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Research Article

Investigating the effect of seasonal changes on the phenolic compounds profile in *Phragmites australis* plant from areas contaminated with heavy metals in the city of Samawah, Iraq

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Abstract

Plants naturally produce various types of secondary metabolites known as polyphenols. The plant's production process of polyphenols can be altered due to environmental stressors such as climate change and the accumulation of heavy metals in the soil or water it relies on. *Phragmites australis*, also called the common reed, is a perennial grass species that thrives in wetland and riparian habitats. This study aims to quantify the impact of seasonal variations and fluctuations in heavy metal concentrations on the production process and yield of plant products in the selected plant. Additionally, it seeks to evaluate the plant's ability to remediate pollutants through phytoremediation. To achieve this objective, soil and plant samples were collected from three distinct locations in the vicinity of Samawah city. Among these, two areas were identified as being polluted with high levels of heavy metals, while the third area was found to be uncontaminated. The sampling was conducted over the course of a year, encompassing all four seasons, in order to assess the impact of seasonal variations. The quantity of heavy metals, specifically lead, chromium, mercury, and cadmium, present in plant samples and the sediment surrounding the plant was determined using atomic absorption spectrometry. Polyphenols were quantified using high-performance liquid chromatography (HPLC), while photosynthesis pigments were determined using spectrophotometry. The findings revealed notable correlations between specific polyphenols and distinct heavy metals, indicating possible interactions and adaptations of the plant in reaction to stress caused by metals. The results showed that Apigenine-7-O-glucoside exhibited the highest polyphenol concentration, which was more than 9000 mg/kg DW in both autumn and spring at the Samaweh site, followed by caffeic acid showed the highest amount (more than 3000 mg/kg DW) in all seasons at the Kader site. Furthermore, the findings indicated that the plant's phytoremediation capacity varies across various seasons and regions. There was no discernible pattern in the levels of polyphenols or the efficacy of heavy metal removal in response to climate change. The findings of this study indicate that the *Phragmatis australis* plant may possess phytoremediation capabilities.

Keywords: Common reed, Environmental contamination, HPLC, Phytoremediation, Al Muthanna

Introduction

Polyphenols are a varied collection of chemical substances that occur naturally and are distinguished by having several phenol units. These substances are categorized as secondary metabolites and are predominantly present in plants (Mohammadi *et al*., 2021; Alizadeh *et al*., 2021). Polyphenols have a crucial role in various aspects of plant biology, including growth, development, defense mechanisms, and the ability to adapt to environmental challenges. Polyphenols possess antioxidant, anti-inflammatory, antibacterial, and anticancer characteristics and regulate several cellular and molecular processes in both humans and animals. They also provide protection against chronic diseases and act as powerful antioxidants (Durazzo *et al*., 2019; Mousavi e*t al*., 2021). Furthermore, they possess the capacity to impact the sensory characteristics, including color, flavor, odor, and stability, of food products (de Araujo *et al*., 2020; Gonzalez *et al*., 2020). Polyphenols are widely present in a variety of plant-based foods, including fruits, vegetables, tea, red wine, and dark chocolate (Mohammad Ghasemi *et al*., 2020**)**.

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The polyphenolic content and composition of plants are influenced by various factors, including plant species, genotype, developmental stage, environmental conditions, and cultivation practices (Maddahi *et al*., 2022). Seasonal variations have been observed to impact the polyphenol composition of plants due to their influence on various environmental factors such as temperature, light intensity, photoperiod, water availability, and nutrient status (Hamouz *et al*., 2018). Numerous studies have documented the occurrence of seasonal fluctuations in polyphenol content within various crops and wild plant species across diverse geographical locations (Chiriac *et al*., 2021; de Medeiros Gomes *et al*., 2021; Sharma *et al*., 2020; Sharifi and Pourakbar, 2016).

Heavy metals, including lead, chromium, mercury, and cadmium, can have a significant effect on the polyphenolic composition of plants when present in the environment. Furthermore, heavy metal contamination can disrupt the enzymatic activity involved in polyphenol biosynthesis, leading to alterations in the types and quantities of polyphenols produced by plants. This can ultimately impact the nutritional value and bioactivity of these plants, affecting their interactions with other organisms in the ecosystem (Goncharuk and Zagoskina, 2023). These metals have the ability to cause oxidative stress, which results in a decrease in chlorophyll content and an increase in the levels of proline, retinol, α-tocopherol, and ascorbic acid in plant tissues (Zengin and Munzuroglu, 2005). It is shown that okra plants (*Abelmoschus esculentus*. L.) possess a significant amount of biomass and exhibit a high capacity to mobilize and accumulate Hg. It is indicated that exposure to mercury (Hg) caused alterations in the phenolic composition of the plants, resulting in an elevation in the levels of chlorogenic acid, rosmaric acid, apigenin, quercetin, and rutin (Mohammadi *et al*., 2021). Another investigation revealed that the levels of phenolic compounds in okra plants treated with Cd were found to have the highest increase in chlorogenic acid (101.91%) and the largest decrease in coumaric acid (60.90%) compared to the control group (Mousavi *et al*., 2021).

The presence of heavy metal contamination is an additional environmental factor that has the potential to exert an influence on the growth, metabolism, and polyphenol production of plants (Zargari *et al*., 2020). Heavy metals are considered to be toxic elements that have the potential to accumulate in both soil and water (Pourakbar *et al*., 2007b). This accumulation can occur as a result of either natural processes or human activities, including but not limited to mining, smelting, industrial operations, agricultural practices, and urban development (Hadia-e-Fatima and Ahmed, 2018). The presence of heavy metals in plants can induce oxidative stress and inflict harm upon plant cells through their interference with crucial physiological processes such as photosynthesis, respiration, enzyme activity, membrane integrity, and nutrient uptake (Abbasi *et al*., 2023; Pourakbar *et al*., 2007a). In response to high levels of heavy metal stress, plants have the ability to engage a range of defense mechanisms, including chelation, sequestration, exclusion, translocation, and detoxification (Mousavi *et al*., 2022). Certain mechanisms are associated with the synthesis or accumulation of polyphenols, which possess the ability to function as antioxidants or metal chelators (Mohammadi *et al*., 2021).

The urban center known as Samawah serves as the administrative and political hub of the Al Muthanna Governorate, situated in the southern region of Iraq. The city is situated along the banks of the Euphrates River and experiences a hot desert climate characterized by elevated temperatures and limited precipitation. The urban area is currently confronted with a multitude of environmental challenges stemming from inadequate infrastructure, sanitation facilities, waste management systems, and water resources (Hussien *et al*., 2020). One of the challenges encountered involves the contamination of soil and water with heavy metals, which can be attributed to the existence of oil refineries, power plants, cement factories, military operations, sewage discharge, and agricultural runoff. The presence of elevated levels of heavy metals in the environment presents a significant concern for both the physical well-being of the local inhabitants and the overall ecological balance.

Phragmites australis, commonly known as the common reed, is a perennial grass species that exhibits growth in wetland and riparian environments across all continents with the exception of Antarctica (Yi *et al*., 2020). The plant exhibits upright stems adorned with elongated and flattened leaves, accompanied by dense plumes of purple-brown flowers (Perna *et al*., 2023). This plant species has the ability to propagate through both rhizomes and seeds, resulting in the formation of dense stands that effectively outcompete and exclude other plant species. The observed potential of this substance lies in its ability to engage in phytoremediation, wherein it demonstrates the capacity to effectively uptake heavy metals and organic pollutants present in both water and soil environments (Rohal *et al*., 2019). Additionally, it serves as a valuable resource for biomass production, paper manufacturing, animal feed, and human consumption (Yi *et al*., 2020).

