

Phytochemical Composition of *Gleditsia triacanthos* L. Grow in Nickel- and Lead-Artificially Contaminated Soil

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Abstract—Plants in the Fabaceae family are rich in phytochemicals, and their composition can be influenced by environmental factors such as heavy metals. A factorial experiment using a completely randomized design was conducted in an open field in an open field in the Koya district, Erbil, Iraq to evaluate the effects of soil contamination with NiCl₂ or PbCl₂ (0, 15, 30, and 45 mg/kg soil) on eight phytochemicals in *Gleditsia triacanthos* L. leaves. Three extraction solvents – water, methanol, and ethyl acetate were compared. Methanol extracted the highest amounts of phenols, flavonoids, glycosides, and alkaloids, while ethyl acetate was more effective for steroids, terpenes, and tannins. Water gave the highest saponin content. Nickel contamination significantly increased phenols and terpenes but reduced flavonoids, glycosides, steroids, and saponins across all solvents. Lead contamination increased flavonoids in all extracts, reduced glycosides in water and methanol extracts, increased methanolic terpenes, and decreased saponins in water extracts. The highest phenol (62.14 mg/g) and alkaloid (3.05 mg/g) contents were obtained with methanol under the Ni45Pb30 treatment. Maximum flavonoid (17.31 mg/g), glycoside (2.44 mg/g), and terpene (5.95 mg/g) levels were recorded under the Ni15Pb30, Ni0Pb0, and Ni30Pb45 treatments, respectively. Overall, nickel and lead contamination significantly altered the phytochemical composition of *G. triacanthos* L. leaves.

Index Terms—Extraction solvents, Fabaceae, Heavy metal, Lead and Nickel, Phytochemicals.

I. INTRODUCTION

The honey locust (*Gleditsia triacanthos*) is a Fabaceae tree commonly used in landscaping throughout much of Iraq; it plays a crucial in carbon restoration, soil stabilization, landfill usage, and the re-vegetation of wastelands. It generates significant biomass and can concentrate substantial amounts of heavy metals in its aerial parts. This plant can

thrive in various stressful environmental conditions, with the exception of frost; it grows rapidly, can mature, and yield seeds within 6–7 months. These species generate phytochemicals or compounds with pharmacological activity that are extensively utilized in human therapy, veterinary practices, agriculture, scientific research, and numerous other fields (Dana, et al., 2022).

Phytochemicals, also known as secondary metabolites, are natural chemical compound generated by living organisms, commonly found in nature, which typically exhibit pharmacological or biological activity. Phytochemicals from the Fabaceae family hold significant industrial and pharmacological value, serving as a plentiful source of phenols, flavonoids, alkaloids, tannins, terpenoids, steroids, glycosides, and saponins, with their application becoming more prevalent over time (Usman, et al., 2022).

Minerals play a varied role in the metabolism of medicinal plants. The intensity of lack of these leads to a variety of impacts on plant metabolism. Every detail of plant biochemistry, anatomy, physiology, and so on is influenced by the mineral nutrient composition of soils. The levels of these minerals in the soil are crucial in secondary plant metabolism, as the compounds of secondary metabolites differ among plants based on their growth conditions, including heavy metal soil content, plant species, plant location, extraction technique, soil topography, and plant age (Emenike and Chukwuebuka, 2021). Some heavy metals are essential for basic biological functions. Nonetheless, an overload of heavy metals can cause significant harmful impacts at both the cellular and organismal level (Briffa, Sinagra and Blundell, 2020).

The issue of heavy metals has recently garnered global attention because of their dangers to humans and the environment by contaminating soil, water, and food (Deswal and Laura, 2018). Nickel (Ni) and lead (Pb) are two heavy metals commonly presented in contaminated locations; multiple authors suggest that the toxicity of Pb and Ni may lead to oxidative cellular harm when they generate reactive oxygen species. Nickel is a vital element for plant growth and development (Shahzad, et al., 2018); however, at elevated levels (ranging from 10 to 1000 mg/kg dry weight based on plant sensitivity), it can become harmful to

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plants, resulting in biochemical, physiological, and cellular alterations that lead to significant damage. Pb is not an essential element for plants; it is a toxic heavy metal and a dangerous environmental pollutant (Mehboob, et al., 2018).

To the best of our knowledge, the only previous study investigating the effects of Ni and Pb on *G. triacanthos* L. was conducted by Khudhur and Albarzinji (2022), who examined the influence of Ni and Pb on some of the plant's enzymatic and non-enzymatic content. As a complement to that earlier work, the present study investigates the effects of Ni and Pb on the phytochemical composition of *G. triacanthos* L., thereby providing a broader understanding of the plant's physiological and biochemical responses to heavy metal stress.

