Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-022-04057-6

ORIGINAL ARTICLE

Seasonal Variations in Bioaccumulation and Translocation of Toxic Heavy Metals in the Dominant Vegetables of East Kolkata Wetlands: a Case Study with Suggestive Ecorestorative Strategies

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Accepted: 26 June 2022

Abstract

In recent times, East Kolkata Wetlands (EKW), a designated Ramsar site in the eastern part of megacity Kolkata, has been threatened by toxic heavy metal (HM) pollution. Besides being a natural wetland supporting biodiversity, EKW serves as a significant food basket for the city. For assessing the magnitude of HM pollution in this wetland, the three most cultivated food crops of EKW, namely *Lagenaria siceraria* (bottle gourd), *Abelmoschus esculentus* (ladies' fingers), and *Zea mays* (maize), as well as the ambient soil samples, were collected during premonsoon, monsoon, and postmonsoon for 2 consecutive years (2016 and 2017). Predominant HMs like cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) were analyzed in the roots and edible parts of these plants, as well as in the ambient soil to evaluate the bioaccumulation factor (BF) and translocation factor (TF) of each HM in the three vegetables. It was observed that the HM content in the food crop species followed the order *Z. mays* > *L. siceraria* > *A. esculentus*. HMs accumulated in all three vegetables as per the order Pb > Cd > Cr > Hg. Monsoon seems to be threatening in terms of bioaccumulation and translocation of HMs as both BF and TF were highest in this season irrespective of the plant species. Hence it demands critical monitoring of HM pollution levels in this wetland and subsequent ecorestoration through distinctive plant growth-promoting rhizobacteria (PGPR)-assisted co-cultivation of these food crops with low-metal-accumulating, deep-rooted, high-biomass-yielding, and bioenergy-producing perennial grass species for minimizing HM intake.

Keywords East Kolkata Wetlands · Heavy metals · Bioaccumulation pattern · *Lagenaria siceraria* · *Abelmoschus esculentus* · *Zea mays* · Ecorestoration

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Introduction

In the recent past, population explosion has augmented urbanization, industrialization, and intensive agricultural activities to meet the increasing demand for food, eventually leading to greater anthropogenic stress on the environment. A key challenge faced by modern society today is environmental pollution, of which one of the severe threats is heavy metal (HM) toxicity. Life processes on earth, such as the growth of plants and animals, depend on some metals in trace amounts, which are essential and considered micronutrients. However, certain forms of some metals can be toxic, even in relatively trace concentrations, and therefore, pose risks to the overall health and well-being of plants, animals, and humans [1, 2]. The HMs, viz., arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), and lead (Pb), have no known physiological functions, but depending on their amount in the human body tissues, there is high probability of adverse impact on the overall health [3–6]. In addition to this, these HMs also negatively affect the overall growth and development of cultivable plants, including food crops (e.g., biomass, photosynthetic rate, chlorophyll a and b content) [7]. Unlike organic contaminants, HMs do not have an environmental half-life but persist indefinitely in the environment. They can only be transformed from more toxic to less or non-toxic forms by the action of microorganisms, including bacteria and fungi [8]. HMs entering food via anthropogenic or natural contamination of air, water, or soil are carried in the food chain by plants and animals because they tend to bioaccumulate in the living tissues [9].

The problem of HM pollution is more pronounced in developing countries than in developed nations. India, the second most populous country globally, with enormous and rich biodiversity, is no exception. Environmental HM toxicity affects biodiversity, and the maritime state of West Bengal in the eastern part of India offers incredible biodiversity owing to the presence of two world-renowned biodiversity hotspots, namely the Indian Sundarbans and the East Kolkata Wetlands (EKW). The former is a UNESCO World Heritage Site, and the latter is a designated Ramsar site. EKW provides a unique example of the participation of the local people in the process of its restoration and upgradation and is a world famous model of a wetland, which serves multiple purposes, such as resource recovery, flood control, detoxification of the environment, carbon sequestration, habitat for a variety of flora and fauna, and livelihood support for the local farmers [10, 11]. It has saved the city from colossal construction and maintenance costs associated with wastewater treatment plants. Discharge of wastewater in the EKW sites often contaminates the associated edible flora and fauna with HMs, most of which are often toxic.

