

Article



Impact of Irrigation with Contaminated Water on Heavy Metal Bioaccumulation in Water Chestnut (*Trapa natans* L.)

Mostafa A. Taher ^{1,2}, Ferjeni Zouidi ¹, Pankaj Kumar ³[®], Sami Abou Fayssal ^{4,5}[®], Bashir Adelodun ^{6,7}[®], Madhumita Goala ⁸[®], Vinod Kumar ³[®], Željko Andabaka ⁹[®], Ivan Širić ^{9,*}[®] and Ebrahem M. Eid ^{10,11,*}[®]

- ¹ Biology Department, Faculty of Science and Arts, King Khalid University, Mohail Assir 61321, Saudi Arabia
- ² Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
- ³ Agro-Ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science,
- Gurukula Kangri (Deemed to Be University), Haridwar 249404, Uttarakhand, India
 ⁴ Department of Agronomy, Faculty of Agronomy, University of Forestry, 10 Kliment Ohridski Blvd, 1797 Sofia, Bulgaria
- ⁵ Department of Plant Production, Faculty of Agriculture, Lebanese University, Beirut 1302, Lebanon
- ⁶ Department of Agricultural and Biosystems Engineering, University of Ilorin, PMB 1515, Ilorin 240003, Nigeria
- ⁷ Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 41566, Republic of Korea
- ⁸ Nehru College, Pailapool, Affiliated Assam University, Silchar 788098, Assam, India
- ⁹ Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia
- ¹⁰ Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia
- ¹¹ Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
- Correspondence: isiric@agr.hr (I.Š.); ebrahem.eid@sci.kfs.edu.eg (E.M.E.)

Abstract: This study investigated the monitoring of six heavy metals (Cd, Cr, Cu, Fe, Mn, and Zn) in pond water, sludge, and cultivated water chestnut (Trapa natans L.) crops in Saharanpur district of Uttar Pradesh, India. For this purpose, samples of pond water, sludge, and T. natans plant (nut, shoot, and root) were collected in November of 2021 and 2022 from three zones (Zone 1: agricultural area irrigated with borewell water, Zone 2: urban area irrigated with municipal wastewater, and Zone 3: rural area irrigated with mixed type of wastewater) and subsequently analyzed for heavy metal concentration using atomic absorption spectroscopy. The results showed that the physicochemical and heavy metal characteristics of pond water and sludge were significantly (p < 0.05) higher in Zone 2 and 3 than to those in Zone 1. The concentration of heavy metals in T. natans root was comparatively high followed by shoot and nut parts. The bioaccumulation factor (BAF) was maximum while using pond water as a reference medium compared to pond sludge. Overall, the increasing order of heavy metals in T. natans was observed as Cd < Cr < Cu < Zn < Mn < Fe. In the edible part (nut) of *T. natans*, the average contents of Cd $(0.005 \pm 0.002 \text{ mg/kg})$, Cr $(0.134 \pm 0.009 \text{ mg/kg})$, Cu $(1.043 \pm 0.104 \text{ mg/kg})$, Fe $(9.589 \pm 0.957 \text{ mg/kg})$, Mn (4.326 ± 0.753 mg/kg), and Zn (1.540 ± 0.537 mg/kg) were comparatively less than shoot and root parts. Overall, the results revealed that T. natans irrigated with contaminated irrigation supplies at Zone 2 and 3 showed the highest BAF of heavy metals than Zone 1. Because the heavy metal concentrations in edible parts of T. natans did not exceed the threshold limits, the contaminated water sources in the Saharanpur region of India should be used in a safe and controlled manner.

Keywords: fruit contamination; health risk; irrigation water; pollution; toxic elements; wastewater reuse

1. Introduction

Agricultural production and trading, and industrial production are the economic mainstays of many countries. Developing countries are often engaged in the intensification of both agricultural and industrial productions considering their large population [1]. Despite the positive impacts of these production activities, especially industrial production on the economy, there are consequences of environmental pollution. This can be pronounced by the large disposal of wastewater into rivers and canals, thus resulting in water pollution via different types of contaminants, e.g., microbes, fungi, pesticide residues, heavy metals,



Citation: Taher, M.A.; Zouidi, F.; Kumar, P.; Abou Fayssal, S.; Adelodun, B.; Goala, M.; Kumar, V.; Andabaka, Ž.; Širić, I.; Eid, E.M. Impact of Irrigation with Contaminated Water on Heavy Metal Bioaccumulation in Water Chestnut (*Trapa natans* L.). *Horticulturae* **2023**, *9*, 190. https://doi.org/10.3390/ horticulturae9020190

Academic Editor: Lucia Bortolini

Received: 29 December 2022 Revised: 20 January 2023 Accepted: 27 January 2023 Published: 2 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). etc. Further, these industrial wastewaters end up being used to supplement agricultural production through inland irrigation [2,3]. Recently, a study evaluated the soil heavy metal bioaccumulation (transfer of heavy metals into vegetative parts of cultivated rice plants) originating from Kali River irrigation water in which paper mill effluents are liberated. The findings showed a significant accumulation of potentially toxic elements in rice fields, with only health risks in the PR-121 rice variety, which indicates a minor accumulation in the edible part of the crop [4]. Heavy metals bioaccumulation in human-consumed crops can lead to health risks varying from diarrhea to anemia, mental and central nervous dysfunction, childbirth complications, and cancer [4]. Thus, adequate monitoring of water resources either for direct consumption or to be used for agricultural production is crucial to avoid contracting transmissible diseases such as cholera and typhoid [5,6].