II. MATERIALS AND METHODS

A. Plant Resources and Growth Condition

The mature seed pods of *G. triacanthos* L. (Fig. 1) were gathered from trees that are 10 years old on March 2, 2021, at Koya University Campus (Koya city, Erbil, Iraq (44°38' E, 36°44' N and an altitude of 570 m above sea level). The seeds were immersed in tap water at room temperature for 24 h. An experiment using a completely randomized design (Factorial CRD) with three replicates was performed, where the first factor involved the types of heavy metal (Ni and Pb), and the second factor consisted of four concentrations of each element. On 25 March 2021, the seeds were cultivated in black polyethylene bags (12 cm diameter and 25 cm height) containing 3 kg of loamy soil (Ec 0.3, pH 8.2, O.M. 0.6%, Ni 220 ppm, and Pb 9 ppm) mixed with Ni (0, 15, 30 or 45 mg $\text{NiCl}_2 \cdot \text{kg}^{-1}$ soil) based on the total weight of the added salts NiCl_2 and PbCl_2 , identified as Ni_0 , Ni_{15} , Ni_{30} and Ni_{45} or Pb (0, 15, 30 or 45 mg $\text{PbCl}_2 \cdot \text{kg}^{-1}$ soil) labeled as Pb_0 , Pb_{15} , Pb_{30} and Pb_{45} along with their interactions. The research was carried out in ambient environmental conditions with temperature ranging from 14 to 39°C and relative humidity between 19% and 64%. The bags were adequately irrigated with tap water to ensure potentially uniform soil moisture levels. To prevent loss of nutrients and trace elements out of the experimental units, unperforated polythene sheets were placed under each one.

Five months post-seed germination, samples were collected from growing seedlings to identify certain



Fig. 1. *Gleditsia triacanthos* L. (a) seeds, (b) stalks.

qualitative and quantitative phytochemicals as outlined by Lahare, et al., (2022).

B. Qualitative Evaluation of the Phytochemical Composition

A preliminary qualitative assessment of phytochemicals was carried out following standard protocols (Figs. 2 and 3).

Phenols were tested using ferric chloride

The test samples received 3–4 drops of 0.1% (v/v) ferric chloride solution. The appearance of a brownish green or blue color indicated the presence of phenolic compounds.

Wagner's test for alkaloids

The filtrate was combined with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown or reddish deposit indicates the existence of alkaloids.

Ferric chloride test for flavonoids

Several drops of ferric chloride solution were mixed with the test solution, and the presence of flavonoids compounds was indicated by a bright green color.

Liebermann-Burchard test for sterols

The solution was treated with a few drops of acetic anhydride (Liebermann-Burchard) and mixed duly. Concentrated sulphuric acid was added from the edges of the test tube. The existence of sterol was verified by the appearance of a brown ring at the junction of the two layers and the green color of the upper layer.

Tannin test

A crude solution was mixed with 2 mL of 2% FeCl_3 solution. The presence of polyphenols and tannins is confirmed by appearing of a blue-green or blue-black coloration.

Keller-Kiliani test for glycosides

Two mL of glacial acetic acid which contain 1–2 drops of 2% of FeCl_3 solution was mixed with the crude extract. Next, the mixture was transferred into another test tube contains 2 mL of concentrated H_2SO_4 . The emergence of a brown ring at the interface confirms the presence of glycosides.

Salkowski test for terpenoids

A few drops of concentrated sulphuric acid were added slowly to the test solution, shaken appropriately, and let it at room temperature. The presence of a lower yellow layer was the confirm to exist of terpenoids.

Test for saponins

Five mL of distilled water was mixed with the crude extract within a test tube and shaken strongly for 30 s. Stable foam (1 cm height) formation even after 30 min is considered as a suggestion for the presence of saponins.

C. Quantitative Determination of the Phytochemicals Content

To estimate the phytochemicals quantitatively, the following extraction method was used: 5 g of leaf powder was mixed with 50 mL of solvent (water, methanol, or ethyl acetate). The mixture was sonicated at 500 W for 2 h. It was then filtered through 0.45 μm filter paper. The filtrate was dried using a freeze dryer. The resulting crude extract

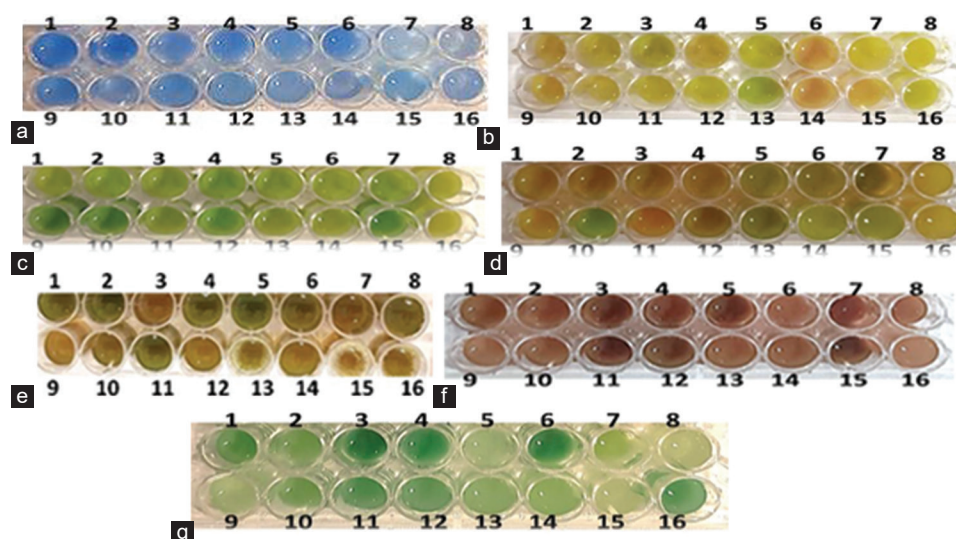


Fig. 2. Methanol extraction of (a) total phenols, (b) total flavonoids, (c) total glycosides, (d) total alkaloids, (e) total steroids, (f) total terpenes, and (g) total tannins content of *Gleditsia triacanthos* leaves, where 1. Ni0×Pb0, 2. Ni0×Pb15, 3. Ni0×Pb30, 4. Ni0×Pb45, 5. Ni15×Pb0, 6. Ni15×Pb15, 7. Ni15×Pb30, 8. Ni15×Pb45, 9. Ni30×Pb0, 10. Ni30×Pb15, 11. Ni30×Pb30, 12. Ni30×Pb45, 13. Ni45×Pb0, 14. Ni45×Pb15, 15. Ni45×Pb30, 16. Ni45×Pb45 mg/kg soil.

