J. Plantarum., 5(SI): 35-53

ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

PHYTOACCUMULATION OF METALS BY AQUATIC MACROPHYTE; *Pistia stratiotes* L. (WATER LETTUCE)

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Received on: 15-06-2023; Reviewed on: 21-08-2023; Accepted on: 07-10-2023; Published on: 28-11-2023

Abstract

Macrophytes have been used for heavy metal removal from a variety of sources. Aquatic macrophytes such as water lettuce, is one of the most commonly used plants in constructed wetlands because of its fast growth rate and large uptake of nutrients and contaminants. It is generally known that aquatic macrophytes in wetlands can accumulate heavy metals. Therefore, an experiment was designed to check the metal tolerance capacity by using different concentrations i.e., 0 ppm, 2 ppm, 4 ppm and 6 ppm of nutrient cadmium chloride and copper sulphate solutions. Various physico-chemical parameters like pH, ECe and TDS were observed in the concentrations. The high value of pH (8.9), ECe (645 µs cm^{-1}) and TDS (350 mg L^{-1}) was found in the highest concentration of nutrient solution (6 ppm). These plants were grown in different concentrations of nutrient solution for 15, 30 and 45 days. Morphological parameters like root length, leave length, dry weight, fresh weight and number of leaves were also observed after 15, 30, 45 days respectively. Best metal uptake was observed in 6 ppm nutrient solution while in control (0 ppm) a reduced amount of uptake of metals was observed. The maximum root and leave length (30 g) were found in case of control concentration (0 ppm) because having no salt. The excess amount of metals reduced the growth parameters. Therefore, minimum growth was observed in 6 ppm nutrient solution. Order of metal uptake was Cd > Cu. Uptake of Cd in roots was higher (134 mg Kg^{-1}) than Cu (50 mg Kg^{-1}). Roots accumulated highest amount of heavy metals in 6 ppm nutrient solution, Cd (187 mg Kg^{-1}) than leaves (98 mg Kg^{-1}) because it was completely submerged into nutrient solutions. Similarly maximum amount of Cu uptake via roots was observed in 6 ppm nutrient solution (100 mg Kg^{-1}) than 0 ppm. Translocation factor of Cadmium and Copper was less than 1. The purpose of this study to evaluate the effect of heavy metals on the growth parameters of plant *Pistia stratiotes* L. in different concentration of nutrient solution as well this present research aims to assess *P. stratiotes'* (as a phytoremediator plant) capability of cadmium (Cd), copper (Cu), and a translocation factor from wastewaters (effluent).

Keywords: Heavy metal toxicity, Phytoaccumulation, *Pistia stratiotes* L.

Introduction

Pistia stratiotes L. (Araceae), water lettuce, is a floating macrophyte that spreads swiftly vegetatively. The tropics and subtropics host *P. stratiotes* L. as well it grows throughout Africa. KwaZulu-Natal, South Africa, first documented it in 1865 (Yuldashev, Abdullaev, Buriyev, & Tajiyev, 2021). Water lettuce exhibits a broad distribution throughout many aquatic habitats in Pakistan, including stagnant water bodies, lakes, marshy areas, ponds and reservoirs (Abd Hamid, Ismail,Mansor, 2021). Among the vast number of plant species, aquatic macrophytes play a crucial role in the field of phytoremediation (Nahar & Hoque, 2021).

There is a huge capacity for metal accumulation in aquatic macrophytes; concentrations can reach 100,000 times greater than in the surrounding water (Khalid, Ganjo, 2021). Heavy metals can be removed from a wide range of environments by using these macrophytes. Because of their rapid growth and high capacity to absorb nutrients and pollutants, aquatic macrophytes like water hyacinth are frequently employed in artificial wetlands (Ansari, Naeem, Gill, AlZuaibr, 2020). *P. stratiotes* L. was examined for resilience and metal sequestration for phytoremediating wastewater and natural water bodies contaminated with eight potentially dangerous (Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) trace elements (Ali *et al*., 2020). Phytoremediation often employs plants with high phytomass, fast growth, and significant hyper-accumulation capability (Chaney *et al.*, 2020). Heavy metals are notoriously difficult to remove from the environment and tend to bioaccumulate in soils and plant life. Consumption of plants containing heavy metals has been linked to a variety of chronic health issues in humans (Alengebawy, Abdelkhalek, Qureshi, Wang, 2021).Heavy metals have an effect on aquatic species because of the accidental mixing of contaminants from different point sources. Pollutants in the aquatic environment come from a wide range of human activities, including manufacturing, agriculture, and even household cleaning products (Elgarahy, Elwakeel, Mohammad, Elshoubaky, 2021). Due to their hazardous effects, longevity, and tendency to accumulate within organisms, pesticides, heavy metals, and detergents are the primary focus of aquatic environment issues (Shah, 2021).

Heavy metals comprise a group of 19 elements that exhibit comparable chemical and physical properties, distinguishing them from the remaining 97 elements (Tauqeer *et al*., 2021). The production of hazardous chemical sludge by current treatment procedures makes disposal or treatment costly and ecologically unfavorable. Thus, cost-effective and ecologically safe heavy metal removal is essential to achieve an environmentally safe level (Behera, Nayak, Banerjee, Chakrabortty, Tripathy, 2021). Cadmium is considered to be phytotoxic since it inhibits processes essential to plant growth such as respiration, photosynthesis, and nutrient absorption.

Additionally, it slows down the production of new cells, slows down root growth, inhibits antioxidant enzyme activity, and causes oxidative stress within cells (Goncharu, Zagoskina, 2023). Plant growth suppression, leaf chlorosis, and root necrosis are all attributable to the biochemical, physical, and genetic changes induced by Cd. Lead (Pb), like cadmium (Cd), has phytotoxic characteristics that reduce plant chlorophyll and so impair photosynthesis. Lead (Pb) inhibits the uptake of magnesium (Mg) and iron (Fe), two metals essential for chlorophyll synthesis, and so affects chloroplast function. This modification impairs the regulation of stomata closure and interferes with critical photosynthesis-related enzymatic activities. (Zulfiqar *et al*., 2022 ; Behl *et al*., 2022).

The relative translocation of metals from the soil to the root and shoot was calculated using the Translocation factor (TF). Analysis of translocation factors (TFs) helps predict how well a plant will perform in phytoremediation. The TF number represents a plant's capacity to translocate metals from the root to the shoot. When the TF value is larger than one, it means that the plant is able to efficiently translocate metals from the root to the shoot. When the TF value is negative, however, it indicates that the plant is accumulating metals in its roots rather than its leaves and stems (Hossain, Rakib, Jolly, Rahman, 2021; Azab, Hegazy, 2020). The current research set out to assess the macrophyte *P. stratiotes* has capacity to absorb metals and to determine whether or not it is resistant to metals.