This study investigated the impact of seasonal variations on the levels of polyphenols in the *Phragmites australis* plant from Samawah, Iraq, which is exposed to high levels of heavy metal contamination. The halophytic plant *P. australis* thrives in saline and alkaline soils and possesses a significant amount of polyphenols and antioxidants (Yun *et al*., 2019). This plant possesses the ability to endure and accumulate significant amounts of heavy metals. It serves as a bioindicator and phytoremediators for local heavy metal pollution. Nevertheless, there is limited knowledge regarding the impact of seasonal variations and heavy metal pollution on the polyphenol composition and

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health benefits of these plants. This study sought to accomplish the following objectives: (1) quantifying and analyzing the polyphenol content and composition of *P. australis* plants grown in areas of Samawah, Iraq, that are contaminated with heavy metals; (2) investigating the connection between the polyphenol profile and the plant's heavy metal content, as well as the composition of the underlying sediment; and (3) exploring the correlation between the polyphenol profile and the levels of chlorophyll I, ll, and carotene in the plant. The study aimed to investigate the seasonal changes and the influence of heavy metals on the polyphenol profiles of the *P. australis* plant. Additionally, it sought to determine the possible health benefits of these plants as natural antioxidants and their ability to mitigate heavy metal pollution in the region.

Materials and methods

Sampling sites: Plants were collected in three regions in Iraq (Table 1). Out of these three areas, two of them were contaminated with heavy metals, and one area was selected as a control. The plants were naturally present in these areas and were not cultivated by the researcher.

The first site that was sampled was the Rumaitha area (Figure 1). This area, which is located in the Mothni province of Iraq, is located in the south of the country. Sampling of plants was done in an area close to the Rumaitha water project, which studies show is an area contaminated with heavy metals (Dalil and Al-Fanharawi, 2023). The second site that was sampled is Samawah, which is located in the same Muthani province. Sampling was done in an area near the Al-Samawah Oil Refinery, which is polluted with heavy metals. The third site, which is a non-polluted control, was taken from Al-Khader district near the bridge, Al-Khader center, so that the results of other sites can be evaluated by comparing the control samples.

Sampling method: The sampling was done in four seasons: autumn (September to November 2022), winter (December 2022 to February 2023), spring (March to May 2023), and summer (June to August 2023). Two of the sites were contaminated with heavy metals due to human activity or other unknown causes, and one was considered as a control. The sampling was done once a season for polyphenols and twice a season for heavy metals, in autumn and winter. At each site, five random quadrats of 1 m x 1 m were established, and all the plants within each quadrat were harvested. The plants were washed with distilled water and dried with filter paper. The plant samples were then oven-dried at 60°C for 48 hours and ground into fine powder using a mortar and pestle. The sediment samples were air-dried, sieved through a 2 mm mesh, and homogenized. The amount of polyphenols and heavy metals (mercury, cadmium, lead and chromium) in each plant sample was determined by standard methods (Mohammadi *et al*., 2021).

The polyphenol content and composition of the plant samples were determined by high-performance liquid chromatography (HPLC) (Cui *et al*., 1999; Preet and Chand Gupta, 2018). The separation was performed on a C18 column (250 mm × 4.6 mm, 5 µm) at 25°C, with a mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was as follows: 0-5 min, 5% B; 5-15 min, 5-15% B; 15-25 min, 15-25% B; 25-35 min, 25-35% B; 35-45 min, 35-45% B; 45-55 min, 45-100% B; 55-65 min, 100% B; 65-70 min, 100-5% B; and 70-80 min, 5% B. The flow rate was 1 mL/min, and the injection volume was 20 µL. The detection wavelength was 280 nm for gallic acid, protocatechuic acid, tamarixtein, rosmaric acid, caffeic acid, hesperidin, chlorogenic acid, p-coumaric acid, carsnoic acid, and vanillic acid; 330 nm for naringin, rutin, quercetin, kaempferol, quercetin-3-O-rutinoside, apigenin-7-O-glucoside, and naringin-O-glucoside. The identification and quantification of the polyphenols were based on the comparison of the retention times and peak areas with the standards. The total polyphenol content was calculated as the sum of the individual polyphenols.

The heavy metal content of the plant and sediment samples was determined by atomic absorption spectrometry (AAS) according to the method of reference (Ferreira *et al*., 2018). The plant samples were digested with a mixture of nitric acid and perchloric acid in a microwave oven. The sediment samples were digested with a mixture of nitric acid, hydrochloric acid, and hydrofluoric acid on a hot plate. The digested samples were diluted with deionized water and filtered through a 0.45 µm membrane. The AAS system consisted of a graphite furnace, a hollow cathode lamp, and software for data acquisition and processing. The wavelengths and the detection limits for the heavy metals were as follows: lead (Pb), 217.0 nm, 0.01 mg/L; chromium (Cr), 357.9 nm, 0.005 mg/L; mercury (Hg), 253.7 nm, 0.001 mg/L; and cadmium (Cd), 228.8 nm, 0.002 mg/L. The calibration curves were prepared using standard solutions of the heavy metals. Quality control was ensured by using blanks, duplicates, and certified reference materials.

To evaluate whether our plant has phytoremediation properties against heavy metal stress, the ratio of the heavy metal concentration in the aerial parts of the plant to the concentration of heavy metal in the sediment, called the bioconcentration factor, has been used. For plants that accumulate heavy metals, the ratio of metal concentration in the plant to metal concentration in the soil (or sediment) is greater than 1. The ratio for non-accumulating plants is less than 1, while for indicator plants, it is approximately 1 (Chitimus *et al*., 2023; Yan *et al*., 2012).

The chlorophyll a and b and carotene content of the plant samples were determined by spectrophotometry (Chazaux *et al*., 2021; Marr *et al*., 1995). The plant samples were extracted with 80% acetone in a dark room. The absorbance of the extracts was measured at 470 nm, 646.8 nm, and 663.2 nm using a spectrophotometer. The chlorophyll a and b and

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Table 1. Geographical coordinates and distances between the sampling areas

Area	Latitude	Longitude	Distance from Samawah
Rumaitha	31.530921° N	45.195597° E	9 km
Samawah	31.320531° N	45.279943° E	0 km
Kadher	31.194251° N	45.265448° E	15 km