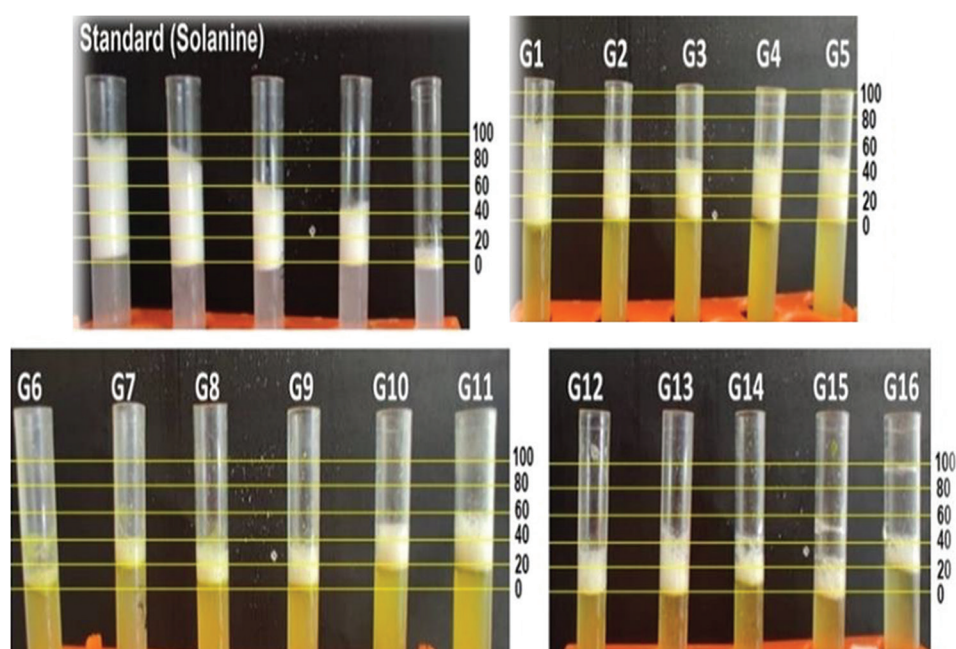


Fig. 3. Water extraction of total saponin content of *Gleditsia triacanthos* leaves, where 1. Ni0×Pb0, 2. Ni0×Pb15, 3. Ni0×Pb30, 4. Ni0×Pb45, 5. Ni15×Pb0, 6. Ni15×Pb15, 7. Ni15×Pb30, 8. Ni15×Pb45, 9. Ni30×Pb0, 10. Ni30×Pb15, 11. Ni30×Pb30, 12. Ni30×Pb45, 13. Ni45×Pb0, 14. Ni45×Pb15, 15. Ni45×Pb30, 16. Ni45×Pb45 mg/kg soil.

was redissolved in solvent to achieve a 1000 ppm stock solution.

Folin–Ciocalteu method was used for determine the total phenolic content. Total alkaloid content by Wagner's test reagent. Total flavonoid content was measured as per the Dowd method. Total glycoside content using 80% phenol solution spectrophotometry at 490 nm. Total steroid content determined by the Liebermann-Burchard test spectrophotometry at 565 nm. Total tannins content by the Braemer test by methanolic and ethyl acetate

spectrophotometry at 490 nm. The content of total terpenes was determined by the Salkowski test using methanolic and ethyl acetate spectrophotometry at 550 nm. Total saponin content using ethanol and diethyl ether.

D. Data Statistical Analysis

The experiment was conducted according to a factorial completely randomized design. Each treatment was replicated 3 times, and 10 bags were considered as an experimental unit. The SAS statistical program version 9.1 was used to compare

treatment means using the analysis of variance. The means of the treatments were compared using Duncan's multiple range test ($p \leq 0.05$) (Al-Mohammadi and Al-Mohammadi, 2002).

III. RESULTS AND DISCUSSION

A. Qualitative Determination of the Phytochemical Constituents

The qualitative analysis for the phytochemicals of *G. triacanthos* showed the existence of many bioactive compounds. Table I represents the secondary metabolites: Phenolic, alkaloid, flavonoid, glycoside, steroid, tannins, terpenes, and saponin, which extracted from the dry leaves by water, methanol, and ethyl acetate as solvents. Each of the methanol and ethyl acetate solvents led to the presence of all the tested phytochemicals, except for saponins that present only for water extract, where methanol was superior to water and ethyl-acetate extracts for extracting total phenols, alkaloids, flavonoids, glycoside, steroid and tannins, whereas they had same activity in extracting total terpenes. Existing of these phytochemicals is previously confirmed by Abou Zeid, et al. (2018) for sterols and triterpenes, Mohammed, et al., (2014) for flavone glycosides and two flavone aglycones, and Lalitha, Ajmeera and Madhavi (2025) for alkaloids, flavonoids, terpenoids and tannins in the methanolic extract.