Materials and Methods

Survey of Ponds and Sampling

The survey of Sheikhupura, situated at coordinates of 31°05'16.32" N, 74°28'36.20" E in Pakistan, was successfully conducted. Samples were gathered and carefully placed in plastic bags. The precise location was recorded using a digital GPS device (Model: Etrex H Garmin, Taiwan).

Collection of Pistia stratiotes L.

P. stratiotes L. (Water lettuce) was drained from its source, a pond in Sheikhupura, using plastic bags. The plants have been donated to the University of Education's Botany Department in Lahore's Township. At first, these plants were brought to the wire house and grown in nurseries there. After that, they moved them to the lab where they would participate in numerous experiments.

Experimental Setup

The experiment in the wire house was set up using a completely randomized factorial design (Hussain *et al.*, 2022). One to two weeks of growing plants were done in plastic containers. Once that time period had passed *P. stratiotes* L. was transplanted out of their pots into nutrient solutions that having different concentrations i.e., 0 ppm, 2 ppm, 4 ppm, and 6 ppm of $CuSO_4$ and $CdCl_2$. These solutions comprise of four concentrations i.e., 0ppm, 2ppm, 4ppm and 6ppm with three replications of each concentration. There were 36 pots used to conduct experiments with different concentration of salts i.e., $CuSO_4$ and $CdCl_2$ as given below.

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Conc.	After 15 days	After 30 days	After 45 days	Total replicates	Total
0ppm	R1	R1	R1	9	36
	R2	R2	R2		
	R3	R3	R3		
2ppm	R1	R1	R1	9	
	R2	R2	R2		
	R3	R3	R3		
4ppm	R1	R1	R1	9	
	R2	R2	R2		
	R3	R3	R3		
6ppm	R1	R1	R1	9	
	R2	R2	R2		
	R3	R3	R3		

Chemical Analysis

Determination of pH

pH meter (Model: HI 9811-5) usage to ascertain nutritional needs and regulate pH in the solution. The pH meter's knob should be dipped in clean water to remove any dirt or dust that may have accumulated there. After the pH meter was calibrated, it was placed in the nutrient solution for a reading. Each time it was used, this pH meter was washed thoroughly in distilled water.

Determination of Electrical Conductivity

With the use of an EC meter, the electric conductivity of nutrient solutions like 2, 4, and 6 ppm nutritional solution was determined. The EC meter's knob should be cleaned by dipping it in distilled water before each usage to eliminate any pollutants that may have attached to it. Dip the EC meter into the sample of nutrient solution to determine its EC. I used distilled water to clean the EC meter's knob before dipping the electrode into another sample. Dissolved solids in the nutrient solution are quantified by their electric conductivity.

Determination of Total Dissolved Solids (TDS)

To measure the TDS of the nutritional solution, an auto-ranging portable microcontroller was used. The TDS was determined by placing an electrode in the nutrient solution. The true TDS of the salt solution was determined after rinsing the electrode extensively with distilled water to remove any impurities. The total dissolved solids (TDS) of a liquid were the sum of the masses of all molecules, ions, and micro-granular suspended particles found there. TDS levels were commonly reported in a range from 0.5 to 1.5 times that, in parts per million (ppm).

Preparation of Copper Sulphate Solution ($CuSO_4$)

The copper sulfate stock solution was made by dissolving 2, 4, and 6 grams of the chemical into one liter of pure water. Different concentrations (2 ppm, 4 ppm, and 6 ppm) were made from this stock solution (Momanyi, Nduko, Omwamba, 2022).

Preparation of Cadmium Chloride Solution ($CdCl_2$)

The stock solution of cadmium chloride was prepared by dissolving 2 g, 4 g and 6 g of cadmium chloride in 1000 ml of distilled water (Gao, 2020). From this stock solution different concentration such as 2 ppm, 4 ppm and 6 ppm were prepared respectively.

Morphological Parameters

Various morphological characteristics, including root length, leaf length, number of leaves, fresh weight, and dry weight, were examined as part of the experimental setup (Youssef, 2021). These parameters were observed both before and after the experimental procedures were conducted.

Samples Digestion

Preparation of Plant Sample for Acid **Digestion**

Plants were taken at 15, 30 and, 45 days post-experiment, and their roots and leaves were used independently for heavy metal analysis. The roots and leaves of the plants were washed under running water to remove any remaining dirt or debris. Blotting paper was used to dry the plants at room temperature. Scale measurements in centimeters were taken of the stems, leaves, and roots. Using a fresh weight scale, measure out a sample of the plant's roots and leaves separately. Roots and leaves were harvested at their fresh weight and then dried in an electric oven at 80 ℃. Pestle and mortar were used to reduce the dried leaves and roots to a powder. For later examination, each sample was sealed in a plastic bag.

Digestion of Plant Material

Root and leaf samples were taken from the plants to analyze the water accumulation trend at each level of the plant. After harvesting, the crop was desiccated using an electrical furnace set at 80 degrees Celsius. The duration of this desiccation process was 24 hours. The sample was ground or crushed using a pestle and mortar after it was removed from the oven. After the samples were crushed, they were stored in plastic bags. A flask containing 5 ml of nitric acid (HNO_3) and 15 ml of perchloric acid ($HClO_4$) was used to digest 1 gram of dried plant material in a 1:3 ratio. During this period of digestion, the flask was placed on a hot plate. It was left to cool once the volume had decreased by half. Whatman filter paper was used to filter the digested sample in a measuring cylinder. To get the volume of the filtrate up to 50 ml, distilled water was added to it (Sultana, 2020; Victor *et al*., 2016). Heavy metal concentration testing required setting up the samples properly. After being digested, the samples were transferred to 100 ml plastic bottles.

Estimation of Toxic metals

The heavy metal analysis was conducted using an Atomic Absorption Spectrophotometer (GBC SAVAANT AA Australia). The operational manual provided clear guidelines for instrument calibration settings, which were strictly adhered to for the accurate determination of elemental assays (El-Said, El Zokm, El Sayed, El Ashmawy, Shreadah, 2020).

Analysis of Toxic metals

The metal concentration data was acquired in hard copy format from the Atomic Absorption Spectrophotometer. The metal concentration was expressed in parts per million (ppm) (Orosun *et al*., 2020).