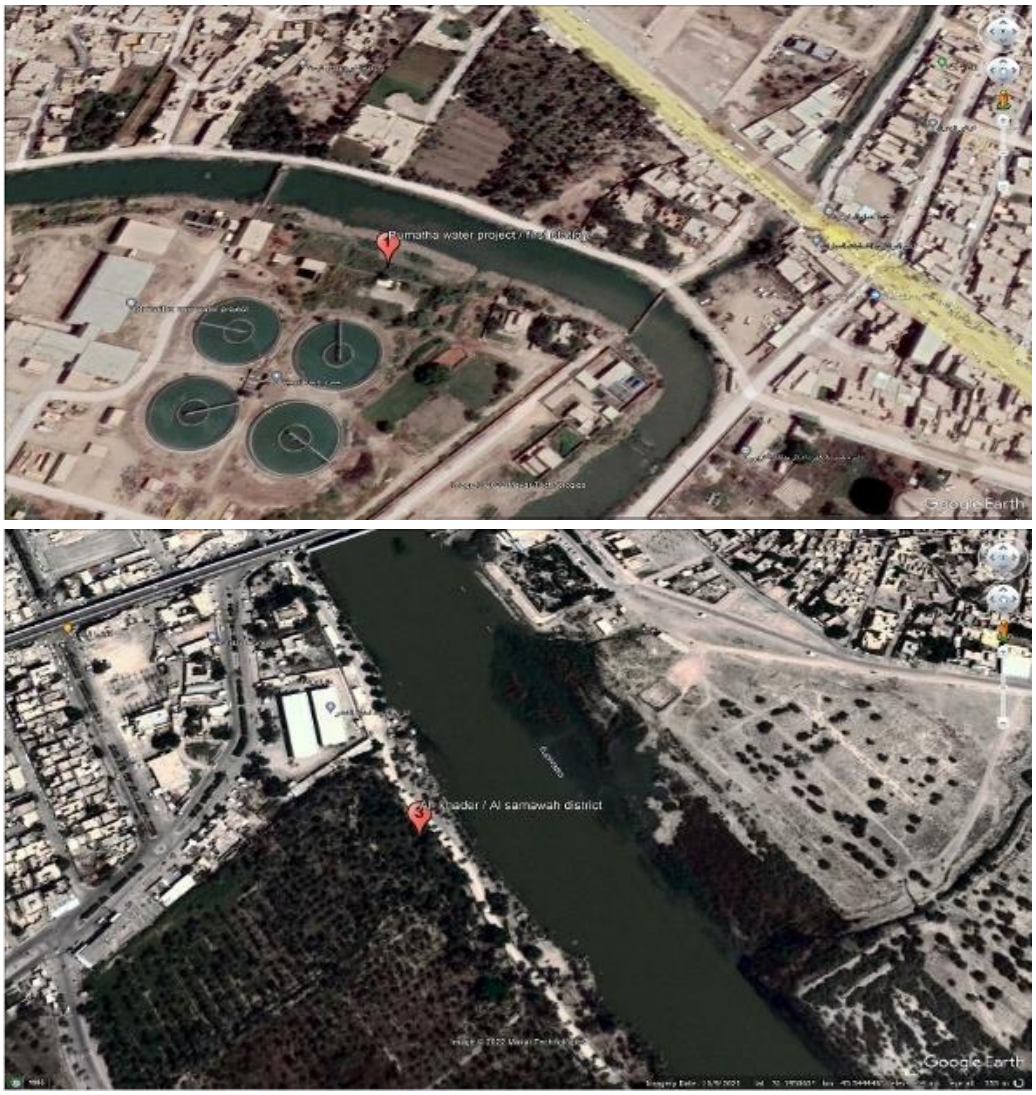


Figure 1. Sampling locations in Iraq. Upper: Rumatha water project, Lower: Samawah-Al-khader district



Figure 2. *Phragmites australis* also known as common reed. Photo from Unsplash

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carotene content were calculated using the following equations (Lichtenthaler, 1987):

Chl a (µg/mL) = 12.21 (A663.2) − 2.81 (A646.8)

Chl b (µg/mL) = 20.13 (A646.8) − 5.03(A663.2)

Total Chl (µg/mL) = 17.32 (A646.8) + 7.18 (A663.2)

$Cx{+}c$ (µg/mL) = 1000 (A470) −3.27 (Chl a (µg/mL) −104 (Chl b (µg/mL)) 229

The climatic conditions of the sampling areas, including the rainfall and the sediment pH, were recorded using a rain gauge and a pH meter, respectively.

The data were analyzed using the SPSS software (version 26). The descriptive statistics, such as the mean, standard deviation, minimum, and maximum, were calculated for each variable. The normality and homogeneity of the data were tested using the Shapiro-Wilk and Levene tests, respectively. The differences among the seasons and the areas were tested using the two-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. The relationships among the variables were tested using the Pearson correlation coefficient. The significance level was set at 0.05.

Results

Physical and chemical properties of sampled regions: Data pertaining to the physical and chemical characteristics of the sampling sites were collected during the sampling process (Table 2). The table below displays the pH levels of the sediment and water at the sampling location, the temperature of the sediment and water, and the average precipitation for the entire season.

The results of heavy metal measurements in surrounded sediment: The tables provided contain the measured concentrations of heavy metals in the bottom sediments of plant growth areas (Table 3). There is a notable variation in the mercury content found in sediments across various seasons. One possible explanation for this could be the variation in human activity levels in the surveyed regions during different seasons.

The result of heavy metal measurements in plant samples: The table below displays the findings of heavy metal measurements conducted on the shoot plant and during various seasons. It is well established that the rate of removal of lead, chromium, and mercury heavy metals from polluted areas is higher compared to the control area. However, the amount of cadmium accumulation is unexpectedly higher in non-polluted areas than in polluted areas. The decrease in the plant's resistance to cadmium removal can be attributed to various causes, including the stress induced by the presence of the contaminated metal.

Based on the findings presented in Table 5, the Samawah site exhibited the greatest quantity of lead metal, with its highest concentration observed during the winter. Regarding chromium metal, it is found in greater abundance at the Samawah site compared to other sites. However, its peak concentration was observed during the summer. In regard to mercury metal, it is notably more abundant at the Rumaitha site, with its peak concentration observed during spring. As previously stated, the concentration of cadmium metal in the control area is significantly greater than in the other two sites, which is contrary to the anticipated results (Table 4). Moreover, the highest level of cadmium was recorded during the summer.

The amount of polyphenols measured in the plant: The table below and Figure 3 display the polyphenolic composition of the plant during various seasons. The quantity of polyphenols varies across seasons and regions, which can be attributed to the plant's metabolic alterations in response to different environmental factors such as temperature, environmental acidity, humidity, and even the stress caused by heavy metal pollution.

Based on the data presented in Tables 6 and 7, Apigenine-7-O-glucoside exhibited the highest concentration of polyphenol, exceeding 9000 mg per kilogram of dry plant weight during both the fall and spring seasons at the Samawah site. Commenting on the lowest amount of polyphenol is not feasible due to the presence of values below the device's threshold, which were not reported numerically.

The amount of chlorophyll a and b and total carotenoids in plant: Chlorophyll and carotene are common metabolites found in most plants. Table 8 displays the quantities of these substances present in the plant. The concentration of these substances can fluctuate depending on the impact of sunlight and other environmental factors.

According to the data provided in the table, the cumulative quantity of chlorophyll a exceeds that of chlorophyll b and carotenoids. The control area and autumn season exhibited the highest concentration of chlorophyll a, measuring 730.5 µg/mL. Conversely, the Samawah area and winter season displayed the lowest concentration, measuring 526.5 µg/mL. Winter in the Samawah region has the highest value of chlorophyll b, while autumn in the control region has the lowest value (250 and 141 µg/mL). Winter and control areas exhibit the highest concentration of total carotenoids, while the lowest concentration is observed in the same season but in the Samawah area (389 and 289 µg/mL).