The present study was carried out to assess the magnitude of HM pollution in EKW, a complex of natural and artificial wetlands lying east of the metropolitan city of Kolkata, West Bengal, in India. The city of Kolkata, with a total area of 1886.67 km^2 (out of which 206.08 km^2 is within the Kolkata Municipal Corporation (KMC) area) (https://en.wikipedia.org/wiki/Kolkata), sustains ~ 15.13 billion people, as reviewed in 2022, due to which the population density is enormously large (https://worldpopulationreview.com/world-cities/kolkata-population). The wetlands cover 125 km^2 and include marshes and meadows as well as sewage farms and settling ponds. The wetlands are used to treat or absorb the sewage wastes of Kolkata, and the various nutrients contained in the wastewater simultaneously sustain pisciculture and agriculture in adjoining fish farms and farmlands, respectively. In EKW, the total fresh vegetables harvested daily are about 150 tons and fish cultivated per year is 10,500 tons [12]. The EKW was designated a “wetland of international importance” under the Ramsar Convention on 19 August 2002. This Ramsar site consists of 264 fish ponds managing wastewater aquaculture, horticultural plots, agricultural land, and residential area [13]. It is a unique resource recovery ecosystem where pond-effluent-based rice cultivation, garbage-based vegetable farming, sewage-fed aquaculture, and agriculture are practiced [14]. The presence of and seasonal variations in toxic HMs like Cd, Cr, Pb, and Hg have been reported by Dutta et al. [15] in the tissues of three commonly cultivated edible carp variety of fishes, namely Rohu (*Labeo rohita*), Catla (*Catla catla*), and Nile tilapia (*Oreochromis niloticus*), cultivated in EKW ponds (locally known as “bheries”).

Around 100 different plant species have been recorded in and around the EKW [16]. A variety of vegetables are farmed here, including bottle gourd, ladies’ fingers, cauliflower, corn, eggplant, pumpkin, and sacred basil [17]. In addition, vast tracts of land are dedicated to paddy cultivation [17]. The three commonly used vegetables, namely *Lagenaria siceraria*, *Abelmoschus esculentus*, and *Zea mays*, along with their ambient soil, were collected from the Dhapa waste dumping site in EKW through three seasons (premonsoon, monsoon, and postmonsoon) in 2 consecutive years 2016 and 2017, and the selected HM contents of Cd, Cr, Pb, and Hg were analyzed in the edible parts of these plants along with their root systems.

L. siceraria (bottle gourd), possibly the first domesticated vegetable species, is used as food and medicine and for other uses like making utensils and musical instruments [18]. *A. esculentus* (ladies’ fingers) is also an economically important vegetable crop, quite popular in India because of the ease of cultivation, dependable yield, and adaptability to varying moisture conditions [19, 20]. *Z. mays* (corn) is a tall monoecious annual grass having high calorific value and nutritional content. It is a staple diet of many inhabitants worldwide [21, 22].

Vegetables are essential dietary constituents and contain water, carbohydrates, proteins, vitamins, minerals, phenolic compounds, volatiles, and antioxidants [23]. Vegetables are connected to improved human health in terms of better vision, reduced risk of heart-related ailments, controlling blood sugar, and gastrointestinal health. Some vegetables have potent antioxidants, which protect the body from reactive oxygen species (ROS). Each vegetable has a unique combination of phytochemicals, and hence only a variety of vegetables can ensure better health benefits.

Like fishes, intake of contaminated vegetables and fruits is a risky affair for the health of individuals [24, 25]. Plants can uptake and bioaccumulate HMs from the contaminated ambient soil through their roots as well as absorb the airborne metal deposits on their aboveground parts [26, 27]. Another cause of contamination can be irrigation with contaminated water, such as wastewater from sewage canals [28, 29]. A fundamental matter of grave concern is that these vegetables can be a prolific accumulator of HMs, especially from soil [30], providing an easy entry of these toxic HMs into the food chain. It is well established that HMs exist in the soil both as soluble forms and in combined forms, as salt complexes. Indeed, only soluble, exchangeable, and chelated metal species in the soils are mobile, and therefore, they are made available to the plants [31, 32]. However, HM bioaccumulation in plants depends on vegetable species, growth stages, soil composition, geographic and atmospheric conditions, and metal types and their bioavailability [32–34]. Unknowingly, people consume vegetables with high HM content, which, when exceeding the permissible limit, impacts their health leading to various medical disorders [25]. There exists a correlation between the concentrations of HMs in the vegetables with the corresponding soil HM content [35].

The present study aims to investigate whether *L. siceraria*, *A. esculentus*, and *Z. mays* could accumulate biologically available forms of selected HM species (like Cd, Cr, Pb, and Hg) from ambient soil in their roots and edible parts, which might be a direct pathway for HM incorporation into the human food chain, causing many adverse health effects. A few mitigating measures to lessen such toxic metal accumulation within dietary vegetable species through co-cultivation with deep-rooted, high-biomass-producing, and bioenergy-generating perennial grass species [36], along with targeted inoculation of vegetable roots/rhizosphere soils with multi-metallotolerant and HM-accumulating plant growth-promoting rhizobacteria (PGPR) [37], have been proposed.