Heavy metals = Concentration of metals X 1000/Weight of samples

Translocation factors

Copper and Cadmium translocation from shoot to root was measured by TF, which is given below:

TF= C shoot/C root

Where C shoot and C root are metals concentration in the shoot ($mgkg^{-1}$) and root of plant ($mgkg^{-1}$), respectively. TF>1 represent that translocation of metals effectively was made to the shoot from root (Singh, Karwadiya, Srivastava, Patra, & Venugopalan, 2022).

Statistical analysis

To determine the statistical significance of the observed mean values for the chosen parameters, the data was subjected to statistical analysis using SPSS (Statistical Package for the Social Sciences) software, specifically version 20.0. The analysis involved performing an F test.

RESULTS

Zero Analysis of Nutrient solution

Combination of salt prepared by CdCl2 and CuSO4, two different concentrations of nutrient solution. Zero analysis of different parameters like pH, EC and TDS was recorded in table 4.1. The amount of heavy metals was increased with increasing the concentrations of nutrient solution. As the concentration of nutrient solution increased, the value of pH, EC and TDS also increased. In table 4.1 pH of control (0 ppm) was recorded as 7.5 lower than the pH of 6ppm nutrient solution i.e., 8.9. The value of pH helps to observe the nutrient solution was acidic or basic.

High value of EC indicated that maximum amount of dissolve salt was present in nutrient solution. In control solution the value of EC was observed low 590 mg L^{-1}. In 6ppm salt solution high value of EC i.e., 645 µs cm^{-1} was observed. High value of Total dissolved substance indicated that high amount of salt suspended in the nutrient solution. High value of TDS was observed in 6ppm nutrient solution i.e., 350 mg L^{-1}. Lowest value of TDS was observed in control concentration i.e., 290 mg L^{-1} because fewer amounts of total dissolved substances were observed.

Table 1: Determination of pH, EC and TDS of different concentration of salt

Salt.	Parameters	Concentrations			
		0ppm	**2ppm**	**4ppm**	**6ppm**
$CdCl_2 + CuSO_4$	pH	7.5	7.9	8.2	8.4
	EC (µs cm^{-1})	590	610	635	645
	TDS (mg L^{-1})	290	305	335	350

0ppm = control solution
2ppm= 2g of CdCl2 + CuSO4 and 2000ml distilled water
4ppm=4g of CdCl2 + CuSO4 and 2000ml distilled water
6ppm=6g of CdCl2 + CuSO4 and 2000ml distilled water
Values are mean ± Standard deviation from 4 replicates
Values are significant (S) at P≤ 0.05 according to F test

Experiment with *Pistia Stratiotes* L.

The morphological growth characteristics of *P. stratiotes* L. plants at 45 days of age, were evaluated under various concentrations of salt solutions, as depicted in the subsequent experimental arrangement. Two to three plants were kept in each pot.

Study of Morphological Parameters after 15 days of experiment

After 15 days, old plants grown in 0, 2, 4, and 6 ppm nutrient solutions were compared for their morphological properties. Leaves at 0 ppm had a fresh weight of 60 g, but those at 6 ppm weighed just 20 g. When comparing 2 ppm and 4 ppm salt solutions, the fresh weight of leaves in 2 ppm was 40 g greater than 4 ppm, or 35 g. Roots grown in a nutritional solution of 6 ppm had a fresh weight of 19 g, 30 g less than those grown in a control solution of 0 ppm. Roots at 2 ppm weighed in at 21 g, whereas those at 4 ppm weighed in at 20 g.

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Fig 1: *Pistia stratiotes* L. grown in different concentrations of nutrient solutions After 45 days of experiment.

Leaves in the control group had a dry weight of 7 g, while roots in the various treatment groups ranged from 1, 1.2, 1, and 1 g when exposed to 0, 2, 4, and 6 ppm, respectively. The longest roots measured 30 centimeters deep at 0 parts per trillion. The 2ppm and 4ppm roots were 22 and 20 cm long, respectively. In the control group, 27 leaves were counted at most, while only 15 were counted in the 6 ppm group. The control group had 93 roots, but the 4 ppm group only had 80. Zero parts per million yielded the longest leaves. The leaf dry weights in the 2 and 4 ppm treatments were 1.2 and 1 g, respectively.

Study of Morphological Parameters after 30 days of experiment

The morphological features of water lettuce plants were studied after a period of 30 days. The fresh weight of leaves in the control group exhibited an increase of 53 g compared to the group treated with a 6 ppm nutritional solution, which had a fresh weight of 23 g. The fresh weight of leaves at concentrations of 2 and 4 parts per million (ppm) was recorded as 30 and 28 grams, respectively. The fresh weight of roots at 0 ppm exhibited an increase of 22 g compared to the weight at 6 ppm, whereas at 6 ppm, the weight was 11 g. The fresh weight of roots was measured to be 12 g and 13 g in nutritional solutions with concentrations of 2 ppm and 4 ppm, respectively. The dry weight of leaves at concentrations of 0, 2, 4, and 6 parts per million (ppm) were measured and found to be 6.95, 4.4, 5.9, and 3 grams, respectively. The dry weight of roots in the 6 ppm treatment exhibited a reduction of 2.35 g compared to the control treatment, which had a dry weight of 3.84 g. The control group exhibited the greatest root, measuring 24 cm, whereas the group exposed to 6 ppm displayed a root size of 16.5 cm. The highest length of leave observed was 11 cm in the 0 ppm condition. The length of leaves observed in a solution with a concentration of 4 parts per million (ppm) was measured to be 7 cm, while in a solution with a concentration of 6 ppm, the length of leaves was found to be 6.5 cm.