The plant capacity to produce the expected favorable biological response is demonstrated by the presence of a broad variety of phytochemical. In addition, the most important factor in extracting secondary plant metabolites is the polarity of the solvents. The chemical composition for the pharmacological and pathological discovery of medicinal drugs can be obtained through the qualitative phytochemical screening of plant extracts (Ushie, et al., 2022). In comparison to water and ethyl acetate, methanol has better capabilities for extracting chemical compounds, where methanol has the ability to extract polar molecules like phenolics and tannins as well as non-polar chemicals like steroids and terpenoids (Table I).

B. Quantitative Determination of the Phytochemical Constituents

From the results of Table II, it is shown that the methanol was superior compare to water and ethyl acetate in extracting

total phenolic content, followed by water, then ethyl acetate solvents. Result of Table II show that phenolic content increased with increasing Ni concentration for all extraction solvents. The highest phenolic content was extracted from methanol solvent (44.76 mg/g), then by water (9.54 mg/g), and finally by ethyl acetate (6.27 mg/g), respectively, for the 45 mg/kg NiCl_2 treatment, while the lowest values were recorded by non-treated plants which were 34.08, 5.48 and 2.56 mg/g for the methanol, water and ethyl-acetate, respectively. Conversely, phenolic content decreased with lead (Pb) application, where the highest phenolic content value was for Pb30 (41.35 mg/g) when extracted with methanol, water (7.99 mg/g), and ethyl-acetate (4.25 mg/g) when no Pb was added, while the lowest values were found by same solvents order (30.76, 6.73 mg/g) at 15 mg/kg PbCl_2 and (4.02 mg/g) when 30 mg/kg PbCl_2 was used. Most of the interaction treatments between high Ni and high Pb increased the plant content of phenolic for water, methanol, and ethyl acetate solvents.

Alkaloid content increased with increasing Ni concentration only by methanol solvent extraction, while it decreased with water and ethyl acetate solvents. The highest alkaloid content was extracted by methanol (2.20 mg/g) at 45 mg/kg NiCl_2 concentration, while for water and ethyl acetate (0.70 and 0.142) mg/g they were recorded at no Ni treatment. The lowest value of alkaloid extracted by methanol (1.66 mg/g) registered in the untreated plants, while for water and ethyl- acetate solvents (0.046 and 0.093 mg/g) were recorded for the 45 mg/kg NiCl_2 concentration. However, the alkaloid content increased significantly with higher Pb concentration (0.073 and 0.151 mg/g) where extracted by water and ethyl acetate solvents, whereas it decreased significantly at the 15 mg/kg Pb concentration where methanol solvent was used (1.489 mg/g). Interactions between Ni and Pb recorded different response, were total alkaloid extracted by water and ethyl-acetate increased in the control treatment (Ni0 Pb0) and interactions of Ni15 with Pb30 and pb45 and the interaction Ni30Pb0. The methanol extraction solvent increased total alkaloids to (3.05 mg/g) in the interaction treatment Ni45Pb30, significantly compared to all other treatments (Table II).

Methanol extraction records higher flavonoid content, followed by water, then ethyl acetate solvents. Flavonoids

TABLE I

PRELIMINARY INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS IN WATER, METHANOL AND ETHYL-ACETATE EXTRACTS OF THE *GLEDISTIA TRIACANTHOS* LEAVES

Phytochemical constituents	Name of the test	Observation	Extract solvent		
			Water	Methanol	Ethyl-acetate
Phenols	Folin - Ciocalteu method	Blue color	+++	++++	++
Alkaloids	Wagner's test	Brown colored	±	++	+
Flavonoids	Dowd method	Green color	++	+++	+
Glycosides	Phenol-sulfuric acid solution	Yellow color	±	+	±
Steroid	Liebermann - Burchard test	Dark green color	—	+	+
Tannins	Braemer test	Dark green color	—	+	±
Terpenoids	Salkowski test	Light brown-orange color	—	++	++
Saponins	Foam test	Formation of foam	++++	—	—

“+” Present, “—” Absent (++++ (Very High) +++ (High), ++ (Moderate) + (low) ± (negligible) and - (Nil))

TABLE II

EFFECTS OF NICKEL, LEAD AND THEIR INTERACTIONS ON TOTAL PHENOLIC AND ALKALOID CONTENT (MG.G⁻¹ DRY WEIGHT) OF *GLEDITSIA TRIACANTHOS* LEAVES USING DIFFERENT EXTRACTION SOLVENTS

