EVALUATION AND TREATMENT OF PB, CD, AND NI BY USING FOUR WATER PLANTS BELONGING TO (*CERATOPHYLLUM* SPP.) FOR WASTEWATER IN TIKRIT TEACHING HOSPITAL

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Abstract: Four species of *Ceratophyllum* (*Ceratophyllum demersum*, *Ceratophyllum echinatum*, *Ceratophyllum muricatum*, and *Ceratophyllum submersum*) were exposed to high concentrations of Pb, Cd, and Ni in a water growing solution to determine their suitability for phytoremediation. All administered heavy metals had very poor translocation ratios to upper plant parts; as a result, metal uptake was restricted to the roots, especially Pb. The amount of metal in the nutrient solution and the genus of the water plants affected the species' capacity to extract and translocate Pb, Cd, and Ni. Comparing the examined species to comparable irrigation trials in the scientific literature, we find that their capacity to accumulate Cd in leaves is among the highest ever recorded. It was established which root development was preferable when encouraged by Cd. This species-specific reaction might be a component of a Cd resistance mechanism. The study showed a low level of dissolved oxygen and high concentrations of vital requirement for oxygen note that the level of DO decreases and BOD and COD increase annually due to the accumulation of pollutants in the river and the increase in population has been removed high levels of pollution with heavy metals using aquatic plants.

Key words: Ceratophyllum, Pb, Cd, Ni, Phytoremediation, Pollution

Introduction

The fast advancement of worldwide industrialization brought heavy metals to light as pervasive contaminants in the environment. The environment of the biosphere naturally contains components known as heavy metals, but human activity has concentrated and extracted these elements. The yearly global discharge of heavy metals over the past fifty years has been 22,000 t for cadmium and 783,000 t for lead [1]. Activities including metalliferous mineral extraction and extraction

[2, 3], agriculture, and industry [4], wastewater sludge from plants [5], burning of fossil fuels [6, pollutants from tires and brake linings [7], military operations and warfare, are common sources of heavy metals. produced several polluted locations, such as oil leaks from the Tikrit Teaching Hospital toward Tigris Using plants and the microorganisms they are linked with to remove, the transfer, equilibrium, and/or degrade pollutants from soil, sediment, water, and air is known as phytoremediation [7, 8]. This is an effective, ecological cleaning method that may be used for both organic and inorganic contaminants. Because it is done in situ and using solar energy, phytoremediation is substantially less expensive than the usual, more established remediation techniques (soil being washed, excavation, incineration, and pump-and-treat systems). One type of phytoremediation that is used to clean up heavy metal contamination is called phytoremediation. It involves using plants to absorb contaminants and store them in their tissues before harvesting the plant material. The aimed is to reduce the amount of heavy metals in contaminated water to levels that are suitable for the environment. Evaluates and treats the released wastewater from Tikrit Teaching Hospital.

Various phytotechnologies employ various species of plants. Fast-growing species, high plant matter, competitiveness, tolerance to a specific type of pollution, high plant absorption, translocation, and accumulation of heavy metals in harvestable plant tissues are all necessary qualities of a successful phytoextractor [7]. Despite their high accumulation properties, hyperaccumulator species are generally unsuitable for phytoremediation, even though they are valuable models for studying the biological processes behind metal accumulation and tolerance. They frequently develop slowly and produce little biomass. Fast-growing, high-biomass species that can extract substantial amounts of heavy metals despite having low accumulation offer an alternative to using hyperaccumulator species. There appears to be

a lot of promise for using trees as vegetation cover for phytoremediation of heavy metal-contaminated land [10]. Trees with water features are potential energy crops that can remove significant levels of heavy metals from soil. *Ceratophyllum* species are easily propagated, reach high biomass levels, and can be coppiced often to yield a maximum of 10-15 dry material t per hectare year-1 [11]. The employment of water plant specimens as in-place phytoextractor plant species is increased by their wide, spreading roots and invasive growth tactics [12]. Water plants have a long history of being utilized in developed nations in Iraq for the phytoremediation of various contaminants, both inorganic and organic [7]. This research aimed to assess and treat the Tikrit Teaching Hospital's released wastewater. Pb and Cd seem to be the most hazardous heavy metals to the environment. These three metals were selected for screening because excessive amounts of Ni exceeding recognized limits are uncommon in Tikrit. Although water plants' remediation potential has been extensively studied [10, 13–19], this paper's detailed data on particular species are lacking. These species were selected for a phytoremediation experiment because genetic variation has a potentially significant impact. The selection of aquatic research allowed for the creation of regulated environments, particularly in the root development media items. This method of study can offer a selection between the species under investigation based solely on genetic features.

A global genus of flowering plants, *Ceratophyllum* was recognized as having four existing species in 2016[2]. These species of plants are typically found in quiet streams, ponds, and marshes in both tropical and temperate regions. It is the only existing genus in the Ceratophyllaceae family, which is the only living family in the Ceratophyllales order [3].[4] Usually referred to as coontails or hornworts, while hornwort is also used to refer to unrelated plants in the Anthocerotophyta division.

Ceratophyllum grows entirely submerged in water, typically floats on the surface, but not constantly, and is drought-tolerant. The plant stems have a maximum length of 1-3 meters. They grow narrow, usually highly branching rings of brilliant green leaves at regular intervals along the stem's nodes. In certain species, the forked leaves feel brittle and hard to the touch, whereas in others they are softer. Especially in the embryonic stage, roots are completely absent[5], but occasionally can produce modified leaves that resemble roots and serve as the plant's anchor. Moreover absent are stomata.[6] All the male and female flowers are on the same plant, and the flowers themselves are tiny and barely noticeable.