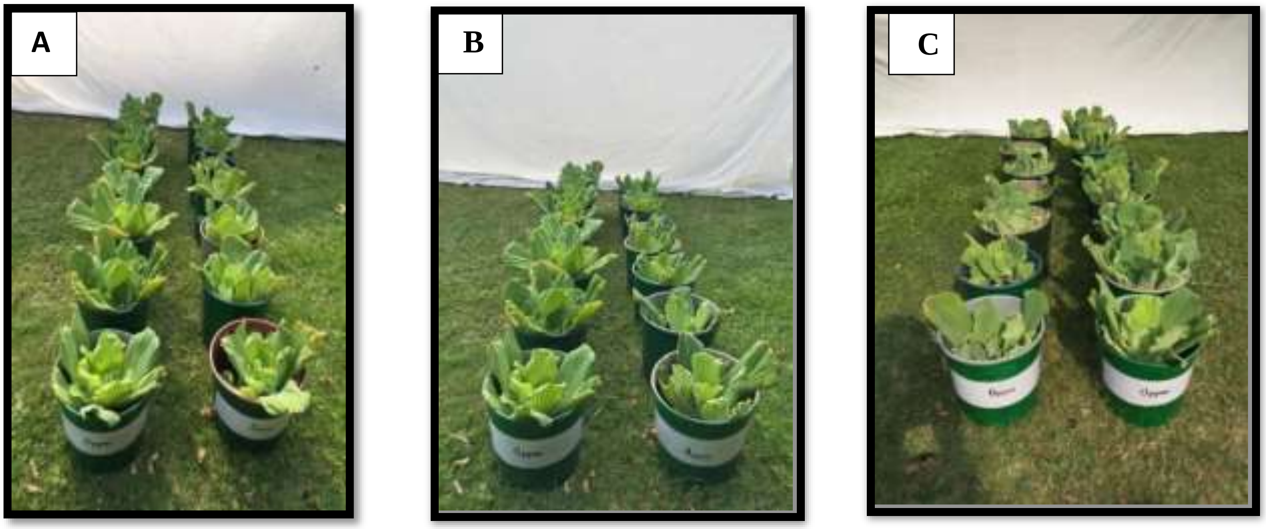


Fig 2: *P. stratiotes* L. grown in different concentrations of nutrient solution after 15 days of experiment. (A) Comparison of nutrient solution concentrations i.e., 0 ppm with 2 ppm (B) comparison of nutrient solution concentrations i.e., 0 ppm with 4 ppm (C) comparison of nutrient solution concentrations i.e., 0 ppm with 6 ppm

Fig 3: *Pistia stratiotes* L. grown in different concentrations of nutrient solution after 30 days of experiment. (A) comparison of 0 ppm(control) with 2 ppm nutrient solution (B) 0 ppm(control) compared with 4 ppm nutrient solution (C) 0 ppm(control) compared with 6 ppm nutrient solution

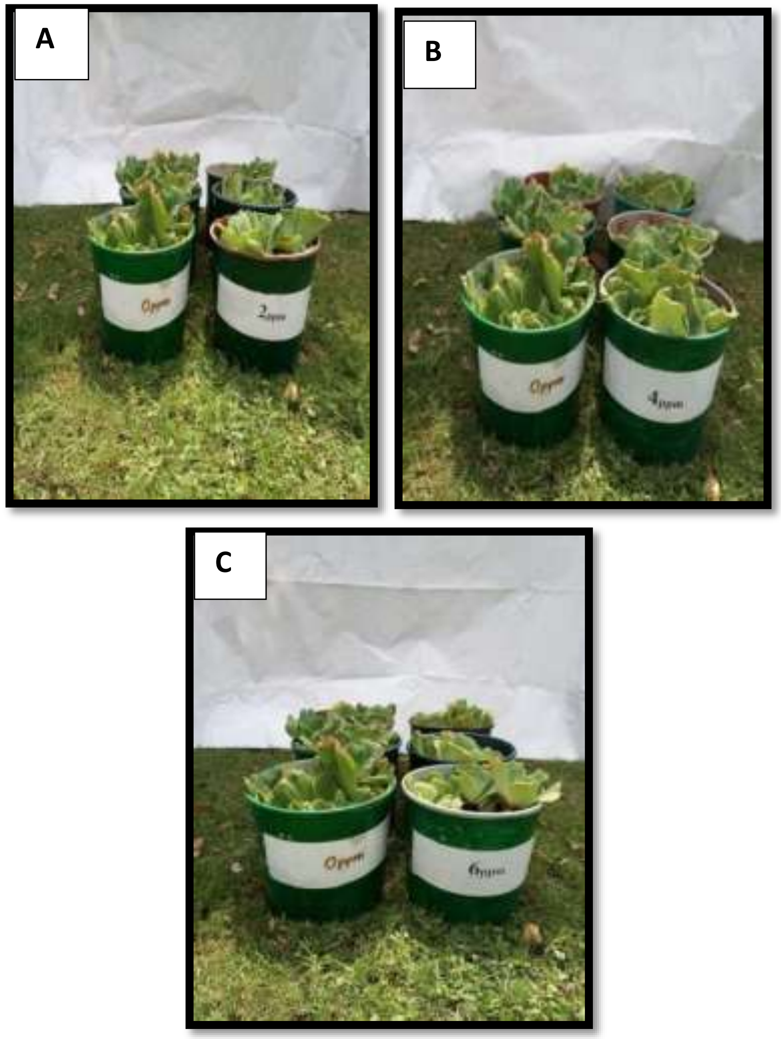


Fig 4: ***Pistia stratiotes*** **L. grown in different concentrations of nutrient solution after 45 days of experiment (A) comparison of 0ppm (control) with 2ppm nutrient solution (B) 0 ppm (control) comparison with 4 ppm nutrient solution (C) 0 ppm (control) compared with 6 ppm nutrient solution.**

Fig 5: Harvested plants of *Pistia stratiotes* L. grown in different concentrations of nutrientsolution after 45 days of experiment.

Study of Morphological Parameters after 45 days of experiment

P. stratiotes L., grown at different concentrations for 45 days, showed that the control had the lightest fresh weight of leaves (26 g). The density of a nutritious solution with 6 ppm was used to weigh 9.76 grams of fresh leaves. The 2 ppm and 4 ppm fresh weights of the leaves were 25 and 20 g. In terms of fresh weight, the roots in the 0 ppm group were 9 g greater than those in the 6 ppm group. Roots between 2 and 4 mm in diameter showed a calculated to be 7g and 4 g. When the leaves were dry, the difference was 8.43 g, compared to 6 ppm. In the 2 ppm and 4 ppm treatments, the dry weight of the leaves was 6.08 g and 5.36 g, respectively. The dry weights of roots with 0, 2, 4, and 6 ppm were 2.91 g, 3.23 g, 3.09 g, and 2.22 g, respectively. The control sample has the longest roots. The control group had 9 cm long leaves, but the 6 ppm group only had 5 cm long leaves. The control group had 29 leaves counted, but the 6 ppm group only had 18. The control group had a maximum of 70 roots, while the 6 ppm group had only 39.