Analysis results

Investigation of seasonal variations in polyphenols, chlorophyll and heavy metal content: A two-way ANOVA test was employed to determine if there were significant variations in the levels of polyphenols, chlorophyll and heavy metals collected by the plant across different seasons and sampled sites. Subsequently, Tukey Post-Hoc was conducted to assess the statistical significance of the dependent variables in relation to the independent factors, namely seasons and sites. Based on the findings, there is no discernible pattern in the fluctuations of polyphenols, chlorophylls, and heavy metals. This lack of consistency can be

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Table 2. pH and temperature of each site for sediment and water and average rain amount in the whole season (°C)

Season	Site	pH		Temperature				Rain amount (mm)
				Sediment		Water		
		Sediment	Water	Min	Max	Min	Max	
Autumn	Rumaitha	7.4±0.01	7.1±0.12	34±0.01	37±0.14	17±0.03	26±0.07	17.2±0.09
	Samawah	7.2±0.02	7.8±0.07	33±0.13	35±0.11	19±0.01	28±0.04	16.5±0.10
	Kadher	7.6±0.01	8.1±0.05	31±0.04	34±0.09	16±0.02	29±0.03	11.3±0.12
Winter	Rumaitha	7.2±0.03	7.4±0.04	28±0.11	29±0.05	12±0.02	18±0.04	16.9±0.07
	Samawah	7.6±010	8.4±0.09	26±0.21	27±0.10	14±0.06	19±0.08	15.7±0.01
	Kadher	8.1±0.11	7.9±0.13	25±0.13	29±0.16	11±0.08	17±0.03	13.4±0.09
Spring	Rumaitha	6.9±0.03	6.7±0.08	30±0.23	34±0.12	18±0.07	26±0.01	10.1±0.12
	Samawah	7.1±0.09	7.5±0.04	31±0.10	36±0.23	21±0.05	28±0.02	8.3±0.05
	Kadher	8.3±0.07	7.7±0.03	33±0.20	34±0.31	19±0.02	30±0.07	4.3±0.01
Summer	Rumaitha	7.1±0.11	6.6±0.08	35±0.14	37±0.01	28±0.01	31±0.05	0±0.01
	Samawah	8.4±0.06	6.9±0.04	36±0.16	38±0.05	29±0.05	33±0.03	0±0.04
	Kadher	6.9±0.01	7.3±0.07	34±0.01	37±0.06	31±0.03	37±0.04	0±0.07

Table 3. The concentration of heavy metals in sediment samples

Season	Site	Pb (ppm)	Cr (ppm)	Hg (ppm)	Cd (ppm)
Autumn	Rumaitha	25.25±0.11	12.50±0.10	0.68±0.001	0.60±0.001
	Samawah	19.75±0.08	12.00±0.06	0.76±0.003	0.71±0.003
	Kadher	19.75±0.13	12.25±0.05	0.72±0.006	0.65±0.005
Winter	Rumaitha	22.8±0.09	22.95±0.02	0.006±0.0001	2.40±0.001
	Samawah	16.55±0.10	21.65±0.13	0.011±0.0001	1.45±0.006
	Kadher	12.7±0.05	21.95±0.10	0.005±0.0001	1.50±0.002
Spring	Rumaitha	20.6±0.13	22.50±0.05	0.005±0.0002	1.91±0.001
	Samawah	16.25±0.07	26.00±0.13	0.010±0.0001	1.24±0.003
	Kadher	11.55±014	25.50±0.04	0.005±0.0005	1.62±0.001
Summer	Rumaitha	21.75±0.05	25.41±0.06	0.007±0.0006	1.98±0.002
	Samawah	14.95±0.06	21.92±0.09	0.010±0.0005	1.72±0.001
	Kadher	16.25±0.11	23.40±0.05	0.008±0.0002	1.73±0.005

Table 4. The results of the analysis of the variance of the heavy metals in the aerial parts of the plant and photosynthetic pigments.

Source of variation	df	Mean of square						
		Pb	Cr	Hg	Cd	Chl a	Chl b	Total Car
Season	3	0.206*	1.040*	0.106*	0.267*	10633.14*	5916.85*	1729.29*
Site	2	805.44*	526.45*	46.67*	34.75*	64688.36*	8248.59*	2543.36*
Season×Site	6	1.536*	0.509*	0.084*	0.276*	2055.25*	655.99*	1634.95*
Experimental error	24	0.024	0.0005	0	0.009	17.528	13.222	8.417

*** show significance at the P ≤ 0.05 levels**

Table 5. The concentration of heavy metals in the aerial parts of the plant

Season	Site	Pb (ppm)	Cr (ppm)	Hg (ppm)	Cd (ppm)
Autumn	Rumaitha	14.425±0.09	6.01±0.042	1.33±0.005	0.061±0.001
	Samawah	18.25±0.11	13.68±0.091	0.875±0.007	0.050±0.003
	Kadher	2.06±0.13	0.29±0.005	0.009±0.0003	2.891±0.004
Winter	Rumaitha	14.50±0.05	7.075±0.032	1.16±0.009	0.065±0.009
	Samawah	18.33±0.10	13.44±0.040	0.8±0.001	0.065±0.005
	Kadher	1.765±0.12	0.24±0.001	0.008±0.004	2.361±0.001
Spring	Rumaitha	14.91±0.08	6.045±0.002	1.90±0.006	0.062±0.002
	Samawah	17.39±0.05	13.55±0.101	0.99±0.004	0.055±0.001
	Kadher	2.84±0.01	0.395±0.004	0.009±0.001	3.17±0.009
Summer	Rumaitha	15.84±0.07	6.965±0.071	1.58±0.004	0.065±0.007
	Samawah	17.02±0.06	14.41±0.091	0.92±0.006	0.051±0.004
	Kadher	2.725±0.03	0.49±0.009	0.007±0.0001	3.411±0.001

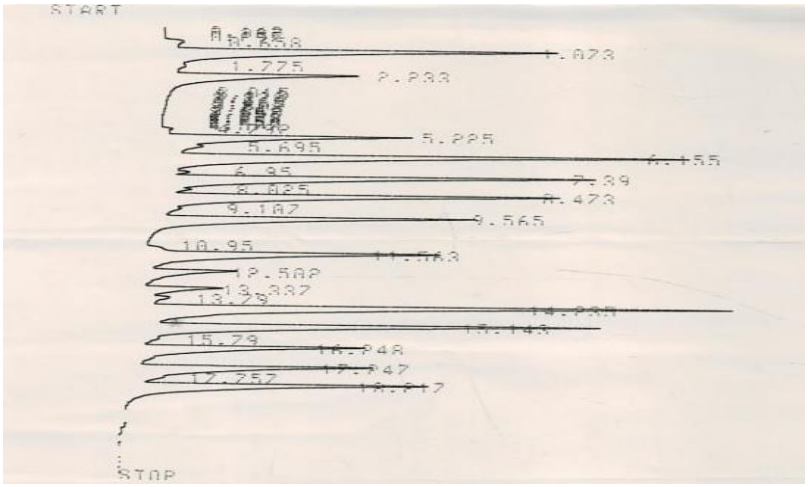
attributed to the influence of various environmental and internal factors that affect the plant at different points in time.

The correlation between polyphenols and

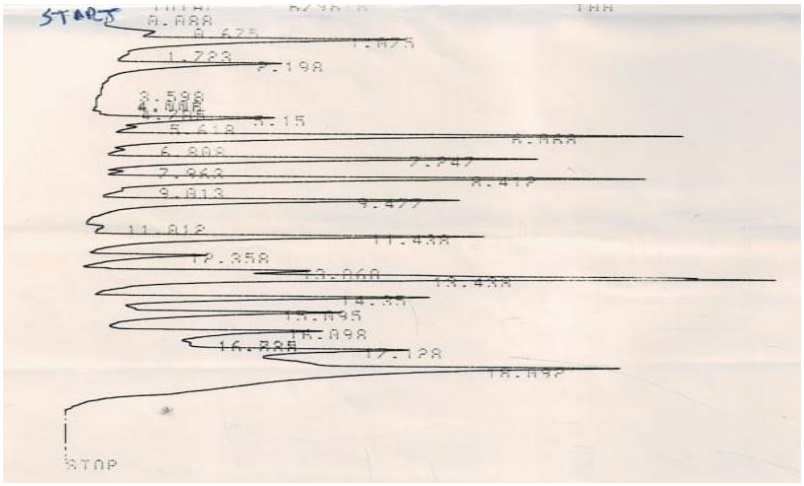
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Rumaitha