Ceratophyllum is thought to be unique enough to merit the Ceratophyllaceae family. It was included in the Cronquist system as a member of Nymphaeales and was thought to be connected to Nymphaeaceae, but more recent studies have revealed that it is not very similar to any other family of living plants. A more recent study indicates that it is the sister group to the eudicots, contrary to some early molecular phylogenies which stated it was a sister group to all other angiosperms. The family was categorized by the APG III system within the Ceratophyllales order.[3][11][12]

Plant species

Ceratophyllum australe Griseb.

Ceratophyllum demersum L. (rigid/common hornwort) – cosmopolitan

Ceratophyllum echinatum A.Gray (spiny hornwort) – North America

Ceratophyllum muricatum Cham. (prickly hornwort) – Near-cosmopolitan

Ceratophyllum platyacanthum Cham. – Europe and Asia

Ceratophyllum submersum L. (soft/tropical hornwort) – Europe, Middle-East, Central Asia, northern and central Africa, Florida, and Dominican Republic

Ceratophyllum tanaiticum Sapjegin

Materials and methods:

The Tikrit Teaching Hospital is located close to the Tigris River and its effluent contains a high concentration of heavy metals and other pollutants.

Photo 1: 1: Tikrit teaching hospital in Salahaldin government, 2. the drain of wastewater of Tikrit teaching hospital in Tigris.

Four species of water plants *Ceratophyllum demersum*, *Ceratophyllum echinatum*, *Ceratophyllum muricatum*, and *Ceratophyllum submersum* constituted the experimental material. Tigris in Tikrit

supplied these species. The species selected for screening were the outcome of a protracted process of identifying genes for high biomass production. Native *Ceratophyllum demersum, Ceratophyllum echinatum, Ceratophyllum muricatum,* and *Ceratophyllum submersum* are some of the three species that have been chosen because they may be crucial for adaptation to the environment and the results of prospective in vivo studies. It was decided to evaluate for species of this plant genus.

Photo 2: *Ceratophyllum* species are used under study and distributed in the Tigris River.
1. Ceratophyllum demersum 2.Ceratophyllum echinatum
3.Ceratophyllum muricatum 4.Ceratophyllum submersum

Physical and chemical characteristics

1. Electrical conductivity: Use a multi parameter Lovibond model CON200 to measure the electrical conductivity of samples after calibrating the device and express the results in microcemines per centimeter.

2. pH Degree: Use a pH meter type consort C350 after calibrating the device with pH solutions with pH 4, 7 and 9.

3. Bio requirement for oxygen: I used the same method of measuring dissolved oxygen has been filled bottles size of 250 ml of the sample and then transferred to the laboratory and kept for five days in a water bath temperature of 25 ° C and expressed the results in milligrams per liter.

4. Turbidity: The turbidity of water was measured by the Hannah LP2000 hardness measuring device, and the solutions are expressed in the total turbidity unit in a specific unit.

5. Measurement of total dissolved substances: The dissolved substances were measured depending on the mentioned method by filtering 200 ml of the sample with Wattman type filter paper and collecting the filtrate in a known weight and the filtrate was evaporated in an oven with a temperature of 105 ° C for 24 hours and then it was weighed.

6. Determination of total solid suspended materials: The suspended solids were measured by filtration of ml of the sample on 0.45 μm filter paper known weight and then evaporation of the filtrate in an oven with a temperature of 103-105 °C for 24 hours and then it was weighed

Using the water culture approach, water plants were grown in nutrient solutions in a semi-controlled environment (greenhouse effect). The actual temperature remained below 30℃. Natural illumination varied according to the amount of light outdoors. Permanent aeration was employed in the solutions to provide adequate mixing and delivery of O2. The cuttings were put on a linoleum cover that had been perforated. Each hydroponic pot held 40 liters of liquid. In each pot, eighteen water plant woody cuttings (20 cm long) containing one shoot per cutting were cultivated. There were 36 woody cuttings (two pots) in each treatment. Every two weeks, the distribution of pot was switched up and randomized. Further, fresh solutions were included every two weeks. Three distinct metals (Cd, Ni, and Pb) were applied to the plants individually at both concentrations (10-4 M and 10-5 M) in a solution at maximum strength.

The mass/volume units (µg/ml) for these quantities are as follows: Cd is 11.24 µg/ml and 1.12 µg/ml; Ni is 5.87 µg/ml and 0.59 µg/ml; Pb is 20.72 µg/ml and 2.07 µg/ml. Plants under control were grown in full-strength, metal-free Hoagland nutrient solution. Pb may become physically unavailable to the plant due to the separation of Pb with other salts from the nutritional solution. Consequently, EDTA (ethylene diamine tetraacetic acid) was used to chelate lead to create a soluble complex. Pb (10-4 M and 10-5 M, provided as Pb-EDTA), Ni (10-4 M and 10-5 M, provided as NiSO4•6H2O), and Cd (10-4 M and 10-5 M, provided as CdCl2•H2O) were the metals utilized. pH solutions were maintained at 5.0. After 70 days, the plants that had received Cd and Ni treatments were measured, examined, and harvested. After 95 days, the plants treated with lead were harvested and examined.