Determination of heavy metals

The concentrations of heavy metals in the nutrition solution were measured with an Atomic Absorption Spectrophotometer (AAS). The aquatic plant *P. stratiotes* L. was supplemented with Cd and Cu in the form of nutrient solution. The uptake of metals followed the pattern Cd > Cu. Heavy metals were taken up by the roots and leaves of plants. When compared to leaf absorption, root absorption of metals was much greater. Due to their elevated position on the plant, leaves have the lowest metal absorption rates. Plants 45 days old had the highest metal concentration, followed by plants 15 days old. Table 4.2 shows the total amount of metals found in the various *P. stratiotes* L. plant portions after 15, 30, and 45 days of the experiment.

Table 2: The morphological growth attributes of 45-day-old *Pistia stratiotes* L. plants wereexamined under diverse concentrations of nutrient solutions

Parameters	Conc.	Days After 15 days	After 30 days	After 45 days	Effect of concentration on various parameters
Root Length	0ppm	30±0.76	24±2.08	22±3.60	S
	2ppm	22±1.04	20±2.51	17±3.77	S
	4ppm	20±1.26	18.5±2.84	15±4.04	NS
	6ppm	18±2.08	16.5±2.92	13±4.50	NS
Leave Length (cm)	0ppm	13±0.72	11±1.52	9±2.64	NS
	2ppm	7±0.90	8±1.80	5±3.0	NS
	4ppm	5.5±1.10	7±2.08	7±3.05	NS
	6ppm	4±1.52	6.5±2.36	5±3.51	NS
Fresh Weight of Leaves(g)	0ppm	60±5.0	53±6.65	26±6.60	S
	2ppm	40±5.7	30±7.0	25±6.37	S
	4ppm	35±5.56	28±7.23	20±7.28	S
	6ppm	20±5.13	23±7.50	9.76±6.6	S
Fresh Weight of roots (g)	0ppm	30±1.15	22±3.21	9±5.08	S
	2ppm	21±2.30	12±3.21	7±4.57	S
	4ppm	20±2.51	13±3.60	4±4.91	S
	6ppm	19±2.64	11±4.35	5±2.74	S
No. of roots	0ppm	93±3.51	91±6.08	70±7.63	S
	2ppm	84±4.50	82±6.42	50±9.01	S
	4ppm	80±5.50	78±6.55	40±10.0	S
	6ppm	70±5.77	69±7.09	39±10.96	S
No. of leaves	0ppm	27±1.73	30±3.21	29±4.58	NS
	2ppm	20±2.0	22±3.78	20±5.03	NS
	4ppm	18±2.08	20±4.16	18±5.56	NS
	6ppm	15±2.88	19±4.35	15±7.63	NS
Dry Weight of Leaves (g)	0ppm	7±1.52	6.95±1.43	8.43±1.95	NS
	2ppm	4±1.12	4.4±1.24	6.08±1.17	NS
	4ppm	5±1.25	5.69±1.35	5.36±1.24	S
	6ppm	5±1.32	3±1.41	4.4±0.53	NS
	0ppm	1±0.25	3.84±0.93	2.91±0.73	S

P. stratiotes L., a control plant 15 days old, showed the lowest levels of heavy metals. Roots exposed to 6 ppm nutritional solution had 134 times as much Cd as those exposed to the control solution (50 mg Kg^{-1}). Leaves with 0 ppm of Cd had 20 mg Kg^{-1}, while those with (6 ppm) contained 71 mg Kg^{-1}. Roots with 2 ppm Cd had 77 mg Kg^{-1}, while those with 4 ppm Cd contained 101 mg Kg^{-1}. Compared to 2 ppm leaves, 4 ppm leaves had much more Cd. Cu content in 6 ppm nutritional solution was greater in leaves (45 mg Kg^{-1}) than in the control (0 mg Kg^{-1}) and in roots (70 mg Kg^{-1}) than in 0 ppm (0 mg Kg^{-1}). Cd levels in leaves increased to 50 mg Kg^{-1} at 4 ppm, much higher than the concentration in leaves at 2 ppm.

Water cabbage plants that were 30 days old grew at a slower rate than those that were 15 days old. Cd concentrations of 187 mg Kg^{-1} in roots and 98 mg Kg^{-1} in leaves were found in plants exposed to a nutrition solution containing 6 ppm Cd. Cd levels in the roots were 55 mg Kg^{-1} and in the leaves, they were 28 mg Kg^{-1}. Roots exposed to 4 ppm Cd absorbed more of the metal than those exposed to 2 ppm. Copper (6 ppm, or 100 mg Kg^{-1}) was taken up by the roots of water lettuce at a much higher rate than at 0 ppm. With the in, copper content was 65 mg Kg^{-1}. The quantity of Cu in the leaves of the control plants was 15 mg Kg^{-1}, while the amount in the roots was 26 mg Kg^{-1}. Roots exposed to 4 ppm Cu had an uptake 77 mg Kg^{-1} more than roots exposed to 2 ppm Cu.

The content of nutrient solution was found to have a direct correlation with the accumulation of heavy metals in plants older than 45 days. Lower than 6 ppm leaves, 0 ppm leaves have a Cd concentration of 40 mg Kg^{-1}. Roots exposed to a nutrient solution of 6 ppm collected a maximum of 250 mg Kg^{-1} more Cd than roots exposed to a nutrient solution of 0 ppm. More Cd was taken up by roots in a 2 ppm nutrient solution than in a 4 ppm solution. There was 101 mg Kg^{-1} of Cu in leaves at 4 ppm, and 79 mg Kg^{-1} in leaves at 2 ppm. Roots (6 ppm), which is equivalent to 144 mg Kg^{-1}, contained the most copper, while leaves (0 ppm), which contained the least, contained only 30 mg Kg^{-1}. More Cu is taken up by the roots at 2 ppm than at 0 ppm. Cu concentrations in 6 ppm leaves were 80 mg Kg^{-1}, while control root concentrations were 43 mg Kg^{-1}. Plant growth may be stunted if excessive metals are taken in. Heavy metals are essential for plant growth, but when they accumulate to hazardous levels, it can stunt the plant's development. Inhibition of cytoplasmic enzymes and oxidative stress-related damage to cellular structures are two examples of the direct toxic effects that can be induced by exposure to high metal concentrations. Plant development slowed as a result of the stress.