Ponds are stagnant water reservoirs formed naturally or by human activity. It is a breeding ground for plants, fish, insects, and amphibians. Moreover, in some countries, e.g., Bangladesh, ponds contribute to 15% of household drinking water and 20–25% of produced fish, thus contributing to the national economy [7]. Due to the unsafe disposal of industrial wastewater into ponds, their water becomes rich in heavy metals, e.g., cadmium, chromium, copper, lead, nickel, and zinc, which are the most deleterious metals for all forms of life. Ponds have been considered, for many decades, as the perfect treatment for stormwater via sedimentation [8]. Whereas, sedimentary ponds near industrial areas are the main source of groundwater and soil pollution [9] as potentially toxic elements are highly available and have a higher potential to accumulate in bottom sediments than in natural ones [10]. Golub and Piekutin [9] outlined that industrial wastewater discharged into sedimentary ponds is sometimes highly saline contributing to the salinization of surface and groundwater sources. Therefore, sediment contamination may originate from anthropogenic activities and/or natural geogenic sources [11]. Heavy metal accumulation in sediments can be transferred easily to wild crops like macrophytes. In this context, Ramachandra et al. [12] reported high heavy metal bioaccumulation by *Typha augustata*, a wetland and pond macrophyte collected from Bellandur lake, exceeding the critical limits of metals in crops.

Water chestnut (*Trapa natans* L.; Trapaceae family) or commonly called "Singhada" is an annual aquatic plant that grows on freshwater rich in nutrients and has a strong submerged stem that can reach depths of 4.5 m. This plant is considered an ecological threat as it reduces oxygen levels in lakes, ponds, and wetlands; thus, resulting in the death of aquatic life, especially fish and other competitive plants. Also, this plant is widely cultivated for its nuts which are consumed as fruit. Despite that, it has been grown in ponds and outdoor water gardens for its bioremediation potential via bioaccumulating toxic heavy metals in stagnant water [13]. Optimum pH of 6.7–8.2 and alkalinity of 12–128 mg/L are crucial for water chestnut growth and reproduction [14]. *T. natans* is well appreciated in Asian cuisine and especially in India. Its nuts can be dried and ground into baking flour, eaten raw, boiled, fried, grilled, pickled, candied, or added to salads [15]. Water chestnut is low in calories, rich in vitamin B6 and vitamin E, and a great source of fiber which can help reduce blood sugar and cholesterol levels [16]. It has been also vastly used in ancient times to treat sunburn and rheumatism [13].

The contamination of stagnant (ponds and wetlands) and river water with heavy metals and fluoride residues have been largely reported in several Indian districts and states, among them are Saharanpur district and Uttarakhand state [17,18] which outlines the need for restoration frameworks and policies implementation [19]. Several investigations have been conducted to assess heavy metal bioaccumulation by crops irrigated with contaminated river water in the Saharanpur district [3,20,21]. However, this is the first study that aims to: (a) assess the water quality and heavy metals pollution in pond water; (b) assess the heavy metals contamination in pond sludge; (c) biomonitoring the health risk associated with heavy metal bioaccumulation in *T. natans* crop. Also, the comparative impact of different irrigation sources was studied on pond water, sludge, and cultivated *T. natans*.

2. Materials and Methods

2.1. Description of the Study Area

The current study was conducted in the Saharanpur district of Uttar Pradesh, India. The district is the northernmost district of Uttar Pradesh (Latitude: 29.53° N and Longitude: 77.40° E). The district spans 4738 km² and its economy is primarily agricultural, with sugar cane, wheat, maize, mango, and rice being the most important crops grown [22]. There are also numerous small-scale industries in the district, primarily manufacturing, wood, and paper products. Saharanpur is also well-known for its wood carving and furniture production. Additionally, fish farming and aquatic macrophyte cultivation (lotus, water chestnut, etc.) are the main agri-businesses of the local farmers. Small-scale farmers convert their lands to mini-ponds (submerged fields) and cultivate water chestnuts [23]. The study area was divided into three zones based on the pond-irrigation source including, Zone 1 (borewell water), Zone 2 (secondarily treated municipal wastewater), and Zone 3 (mixed-type; borewell and wastewater), respectively. Figure 1a shows the locations of the selected sampling sites for water chestnut, water, and pond sediment sample collection.



Figure 1. (a) Map view of Saharanpur district depicting sampling zones and (b) layout for collection of water chestnut, sediment, and water samples from ponds.