Samawah



Kadhar

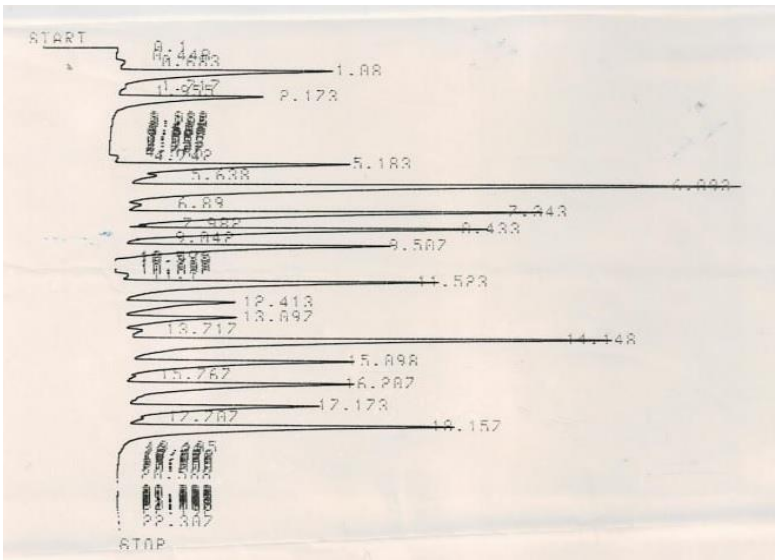


Figure 3. HPCL diagram of polyphenolic compounds of *Phragmites australis* collected from Rumaitha, Samawah and Kadhar.

chlorophyll and the total uptake of heavy metals by plants: Pearson's correlation test was employed to assess the correlation between the fluctuations in polyphenols and chlorophyll and the overall absorption of heavy metals by the plant. The significance thresholds were classified into two categories: Highly pertinent and pertinent. The findings indicated that the polyphenols and chlorophyll listed in Table 9 exhibit a direct and substantial correlation with heavy metals. This suggests that these specific compounds might play

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Table 6. The results of the analysis of variance of the phenolic compounds in the aerial parts of the plant

Source of variation	df	Mean Square							
		Gallic acid	Protocatechuic acid	Tamarixtein	Rosmaric acid	Caffeic acid	Hesperidin	Chlorogenic acid	p-coumaric acid
Season	3	71638.5*	59131467.6*	1592.5*	57930.17*	132855.3*	334910.9*	11041.8*	19864.91*
Site	2	9326302.2*	93730787.1*	756.61*	3758869.3*	9966294.3*	3902232.8*	3322765.4*	12389198.5*
Season×Site	6	10826.4*	57982436.1*	1592.5*	4392.17*	40582.5*	152546.9*	74529.2*	19864.91*
Experimental error	24	18.111	1361182.250	18.722	6.861	561.917	302.167	316.694	3.611

Continue of table 6.

Source of variation	df	Mean Square								
		Carsnoic acid	Vanillic acid	Naringin	Rutin	Quercetin	Kaempferol	Quercetin - 3-O-rutinoside	Apigenine-7-O-glucoside	Naringin-O-glucoside
Season	3	268102.8*	99734.9*	26588.9*	337708.4*	106956.5*	17628.7*	8225.5*	19845313.2*	137431.7*
Site	2	2144014.7*	7796946.0*	618869.3*	5628847.0*	179691.1*	1917143.6*	2096101.5*	67065843.5*	1230613.7*
Season×Site	6	74179.0*	56437.3*	18158.8*	222839.5*	91404.9*	34170.3*	104463.1*	18355681.1*	137431.7*
Experimental error	24	11.694	12.917	327.88	18.472	38.972	22.028	21.500	1052.083	5.167

*** show significance at the P ≤ 0.05 levels.**

Table 7. The results of measuring polyphenols in plants sampled from three areas and in different seasons (mg/kg DW). ND indicates that the amount of phenol in the plant is below the threshold of the device and cannot be identified and measured.

Season	Site	Gallic acid	Protocatechuic acid	Tamarixtein	Rosmaric acid	Caffeic acid	Hesperidin	Chlorogenic acid	p-coumaric acid
Autumn	Rumaitha	671.14	nd	856.36	757.66	1895.26	1721.42	1718.1	Nd
	Samawah	686.71	1156.35	nd	666.37	2283.63	1905.78	1747.20	1856.20
	Kadhar	2216.98	363.46	nd	1607.85	3762.07	2917.35	2748.29	Nd
Winter	Rumaitha	745.81	nd	756.61	865.20	1821.76	1848.87	1503.41	Nd
	Samawah	852.40	1885.37	nd	712.68	2505.37	1216.89	1803.90	1565.87
	Kadhar	2451.70	546.13	nd	1806.14	3634.04	2234.79	2708.23	Nd
Spring	Rumaitha	612.89	nd	799.66	812.24	1899.78	1678.89	1823.45	Nd
	Samawah	712.47	1245.32	nd	754.61	2145.32	1856.80	1653.99	1745.89
	Kadhar	2145.76	456.12	nd	1756.19	3654.01	2894.78	2698.21	Nd
Summer	Rumaitha	785.60	nd	812.30	963.11	1723.10	1756.55	1863.22	Nd
	Samawah	854.50	1325.44	nd	799.50	2168.40	1796.43	1745.52	1863.64
	Kadhar	2245.80	563.12	nd	1853.11	3326.88	2756.79	2436.89	Nd

Continue of table 7.

Season	Site	Carsnoic acid	Vanillic acid	Naringin	Rutin	Quercetin	Kaempferol	Quercetin - 3-O-rutinoside	Apigenine-7-O-glucoside	Naringin-O-glucoside
Autumn	Rumaitha	1107.86	Nd	456.36	2063.76	1518.63	713.05	742.40	978.08	Nd
	Samawah	1442.08	1373.71	688.71	1326.45	1619.30	1522.67	1950.22	9018.06	Nd
	Kadhar	453.11	1218.94	1088.64	3096.60	1542.65	1663.34	1129.88	1876.46	754.63
Winter	Rumaitha	1426.91	Nd	564.29	2757.37	1643.26	852.31	963.29	965.74	Nd
	Samawah	1899.80	1886.21	772.93	1896.86	1785.34	1456.85	1485.40	854.26	Nd
	Kadhar	677.47	1328.18	923.17	2656.36	1969.67	1323.10	1452.26	1635.14	Nd
Spring	Rumaitha	1056.90	Nd	546.20	2147.32	1463.21	789.37	923.20	896.76	Nd
	Samawah	1399.85	1456.20	789.90	1256.89	1745.30	1456.89	1854.47	9156.23	Nd
	Kadhar	624.45	1178.10	989.10	2956.30	1489.65	1563.11	1248.27	1789.10	699.78
Summer	Rumaitha	1145.60	Nd	635.24	2247.50	1564.20	801.63	988.44	799.82	Nd
	Samawah	1256.33	1325.78	965.33	1369.90	1963.44	1365.30	1963.50	2456.78	Nd
	Kadhar	752.99	1236.45	1020.35	2463.40	1345.66	1489.45	1347.27	1789.10	756.23

a crucial role in the plant's ability to absorb heavy metals. Additionally, the figures 4 to 7 display the data scatter images.

Investigation of phytoremediation of heavy

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Table 8. The amount of chlorophyll and total carotenoids of plants in different seasons (µg/mL).