Because of established chlorosis and necrosis that threatened the plants' viability, plants treated with Cd and Ni were harvested 25 days sooner than expected. When Cd and Ni at 10-4 M were administered, symptoms became apparent. The plant material was dried, rinsed in deionized water, and processed for analysis using industry standards for water and wastewater analysis [20].

Following mixing and heating with H2O2, drying at 450ºC, and treatment with 25% HCl, the amounts of heavy metals were ascertained. Atomic absorption spectrophotometry was used to measure the concentrations of Cd, Ni, and Pb in prepared solutions (AAS). Roots, shoots, and leaves were tested for Cd, Ni, and Pb levels. Shoot material was made up solely of stems devoid of leaves. Two sets of leaf samples were formed. The seven smallest leaves at the top of the stalk made up the "Upper leaves" sample; the remaining leaves made up the "Lower leaves" sample. Due to detection limits, the levels of Cd, Ni, and Pb in control plants were excluded from the statistical analysis. Starting from the lowest concentration with a given value of 1, the distribution of metals inside the plant was expressed as the [Element]root: [Element]shoot: [Element]lower leaves: [Element]upper leaves ratio, which is displayed in Table 3. Duncan's Multiple Range Test was used to perform statistical analyses at significant levels of p 80% (species 1), > 70% (species 2 and 3), and > 50% (species 4).

Results:

The high concentration of BOD indicates an increase in the concentration of organic and untreated substances released from Tikrit Teaching Hospital to the Tigris River directly, and this increases organic and bacterial contamination and thus increases diseases because most of the bacteria issued by hospitals are resistant to antibiotics, as well as the spread of odors and distortion of the river landscape and the beauty of the water in the river. Despite these high levels of pollutants in the river, the water specifications are still within the permissible level within the regulations for the protection of rivers and water from pollution No. (25-A1) and (25-B1) for the year 1967 Directorate of Human Environmental Protection in Iraq. Also, the concentration of impurities is within the permissible range within the river and water protection system and can describe wastewater as well as nitrates, phosphates, and BOD within the permissible limits as in Table 1.

These high levels of nitrates are due to increased activities inside hospitals and because of cleaning powders. The high concentration of hardness in wastewater from the water of the Tigris River due to the torrential rains carrying the topsoil, the melting of gypsum and limestone rocks, as well as the high chlorides as a result of the discharge of liquid waste from the hospital. The wastewater estuaries are the main source of deteriorating water quality for the Tigris River within the city of Tikrit, one of which is the mouth of the Tikrit Teaching Hospital, which is supposed to clean the river water from solid accumulations and plants that hinder the speed of water flow in the river and remove pollutants to help flow and thus increase the permanent purification of water, as well as studying the amount of heavy elements in the water and developing appropriate solutions to treat them, whether environmentally friendly biological methods, nano-methods or artificial mud treatment method.

The study showed a low level of dissolved oxygen and high concentrations of a vital requirement for oxygen (Dulie and Al-Qazzaz, 1997) note that the level of DO decreases and BOD and COD increase annually due to the accumulation of pollutants in the river and the increase in population has been removed high levels of pollution with heavy metals using aquatic plants.

Table 1: the results of ecological parameters for wastewater of Tikrit teaching hospital outcome in Tigris

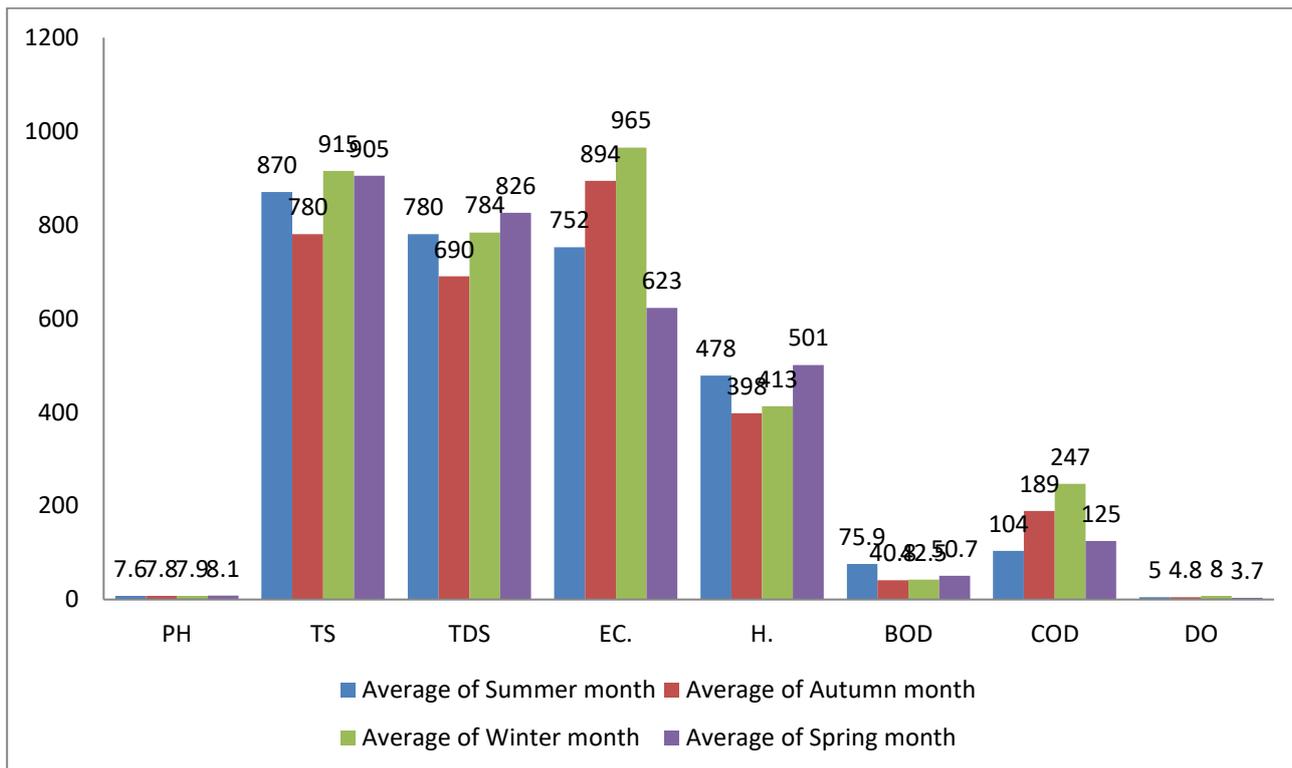| Parameters	PH	TS	TDS	EC.	H.	BOD	COD	DO
Average of Summer month	7.6	870	780	752	478	75.9	104	5.0
Average of Autumn month	7.8	780	690	894	398	40.8	189	4.8
Average of Winter month	7.9	915	784	965	413	42.5	247	8.0
Average of Spring month	8.1	905	826	623	501	50.7	125	3.7