2.2. Sample Collection

For the present study, the pond water, sludge, and *T. natans* plant samples were collected in November 2021 and 2022. The samples were collected with the help of native farmers and field workers. During the sample collection, protective gear such as a lab coat, gloves, and safety glasses were used. The pond water samples were collected in a polyvinyl chloride (PVC) container of 5 L capacity. Similarly, the pond sludge sample was collected in 2 kg capacity transparent and zip-locking polyethylene bags. For plant sample collection, the matured vines of *T. natans* were selected and taken out carefully from the pond. The vines were washed with tap water and segregated by cutting them into nut (fruit), shoot (stem and leaves), and root parts. The plant samples were also collected in polyethylene bags. All samples were immediately transported to the laboratory for further processing and analysis. A total of five representative samples were collected from each pond (for water, sludge, and plant) and mixed to obtain a single identical sample (twice a month). In this way, two identical samples were collected from five ponds of each zone i.e., starting and end of November months of 2021 and 2022, separately. Thus, twenty identical samples were collected from each zone and then analyzed separately. In this manner, a total of 60 samples were collected in two years for each pond water, sludge, and plant tissue. Sample containers and bags were carefully labeled with the date and time of collection, collection site, and any other relevant information. The pond water and sludge samples were placed at 4 °C while plant tissue samples were oven dried (at 60 °C for 3–5 h) and stored until further analyses.

2.3. Chemical Analyses

The pond water and sludge samples were analyzed for selected physicochemical and heavy metal parameters including, pH, electrical conductivity (EC: dS/m), total dissolved solids (TDS: mg/L), total suspended solids (TSS: mg/L), biochemical oxygen demand (BOD: mg/L), chemical oxygen demand (COD: mg/L), total nitrogen (TN: mg/L or g/kg), total phosphorus (TP: mg/L or g/kg), organic matter (OM: g/kg), cadmium (Cd: mg/L or mg/kg), chromium (Cr: mg/L or mg/kg), copper (Cu: mg/L or mg/kg), iron (Fe: mg/L or mg/kg), manganese (Mn: mg/L or mg/kg), and zinc (Zn: mg/L or mg/kg). For this, standard protocols were adopted as prescribed by APHA [24] and AOAC [25]. Purposely, microprocessor-based digitally calibrated multipurpose meters were used for pH-EC-TDS (1611, ESICO, Parwanoo, India). TSS measurement was done based on a pre-weighed filter at oven drying (105 °C) method. BOD and COD were determined by following Winkler's dissolved oxygen and open acid-reflux methods, respectively [3]. TN was determined by Kjeldahl's assembly [26] while the contents of TP were measured using acid-digestion and spectrophotometric (60 Cary, Agilent Technologies, Santa Clara, CA, USA) methods, respectively. The contents of heavy metals in pond water, sludge, and plant samples were digested in di-acid solution (HNO₃ and HClO₄; 3:1) and then determined using atomic absorption spectroscopy (AAS, Analyst 800, PerkinElmer, Waltham, MA, USA). All chemicals and reagents were procured from an authentic source (Merck Pvt. Ltd., New Delhi, India).

2.4. Data Analysis and Software

The data obtained in this study were analyzed using principal component analysis (PCA) to understand the interrelationship between sampling zones and selected parameters of collected pond water and sludge samples. Purposely, the multivariate toolkit package of OriginPro (Version 2022, OriginLab, Northampton, MA, USA) was used for the PCA computations. Moreover, the heavy metal bioaccumulation potential of *T. natans* was assessed using the bioaccumulation factor (BAF) index. BAF is used to estimate heavy metal concentration in an organism in relation to the amount present in the environment. They are used to assess a chemical's perceived hazard or potential to an organism or population. Environmental risk assessments, environmental monitoring, and environmental regulation all make use of bioaccumulation factors [27]. The form of the index is given in Equation (1):

$$Bioaccumulation factor (BAF) = PP/RM$$
(1)

where, PP and RM indicate the heavy metal concentration (mg/kg) in plant parts (nut, shoot, and root) with respect to concentration in the reference medium i.e., pond water (mg/L) or sludge (mg/kg), respectively. The data were also analyzed for statistical significance using a one-way analysis of variance (ANOVA) test. The mean comparison was carried out using Tukey's post-hoc test. In this, the level of all statistical tests was adjusted to a probability (*p*) < F value of 0.05 (95% confidence interval). Moreover, Microsoft Excel 2019 (Microsoft, Redmond, WA, USA) software was used for data visualizations.

3. Results and Discussion

3.1. Status of Water Quality and Heavy Metals Pollution in Pond Water

The results of the physicochemical and heavy metal analyses of pond water collected from the studied zones are presented in Table 1. The analyses indicated that Zone 3 had a significantly (p < 0.05) higher pH (7.89 \pm 0.03) than the agricultural and urban zones. However, all zones showed an almost neutral pH. ANOVA test outlined a significant increase (p < 0.05) in almost all parameters in Zone 2 in comparison with the remaining zones. In particular, the following physicochemical and heavy metal parameters were significantly higher (p < 0.05) in pond water of Zone 2: EC (0.93 \pm 0.04 dS/m), TDS (2508.60 \pm 68.29 mg/L), TSS (913.04 \pm 31.60 mg/L), BOD (383.45 \pm 12.24 mg/L), COD (1108.59 \pm 66.17 mg/L), TN (24.60 \pm 3.81 mg/L), TP (9.32 \pm 014 mg/L), Fe (1.822 \pm 0.124 mg/L), Mn (0.290 \pm 0.019 mg/L)