Season	Site	Chl(a)	Chl(b)	Total carotenoids
Autumn	Rumaitha	708±10.01	185±3.21	363±14.12
	Samawah	566±5.01	197±4.11	376±11.01
	Kadhar	731±6.09	141±2.07	369±7.01
Winter	Rumaitha	598±13.01	217±6.98	324±5.09
	Samawah	527±11.65	250±4.76	289±4.19
	Kadhar	635±10.50	221±7.01	389±2.98
Spring	Rumaitha	649±9.75	197±6.98	331±3.08
	Samawah	532±8.99	220±3.87	349±4.10
	Kadhar	725±7.54	156±3.76	353±6.11
Summer	Rumaitha	679±6.98	156±5.21	328±3.49
	Samawah	582±13.21	216±4.76	351±8.40
	Kadhar	677±11.21	157±3.87	347±4.87

Table 9. Summary of correlated polyphenols and chlorophyll with heavy metal uptake. A single asterisk shows a high correlation (P<0.001), and two asterisks show a significant correlation (P<0.05). Other measured polyphenols show no correlation with HM uptake (P<0.05)

Heavy metals	Related polyphenols	Significance level
Pb	Hesperidin*	<0.001
	Carnosic acid*	<0.001
Cr	Hesperidin*	<0.001
	Carnosic acid*	<0.001
	Chlorophyll b**	0.022
Hg	Hesperidin**	0.039
Cd	Gallic acid*	<0.001
	Rosmaric acid*	<0.001
	Caffeic acid*	<0.001
	Chlorogenic acid*	<0.001
	Naringin**	0.003
	Rutin**	0.012
	Chlorophyll a**	0.039

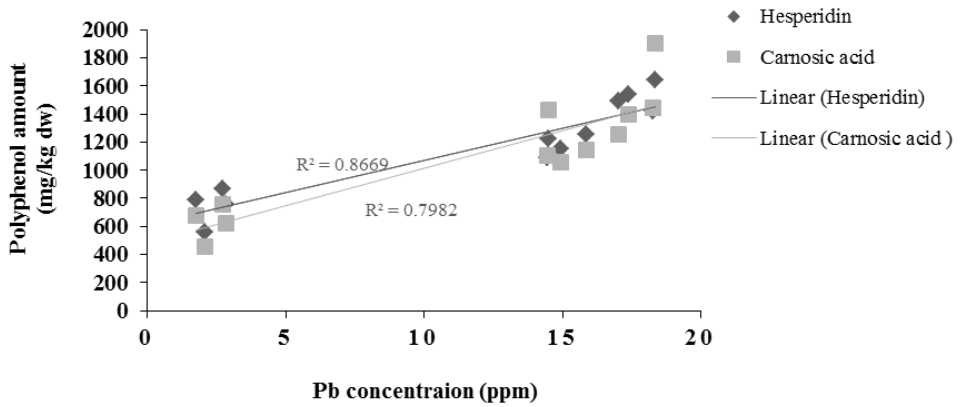


Figure 4. Correlation between lead (Pb) and polyphenols. Both Hesperidin and Carnosic acid are highly correlated with Pb (P <0.001).

metals: Figures 4 to 11 present the outcomes of computing the bioaccumulation factor using the ratio of heavy metal accumulation in the above-ground parts of the plant to the heavy metal concentration in the soil. The findings indicate that this plant demonstrates distinct characteristics in varying environments. The bioaccumulation factor (BF) of the plant for the heavy metal lead in the Samawah site is consistently above 1, except during autumn when it is close to 1. However, in different regions and time periods, this variable is below 1. For chrome metal, a value greater than 1 is observed exclusively at the Samawah site during the autumn. However, when considering mercury metal, it is evident that the value exceeds 1 at the Rumaitha site during all seasons. In the Samawah site, the number of something is typically less than 1 in autumn but greater than 1 in all other seasons. In spring, this number can even reach as high as 380. The values of this factor in the control area and during the winter and spring seasons exhibit values greater than 1, whereas during the summer it

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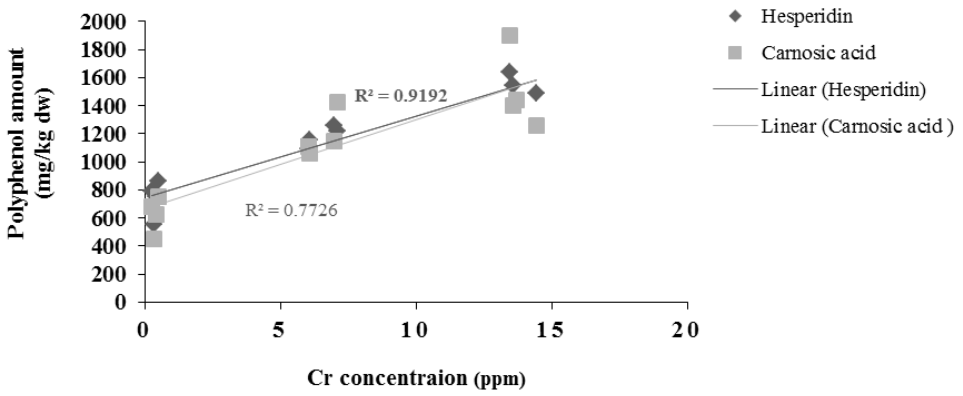


Figure 5. Correlation between Chromium (Cr) and polyphenols. Both Hesperidin and Carnosic acid are highly correlated with Cr (P <0.001).

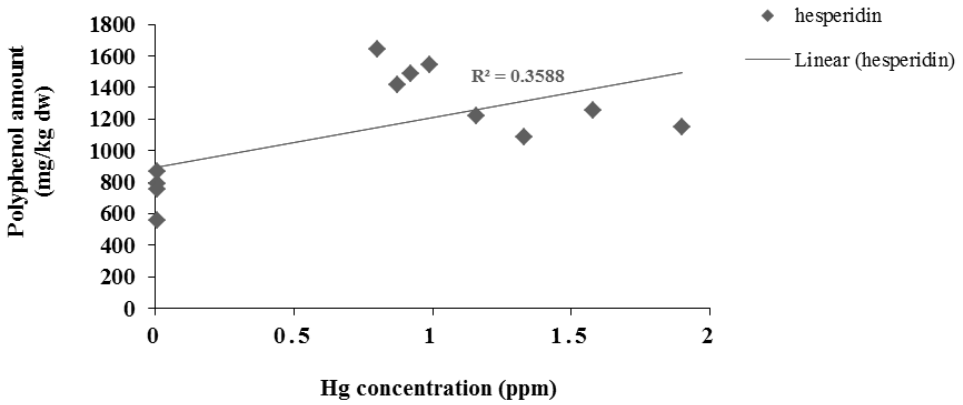


Figure 6. Correlation between mercury (Hg) and hesperidin. The correlation between these variables are significant (P<0.05).

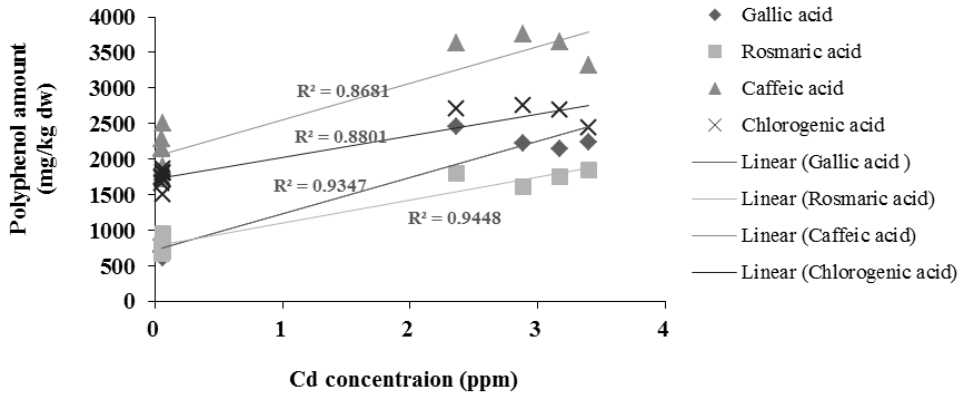


Figure 7. Correlation between cadmium (Cd) and polyphenols. The polyphenols shown in this figure are highly correlated with cadmium (P <0.001). However, two other polyphenols, Naringin and Rutin are also significantly correlated (P < 0.05)

approaches a value of 1. For cadmium, the factor exceeds 1 solely in the control area and across all seasons, with its peak value occurring during autumn.