Fig1: showed the seasonal variation of water parameters.

For species 3 (10-4 M) and 4 (both applied Pb concentrations), Pb-EDTA treatment resulted in a statistically significant reduction in shoot mass (Table 2). Moreover, 10-4 M of Pb accelerated the development of species 1. In contrast to the shoot mass, species 1, 3, and 4's Cd treatment resulted in a much larger mass of roots (Table 3). In the presence of greater Cd concentrations (10-4 M), the weight of the roots of species 1 and 4 rose, but the weight of the roots of species 3 decreased (10-5 M). There was no discernible difference in the root mass of treated and control plants for any species that received a Ni dose of 10-5 M (Table 4). But for all species but No. 4, the suppression of root growth caused by 10-4 M concentrations of nickel was noticeable and noteworthy. Specific to each species, root development responded differently to Pb-EDTA treatment. When compared to control plants, Species 1 exhibited a statistically significant increase in root mass for both Pb-EDTA doses (Table 3). While species 3 and 4 showed a statistically significant decrease in the root mass of treated plants, species 2 showed no response to treatment. Compared to plants treated with a lower concentration of 10-5 M of Cd, the plants treated with 10-4 M of Cd had significantly increased Cd concentrations in their roots, shoots, and leaves.

Table 2: the concentration (gram) of Pb, Cd, and Ni in shoot mass.

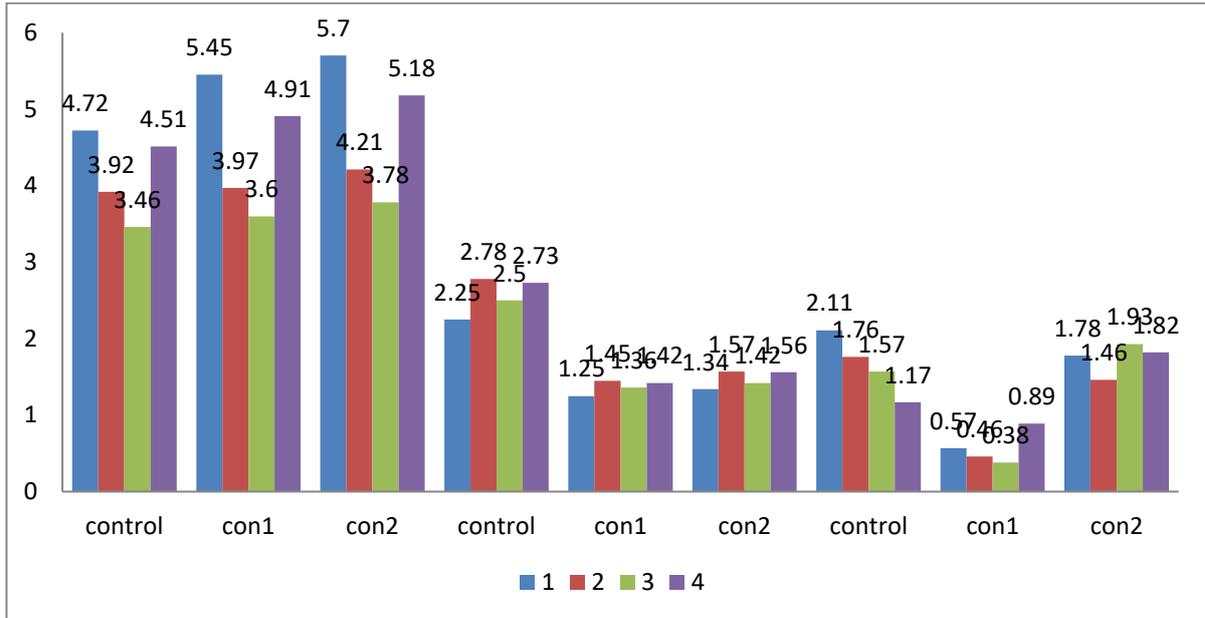| Heavy metals treatment		Pb			Cd			Ni		
		control	10^4 m/l	10^5 m/l	control	10^4 m/l	10^5 m/l	control	10^4 m/l	10^5 m/l
Species *Ceratophyllum*	*C.demersum*	4.72b	5.45a	5.70b	2.25a	1.25d	1.34d	2.11a	0.57e	1.78a
	C.echinatum	3.92c	3.97c	4.21b	2.78b	1.45e	1.57d	1.76b	0.46e	1.46c
	C.muricatum	3.46d	3.60f	3.78e	2.50b	1.36d	1.42d	1.57b	0.38e	1.93d
	C.submersum	4.51a	4.91c	5.18b	2.73a	1.42c	1.56b	1.17a	0.89d	1.82a

Fig 2: concentration of Pb, Cd, and Ni in four root water plants (*Ceratophyllum* spp.)