and Zn ($0.315 \pm 0.044 \text{ mg/L}$). Though, the contents of Cd, Cr, and Cu were significantly (p < 0.05) higher in Zones 2 and 3 compared to Zone 1 with values slightly higher in Zone 2 than in Zone 3 (0.032 ± 0.010 and $0.023 \pm 0.006 \text{ mg/L}$; 0.026 ± 0.009 and $0.020 \pm 0.007 \text{ mg/L}$; 0.157 ± 0.011 and $0.119 \pm 0.043 \text{ mg/L}$, respectively). Herein, the levels of TDS were below the standard safe limit in the pond water of Zone 1; whereas, it exceeded them in Zones 2 and 3. Similarly, the concentrations of Cu, Fe, Mn, and Zn in pond water of all zones were below the safe standard limits outlining a low accumulation of heavy metals, except for Cd content. The latter exceeded the standard safe limit in the three studied zones. Additionally, all zones depicted extremely high TSS, BOD, and COD exceeding the safe standard limits set. Based on the PCA results, the pond water quality data were transformed into two principal components namely PC1 and PC2 having a variance of 99.83 and 0.13%, respectively. The vector lengths of corresponding zones, given in Figure 2a, suggested that COD, TDS, TSS, and BOD were the most dominating parameters at Zone 2 followed by Zone 3 and Zone 1, respectively.

Table 1. Average characteristics of pond water collected from selected sampling zones of the study area in two years (2021 and 2022).

Parameters	Sampling Zone ^			Avorago	<u> </u>
	Zone 1 (Agricultural)	Zone 2 (Urban)	Zone 3 (Rural)	Avelage	Standard Limit "
pН	$7.72\pm0.05\mathrm{b}$	$7.34\pm0.07~\mathrm{a}$	$7.89\pm0.03~\mathrm{c}$	7.88 ± 0.54	5.50-9.00
Electrical Conductivity (EC: dS/m)	0.57 ± 0.09 a	$0.93\pm0.04~\mathrm{b}$	0.54 ± 0.05 a	0.68 ± 0.22	-
Total Dissolved Solids (TDS: mg/L)	1629.08 ± 297.10 a	$2508.60 \pm 68.29 \text{ c}$	$2156.11 \pm 79.04 \mathrm{b}$	2097.93 ± 442.64	1900.00
Total Suspended Solids (TSS: mg/L)	736.22 ± 10.25 a	$913.04 \pm 31.60 \text{ c}$	$825.84 \pm 26.92 \mathrm{b}$	825.03 ± 88.41	-
Biological Oxygen Demand (BOD: mg/L)	191.56 ± 8.86 a	$383.45 \pm 12.24 \text{ c}$	$269.03 \pm 10.05 \mathrm{b}$	281.35 ± 96.54	100.00
Chemical Oxygen Demand (COD: mg/L)	677.06 ± 27.01 a	$1108.59 \pm 66.17 \text{ c}$	$820.20 \pm 42.93 \mathrm{b}$	868.62 ± 219.80	250.00
Total Nitrogen (TN: mg/L)	10.88 ± 2.04 a	$24.60 \pm 3.81 \text{ c}$	$18.51 \pm 1.65 \mathrm{b}$	18.00 ± 6.87	-
Total Phosphorus (TP: mg/L)	4.36 ± 0.53 a	$9.32\pm0.14~\mathrm{c}$	$7.02\pm0.32~\mathrm{b}$	6.90 ± 2.48	-
Cadmium (Cd: mg/L)	0.014 ± 0.003 a	$0.032 \pm 0.010 \text{ b}$	$0.023 \pm 0.006 \mathrm{b}$	0.023 ± 0.009	2.000
Chromium (Cr: mg/L)	0.011 ± 0.005 a	$0.026 \pm 0.009 \text{ b}$	$0.020 \pm 0.007 \mathrm{b}$	0.019 ± 0.008	2.000
Copper (Cu: mg/L)	0.106 ± 0.017 a	$0.157 \pm 0.011 \text{ b}$	$0.119 \pm 0.043 \mathrm{b}$	0.127 ± 0.027	3.000
Iron (Fe: mg/L)	0.845 ± 0.090 a	$1.822 \pm 0.124 \text{ c}$	$1.250 \pm 0.065 \mathrm{b}$	1.306 ± 0.491	3.000
Manganese (Mn: mg/L)	0.132 ± 0.007 a	$0.290 \pm 0.019 \text{ c}$	$0.226 \pm 0.030 \mathrm{b}$	0.216 ± 0.079	2.000
Zinc (Zn: mg/L)	0.207 ± 0.060 a	$0.315\pm0.044\mathrm{b}$	0.280 ± 0.052 a	0.267 ± 0.055	5.000

[^] Values are mean \pm standard deviation of twenty samples; *: Bureau of Indian Standards (BIS) and Central Pollution Control Board of India (CPCB) standards for surface disposal and inland irrigation [4]; The same letters (a–c) indicate no significant difference in the properties of pond water among sampling zones at p < 0.05. -: Not available.