Materials and Methods

The present research work was carried out through three seasons, namely premonsoon, monsoon, and postmonsoon, for 2 consecutive years (2016 and 2017). The entire research methodology is divided into four sections, as highlighted.

i) Selection of the study site
ii) Sampling and processing of selected vegetable species and ambient soil
iii) Spectrophotometric analysis of selected HMs
iv) Determining the potential for HM bioaccumulation and translocation

Study Site Selection

Dhapa (22° 32′ 17.82″N; 88° 25′ 59.19″E), a municipal solid waste (MSW) dumping station in EKW (22° 33′ 13.40″N; 88° 26′ 41.34″E), was selected as the sampling site, which is located in the eastern part of Kolkata, West Bengal. The city of Kolkata is the capital of West Bengal, a maritime state in the northeast part of the Indian subcontinent (Fig. 1).

Sample Collection and Processing

Three common vegetables were chosen for the present study, namely *L. siceraria*, *A. esculentus*, and *Z. mays*, as represented in Fig. 2. The edible parts of *L. siceraria*, *A. esculentus*, and *Z. mays*, their root system, and the ambient soil samples were obtained by sampling from the EKW during the premonsoon, monsoon, and postmonsoon seasons in the years 2016 and 2017. All the selected vegetable samples were washed with deionized water and oven-dried at 60 °C overnight.

Heavy Metal Analysis Using Atomic Absorption Spectrometry

The vegetable samples, including the roots and edible parts, were powdered and weighed to determine the HM concentrations using atomic absorption spectrometry (AAS). Each dried sample (1 g on a dry weight basis) was digested with a mixture of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), followed by the addition of hydrochloric acid (HCl) [38]. The digested samples were analyzed for Cd, Cr, Pb, and Hg against a standard concentration of each metal on a PerkinElmer Atomic Absorption Spectrophotometer (Model 3030) equipped with an HGA-500 graphite furnace atomizer and a deuterium background corrector. The blank correction was done to bring accuracy to the results. The methodology of HM assessment in

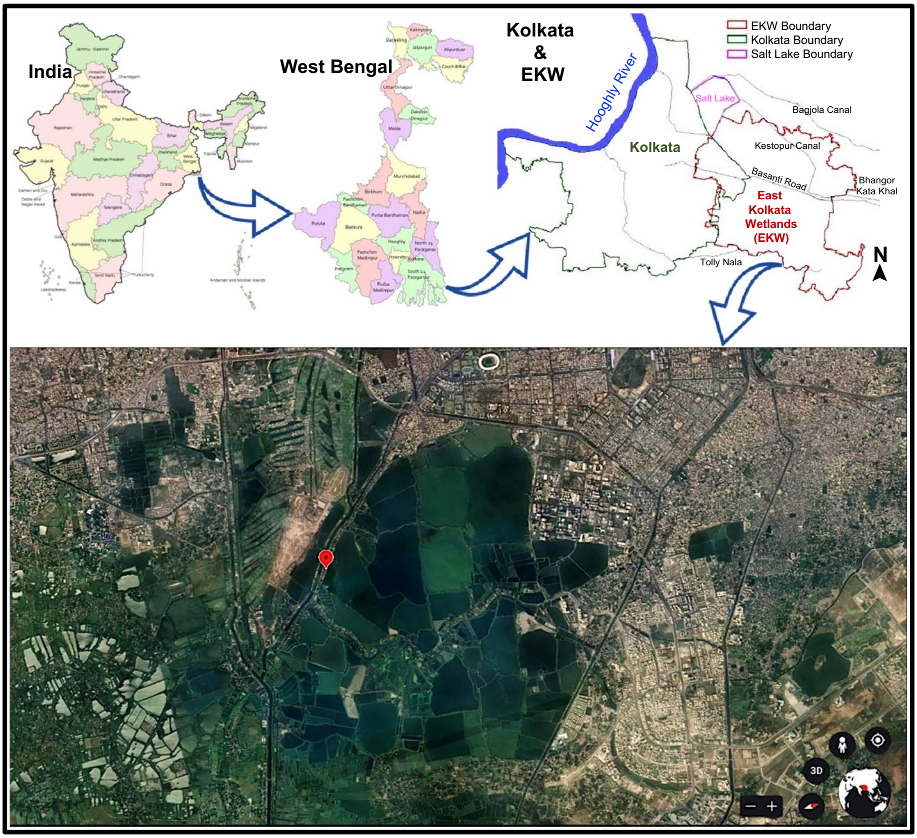

Fig. 1 Map showing the location of the study site, Dhapa (indicated with a red balloon) in East Kolkata Wetlands (22° 32′ 17.82″N; 88° 25′ 59.19″E), situated in the eastern fringe of the metropolitan city of Kolkata, West Bengal, India (source: Google Earth; earth.google.com/web/)