Treatment	Total phenols			Total alkaloids		
	Extraction solvent					
	Water	Methanol	Ethyl- acetate	Water	Methanol	Ethyl- acetate
Nickel concentration (mg/kg soil)						
Ni 0	5.48 ^{b*}	34.08 ^c	2.56 ^d	0.070 ^a	1.66 ^c	0.142 ^a
Ni 15	5.49 ^b	33.77 ^c	2.80 ^c	0.069 ^a	1.63 ^d	0.140 ^a
Ni 30	8.90 ^a	41.38 ^b	3.78 ^b	0.062 ^b	2.01 ^b	0.129 ^b
Ni 45	9.54 ^a	44.76 ^a	6.27 ^a	0.046 ^c	2.20 ^a	0.093 ^c
Lead concentration (mg/kg soil)						
Pb 0	7.89 ^a	41.04 ^{ab}	4.25 ^a	0.063 ^b	2.00 ^{ab}	0.128 ^b
Pb 15	6.73 ^b	30.76 ^c	4.12 ^{ab}	0.053 ^d	1.489 ^c	0.106 ^d
Pb 30	7.99 ^a	41.35 ^a	3.01 ^c	0.059 ^c	2.022 ^a	0.119 ^c
Pb 45	6.80 ^b	40.84 ^b	4.02 ^b	0.073 ^a	1.994 ^b	0.151 ^a
Interaction between concentrations of Ni and Pb						
Ni0 Pb0	6.15 ^{cd}	38.95 ^c	3.01 ^{fg}	0.081 ^a	1.88 ^{de}	0.163 ^a
Ni0 Pb15	5.86 ^{cd}	36.11 ^h	2.89 ^g	0.075 ^b	1.74 ^g	0.152 ^c
Ni0 Pb30	4.95 ^{cd}	30.32 ^{jk}	2.16 ^h	0.064 ^c	1.51 ⁱ	0.120 ^f
Ni0 Pb45	4.96 ^{cd}	30.95 ^j	2.17 ^h	0.062 ^c	1.51 ⁱ	0.131 ^c
Ni15 Pb0	4.09 ^d	26.98 ⁱ	2.22 ^h	0.053 ^d	1.32 ^k	0.112 ^g
Ni15 Pb15	5.06 ^{cd}	30.14 ^k	2.52 ^h	0.063 ^c	1.45 ^j	0.125 ^{ef}
Ni15 Pb30	6.64 ^c	39.85 ^d	3.30 ^f	0.081 ^a	1.93 ^d	0.164 ^a
Ni15 Pb45	6.18 ^{cd}	38.12 ^f	3.17 ^{fg}	0.080 ^a	1.84 ^{ef}	0.160 ^{ab}
Ni30 Pb0	10.41 ^{ab}	59.10 ^b	2.98 ^{fg}	0.081 ^a	2.89 ^b	0.162 ^a
Ni30 Pb15	10.08 ^{ab}	36.15 ^h	4.05 ^c	0.029 ^g	1.74 ^g	0.060 ^j
Ni30 Pb30	9.06 ^b	33.10 ⁱ	2.39 ^h	0.064 ^c	1.60 ^h	0.140 ^d
Ni30 Pb45	6.04 ^{cd}	37.16 ^g	5.68 ^c	0.074 ^b	1.82 ^f	0.154 ^{bc}
Ni45 Pb0	10.91 ^{ab}	39.14 ^c	8.81 ^a	0.036 ^f	1.92 ^d	0.073 ⁱ
Ni45 Pb15	5.92 ^{cd}	20.63 ^m	7.0 ^b	0.044 ^c	1.03 ^l	0.090 ^h
Ni45 Pb30	11.29 ^a	62.14 ^a	4.20 ^c	0.026 ^g	3.05 ^a	0.052 ^k
Ni45 Pb45	10.03 ^{ab}	57.14 ^c	5.07 ^d	0.077 ^{ab}	2.82 ^c	0.157 ^c

*Within columns, means followed by the same letters are not significantly different at $p \leq 0.05$ according to the Duncan test

decreased with increasing the Ni element concentration by all solvents used. The highest value of the flavonoid was extractions was recorded at no Ni added treatments (14.89, 3.79, and 2.30) mg/g were registered in the control treatment, and the lowest content were recorded at higher Ni concentrations. However, the flavonoid content increased significantly at 45 mg/kg soil PbCl₂ to 15.55, 4.12, and 1.41 mg/g for methanol, water, and ethyl acetate solvents, respectively. The interactions between the elements Ni and Pb, with increased concentrations to 45 mg/kg of NiCl₂ or PbCl₂ for two solvents (methanol and ethyl acetate), decreased the flavonoids content, but it was increased with increasing nickel and lead concentrations only for the water solvent, as seen in Table III.

Glycoside content (Table III) increased significantly at 15 mg/kg NiCl₂ to 1.34 and 0.83 mg/g when water and ethyl acetate were used as solvents, and the concentration 45 mg/kg NiCl₂ decreased the glycoside content significantly to 0.49 and 0.46 mg/g, respectively, whereas the 30 mg/kg concentration was superior in increasing the glycoside content significantly to 1.44 mg/g with methanol solvent. Glycoside content decreased with increasing Pb concentration when water and methanol solvents were used, to reach 0.43 and

TABLE III

EFFECTS OF NICKEL, LEAD AND THEIR INTERACTIONS ON FLAVONOID AND GLYCOSIDES CONTENT (MG.G⁻¹ DRY WEIGHT) OF *GLEDITSIA TRIACANTHOS* LEAVES WITH DIFFERENT EXTRACTION SOLVENTS