Table 3: the concentration (gram) of Pb, Cd, and Ni in root mass.

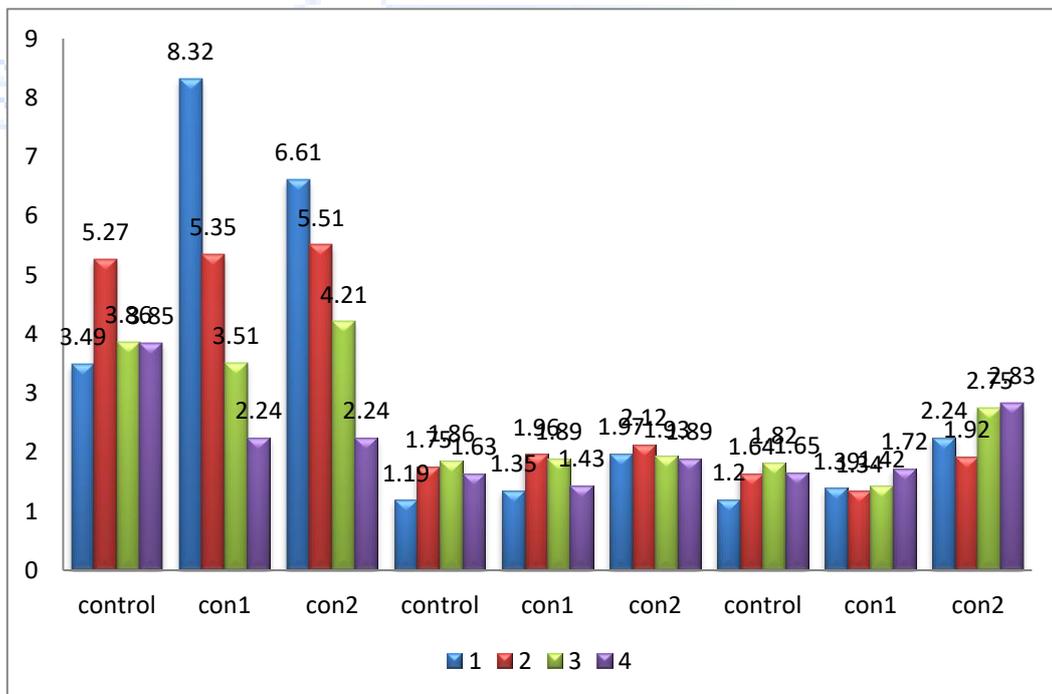| Heavy metals treatment		Pb			Cd			Ni		
		control	$10^{4\,m/l}$	10^{5} m/l	control	$10^{4\,m/l}$	10^{5} m/l	control	$10^{4\,m/l}$	10^{5} m/l
Species *Ceratophyllum*	*C.demersum*	3.49d	8.32a	6.61b	1.19c	1.35a	1.97c	1.20a	1.39e	2.24a
	C.echinatum	5.27e	5.35e	5.51c	1.75d	1.96g	2.12E	1.64b	1.34e	2.92b
	C.muricatum	3.86d	3.51g	4.21e	1.86d	1.89d	1.93b	1.82b	1.42e	2.75c
	C.submersum	3.85f	2.75i	2.24h	1.63f	1.43a	1.86c	1.65d	1.72d	2.83b

Fig 3: the concentration (gram) of Pb, Cd, and Ni in root mass.

Table 4: the concentration (gram) of Pb, Cd, and Ni in root: shoot: lower leaves: upper leaves ratio.

Species			*C.demersum*				*C.echinatum*				*C.muricatum*				*C.submersum*			
			R	S	L	U	R	S	L	U	R	S	L	U	R	S	L	U
Heavy metals	Pb	$10^{4\ m/l}$	31.2	1.8	1.6	1.4	65.7	7.5	3.1	2.1	28.7	1.5	1.8	1.9	9.6	1.4	1.6	1.7
		$10^5_{\ m/l}$	18.9	1.9	1.7	1.5	41.9	3.4	3.5	1.2	38.6	1.6	1.9	2.1	10.1	1.3	1.8	1.4
	Cd	$10^{4\ m/l}$	63.5	1.3	3.9	4.5	60.3	1.2	3.1	3.5	68.8	1.3	5.2	3.6	20.2	1.7	4.3	4.9
		$10^5_{\ m/l}$	86.9	1.2	2.7	5.2	92.8	1.8	3.8	2.7	59.7	1.5	3.6	2.7	6.6	1.8	3.8	2.9
	Ni	$10^{4\ m/l}$	41.8	1.6	1.9	3.7	75.6	3.9	3.7	2.8	45.2	1.4	4.1	2.3	10.9	1.8	2.7	2.7
		$10^5_{\ m/l}$	49.9	1.7	2.1	2.1	65.4	2.7	3.6	2.3	55.7	1.1	3.4	1.9	8.2	1.6	1.9	2.4