Figure 2. Principal component (PC) biplots for the interrelationship between sampling zones and characteristics of pond (**a**) water and (**b**) sludge samples.

These findings may be related to the excessive use of fertilizers in agricultural lands by farmers aiming to increase crop yields, which results in water source pollution. Besides that, a recent study showed that the liberation of municipal, wood, and paper mill wastewater [4] based in the Saharanpur region in water canals increases the pollutants levels posing serious health risks. The nearby wood and paper industries in the Saharanpur region are regarded as a source of highly contaminated wastewater that has been released into the wild without any pre-treatment [28,29]. In a report, Laohaprapanon et al. [30] stated that wood industry-based waters have high total organic carbon (1376 \pm 62 mg/L) and COD (4380 \pm 94 mg/L)

levels of extreme environmental toxicity even if heavily diluted. Similarly, the study on rice fields irrigated with paper mill wastewater in the Saharanpur district revealed the serious pollution of such waters [4]. Zones 1 and 3 showed a strong positive correlation with increased TSS in pond water (Figure 2a) matching in the same PCA quadrant. The data were categorized into two principal components (PC1 and PC2) having variances of 99.83 and 0.13% respectively. The axial biplot showed that the axial lengths of Zone 2 were strongly correlated with selected parameters including BOD, COD, and TDS showing their highest concentration. On the other hand, axial lengths of Zone 1 and Zone 3 showed a strong correlation with TSS values. Rey-Romero et al. [31] related agricultural activities and increased TSS in the surface water of the Jordan river catchment in Colombia. Urban surface runoffs showed high TSS concentrations with coarse particle size in a recent study by Zhao et al. [32] which confirm the results of PCA. Similarly, Zone 2 showed a strong positive correlation with increased TDS and COD in pond water. Industrial wastewaters outline high COD and TDS levels due to the presence of inorganic compounds susceptible to oxidation which may originate from plant decay resulting from different industrial applications [33]. Thus, it was observed from the above results that excessive urban, rural, and industrial activities in Zone 2 and 3 might have resulted in increased pollution levels of pond water used to cultivate T. natans.

3.2. Status of Heavy Metals in Pond Sludge

Pond sludge is a layer of organic material that accumulates on the bottom of a pond (dead plants, leaves, and other debris). It is made up of decaying organic matter and may contain minerals and other substances. Pond sludge can become a problem when it accumulates too many substances and begins to affect the pond's water quality and clarity [34]. For *T. natans* cultivation, pond maintenance, such as aeration and bottom cleaning, can aid in the prevention and management of pond sludge and thereby better crop yields. In this study, the results of the physicochemical and heavy metal analysis of pond sludge in the studied zones are shown in Table 2. Herein, EC and OM showed no significant difference in pond sludge of the three studied zones (ranging between 3.04 and 3.11 dS/m, and 5.72 and 6.13 g/kg, respectively). Pond sludge of all zones showed a neutral pH range $(7.46 \pm 0.17 - 7.75 \pm 0.06)$. Specifically, the urban areas (Zone 2) had significantly (p < 0.05) higher contents of TN, TP, Cd, Cr, Cu, Fe, Mn, and Zn than Zones 1 and 3 (0.37 ± 0.02 g/kg, 0.25 ± 0.04 g/kg, 0.125 ± 0.016 mg/kg, 2.443 ± 0.120 mg/kg, 5.854 ± 0.315 mg/kg, 12.406 ± 2.620 mg/kg, 1.210 ± 0.105 mg/kg, and 8.621 ± 1.431 mg/kg, respectively). High levels of TN and TP can lead to excessive algae growth, as well as an increase in the number of weeds in the pond. It can also increase the risk of water pollution, as TP can cause toxic algal blooms which can poison the water [35]. However, no such algal bloom and weeds have been reported in any pond of this study as farmers regularly cleaned pond surfaces. Increased heavy metals in pond sludge of urban areas may be a result of possible accumulation [36] posing serious health risks when used to irrigate plants destinated for human consumption [37]. The variances of PC1 and PC2 for the interrelationship between sampling zones and physicochemical and heavy metal characteristics of pond sludge were noted as 96.55 and 3.42%, respectively. Also, Zones 1 and 3 fitting in the same PCA quadrant were negatively correlated with TN, TP, Cd, Cr, and Mn (Figure 2b) which outlines a lower risk associated with their pond sludges. Whereas, Zone 2 was strongly positively correlated with high Cu, Fe, and Zn contents in its pond sludge.