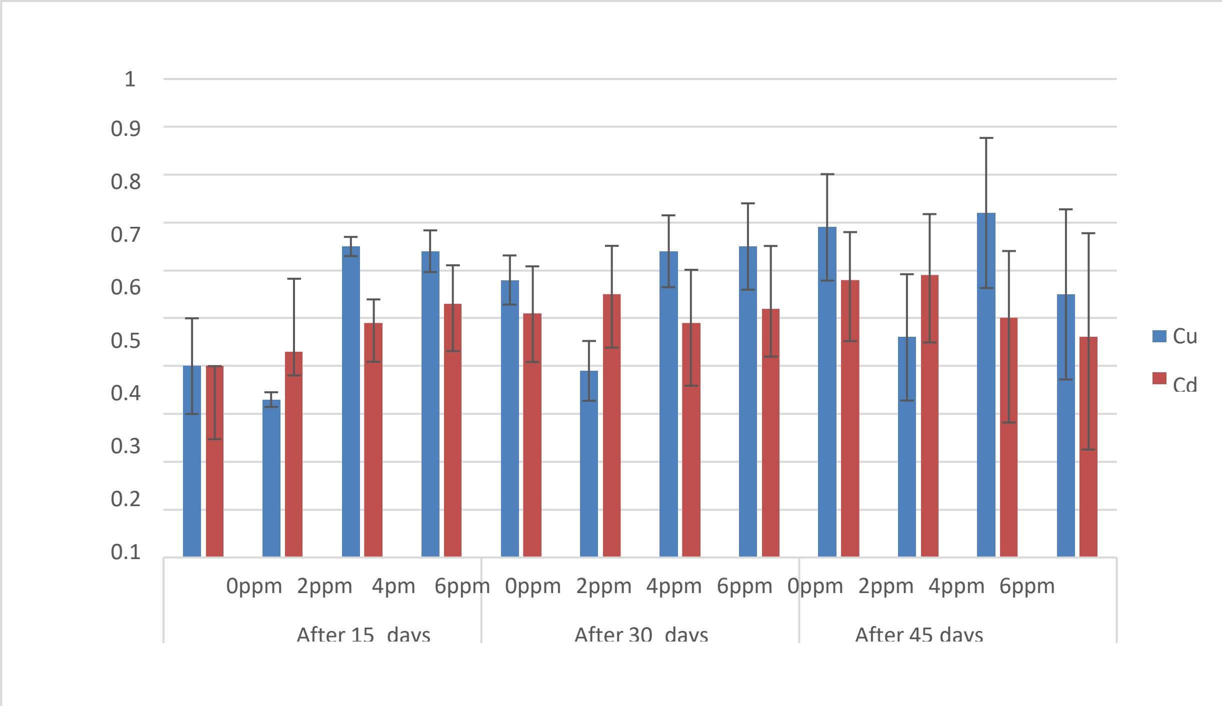
Table 3: The quantity of heavy metals present in the leaves and roots of 45-day-old *Pistia stratiotes* L. plants cultivated under various concentrations of nutrient solutions was assessed.

Days	Conc.	Plants Parts	Metals (mg Kg^{-1}) Cd	Cu	Effect of concentration on heavy metals
After 15 days		Roots	50±0.58	20±0.58	NS
	0ppm	Leaves	20±1.0	08±1.15	NS
		Roots	77±1.52	45±1.73	S
	2ppm	Leaves	33±2.08	15±2.0	S
		Roots	101±3.78	58±2.08	S
	4ppm	Leaves	50±4.04	38±2.52	S
		Roots	134±4.50	70±2.88	S
	6ppm	Leaves	71±4.72	45±3.21	NS
After 30 days		Roots	55±5.03	26±3.51	NS
	0ppm	Leaves	28±5.56	15±4.04	NS
		Roots	100±6.02	67±4.73	S
	2ppm	Leaves	55±6.42	26±5.03	NS
		Roots	159±6.65	77±5.13	S
	4ppm	Leaves	78±6.80	49±5.57	NS
		Roots	187±7.37	100±6.24	S
	6ppm	Leaves	98±8.14	65±7.09	NS

Fig. 5 Sodium ions in shoot and root, Potassium ions in shoot and root and membrane permeability of two cultivars of pea (Pisum sativum L.) grown for 29 days under salt stress and Zinc sulfate (500 and 1000 ppm).

Translocation Factor (TF)

Translocation factor was recorded in roots and leaves of the plant *P. stratiotes* L. Results shows that TF<1. Cd and Cu translocate from the plant parts i.e., roots and leaves accurately. The maximum rate of translocation factor was 0.24 with the 45 days of old plants in 6ppm nutrient solution. Translocation factors were documented after 15, 30, and 45 days at varying levels, namely 0, 2, 4, and 6 ppm, as depicted in graph 1



Graph 1: Translocation agents of 45-day-old *Pistia stratiotes* L. plants cultivated in varying levels of

Discussion

In the present research, *P. stratiotes* L. was grown in a range of nutrient concentrations to assess its response to those variations. These results imply that as concentrations of nutrients in the solution increased, so did the pH. The pH in the 0 ppm control group was low (7.5), but in the 6 ppm nutrition solution it was high (8.4). Alkaline nutrition solutions were indicated by the highest pH values (Tang, Awa, Hadibarata, 2020). Chlorination, which is used to purify drinking water, does not work well with pH levels above 8.0 (Nti, Buamah, & Atebiya, 2021).

According to the data shown above, a high electrical conductivity value indicates a high concentration of salt in the nutritional solution. Compared to the control group, the EC value was highest in the 6ppm (645 µs cm^{-1}) nutrient solution (Santander, Aroca, Cartes, Vidal, & Cornejo, 2021; Victor *et al.*, 2016). Oruko *et al.* (2021) report similar findings; tannery effluent had the highest EC value compared to the control. The TDS reading for the 6ppm nutrient solution was the highest (350 mg L^{-1}), while the reading for the control (0 ppm) condition was the lowest (290 mg L^{-1}). Several researchers (Shakil *et al.*, 2023) came to this conclusion. It showed that the same outcomes occurred when comparing tannery effluent with the highest documented value of TDS.

Cd and Cu concentrations have an effect on both shoot and root growth. The lengths of the leaves and roots were found to be shorter (6.5 cm, 16.5 cm) respectively when Cd and Cu concentrations in the nutritional solution increased (6 ppm). In contrast to the other concentrations, the control (0 ppm) treatment resulted in the longest roots and leaves (24,9cm). Due to the high concentration of metals, the lowest rates of growth were seen in the most nutrient-rich medium. Latif *et al*. (2020) found that, similar to the findings above, the root and leaf length decreased at the maximum dose of Cd and Cu compared to the control concentration. The effects on root length were generally more pronounced than those on shoot length.