Fig. 2 Representative images of the three selected vegetable species from the sampling site. **a** *Lagenaria siceraria* (bottle gourd). **b** *Abelmoschus esculentus* (ladies' fingers or okra). **c** *Zea mays* (corn/maize). The red arrow indicates the edible plant part for each food crop species under study

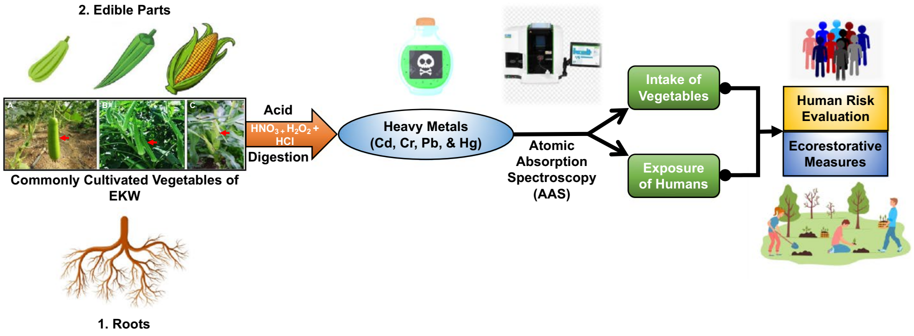

Fig. 3 Flow diagram of the methodology of heavy metal assessment in selected vegetable samples collected from East Kolkata Wetlands. The graphics are not drawn to scale

the selected vegetable samples is illustrated with the help of a flow diagram, as detailed in Fig. 3.

A quantity of approximately 0.5 g of ambient soil samples was digested using a weak acid treatment (i.e., 0.5 N HCl) (37%, Sigma-Aldrich), following the procedure of Malo [39]. Biologically available HM concentrations (Cd, Cr, Pb, and Hg) in the soil samples were analyzed against a standard concentration of each metal in the same instrument as above. For checking the instrument's analytical precision, the samples (around 20% of the total numbers) were chosen randomly and measured in triplicates against internationally used soil Standard Reference Materials (SRMs): NIST SRM 2709, 2710, and 2711. Average recoveries ($n=5$) ranged from 85 to 99% for the analyzed HMs.

Heavy Metal Bioaccumulation and Translocation Potential

Bioaccumulation Factor Estimation

The bioaccumulation factor (BF) is the ratio of HMs within the plant body parts relative to ambient soil. BF of HMs within the roots and edible parts of the selected vegetables from the soil was determined mathematically from the Eqs. (1–2) as given by Mukherjee et al. [37] with some modifications given below:

For roots,

$$\mathrm{BF} = Cr \div Cas. \tag{1}$$

For edible parts,

$$\mathrm{BF} = Cep \div Cas, \tag{2}$$

where *Cr* and *Cep* represent HM concentrations in the roots and edible parts of the food crop species under study, respectively, and *Cas* represents HM concentrations in the ambient soil of the plant species under investigation.

Translocation Factor Estimation

With some modification, the translocation of HMs from the roots to the edible parts of the selected plant species was calculated using Eq. (3), as given by Mukherjee et al. [37]. The translocation factor (TF) is the ratio of HMs in the plant's aboveground biomass (edible parts in this case) relative to roots.

$$\mathrm{TF} = Cep \div Cr, \tag{3}$$

where *Cep* represents HM concentrations in the edible parts of the food crop species under study, and *Cr* represents HM concentrations in the roots of the plant species collected from the study area.

Statistical Analyses

A two-way analysis of variance (ANOVA) was performed to determine if the HM concentration varied significantly between species and seasons; a p value of < 0.01 was considered significant. All analyses were done in triplicates, and the mean values are represented here. The correlation coefficient was calculated to determine the interrelationship between HM concentration in the three selected vegetable species' ambient soil, roots, and edible parts. Microsoft Excel was used to prepare the graphs and SPSS 21.0 (for Windows) was used for the statistical analyses.