Parameters –		A		
	Zone 1 (Agricultural)	Zone 2 (Urban)	Zone 3 (Rural)	Average
pН	7.52 ± 0.23 a	$7.46\pm0.17~\mathrm{a}$	$7.75\pm0.06~\mathrm{b}$	7.58 ± 0.15
Electrical Conductivity (EC: dS/m)	3.04 ± 0.09 a	3.11 ± 0.16 a	3.09 ± 0.12 a	3.08 ± 0.04
Organic Matter (OM: g/kg)	5.72 ± 0.17 a	$6.13\pm0.31~\mathrm{ab}$	6.02 ± 0.24 a	5.96 ± 0.21
Total Nitrogen (TN: g/kg)	0.25 ± 0.03 a	$0.37 \pm 0.02 \text{ c}$	$0.31\pm0.01~{ m b}$	0.31 ± 0.06
Total Phosphorus (TP: g/kg)	$0.18\pm0.02~\mathrm{a}$	0.25 ± 0.04 b	$0.22\pm0.01~\mathrm{b}$	0.22 ± 0.04
Cadmium (Cd: mg/kg)	0.064 ± 0.012 a	$0.125 \pm 0.016 \text{ c}$	$0.098 \pm 0.009 \mathrm{b}$	0.096 ± 0.031
Chromium (Cr: mg/kg)	1.480 ± 0.140 a	$2.443 \pm 0.120 \text{ c}$	$1.910 \pm 0.276 \mathrm{b}$	1.944 ± 0.482
Copper (Cu: mg/kg)	2.031 ± 0.101 a	5.854 ± 0.315 c	$3.722 \pm 0.149 \mathrm{b}$	3.869 ± 1.916
Iron (Fe: mg/kg)	7.517 ± 0.226 a	$12.406 \pm 2.620 \text{ bc}$	$9.416 \pm 0.577 \mathrm{b}$	9.780 ± 2.465
Manganese (Mn: mg/kg)	0.804 ± 0.024 a	$1.210 \pm 0.105 \text{ c}$	$1.154\pm0.046~\mathrm{b}$	1.056 ± 0.220
Zinc (Zn: mg/kg)	4.346 ± 0.330 a	$8.621 \pm 1.431 \text{ c}$	$5.736 \pm 0.529 \text{ b}$	6.234 ± 2.181

Table 2. Average characteristics (mean \pm SD) of pond sludge collected from selected sampling zones of the study area in two years (2021 and 2022).

[^] Values are mean \pm standard deviation of twenty samples; The same letters (a–c) indicate no significant difference in the properties of pond sludge among sampling zones at *p* < 0.05. -: Not available.

Pond sludge is a thick, sediment-like material that accumulates at the bottom of a pond over time. The accumulation of sediment, organic matter, and other pollutants on the bottom of a pond or lake is known as pond sludge pollution. In the current study, such pollutants were possibly emitted by a variety of nearby sources, including agricultural runoff, urban runoff, and industrial wastewater. Such pollutants settle at the pond's bottom, where they can accumulate and cause further environmental harm. In some cases, sludge can contain toxic pollutants like heavy metals or nutrients, which can contribute to eutrophication and other environmental damage [38]. Controlling or eliminating the sources of the pollutants, as well as removing or treating the existing sludge, is required to reduce pond sludge pollution [39]. Therefore, it was concluded that excessive urbanization and industrial activities in the Saharanpur region might have contributed to organic and heavy metal pollution of ponds sludge.

3.3. Concentration and Bioaccumulation of Heavy Metals in Water Chestnut

Table 3 shows the concentrations of six heavy metals (Cd, Cr, Cu, Fe, Mn, and Zn) in the nut, shoot, and root parts of the *T. natans* plant cultivated in selected ponds of the study area during 2021–2022. It was evidenced from the findings that T. natans was capable to accumulate contents of all six heavy metals in their vegetative parts. Specifically, the highest levels of heavy metals were observed in the root followed by the shoot, and nut parts. However, the analyzed levels of heavy metals were significantly (p < 0.05) impacted by the irrigational waters that originated from several sources (i.e., borewell, municipal, and mixed type). In this, the highest significant (p < 0.05) levels of all heavy metals in T. natans plant parts were observed in the case of Zone 2 where maximum urban activities were reported. This contributed to the accumulation of greater heavy metals in municipal wastewater. However, Zone 3 showed moderate contamination of all heavy metals since there were moderate anthropogenic activities. On the other hand, Zone 1 showed the minimum level of heavy metals due to the use of a non-contaminated borewell water supply as supported by a recent study in the same region [4]. In particular, the increasing order of heavy metals (based on their concentration) in nut and root parts of T. natans was identified as Cd < Cr < Cu < Zn < Mn < Fe; however, for shoot parts, the order was slightly different i.e., Cd < Cr < Cu < Mn < Zn < Fe. A previous study also reported that Fe is a maximally absorbed heavy metal by T. natans [40]. Overall, the maximum levels of Cd, Cr, Cu, Fe, Mn, and Zn in *T. natans* reached 0.010 ± 0.002 , 0.187 ± 0.019 , 3.632 ± 0.181 , 89.107 ± 3.639 , 10.791 ± 2.110 , and 7.250 ± 0.215 mg/kg dwt, respectively. Since the only edible part of this plant is the nut, the detected contents of Cd $(0.005 \pm 0.002 \text{ mg/kg})$, Cr $(0.134 \pm 0.009 \text{ mg/kg})$, Cu (1.043 \pm 0.104 mg/kg), Fe (9.589 \pm 0.957 mg/kg), Mn (4.326 \pm 0.753 mg/kg), and Zn $(1.540 \pm 0.537 \text{ mg/kg})$ were comparatively lower than shoot and root parts.