Cd and Cu toxicity effects on shoot and root biomass were evaluated. The biomass was shown to be significantly affected by these heavy metal concentrations. The control group had the maximum leaves and roots by dry weight (2.91, 6.95 g). When exposed to a high concentration (6 ppm) of nutritional solution, leaf and root dry weight dropped (3, 2.35g). Since roots are the most vulnerable component of plants, they react rapidly to cadmium toxicity. Yildirim *et al*. (2021) reported similar outcomes, showing that supplying an excess amount of Cd to the leaves and roots caused a reduction in both the fresh and dry weight of the leaves and roots.

Excessive metal absorption causes stress in the plant's leaves and roots, reducing both its fresh and dry weight. Roots' fresh and dry weight were more affected than leaves' fresh and dry weight. Heavy metals accumulated the most in a nutritional solution of 6 ppm. Compared to Cu, *Pistia stratiotes* L. stores a disproportionately large amount of Cd (Lebrun *et al*., 2023) in its tissues. Because the roots were completely immersed in the nutritional solution, they acquired significant amounts of metals. The findings were also supported by additional research (Nguyen, Sesin, Kisiala, Emery, 2021). High concentrations (6 ppm) showed the highest metal surplus when compared to all other values. Phytoremediation with *P. stratiotes* L. was commonplace (entürk, Eyceyurt Divarc, ztürk, 2022).

According to the latest research, TF < 1. Cd and Cu successfully translocate from roots to leaves. Consistent with these findings are those of (Zemiani, Boldarini, Anami, de Oliveira, & da Silva, 2021). It was also found by Qian *et al*. (2023) using TF that the majority of the metals taken up by the aquatic plants were stored in their roots After 45 days in 6ppm nutrient solution, the plants had their highest translocation factor of 0.24. Heavy metals stunt plant development. Plants grown in environments with a higher concentration of risk factors have slower photosynthetic rates as a result (Mehdizadeh *et al*., 2021).

Conclusion

In this study, water lettuce grown in a fertilizer solution reduced metal levels. Extremely low concentrations of heavy metals in water are no match for water lettuce's amazing ability to filter them out. The results of this study demonstrated that *P. stratiotes* was effective at detoxifying nutrient solution of heavy metals. The optimal growth was seen in a nutrient solution of 6 ppm. Heavy metal pollution significantly reduced root and leaf area as well as other growth metrics. Metals tend to be stored in roots rather than leaves. Cd was more readily absorbed than Cu. The majority of efforts to remove heavy metals relied on phytoremediation. The use of plants for the removal of harmful metals from wastewater is called phytoremediation. It was a method that didn't break the bank financially.

References

Abd Hamid, M., Ismail, S. N. and Mansor, M. 2021. An overview of macrophytes in the tropical wetland ecosystem. *Indonesian Journal of Limnology, 2*(1), 25-34.

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R. and Wang, M. Q. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics,* 9(3): 42.

Ali, S., Abbas, Z., Rizwan, M., Zaheer, I. E., Yavaş, İ., Ünay, A. and Kalderis, D. 2020. Application of floating aquatic plants in phytoremediation of heavy metals polluted water: A review. *Sustainability,* 12(5): 1927.

Ansari, A. A., Naeem, M., Gill, S. S., & AlZuaibr, F. M. 2020. Phytoremediation of contaminated waters: An eco-friendly technology based on aquatic macrophytes application. *The Egyptian Journal of Aquatic Research,* 46(4): 371-376.

Azab, E. and Hegazy, A. K. 2020. Monitoring the efficiency of *Rhazya stricta* L. plants in phytoremediation of heavy metal-contaminated soil. *Plants,* 9(9): 1057.

Behera, M., Nayak, J., Banerjee, S., Chakrabortty, S. and Tripathy, S. K. 2021. A review on the treatment of textile industry waste effluents towards the development of efficient mitigation strategy: An integrated system design approach. *Journal of Environmental Chemical Engineering,* 9(4): 105277.

Behl, T., Kaur, I., Sehgal, A., Singh, S., Sharma, N., Bhatia, S. and Bungau, S. 2022. The dichotomy of nanotechnology as the cutting edge of agriculture: Nano-farming as an asset versus nanotoxicity. *Chemosphere,* 288: 132533.

Chaney, R. L., Li, Y. M., Brown, S. L., Homer, F. A., Malik, M., Angle, J. S. and Chin, M. 2020. Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress *Phytoremediation of contaminated soil and water* (pp. 129-158): CRC press.

El-Said, G. F., El Zokm, G. M., El Sayed, A. A., El Ashmawy, A. A. and Shreadah, M. A. 2020. Anomalous fluctuation of halogens in relation to the pollution status along Lake Mariout, Egypt. *Journal of Chemistry,* 2020: 1-20.

Elgarahy, A., Elwakeel, K., Mohammad, S. and Elshoubaky, G. 2021. A critical review of biosorption of dyes, heavy metals and metalloids from wastewater as an efficient and green process. *Cleaner Engineering and Technology,* 4: 100209.

Gao, B. 2020. Enhanced Nitrogen, Organic Matter and Color Removal from Landfill Leachate by Biological Treatment Processes with Biochar and Zeolite: University of South Florida.

Goncharuk, E. A. and Zagoskina, N. V. 2023. Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on Cadmium. *Molecules,* 28(9): 3921.

Hossain, M. B., Rakib, M. R. J., Jolly, Y. and Rahman, M. 2021. Metals uptake and translocation in salt marsh macrophytes, *Porteresia sp*. from Bangladesh coastal area. *Science of the Total Environment,* 764, 144637.

Hussain, M., Aziz, A., MM, J., Ashraf, M., Wasaya, A., Majeed, M. Z. and Bhatti, A. 2022. Evaluating the phytotoxic effects of some plant species of semi-arid regions. *Plant Cell Biotechnol. Mole. Biol*, 1-18.

Khalid, K. M. and Ganjo, D. G. 2021. Removal of Pb and Zn in municipal wastewater by a consortium of four aquatic plants in vertical subsurface flow constructed wetland (VSF- CW). *International Journal of Environmental Studies*, 78(2): 341-357.

Latif, J., Akhtar, J., Ahmad, I., Mahmood-ur-Rehman, M., Shah, G. M., Zaman, Q. and Saleem, A. 2020. Unraveling the effects of cadmium on growth, physiology and associated health risks of leafy vegetables. *Brazilian Journal of Botany*, 43: 799-811.