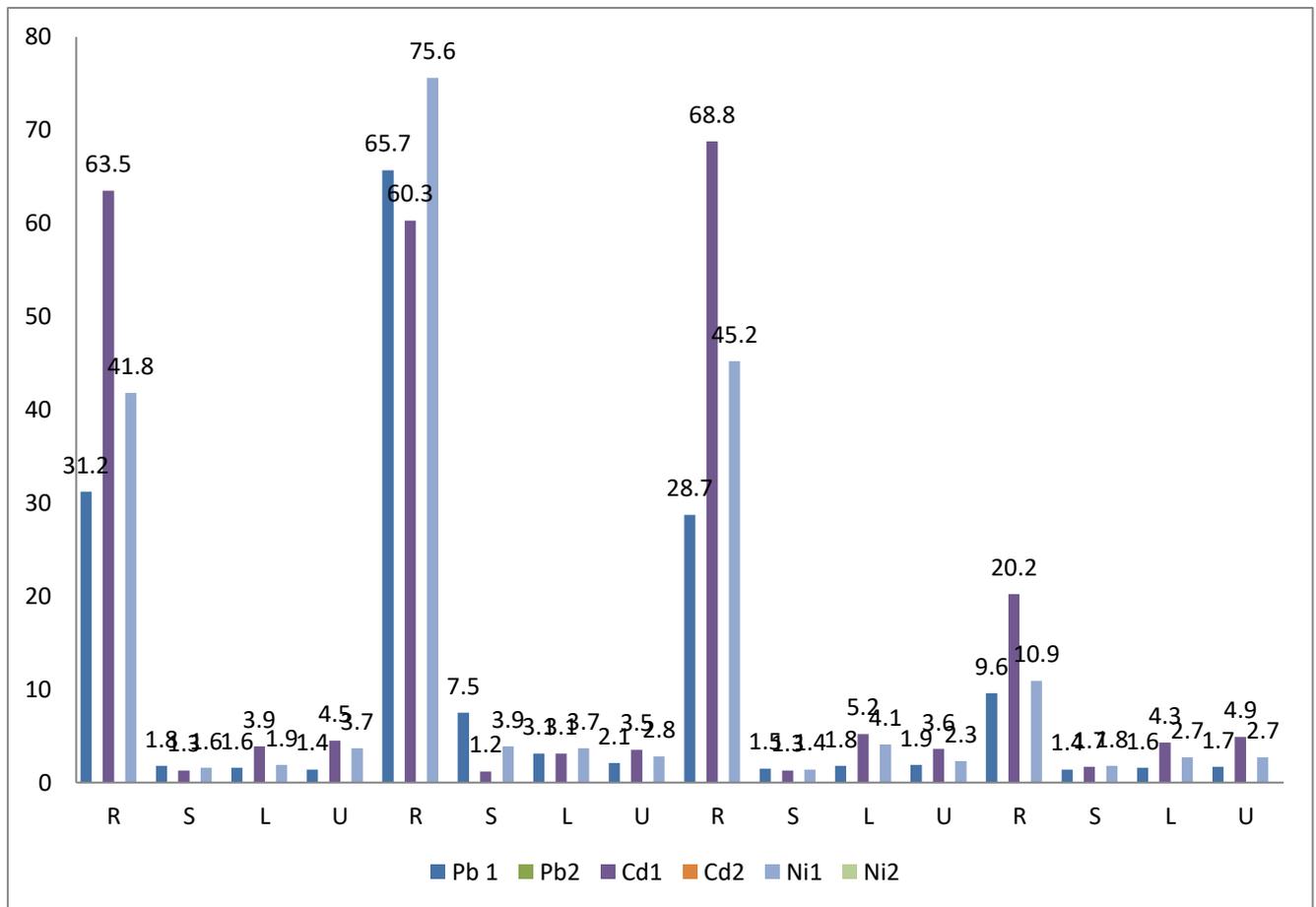R: Root, S: Shoot, U: Upper leaves, L: Lower leaves

Fig4: the concentration (gram) of Pb, Cd, and Ni in root, shoot, lower leaves, and upper leaves ratio.

In comparison to the concentrations of Cd in shoots and leaves, the level of Cd in roots was several dozen times greater. Each species has a different ratio of Cd translocation from root to shoot. Compared to the other species, species 4 exhibited the highest Cd content in its leaves due to its highest Cd translocation level from roots to leaves (Root: Leaf ratio below 10) for both applied Cd doses (Table 4). With a root: leaf ratio of 51.7 and 73.7, species 2 exhibited the lowest translocation level and, consequently, the lowest Cd content in the

leaves. When comparing the accumulation of Cd in roots and shoots across nickel treatments (10-4 M and 10-5 M), the difference in Ni accumulation was much smaller (Figs. 4).

In roots and shoots (10-4 M:10-5 M), the ratio of Cd to Ni was 1.0–3.0, and the ratio of Cd to 6.2–2.1. Except for species 2, there was substantially less nickel translocation from roots to shoots than there was Cd translocation (Table 3). Concentration determined the extent of nickel translocation to the plant's higher regions. In comparison to plants cultivated on 10-5 M of Ni, it was two times greater in plants produced on 10-4 M of Ni. In comparison to the concentrations of Ni in the leaves, the concentration of Ni in the shoots was many times lower (1.7–4.6). For species 1, 3, and 4, the uptake and translocation of nickel to the upper portions of the plants was noticeably less than the translocation of cadmium. As a result, the amount of Ni in the leaves (28.9-148.6 µgNi/g dry weight) was significantly less than the amount of Cd in the leaves (121.0-507.5 µgCd/g dry weight). The plants treated with 10-4 M of PbEDTA exhibited a considerably higher concentration of Pb in their roots, shoots, and leaves as compared to the species treated with 10-5 M of PbEDTA, as illustrated in Figure 4.