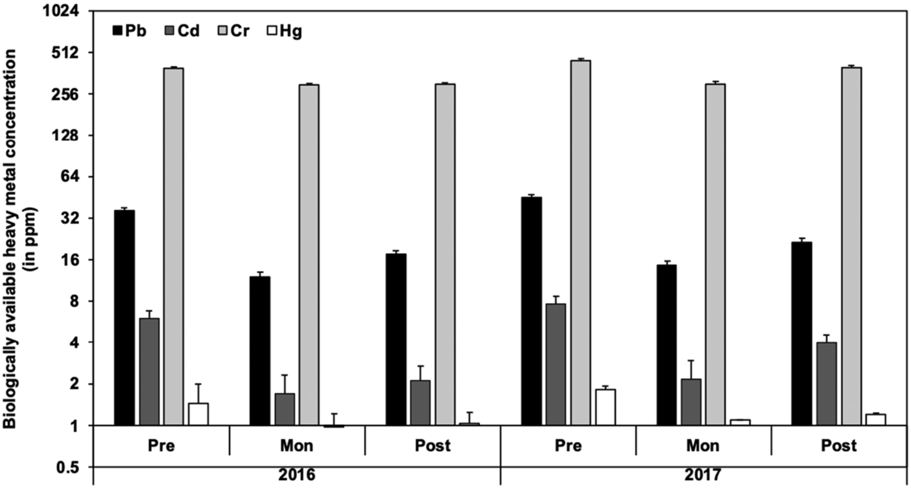

Fig. 4 The concentration of biologically available selective heavy metals (in ppm) in the ambient soil during three seasons in 2016 and 2017. Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon

Results

Biologically Available Heavy Metals in Ambient Soil

The trend of the selected HM concentrations in the ambient soil samples was Cr (298.76–443.85 ppm) > Pb (12.02–45.22 ppm) > Cd (1.69–7.68 ppm) > Hg (0.98–1.81 ppm) for 2016 and 2017 with the highest value in premonsoon, followed by postmonsoon and monsoon, respectively (Fig. 4).

Bioaccumulated Heavy Metals in Belowground Biomass (Roots) and Aboveground Biomass (Edible Parts)

Roots

The trend of HM accumulation was the same in the roots of the three selected vegetable species, which varied as per the order Pb > Cd > Cr > Hg in both the years. For all HMs, the highest values were observed in the three selected vegetable roots during monsoon in both years except for Cr and Hg, where the highest values were observed during 2016 postmonsoon in the case of *L. siceraria*. The HMs (Pb, Cd, Cr, and Hg) in the *L. siceraria* roots ranged from 26.67 to 39.75 ppm, 9.89 to 17.58 ppm, 7.08 to 12.83 ppm, and 4.89 to 6.26 ppm, respectively, during the entire study period in the chosen site. The range of Pb, Cd, Cr, and Hg in the roots of *A. esculentus* was 14.66–30.17 ppm, 5.32–11.24 ppm, 2.76–7.16 ppm, and 0.59–2.45 ppm, respectively, while for *Z. mays*, the range was 33.67–42.39 ppm, 13.97–20.57 ppm, 11.98–19.35 ppm, and 1.37–4.99 ppm for Pb, Cd, Cr, and Hg, respectively. To sum up, the overall seasonal trend of HM bioaccumulation in

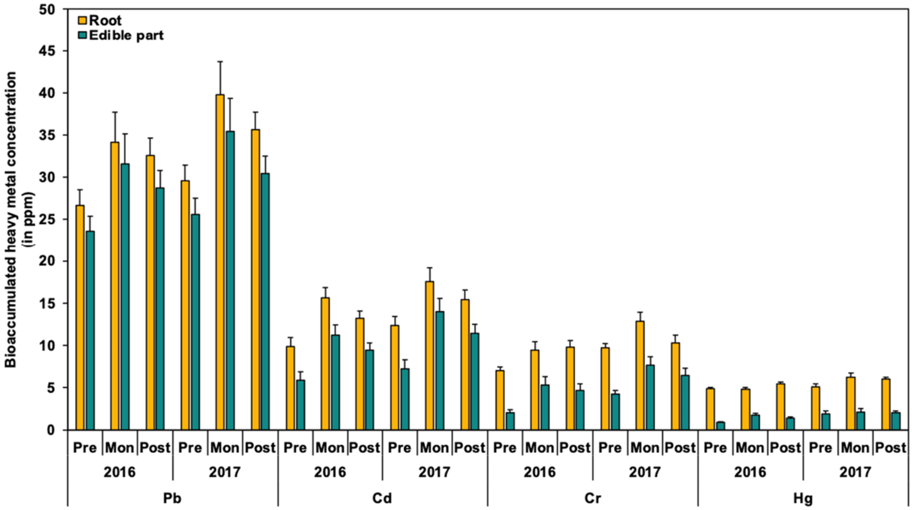

Fig. 5 The concentration of bioaccumulated selective heavy metals (in ppm) in the vegetative and reproductive parts of *L. siceraria* during three seasons in 2016 and 2017. Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon

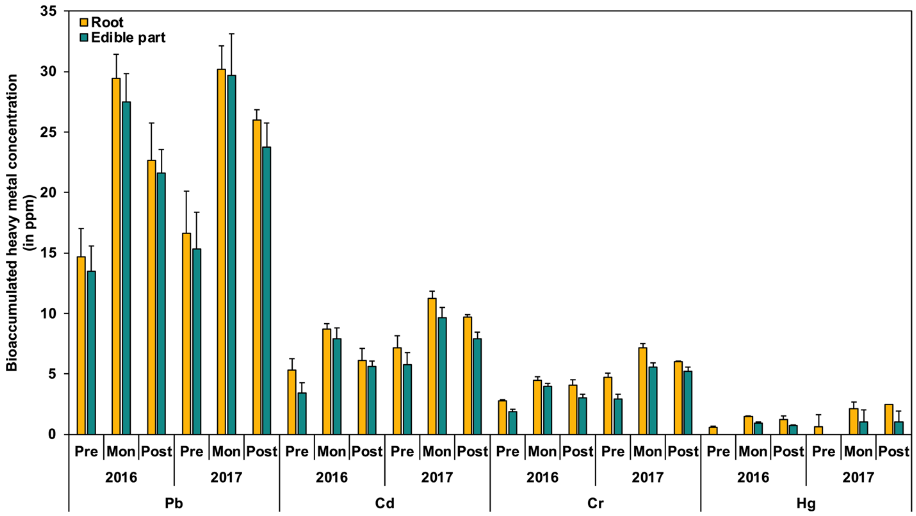

Fig. 6 The concentration of bioaccumulated selective heavy metals (in ppm) in the vegetative and reproductive parts of *A. esculentus* during three seasons in 2016 and 2017. Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon

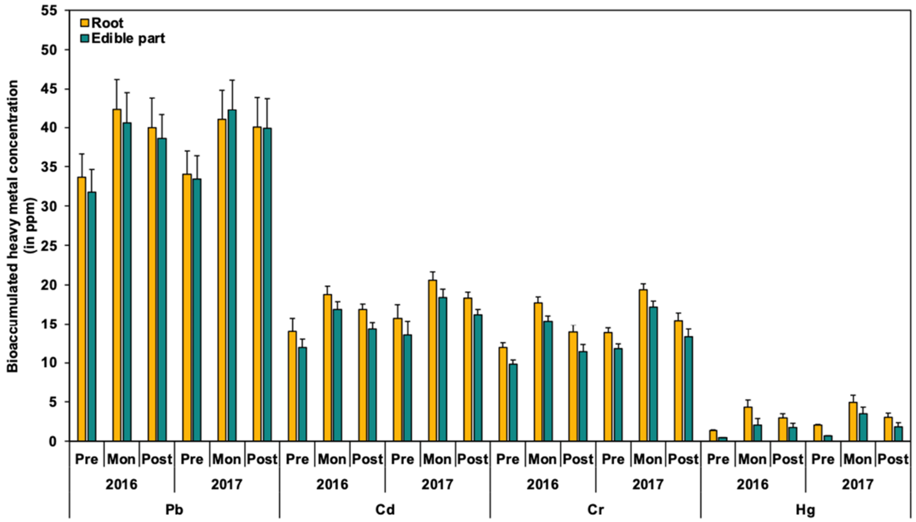

Fig. 7 The concentration of bioaccumulated selective heavy metals (in ppm) in the vegetative and reproductive parts of *Z. mays* during three seasons in 2016 and 2017. Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon

the roots of the three selected food crop species is monsoon > postmonsoon > premonsoon, with exceptions mentioned above.

Edible Parts

The data revealed that out of the four HMs studied in the three selected vegetables, the concentration of the HMs in the edible parts followed the trend Pb > Cd > Cr > Hg, and the highest values were observed during monsoon, followed by postmonsoon and premonsoon, respectively, for both the study years 2016 and 2017 (Figs. 5, 6, 7). Among the selected vegetables, the HM content was found to be highest in *Z. mays* and lowest in *A. esculentus*.

The HMs (Pb, Cd, Cr, and Hg) in edible parts of *L. siceraria* ranged from 23.59 to 35.45 ppm, 5.88 to 13.98 ppm, 2.04 to 7.63 ppm, and 0.88 to 2.04 ppm, respectively, during the entire study period in the chosen site. The range of Pb, Cd, Cr, and Hg in the edible parts of *A. esculentus* was 13.51–29.67 ppm, 3.42–9.64 ppm, 1.86–5.56 ppm, and 0.00–1.04 ppm, respectively, while for *Z. mays*, the range was 31.78–42.33 ppm, 11.93–18.43 ppm, 9.83–17.21 ppm, and 0.45–3.52 ppm for Pb, Cd, Cr, and Hg, respectively.

Bioaccumulation Factor of the Selected Heavy Metals in the Three Vegetable Species

The trend of BF in the three selected vegetables exhibits differences in the accumulation of HMs within roots and edible parts from ambient soil.