Discussion

This study aimed to examine the impact of heavy metal pollution on the polyphenol composition of *Phragmites australis* plants in specific areas of Samawah, Iraq. The findings demonstrated that the polyphenol content and composition were notably affected by the existence and concentration of toxic heavy metals, such as lead (Pb), chromium (Cr), mercury (Hg), and cadmium (Cd), in the sediment. The findings also demonstrated significant associations between certain polyphenols and specific heavy metals, suggesting potential interactions and adaptations of the plant in response to metal-induced stress.

Polyphenols are a heterogeneous collection of secondary metabolites that exhibit a range of biological and ecological roles, including antioxidant,

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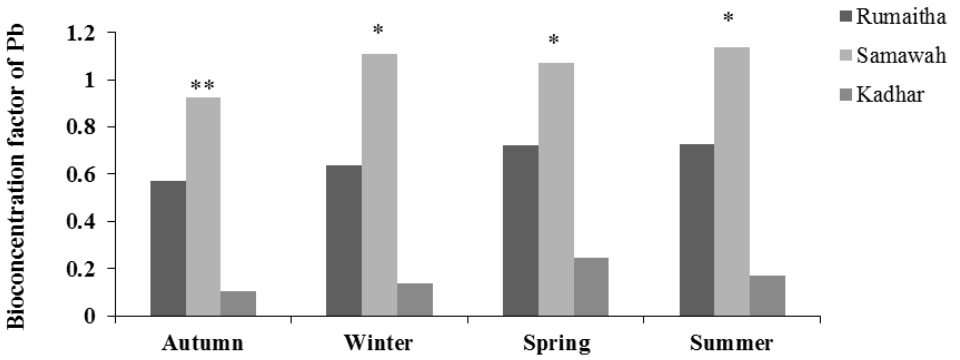


Figure 8. Ratio of lead (Pb) metal concentration in plants to sediment. One asterisk shows the accumulation property of the plant. Two asterisks show the indication property of the plant.

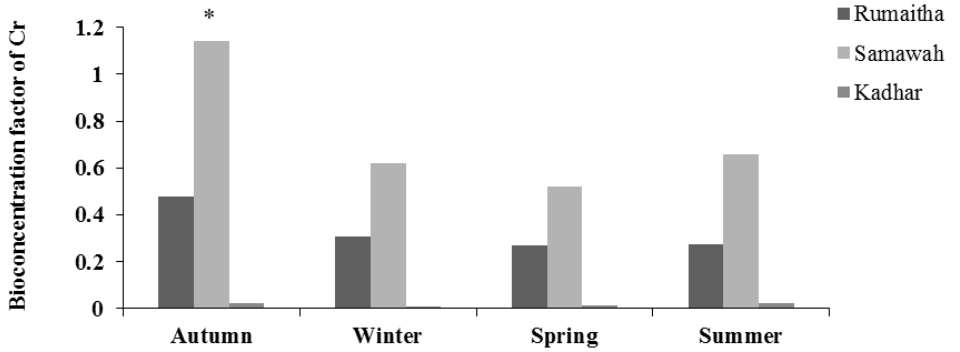


Figure 9. Ratio of chromium (Cr) metal concentration in plant to sediment. One asterisk shows the accumulation property in plant.

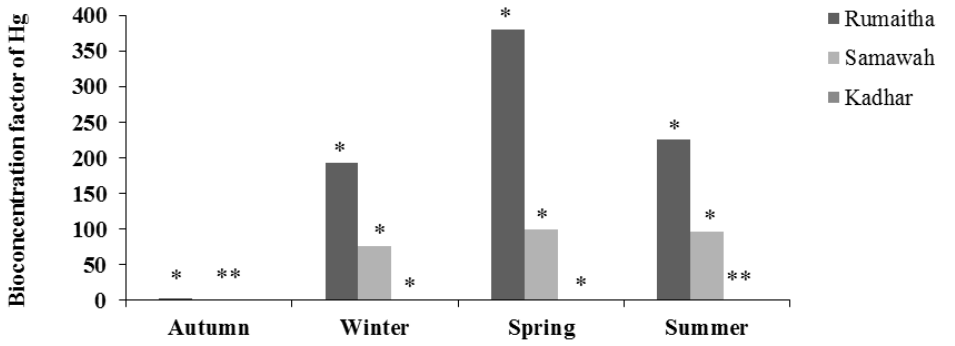


Figure 10. Ratio of mercury (Hg) metal concentration in plant to sediment. One asterisk shows the accumulation property in plant. Two asterisks show the indication property of the plant.

antimicrobial, anti-inflammatory, and metal chelating properties (Sobhani *et al*., 2021). Polyphenols are recognized for their ability to regulate the activity of genes associated with stress response, detoxification, and defense mechanisms. Hence, polyphenols might contribute to the tolerance and accumulation of heavy metals in plants (Sytar *et al*., 2013; Samec *et al*., 2021).

The polyphenol composition of *P. australis* was analyzed using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) in this study. The polyphenols that were examined in the study included various phenolic acids such as gallic acid, protocatechuic acid, rosmaric acid, caffeic acid, chlorogenic acid, p-coumaric acid, carsnoic acid, and vanillic acid. Additionally, flavonoids such as tamarixetin, hesperidin, naringin, rutin, quercetin, kaempferol, quercetin-3-O-rutinoside, apigenin-7-O-glucoside, and naringin-O-glucoside were also

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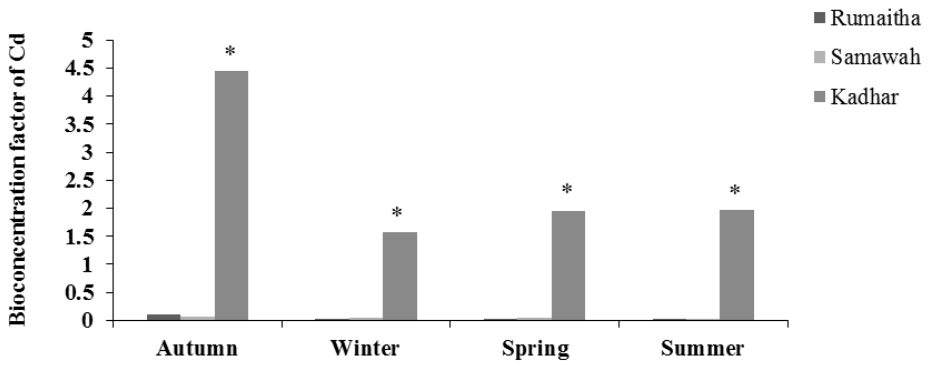


Figure 113. Ratio of cadmium (Cd) metal concentration in plant to sediment. One asterisk shows the accumulation property in plant.

analyzed, along with tannins. The polyphenol content and composition exhibited variations across different sampling sites (with varying degrees of heavy metal contamination). Statistical analyzes about the effect of seasons on the amount of polyphenols were not significant.

The findings demonstrated that the presence of heavy metal contamination in the sediment had a significant impact on both the quantity and composition of polyphenols. The plants from sites with elevated levels of heavy metals, such as Pb, Cr, Hg, and Cd, exhibited a greater polyphenol content. This suggests that polyphenols function as a detoxification mechanism for plants by attaching to metal ions and decreasing their ability to be absorbed and their harmful effects (Borowska *et al*., 2018). The heavy metal contamination caused changes in the polyphenol composition, resulting in an increase in the abundance of certain compounds and a decrease in the abundance of others in the plants from the contaminated sites (Kısa *et al*., 2016). This implies that certain polyphenols exhibit a greater attraction and selectivity towards specific metal ions and that plants regulate their production of polyphenols based on the type and intensity of metal stress (Kruk *et al*., 2022).