For species 3 and 4, the treatment variations were remarkably large; in these species, the Pb concentration ratio (10-4 M:10-5 M) in the roots was 6.3 (species 4) and 7.3 (species 3). In comparison to the levels of Cd and Ni in the roots, the amount of Pb uptake and accumulation in the roots of species 3 and 4 was several times higher. In contrast to the movement of Cd and Ni, the transport of Pb to the upper portions of the plant was notably reduced (Table 3). Lower leaves acquired larger quantities of Pb than upper leaves when 10-4 M of Pb-EDTA was administered. When compared to the concentrations of Cd and Ni in the shoots and leaves, the Pb concentrations were often several times lower. With a few notable exceptions, species variations in metal accumulation in the shoots and leaves for both Ni and Pb were not statistically significant.

Discussion

Species growth responses to treatment varied significantly, according to morphological parameter study. Strong reductions in the shoot mass of the plants treated with 10-4 M of Cd and Ni, along with identified symptoms of necrosis and chlorosis, indicate that these metals' toxic effects at this level of contamination cause a major decline in the viability of the genotypes under investigation. Because the plants treated with Pb-EDTA showed no signs of chlorosis or other metal toxicity symptoms, the hydroponic trial for Pb-EDTA was conducted over a longer length of time. The shoots and leaves were shielded from Pb's hazardous effects by their poor translocation to green plant parts, which is why the Pb-EDTA treatment did not have any negative effects. It is possible to identify species 4 as the genotype that is most bioproduction-tolerant to Cd and Ni treatments. In general, this genotype exhibited the highest shoot mass and the highest bioproduction tolerance to treatments with Cd and Ni. Frequently, there were more differences between the plants within the same species— between *Ceratophyllum demersum, Ceratophyllum echinatum, Ceratophyllum muricatum*, and *Ceratophyllum submersum*, for example—than there were between distinct species. Thus, in line with other studies [13, 14], responses to the treatments were not only species-specific but also genotype- or species-specific. For species 1, 3, and 4, the presence of Cd in the hydroponic fluid promoted root development at various Cd concentrations. Gussarsson (1994) [21] found similar outcomes on trees, albeit with a lower Cd concentration. In that instance, the author proposed that a mechanism for Cd resistance might include the build-up of Cd in fine roots along with a predilection for root growth. On the other hand, root growth was reduced by the Ni and PbEDTA treatments, except for species 1 which received Pb-EDTA treatment. These findings also imply that root growth may respond differently depending on the species to the Ni and Pb-EDTA treatments. The amounts

of accumulated Pb, Ni, and Cd in the roots, shoots, and leaves varied according to genotype. Similarly, genetically driven responses to metal stress were observed by other researchers. The hydroponic experiment conducted by Landberg and Greger (1994) [22] tested ninety-four species of *Ceratophyllum demersum, Ceratophyllum echinatum, Ceratophyllum muricatum*, and *Ceratophyllum submersum*. There were notable differences in metal uptake and tolerance between species. In certain instances, the ability of a species to absorb metal varied by a factor of 80. The propensity of different species to accumulate different metals was more pronounced at larger (10-4 M) concentrations applied to all metals (Cd, Ni, and Pb). The ability to accumulate metal varied more at increasing concentrations. These data imply that the concentration of heavy metals in the growing media affects the clonal-specific ability to accumulate heavy metals. The finding that the translocation ratio of Ni was almost 50% greater at higher applied doses (10-4 M) than at lower concentrations (10-5 M) supports this theory. According to Galardi et al. (2007) [23], Alyssum bertolonii's root and shoot react to nickel concentrations differently; as a result, while the medium's nickel concentration grew, the root's tolerance variation reduced while the shoot's tolerance variation increased.

Translocation of metals from the roots to the top sections was very low for all applied heavy metals, even when roots were rinsed in deionized water, which permits the sorption of one fraction of metals in the apparent space. Metal translocation has been limited to the shoot in many species that are tolerant of metals [24–26]. Exclusion mechanisms are thought to be responsible for the limited uptake of metals by shoots and leaves, most likely to shield photosynthesis from harmful concentrations of heavy metals [3, 27]. For Pb, the lowest transit level was identified. Even though Pb was given chelated with EDTA, which should boost Pb mobility, the translocation ratio of Pb was up to 10 times lower than those of Cd and Ni.

According to several studies, certain synthetic chelates may be able to improve Pb's solubility, mobility, and translocation concerning certain soil fractions [28–36]. According to Huang et al. (1997) [30], the following Pb chelates were the most effective in decreasing order: EDTA > HEDTA > DTPA > EGTA > EDDHA. Even with the drawbacks, Hernández-Allica et al. (2007) [37] note that with careful control over EDTA treatment, metal phytotoxicity can be decreased and metals with limited phytoavailability can be better absorbed. EDTA-metal complexes, on the other hand, can be harmful to plants and soil microorganisms. They can readily enter groundwater due to their high solubility and low degradability, and they can form high-stability complexes with other heavy metals in soil, which increases their toxicity and causes environmental harm. Malkowski et al. (2004) [25] also reported ten-fold more Pb accumulation in maize apical root segments compared to Cd, and higher Cd transfer to shoot compared to Pb, suggesting that the mechanisms of Pb and Cd translocation from root to shoots are distinct.