Treatment	Flavonoid			Glycosides		
	Extraction solvent					
	Water	Methanol	Ethyl- acetate	Water	Methanol	Ethyl- acetate
Nickel concentration (mg/kg soil)						
Ni 0	3.79 ^{a*}	14.89 ^a	2.30 ^a	1.24 ^b	1.39 ^b	0.73 ^b
Ni 15	3.83 ^a	14.59 ^a	2.27 ^b	1.34 ^a	0.48 ^d	0.83 ^a
Ni 30	3.47 ^b	13.12 ^b	0.33 ^d	0.80 ^c	1.44 ^a	0.68 ^c
Ni 45	2.61 ^c	9.38 ^c	0.59 ^c	0.49 ^d	0.81 ^c	0.46 ^d
Lead concentration (mg/kg soil)						
Pb 0	3.44 ^b	12.92 ^b	1.37 ^b	1.22 ^a	1.39 ^a	0.68 ^b
Pb 15	3.03 ^c	10.95 ^c	1.36 ^b	1.15 ^b	1.12 ^b	0.66 ^b
Pb 30	3.10 ^c	12.56 ^b	1.35 ^b	1.07 ^c	0.70 ^d	0.63 ^c
Pb 45	4.12 ^a	15.55 ^a	1.41 ^a	0.43 ^d	0.91 ^c	0.74 ^a
Interaction between concentrations of Ni and Pb						
Ni0 Pb0	4.34 ^a	17.06 ^{ab}	2.61 ^b	1.21 ^e	2.44 ^a	0.75 ^e
Ni0 Pb15	4.32 ^a	15.85 ^c	2.42 ^d	1.32 ^d	1.61 ^b	0.84 ^d
Ni0 Pb30	3.14 ^b	13.31 ^e	2.09 ^e	1.81 ^b	0.53 ⁱ	1.14 ^c
Ni0 Pb45	3.35 ^b	13.35 ^e	2.10 ^e	0.61 ^k	0.99 ^g	0.21 ^j
Ni15 Pb0	3.11 ^b	11.44 ^f	1.81 ^f	1.93 ^a	0.36 ^j	1.24 ^b
Ni15 Pb15	3.61 ^b	13.21 ^e	2.05 ^e	1.73 ^c	0.31 ^{jk}	1.15 ^c
Ni15 Pb30	4.32 ^a	17.31 ^a	2.68 ^a	1.16 ^f	0.34 ^{kl}	0.73 ^e
Ni15 Pb45	4.29 ^a	16.41 ^{bc}	2.54 ^c	0.54 ^l	0.91 ^h	0.20 ^j
Ni30 Pb0	4.25 ^a	16.07 ^c	0.23 ^l	0.94 ^h	1.53 ^c	0.27 ^{hi}
Ni30 Pb15	1.70 ^{de}	5.75 ⁱ	0.34 ^k	0.84 ^j	1.40 ^d	0.30 ^{gh}
Ni30 Pb30	3.49 ^b	14.38 ^d	0.22 ^l	1.03 ^g	1.62 ^b	0.41 ^f
Ni30 Pb45	4.44 ^a	16.26 ^c	0.51 ⁱ	0.39 ^m	1.19 ^f	1.72 ^a
Ni45 Pb0	2.07 ^{cd}	7.12 ^h	0.82 ^g	0.81 ⁱ	1.25 ^c	0.44 ^f
Ni45 Pb15	2.50 ^c	8.99 ^g	0.64 ^h	0.72 ^j	1.16 ^f	0.35 ^g
Ni45 Pb30	1.43 ^c	5.22 ⁱ	0.40 ^j	0.25 ⁿ	0.29 ^k	0.23 ^{ij}
Ni45 Pb45	4.43 ^a	16.18 ^c	0.51 ⁱ	0.19 ^o	0.54 ⁱ	0.81 ^d

*Within columns, means followed by the same letters are not significantly different at $p \leq 0.05$ according to the Duncan test

0.70 mg/g at 45 and 30 mg/kg PbCl₂, whereas higher content was recorded in the plants that not treated with Pb element (1.22 and 1.39 mg/g). Regarding the ether-acetate solvent, a higher Pb concentration 45 mg/kg increased significantly glycoside content to 0.74 mg/g. Interaction treatment Ni15Pb0 increased glycoside content significantly to 1.93 mg/g, whereas lowest value (0.19 mg/g) recorded at the interaction treatment of higher Ni and Pb, where water was used as a solvent. Higher glycoside content at methanol solvent was recorded at the control treatment (2.44 mg/g), and the lowest at the Ni45Pb30 treatment. For ethyl acetate, the interaction treatment Ni30Pb45 recorded a higher value (1.72 mg/g), and the lowest value at the treatment Ni0Pb45 (0.21 mg/g). It is seen that interactions of decreasing Ni and increasing Pb decreased these phytochemicals.

Steroid content decreased significantly with increasing the Ni element concentration by both extract solvents. The highest values of steroid content extracted by methanol and ethyl acetate (0.044 and 0.0672 mg/g) were registered in the control treatment, whereas the lowest values (0.029 and 0.0435 mg/g) were extracted by methanol and ethyl acetate at 45 mg/kg NiCl₂ concentration. However, the steroid content increased with higher Pb concentration for both methanol

and ethyl acetate (0.047 and 0.071 mg/g). Most interaction treatments between Ni and Pb had the same effects of individual elements, where the lowest values of steroids were recorded in the interactions of high Ni and Pb concentrations, except the Ni45Pb45 treatment (Table IV).

Content of tannin increased with the increase in Ni element concentration by two extract solvents. The highest values of tannin content extracted by methanol and ethyl acetate (0.055 and 0.088) mg/g were recognized from the concentration 45 mg/kg NiCl_2 treatment, while the control treatment recorded the lowest values (0.041 and 0.051) mg/g. The Pb concentration 15 mg/kg PbCl_2 records significant lowest tannin values reached 0.037 and 0.061 mg/g for the methanol and ethyl acetate solvents. Interactions between Ni and Pb, with increasing elements to 45 mg/kg NiCl_2 or PbCl_2 concentration, increased tannin content for the two extracted solvents, as it see in Table IV.