The findings also demonstrated significant associations between certain polyphenols and specific heavy metals, suggesting potential interactions and adaptations of the plant in response to metal-induced stress. Pb and Cr exhibited a positive correlation with hesperidin and carnosic acid, while Hg showed a positive correlation with hesperidin. Cd demonstrated a positive correlation with gallic acid, rosmaric acid, caffeic acid, chlorogenic acid, naringin, and rutin. These correlations suggest that these polyphenols play a role in the storage, transportation, and removal of these metals in plant tissues. Furthermore, the findings indicated a positive correlation between chlorophyll a and Cd, and a positive correlation between chlorophyll b and Cr. This suggests that these metals influence the plant's photosynthetic activity, prompting the plant to respond by increasing its chlorophyll content.

The effect of heavy metals on the polyphenolic profile of plants is that the application of heavy metals generally increases the total phenolics in the leaves of plants. However, the specific phenolic compounds present in the plants can vary depending on the type and dose of heavy metal. Chlorogenic acid and rutin are the main phenolic compounds that increase with heavy metal exposure, while caffeic acid, ferulic acid, and vanillic acid decrease in content. The accumulation of phenolic compounds is a universal response to heavy metal stress, and these compounds play a role in the plant's defense against stressors. The synthesis of phenolic compounds may be influenced by the amount of metal accumulated in the plant tissue (Janczak-Pieniazek *et al*., 2023; Kisa *et al*., 2016).

Hesperidin, a bioflavonoid found in citrus fruits, has demonstrated a protective effect against toxicity caused by heavy metals in different organisms. Hesperidin treatment in rats effectively decreased oxidative stress and neurotoxicity biomarkers in the brain, which had been elevated due to cadmium exposure (Khan and Parvez, 2015). In the same manner, hesperidin mitigated the oxidative stress caused by trichloroethylene in Drosophila melanogaster. It restored the levels of antioxidants and decreased the presence of reactive oxygen species (Abolaji *et al*., 2017). Application of hesperidin and chlorogenic acid to *Zea mays* enhanced antioxidant capacity and regulated redox balance in the presence of arsenic stress (Arikan *et al*., 2022). These studies indicate that hesperidin may provide protection against heavy metal toxicity in plants, possibly due to its antioxidant properties.

Carnosic acid, an inherent antioxidant present in rosemary, has demonstrated promising capabilities for alleviating the harmful effects of heavy metal toxicity in plants. This is because it has the capacity to remove reactive oxygen species (ROS) and decrease oxidative stress, which are important factors in the toxicity of heavy metals (Franic and Galic, 2019; Syed *et al*., 2018). In addition, carnosic acid has been discovered to augment the function of antioxidant enzymes such as catalase and superoxide dismutase. These enzymes are vital in safeguarding plants against oxidative damage caused by heavy metals. Hence, the utilization of

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carnosic acid in plant systems holds great potential as a viable approach to mitigate the adverse effects of heavy metal toxicity (Shah *et al.*, 2010).

Gallic acid, a phytochemical, has been discovered to mitigate the toxicity of heavy metals, such as mercury chloride, in plants. This is important because the presence of heavy metals, such as mercury, can cause oxidative stress and cellular damage in plants (Rocha *et al.*, 2019). The capacity of gallic acid to form stable complexes with heavy metals and shield plants from their detrimental impacts is a promising field for additional investigation and potential utilization in phytoremediation endeavors (Syed *et al.*, 2018). However, the specific correlation between the compounds rosmaric acid, caffeic acid, chlorogenic acid, naringin, and rutin and their role in heavy metal toxicity in plants is not directly addressed in studies.

Based on the provided results, this plant exhibits varying characteristics in terms of phytoremediation across different geographical areas and seasons. The phytoremediation capability of this plant can only be observed at the Samawah site when it comes to heavy metal contamination. Indeed, in this specific region and during autumn, the plant serves as a reliable indicator for the presence of lead metal. However, during the other seasons, it is regarded as a substance that accumulates lead. Chrome metal serves as a specific indicator solely at the Samawah site, exclusively during autumn. This plant exhibits a significant capacity for phytoremediation of mercury heavy metal in nearly all regions and throughout the year, except the control area during autumn. This plant serves as an indicator of seasonal changes in autumn and at the Samawah site and as an accumulator of certain properties in the summer of the control area. However, in other seasons and areas, it also exhibits the property of being an accumulator. This plant exhibits the characteristic of being an accumulator of the heavy metal cadmium throughout the year in the control area.

The comparison between the heavy metal concentration in the plant and the sediment samples indicates that the plant can exhibit varying functions in phytoremediation across different environments. Several factors can influence this, such as weather conditions, soil type, and type. The presence of other plants in the vicinity necessitates a thorough examination of this plant's impact on the desired area before utilizing it for phytoremediation, in order to assess its efficacy in purifying the environment. Nevertheless, other studies have demonstrated positive linear correlations between the concentrations of heavy metals in various plant organs and the concentrations in sediment. This suggests that these plant organs have the potential to be used for monitoring pollution in sediment and soil samples (Bonanno and Giudice, 2010; Ciszewski *et al.*, 2013).

The study's findings offer novel perspectives on the impact of heavy metal pollution on the composition of polyphenols in *P. australis*, a prevalent plant species found in wetlands. The findings illustrate the capacity of *P. australis* to serve as a bioindicator and phytoremediator for soil contaminated with heavy metals. Additional research is required to clarify the molecular mechanisms and ecological consequences of polyphenol production and buildup in *P. australis* under varying environmental circumstances.

Conclusion

This study investigates the impact of heavy metal pollution on the polyphenol composition of *Phragmites australis* plants in Samawah, Iraq. The research found that the presence and concentration of toxic heavy metals, such as lead (Pb), chromium (Cr), mercury (Hg), and cadmium (Cd), in the sediment significantly affected the polyphenol content and composition. Polyphenols are known for their antioxidant, antimicrobial, anti-inflammatory, and metal chelating properties, which may contribute to the tolerance and accumulation of heavy metals in plants.

The polyphenol composition of *P. australis* was analyzed using high-performance liquid chromatography (HPLC) and mass spectrometry (MS). The results showed significant associations between certain polyphenols and specific heavy metals, suggesting potential interactions and adaptations of the plant in response to metal-induced stress. Hesperidin, a bioflavonoid found in citrus fruits, has demonstrated protective effects against heavy metal toxicity in various organisms. Carnosic acid, an inherent antioxidant present in rosemary, has demonstrated promising capabilities in mitigating the harmful effects of heavy metal toxicity in plants by removing reactive oxygen species (ROS) and decreasing oxidative stress. Gallic acid, a phytochemical, has been discovered to mitigate the toxicity of heavy metals, such as mercury chloride, in plants. Furthermore, the findings indicated that the plant's phytoremediation capacity varies across different seasons and regions. There was no discernible pattern in the levels of polyphenols or the efficacy of heavy metal removal in response to climate change.

The study's findings offer novel perspectives on the impact of heavy metal pollution on the composition of polyphenols in *P. australis*, a prevalent plant species found in wetlands. The findings illustrate the capacity of *P. australis* to serve as a bioindicator and phytoremediator for soil contaminated with heavy metals. Further research is required to clarify the molecular mechanisms and ecological consequences of polyphenol production and buildup in *P. australis* under varying environmental circumstances.

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