L. siceraria

In the roots of *L. siceraria*, the BF trend in 2016 is Cd > Hg > Pb > Cr in monsoon and postmonsoon, but during premonsoon, a variation in trend was found (Hg > Cd > Pb > Cr). In 2017, the trend is Cd > Hg > Pb > Cr in monsoon, whereas in premonsoon and postmonsoon, the trend is Hg > Cd > Pb > Cr. In the edible parts of *L. siceraria*, the BF trend in 2016 is Cd > Pb > Hg > Cr throughout all the seasons, and the same trend was found in 2017 during the monsoon. However, in 2017, the trend during premonsoon and postmonsoon is Hg > Cd > Pb > Cr and Cd > Hg > Pb > Cr, respectively.

A. esculentus

In *A. esculentus*, the BF trend in roots is Cd > Pb > Hg > Cr during monsoon and postmonsoon in 2016, but the variation in trend during the premonsoon season was observed in the order Cd > Hg > Pb > Cr. In 2017, the trend of BF in roots is Cd > Pb > Hg > Cr during premonsoon and monsoon, whereas during postmonsoon, the trend is Cd > Hg > Pb > Cr. The BF trend in the edible parts of *A. esculentus* is Cd > Pb > Hg > Cr during monsoon and postmonsoon in both the years and Cd > Pb > Cr > Hg during premonsoon of both years, where Hg level remains undetected.

Z. mays

In *Z. mays*, the BF trend in roots is Cd > Hg > Pb > Cr through all seasons in 2016 and 2017. The trend of BF in the edible parts of *Z. mays* is Cd > Pb > Hg > Cr through all

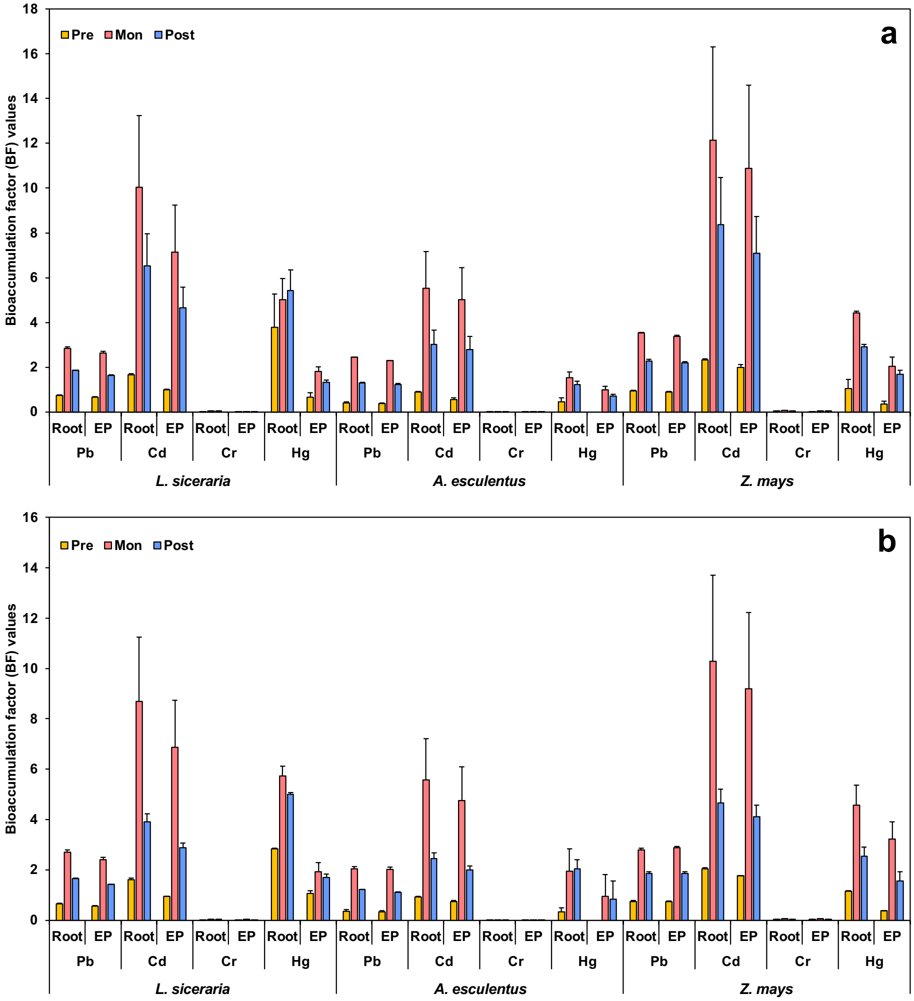

Fig. 8 Bioaccumulation factor of Pb, Cd, Cr, and Hg in roots and edible parts of the three selected vegetable species during three seasons in 2016 (**a**) and 2017 (**b**). Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon; EP, edible parts

seasons in both the years except during monsoon in 2017, when the trend was observed to be in the sequence Cd > Hg > Pb > Cr. The BF of the three food crop species is represented in Fig. 8a and b for 2016 and 2017, respectively.