According to the authors, Pb binds more strongly than Cd in the root apoplast (cell walls). The Free Ion Activity Model (FIAM) states that one of the main factors influencing bioavailability is the activity of a specific metal species in the soil solution [39]. While applying soil amendments like EDTA should raise metal ion activity, there is no certainty that this will happen. The depletion of ions near roots is one of the issues with implementing FIAM, as rhizosphere conditions might not accurately reflect bulk soil conditions [40]. Therefore, studied species need to be examined in outdoor soil conditions following the determination of genotypic references in hydroponic tests. Evaluation of this application's success must take soil conditions into account.

In comparison to other hydroponic screenings, the concentrations of Cd in leaves of *Ceratophyllum demersum* (476.6 µg/g of dry weight), *Ceratophyllum echinatum* (389.0), and *Ceratophyllum muricatum* (507.5), as well as

in shoots of *Ceratophyllum demersum* (338.9), and *Ceratophyllum submersum (*347.0), are among the highest ever published. As stated by Lunackova *et al.* (2003) [19] and Lux *et al.* (2002) [18], The range of Cd concentration (µg/g of dry weight) observed in *Ceratophyllum demersum* varied from 2.0–62.1 in shoots and 3.0-160 in leaves and stems. Only Cosio *et al.* (2006) [15] found a greater concentration of Cd in leaves of *Ceratophyllum* species (519.0 and 584.0 µg/g) using applied concentrations of 50 µM in hydroponic solution. The study found that when treatment was increased to 100µM (10-4 M = 11.24 µg/ml) and 200 µM, the amount of Cd in the leaves decreased. In air-dried soil in the Republic of Tikrit, the maximum allowable content of Cd is 3 µg/g. Studies verify that Cd seldom surpasses these thresholds by more than ten times, and even less frequently by twenty times [41, 42]. Concentrations of Cd have ranged from 4.6 to 64.0 µg/g d.w in Poland's heavily anthropopredited areas [42]. The system, metal phytotoxicity, concentration, and soil bioavailability all affect how much cadmium accumulates in plants. However, the genotypes under investigation undoubtedly can accumulate CD. The biomass output of water plants can reach up to 10-15 dry t ha-1 year-1 [11], and a significant quantity of metal can be removed from contaminated soil.

There can be significant differences between soil phytoremediation and hydroponic phytoremediation. For instance, it was found by Klang-Westin & Eriksson (2003) [17] that the net removal of Cd by Ceratophyllum ranged between 2.6 and 16.5 g Cd ha-1 year-1 in different soil types. It is established that many *Ceratophyllum* species can accumulate Cd in their shoots and leaves [15, 16]. The net removal of metals from the soil is much below these presumptive limitations, and the fluxes of heavy metals in *Ceratophyllum* stands under field circumstances are not thoroughly studied.

One benefit of a low metal translocation ratio from the roots to the higher sections of the plant is that it makes it more difficult for metals to be released into the environment by senescence or herbivores. *Ceratophyllum* species had a much greater metal translocation ratio in situ as compared to hydroponics, according to certain studies, which also note that uptake and translocation ratios in field plants differ from those in hydroponics [3, 16]. These variations might result from the fact that metal uptake and translocation are influenced by root activity in field circumstances, which includes interactions between rootsoil particles, roots-bacteria, and/or roots-mycorrhiza [43–46]. However, hydroponic trials might still serve as a foundation for the possible application of untested or underutilized plant genotypes in phytoremediation [47]. In comparison to soil-grown trials, hydroponic screenings are faster and allow for the differentiation of species that closely resemble those seen in the field [16, 48]. In a developing province like Tikrit, where many polluted sites need to be remedied, the technology of phytoremediation has great potential because it is far less expensive than traditional procedures. In summary, In comparison to their aboveground tissues, all genotypes under investigation accumulated higher quantities of Cd, Ni, and Pb in their roots. The most promising genotype for use in Cd and Ni phytoremediation is species 4, *Ceratophyllum*, or species 0408, and it merits more investigation and field validation. At the applied dose of 10-4 M, the studied species were significantly exposed to the harmful effects of Cd and Ni. The amount of the metal load in the nutritional solution affects a species' capacity to extract and translocate Cd, Ni, and Pb. The amount of extracted heavy metal can be substantial even when the translocation ratio to the upper plant parts is low if the harmful influence of heavy metals does not interfere with biomass production. The levels of Cd deposited in leaves and shoots are among the highest that have been seen in history. Thus, the plants under investigation have promising potential for Cd phytoremediation. It was shown that the presence of Cd in the hydroponic medium increased the root mass. This inclination toward root expansion might be a component of the Cd resistance

mechanism. To ascertain the reason behind the roots' reaction, more investigation is necessary. The selection of specific species, as opposed to species based on environmental features of each contaminated site, should be carried out since the phytoremediation potential of water plants is species. The paper's findings serve as a baseline for judgments to be made in the future regarding the genotypes to be used concerning the element that requires repair. Based on the comparatively low cost of this ecologically benign technology, Tikrit, being a developing country undergoing economic transition, has a significant potential for the potential application of Phytoremediation, which is not yet commercially exploited.

Acknowledgments

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