Terpene content increased significantly to 4.44 and 4.16 mg/g at the Ni concentration 30 mg/kg NiCl_2 in comparison to other treatments, whereas the lowest content extracted by methanol (2.92) mg/g was recorded in the Ni0 treatment, and the treatment 45 mg/kg NiCl_2 (2.57) which extracted by ethyl acetate. However, the terpenes content decreased significantly with increased Pb element to 30 and 45 mg/kg when methanol is the extraction solvent, while it increased significantly to 3.80 mg/g at 45 mg/kg PbCl_2 element concentration for ethyl acetate solvent extraction. The interactions between Ni30 with Pb30 and 15 increased the terpene content to 5.06 and 5.95 mg/g for the methanol solvent and to 4.85 and 5.50 mg/g for the ethyl acetate solvent, significantly to all other treatments Table V.

Saponin content decreased significantly with the exist of Ni and Pb element in the soil, where the highest saponin content for watering extraction (54.14 mg/g) was for the no-Ni treatment, while the lowest content (24.14) mg/g was recorded at the treatment 45 mg/kg NiCl_2 . As similar as to Ni, no PbCl_2 treatment records the highest value of saponin content (46.64 mg/g), significantly compared to all other treatments. At same trend, the interactions between Ni and Pb concentrations decreased significantly saponin content compared the control treatment, as it clear in (Table V).

According to the rule of “like dissolves like” polar solutes generally dissolved better in polar solvents such as water and methanol, while non-polar solutes dissolved better in a non-polar solvents such as ethyl acetate, so for high-performance extraction capacity, closeness of polarity values of the solute and solvents is necessary (Zhang, et al., 2018). One of the most important parameters for the extraction process is polarity, which affects the efficiency of extraction. The most efficient solvent for extracting the chemical compounds in *G. triacanthos* was methanol. Methanol has been shown to be more active in dissolving each of polar and polar intermediate molecules that containing OH or COO groups, such as flavonoids and phenolic (Satrianegara, et al., 2024). According to Thouri, et al. (2017), this particular group has the ability to engage in interactions with ionic or hydrogen bonds, specifically with polar sites or ions originating from the solvent.

TABLE IV
EFFECTS OF NICKEL, LEAD AND THEIR INTERACTIONS ON STEROID AND TANNINS CONTENT (MG.G⁻¹ DRY WEIGHT) OF *GLEDITSIA TRIACANTHOS* LEAVES WITH DIFFERENT EXTRACTION SOLVENTS

Treatment	Steroid		Tannins	
	Extraction solvent			
	Methanol	Ethyl-acetate	Methanol	Ethyl-acetate
Nickel concentration (mg/kg soil)				
Ni 0	0.044 ^{a*}	0.0672 ^a	0.041 ^c	0.051 ^c
Ni 15	0.045 ^a	0.0672 ^a	0.041 ^c	0.051 ^c
Ni 30	0.040 ^b	0.0613 ^b	0.51 ^b	0.092 ^a
Ni 45	0.029 ^c	0.0435 ^c	0.055 ^a	0.088 ^b
Lead concentration (mg/kg soil)				
Pb 0	0.040 ^b	0.060 ^b	0.050 ^a	0.074 ^a
Pb 15	0.032 ^d	0.050 ^d	0.037 ^b	0.061 ^c
Pb 30	0.038 ^c	0.058 ^c	0.051 ^a	0.074 ^a
Pb 45	0.047 ^a	0.071 ^a	0.050 ^a	0.073 ^b
Interaction between concentrations of Ni and Pb				
Ni0 Pb0	0.0515 ^a	0.0735 ^{cd}	0.048 ^{cd}	0.059 ^f
Ni0 Pb15	0.0435 ^c	0.0714 ^d	0.041 ^f	0.055 ^h
Ni0 Pb30	0.0404 ^d	0.0619 ^f	0.038 ^g	0.046 ⁱ
Ni0 Pb45	0.0405 ^d	0.0620 ^f	0.038 ^g	0.046 ⁱ
Ni15 Pb0	0.0360 ^e	0.0540 ^g	0.033 ^h	0.041 ^j
Ni15 Pb15	0.0395 ^d	0.0600 ^f	0.037 ^g	0.046 ⁱ
Ni15 Pb30	0.0525 ^a	0.0795 ^a	0.049 ^c	0.060 ^f
Ni15 Pb45	0.0505 ^{ab}	0.0755 ^{bc}	0.047 ^{cd}	0.057 ^g
Ni30 Pb0	0.0505 ^{ab}	0.0765 ^b	0.072 ^b	0.098 ^b
Ni30 Pb15	0.0185 ^h	0.0275 ^j	0.045 ^e	0.092 ^d
Ni30 Pb30	0.0435 ^c	0.0665 ^e	0.041 ^f	0.085 ^e
Ni30 Pb45	0.0485 ^b	0.0745 ^{bc}	0.046 ^{de}	0.095 ^c
Ni45 Pb0	0.0225 ^g	0.0345 ⁱ	0.048 ^{cd}	0.098 ^b
Ni45 Pb15	0.0275 ^f	0.0415 ^h	0.026 ^j	0.054 ^h
Ni45 Pb30	0.0155 ⁱ	0.0245 ^k	0.076 ^a	0.106 ^a
Ni45 Pb45	0.0485 ^b	0.0735 ^{cd}	0.71	0.094 ^c

*Within columns, means followed by the same letters are not significantly different at $p \leq 0.05$ according to the Duncan test.