Parameters	Chestnut Plant Part —		A		
		Zone 1 (Agricultural)	Zone 2 (Urban)	Zone 3 (Rural)	Average
Cadmium (Cd: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	0.003 ± 0.002 a 0.006 ± 0.001 a 0.008 ± 0.003 a	0.006 ± 0.002 ab 0.008 ± 0.003 ab 0.011 ± 0.001 a	0.005 ± 0.001 a 0.008 ± 0.003 ab 0.010 ± 0.001 a	$\begin{array}{c} 0.005 \pm 0.002 \\ 0.007 \pm 0.001 \\ 0.010 \pm 0.002 \end{array}$
Chromium (Cr: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	0.125 ± 0.006 a 0.152 ± 0.005 a 0.173 ± 0.009 a	$\begin{array}{c} 0.142 \pm 0.007 \ \text{bc} \\ 0.160 \pm 0.008 \ \text{a} \\ 0.209 \pm 0.010 \ \text{c} \end{array}$	$\begin{array}{c} 0.136 \pm 0.007 \text{ b} \\ 0.162 \pm 0.007 \text{ ab} \\ 0.180 \pm 0.005 \text{ b} \end{array}$	$\begin{array}{c} 0.134 \pm 0.009 \\ 0.158 \pm 0.005 \\ 0.187 \pm 0.019 \end{array}$
Copper (Cu: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	0.952 ± 0.048 a 2.705 ± 0.135 a 3.460 ± 0.073 a	$\begin{array}{c} 1.157 \pm 0.053 \text{ c} \\ 3.210 \pm 0.160 \text{ b} \\ 3.820 \pm 0.098 \text{ c} \end{array}$	$\begin{array}{c} 1.021 \pm 0.041 \text{ b} \\ 3.304 \pm 0.165 \text{ b} \\ 3.615 \pm 0.183 \text{ b} \end{array}$	$\begin{array}{c} 1.043 \pm 0.104 \\ 3.073 \pm 0.322 \\ 3.632 \pm 0.181 \end{array}$
Iron (Fe: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	8.533 ± 0.720 a 46.984 \pm 2.349 a 75.002 \pm 4.250 a	$\begin{array}{c} 10.401 \pm 1.020 \text{ bc} \\ 62.550 \pm 3.128 \text{ c} \\ 90.384 \pm 3.019 \text{ b} \end{array}$	$\begin{array}{c} 9.832 \pm 0.290 \text{ b} \\ 50.127 \pm 2.006 \text{ ab} \\ 91.936 \pm 4.597 \text{ b} \end{array}$	$\begin{array}{c} 9.589 \pm 0.957 \\ 53.220 \pm 8.231 \\ 89.107 \pm 3.639 \end{array}$
Manganese (Mn: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	3.530 ± 0.507 a 6.149 ± 0.307 a 8.814 ± 0.441 a	5.028 ± 1.251 bc 10.907 ± 0.545 c 13.012 ± 2.050 b	$\begin{array}{c} 4.420 \pm 0.321 \text{ b} \\ 8.001 \pm 0.400 \text{ b} \\ 10.547 \pm 0.823 \text{ b} \end{array}$	$\begin{array}{c} 4.326 \pm 0.753 \\ 8.352 \pm 2.398 \\ 10.791 \pm 2.110 \end{array}$
Zinc (Zn: mg/kg)	Nut (Without Shell) Shoots (Above Water) Roots (Below Water)	$\begin{array}{c} 0.952 \pm 0.069 \text{ a} \\ 6.087 \pm 1.304 \text{ a} \\ 7.305 \pm 0.092 \text{ a} \end{array}$	$2.003 \pm 0.446 \text{ b}$ $6.260 \pm 0.810 \text{ a}$ $7.432 \pm 0.472 \text{ a}$	$\begin{array}{c} 1.665 \pm 0.120 \text{ b} \\ 6.431 \pm 0.372 \text{ a} \\ 7.012 \pm 0.210 \text{ a} \end{array}$	$\begin{array}{c} 1.540 \pm 0.537 \\ 6.259 \pm 0.172 \\ 7.250 \pm 0.215 \end{array}$

Table 3. Concentrations of six heavy metals in the nut, shoot, and root parts of the *T. natans* plant cultivated in selected zones of the study area.

[^] Values are mean \pm standard deviation of twenty samples; The same letters (a–c) indicate no significant difference in the heavy metal concentration in *T. natans* water among sampling zones at *p* < 0.05.

