووە، لە کاتێکدا لە *G. triacanthos* بە شێوەیەکی بەرچاو زیادی کردووە. لە کۆتاییدا، زیادبوون یان کەمبوونەوەی گشتی لە ناوەڕۆکی هەندێک دژە ئۆکسێنەر لە گەڵاکانی هەموو جۆرەکانی لێکۆڵینەوەدا هەبووە. بەڵام هەندێک جیاوازی تایبەت بە جۆرەکانیش هەبوو، کە ئاماژەیە بۆ میکانیزمی جیاوازی بەرگەگرتن بەرامبەر بە فشاری کانزا دەگمەنەکان لە نێوان ئەم جۆرانەدا.

کاریگەرییەکانی نیکل (Ni)، قورقوشم (Pb)، و کارلێکەکانیان لەسەر پوختەکردنی کۆی چۆرایەتی و چەندایەتی فیتۆکیمیایی جۆراوجۆر (فینۆلیک، فلاڤۆنۆید، گلایکۆساید، ئەلکالۆید، ستیرۆید، تێرپین، تانین، و ساپۆنین) بە بەکارهێنانی سێ توێنەرەوەی جیاواز بینرا شێوازەکانی دەرهێنان (میتانۆل، ئاو، و ئیتیل ئەسیتات پوختە سەرەتایی ئەم فیتۆکیمیاییانە لە هەر سێ جۆرەکەدا 45 ملغم/کغم خاک بوو. ئەنجامەکان ئاماژەیان بەوە کرد کە دەرهێنانی توێنەرەوەی مێتانۆل بەهای بەرزتری بۆ هەموو فیتۆکیمیاییە چۆرایەتی و چەندایەتییەکان بەدەستهێناوە،

جگە لە کۆی ڕێژەی ساپۆنین، کە تەنها زیاتر بووە کاتێک ئاو وەک توێنەر بۆ گەڵاکانی *G. triacanthos*، *L. leucocephala* و *R. pseudoacacia* بەکاردەهێنرێت . دەرهێنانی فیتۆکیمیایی چۆرایەتی و چەندایەتی لە ژێر کاریگەری هۆکارەکانی وەک جەمسەری توێنەر، ڕێژەی توێنەر بۆ ماددە ڕووەکییەکان، قەبارەی تەنۆلکەکانی مادەکان، شێوازی دەرهێنان و پلەی گەرمی بوو. دەرکەوت کە فیتۆکیمیاییەکان ڕاستەوخۆ وابەستەی قۆناغی گەشەکردنی ڕووەکەکە و بەشە تایبەتەکانی ڕووەکەکە و ئەو توێنەرانەی کە بۆ دەرهێنان و جیاکردنەوە بەکاردەهێنرێن. کانزاکان ڕۆڵێکی جۆراوجۆر دەگێڕن لە میتابۆلیزمی ڕووەکی دەرمانیدا، و بوونیان بە پلەی جیاواز دەتوانێت کاریگەری جۆراوجۆری لەسەر میتابۆلیزمی ڕووەکەکان هەبێت. ئەم کاریگەریانە دەتوانن هەم توند بن و هەم جیاواز بن بەپێی زۆری یان کەمی ئەم کانزایانە.

بەکارهێنانی نیکل (Ni)، قورقوشم (Pb) و کارلێکەکانیان لەسەر هەندێک تایبەتمەندی شانە زانی ڕووەکەکان، وەک گەڵا و لق وڕەگ، هەروەها ژمارە و درێژی و پانی دەمیلەکان، دەرکەوت کە زیادی کردوە بەزیادکردنی خەستی 30 و 45 ملغم/کغم خاک لە نەمامەکانی *G. triacanthos*، *L. leucocephala* و *R. pseudoacacia*. هەڵمژینی بەرز و کەڵەکەبوونی کانزا قورسەکان لە ڕەگی ڕووەکی جۆراوجۆردا دەبێتە هۆی گۆڕانکاری ئەناتۆمی جۆراوجۆر کاتێک بەکارهینانی خەستی زۆر نیکل دەبن. بوونی زۆری هەردوو کانزای بنەڕەتی و ناپێویست دەبێتە هۆی فشاری کانزایی لە ڕووەکەکاندا و چەندین کاریگەری ڕاستەوخۆ یان ناڕاستەوخۆ دروست دەکات، کە کاریگەرییان لەسەر نزیکەی هەموو ئەرکە کار ئەندام زانیەکان هەیە.

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