Methanol is highly effective for quantitative extractions because its amphiphilic structure (a polar hydroxyl group and a non-polar carbon chain) allows it to dissolve a wider spectrum of phytochemicals. It extracts polar molecules (phenolics, tannins) and lower-polarity compounds (steroids, terpenoids) better than water or ethyl acetate (Tables II, IV, and V). The results were agreed with Jayashik (2025) and Satrianegara, et al. (2024), plant, that was the highest total phenolic content found in the methanol extract. In another study, Salem, et al. (2011) found that extraction using 80% methanol produced the highest concentration of phenolic and flavonoid compounds, and with the results of Karimkhani et al. (2016), where the optimal extraction of flavonoid glycosides and polar aglycones is achieved using a mixed solvent with water, in this case 70% ethanol.

The decrease in the phytochemical yield with lead exposure may related to the toxicity of lead, which may interfere with plant metabolic processes such as photosynthesis and secondary metabolite production (Chowbey and Rehman, 2025), or changing in enzymatic and non-enzymatic antioxidants such as decreasing catalase enzyme activity, proline content, total carotenoids, and Vitamin C with increasing pb concentration in the soil of growing *G. triacanthos* plants (Khudhur and Albarzinji,

TABLE V

EFFECTS OF NICKEL, LEAD AND THEIR INTERACTIONS ON TERPENES AND SAPONIN CONTENT (MG.G⁻¹ DRY WEIGHT) OF *GLEDITSIA TRIACANTHOS* LEAVES WITH DIFFERENT EXTRACTION SOLVENTS

Treatment	Terpenes		Saponin
	Extraction solvent		
	Methanol	Ethylacetate	
Nickel concentration (mg/kg soil)			
Ni 0	2.92 ^{c*}	3.12 ^c	54.14 ^a
Ni 15	2.87 ^d	3.72 ^b	26.24 ^c
Ni 30	4.44 ^a	4.16 ^a	34.45 ^b
Ni 45	3.22 ^b	2.57 ^d	24.14 ^d
Lead concentration (mg/kg soil)			
Pb 0	3.41 ^b	2.54 ^d	46.64 ^a
Pb 15	3.69 ^a	3.72 ^b	33.50 ^b
Pb 30	3.08 ^d	3.50 ^c	27.89 ^d
Pb 45	3.28 ^c	3.80 ^a	30.93 ^c
Interaction between concentrations of Ni and Pb			
Ni0 Pb0	2.16 ⁿ	2.17 ⁿ	73.13 ^a
Ni0 Pb15	4.23 ^d	4.71 ^c	64.69 ^b
Ni0 Pb30	2.34 ^m	2.62 ^l	38.44 ^e
Ni0 Pb45	2.95 ⁱ	2.97 ⁱ	40.31 ^d
Ni15 Pb0	3.94 ^e	3.91 ^e	42.19 ^c
Ni15 Pb15	3.20 ^h	3.59 ^h	11.22 ⁿ
Ni15 Pb30	2.79 ^k	2.68 ^k	17.81 ^m
Ni15 Pb45	1.56 ^p	4.69 ^d	33.75 ^h
Ni30 Pb0	2.91 ^j	3.67 ^g	32.81 ⁱ
Ni30 Pb15	3.83 ^f	2.59 ^m	37.49 ^f
Ni30 Pb30	5.06 ^b	4.85 ^b	35.63 ^g
Ni30 Pb45	5.95 ^a	5.50 ^a	31.89 ^j
Ni45 Pb0	4.62 ^c	1.48 ^p	38.43 ^e
Ni45 Pb15	3.48 ^g	2.92 ^j	20.63 ^k
Ni45 Pb30	2.13 ^o	3.85 ^f	19.69 ^l
Ni45 Pb45	2.67	2.021 ^o	17.81 ^m

*Within columns, means followed by the same letters are not significantly different at $p \leq 0.05$ according to the Duncan test.

2022). The findings of the quantitative analysis presented that the methanolic extract is higher in phenolics, flavonoids, glycosides, alkaloids compounds compared to the water and ethyl acetate extracts, which coincides with Belkhodja, et al., (2020) results, who found that the methanolic extract is higher in polyphenolic, flavonoid, and condensed tannins compounds. The study results also agree with that of (Sobati-Nasab, Alirezalu and Noruzi, 2021).

IV. CONCLUSION

The research on *G. triacanthos* L. shows that contact with the heavy metals nickel and lead greatly influences the plant's phytochemical makeup using various extraction solvents. Polar solvents can extract a greater quantity of phytochemicals. The increase or decrease in bioactive compounds like phenols, flavonoids, alkaloids, glycosides, steroids, tannins, saponins, and terpenes suggests that environmental pollution may alter the therapeutic potential of the plant. Based on these findings, a future outlook can be established for utilizing alternative extraction solvents such as chloroform, ethanol, acetone, and hexane for quantitative and qualitative analysis of 22 phytochemicals, potentially yielding diverse outcomes.

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