Lebrun, M., Száková, J., Drábek, O., Tejnecký, V., Hough, R. L., Beesley, L. and Trakal, L. 2023. ETDA as a legacy soil chelatant: a comparative study to a more environmentally sensitive alternative for metal removal by *Pistia stratiotes* L. *Environmental Science and Pollution Research*, 1-13.

Mehdizadeh, L., Farsaraei, S. and Moghaddam, M. 2021. Biochar application modified growth and physiological parameters of *Ocimum ciliatum* L. and reduced human risk assessment under cadmium stress. *Journal of Hazardous Materials*, 409: 124954.

Momanyi, M. R., Nduko, J. M. and Omwamba, M. 2022. Effect of hermetic Purdue Improved Crop Storage (PICS) bag on chemical and anti-nutritional properties of common Bean (*Phaseolus vulgaris* L.) varieties during storage. *Current Research in Food Science*, 5: 107-116.

Nahar, K. and Hoque, S. 2021. Phytoremediation to improve eutrophic ecosystem by the floating aquatic macrophyte, water lettuce (*Pistia stratiotes* L.) at lab scale. *The Egyptian Journal of Aquatic Research*, 47(2): 231-237.

Nguyen, T. Q., Sesin, V., Kisiala, A. and Emery, R. N. 2021. Phytohormonal roles in plant responses to heavy metal stress: Implications for using macrophytes in phytoremediation of aquatic ecosystems. *Environmental Toxicology and Chemistry*, 40(1): 7-22.

Nti, S. O., Buamah, R. and Atebiya, J. 2021. Polyaluminium chloride dosing effects on coagulation performance: case study, Barekese, Ghana. *Water Practice and Technology*, 16(4): 1215-1223.

Orosun, M. M., Adewuyi, A. D., Salawu, N. B., Isinkaye, M. O., Orosun, O. R. and Oniku, A. S. 2020. Monte Carlo approach to risks assessment of heavy metals at automobile spare part and recycling market in Ilorin, Nigeria. *Scientific Reports*, 10(1): 22084.

Oruko, R. O., Edokpayi, J. N., Msagati, T. A., Tavengwa, N. T., Ogola, H. J., Ijoma, G. and Odiyo, J. O. 2021. Investigating the chromium status, heavy metal contamination, and ecological risk assessment via tannery waste disposal in sub-Saharan Africa (Kenya and South Africa). *Environmental Science and Pollution Research*, 28: 42135-42149.

Qian, S., Lu, H., Xiong, T., Zhi, Y., Munoz, G., Zhang, C., and Wang, X. 2023. Bioaccumulation of Per-and Polyfluoroalkyl Substances (PFAS) in

Ferns: Effect of PFAS Molecular Structure and Plant Root Characteristics. *Environmental Science & Technology,* 57(11): 4443-4453.

Santander, C., Aroca, R., Cartes, P., Vidal, G. and Cornejo, P. 2021. Aquaporins and cation transporters are differentially regulated by two arbuscular mycorrhizal fungi strains in lettuce cultivars growing under salinity conditions. *Plant Physiology and Biochemistry*, *158*, 396-409.

Şentürk, İ., Eyceyurt Divarcı, N. S. and Öztürk, M. 2022. Phytoremediation of nickel and chromium-containing industrial wastewaters by water lettuce (*Pistia stratiotes*). *International Journal of Phytoremediation*, 1-12.

Shah, S. B. 2021. Heavy metals in the marine environment—an overview. *Heavy Metals in Scleractinian Corals*, 1-26.

Shakil, S., Abbasi, N., Shakoor, M., Ahmad, S., Majid, M., Ali, A. and Farwa, U. 2023. Assessment of physicochemical parameters and trace elements in tannery wastewater treatment facility and associated health risks. *International Journal of Environmental Science and Technology,* 1-14.

Singh, S., Karwadiya, J., Srivastava, S., Patra, P. K. and Venugopalan, V. 2022. Potential of indigenous plant species for phytoremediation of arsenic contaminated water and soil. *Ecological Engineering,* 175: 106476.

Sultana, S. 2020. Nutritional and functional properties of *Moringa oleifera*. *Metabolism open,* 8: 100061.

Tang, K. H. D., Awa, S. H. and Hadibarata, T. 2020. Phytoremediation of copper-contaminated water with *Pistia stratiotes* in surface and distilled water. *Water, Air, & Soil Pollution,* 231(12): 573.

Tauqeer, H. M., Karczewska, A., Lewińska, K., Fatima, M., Khan, S. A., Farhad, M. and Iqbal, M. 2021. Environmental concerns associated with explosives (HMX, TNT, and RDX), heavy metals and metalloids from shooting range soils: prevailing issues, leading management practices, and future perspectives *Handbook of bioremediation* (pp. 569- 590): Elsevier.

Victor, K. K., Ladji, M., Adjiri, A. O., Cyrille, Y. D. A. and Sanogo, T. A. 2016. Bioaccumulation of heavy metals from wastewaters (Pb, Zn, Cd, Cu and Cr) in water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*). *International Journal of ChemTech Research,* 9(2): 189-195.

Yildirim, E., Ekinci, M., Turan, M., Ağar, G., Dursun, A., Kul, R. and Argin, S. 2021. Humic+ Fulvic acid mitigated Cd adverse effects on plant growth, physiology and biochemical properties of garden cress. *Scientific Reports,* 11(1): 8040.

Youssef, N. A. 2021. Changes in the morphological traits and the essential oil content of sweet basil (*Ocimum basilicum* L.) as induced by cadmium and lead treatments. *International Journal of Phytoremediation,* 23(3): 291-299.

Yuldashev, K., Abdullaev, I., Buriyev, S. and Tajiyev, Z. 2021. Use of *Pistia Stratiotes*

(Araceae) in Water Quality Management in Khorezm Region (Uzbekistan). *Annals of the Romanian Society for Cell Biology*, 6175-6185.

Zemiani, A., Boldarini, M. T. B., Anami, M. H., de Oliveira, E. F. and da Silva, A. F. 2021. Tolerance of *Mentha crispa* L.(garden mint) cultivated in cadmium-contaminated oxisol. *Environmental Science and Pollution Research*, 28: 42107-42120.

Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E. A., El-Esawi, M. A., Ishfaq, M. and Maqsood, M. F. 2022. Cadmium toxicity in plants: Recent progress on morpho-physiological effects and remediation strategies. *Journal of Soil Science and Plant Nutrition*, 1-58.