On the other hand, the bioaccumulation factor (BAF > 1) values showed that *T. natans* had good potential to accumulate contents of selected heavy metals from growing environments. Figure 3 shows the BAF value of *T. natans* parts (nut, shoot, and root) based on pond water and sludge qualities. The results showed that higher BAF values for selected heavy metals were related to the use of pond water as a reference medium as compared to pond sludge. Comparatively, Zone 1 showed maximum BAF values followed by Zone 3, while Zone 2 showed minimum BAF values. Also, the root part of *T. natans* depicted maximum BAF values for all heavy metals as compared to nut and shoot parts. The increasing order of BAF values, based on pond water, was identified as: Cd < Cr < Zn < Cu < Mn < Fe; while, based on pond sludge, the increasing order was observed as Cd < Cr < Zn < Cu < Mn < Fe. *T. natans* is an aquatic macrophyte and its root system is submerged in water; therefore, pond sludge had lower contact which eventually resulted in lower BAF values. The higher levels of some potentially toxic heavy metals e.g., Cd and Cr in edible parts of T. natans i.e., nuts might be unsafe for human consumption as they may cause health issues [41]. Previous studies showed that threshold limits of Cd, Cr, Cu, Fe, Mn, and, Fe in fruits should not exceed the standard limits of 0.10, 2.30, 40.00, 425.00, 30.00, and 50.00 mg/kg dwt [42,43]. However, T. natans from all ponds had relatively lower concentrations of these heavy metals. Still, it is recommended to carefully utilize the contaminated water sources for the irrigation of *T. natans* crops.

Pond plants may suffer as a result of high levels of heavy metals. These metals can accumulate in the soil and water, which can be toxic to plants and reduce plant health and growth [4]. Additionally, heavy metals can obstruct the absorption of vital nutrients by plants, causing growth to be stunted or delayed. Furthermore, the presence of heavy metals can reduce the amount of oxygen in the water, depriving the plants of oxygen. Heavy metals can reduce the amount of oxygen in water by forming insoluble compounds with oxygen molecules [40]. This may result in yellowing or even death of the leaves [44]. Heavy metals can consequently significantly lower aquatic plants' productivity when they are present in ponds. Previously, Krivokapić [45] reported eight heavy metals (Pb, Cd, Cu, Zn, Al, Cr, Hg, and As) in fruits of *T. natans* growing in Skadar Lake (Montenegro), Balkans. The report showed that *T. natans* accumulated all eight heavy metals where the increasing order of BAF was as follows: As < Pb < Hg < Cd < Cr < Zn < Cu < Al. Similarly, Babu et al. [46] also analyzed the levels of three heavy metals (Cu, and Fe) in different parts (root, stem, leaf, peel, and kernel) of *T. natans* cultivated in the Lucknow region of Uttar Pradesh state, India.

They found that concentrations of Cu and Fe in *T. natans* fruits ranged between 5.60–8.80 and 135.40–297.60 mg/kg dwt, respectively. These values are much higher than those recorded in the present study. They suggested that it is essential to monitor the levels of potentially toxic heavy metals in the region to ensure the safe edibility of *T. natans* for humans.



Figure 3. Bioaccumulation factor (BAF) of heavy metals based on sampling zones and characteristics of the pond (**a**) water and (**b**) sludge as reference.

4. Conclusions

From the findings of this study, it was concluded that water chestnut (*Trapa natans* L.) cultivated in selected zones of the Saharanpur district of Uttar Pradesh, India showed availability of selected heavy metals (Cd, Cr, Cu, Fe, Mn, and Zn). It was evidenced that *T. natans ponds* irrigated with contaminated water supplies (Zones 2 and 3) showed significantly high (p < 0.05) concentrations of heavy metals in both pond water and sludge samples during two years (2021 and 2022) as compared to the ponds irrigated with borewell water supplies (Zone 1). Overall, the highest concentrations (mg/kg dwt) of heavy metals were reported in root parts as compared to the shoot and nut of *T. natans*. The bioaccumulation factor (BAF) of heavy metals showed higher values (>1) for pond water as compared to pond sludge. Since the concentration of heavy metals did not exceed threshold limits, thus, the wastewater should be used in a safe and controlled manner to irrigate the *T. natans* crop. Further studies on the analysis of other potentially toxic heavy metals in other regions of Uttar Pradesh, India are highly recommended.

Author Contributions: Conceptualization, P.K.; Formal analysis, P.K. and M.G.; Funding acquisition, M.A.T. and F.Z.; Investigation, M.G.; Methodology, P.K.; Project administration, E.M.E.; Resources, V.K.; Software, P.K.; Supervision, E.M.E.; Validation, M.A.T., F.Z., S.A.F., B.A., V.K., Ž.A., I.Š. and E.M.E.; Visualization, P.K.; Writing—original draft, P.K. and S.A.F.; Writing—review & editing, B.A., Ž.A., I.Š. and E.M.E. All authors have read and agreed to the published version of the manuscript.

Funding: Ferjeni Zouidi extends his appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through Grant number (RGP. 1/289/43).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to their host institutes for providing the necessary facilities to conduct this study. This is joint work from the members of the Sustainable Agro-Environment International Research Group (SAEIRG). Ferjeni Zouidi extends his appreciation to the Deanship of Scientific Research at King Khalid University for funding this work under Grant number (RGP. 1/289/43). All individuals included in this section have consented to the acknowledgment.

Conflicts of Interest: The authors declare no conflict of interest.

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