Translocation Factor of the Selected Heavy Metals in the Three Vegetable Species

A pattern of HM translocation from roots to the aerial parts needs to be established in growing plant species, which could benefit the biological monitoring of HM contamination. With BF alone, it is impossible to establish that the vegetables studied are accumulator species for certain metals with underlying health implications. The TF for *L. siceraria* showed the trend

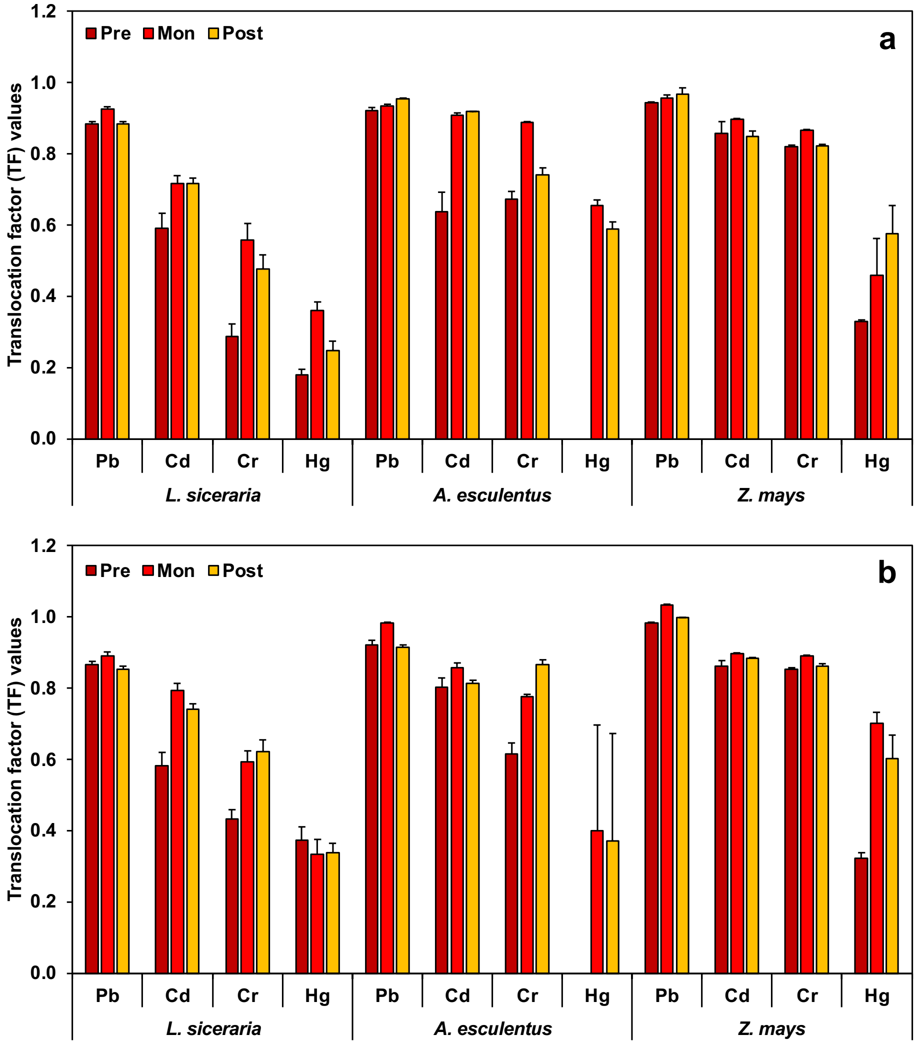

Fig. 9 Translocation factor of Pb, Cd, Cr, and Hg in edible parts of the three selected vegetable species during three seasons in 2016 (**a**) and 2017 (**b**). Error bars indicate standard deviation from the mean of triplicate values. Pre, premonsoon; Mon, monsoon; Post, postmonsoon

Pb > Cd > Cr > Hg for 2016 and 2017 through all seasons. *A. esculentus* and *Z. mays* also exhibited a similar trend for both years through all seasons (Fig. 9a and b). It is noteworthy that the TF values were found to be lower than the BF values in all selected plant species in both the years for all the HMs.

Table 1 Analysis of variance of heavy metal concentrations in the roots and edible parts of the selected vegetables between species and seasons in 2016 and 2017

Heavy metals	Years	Vegetative and reproductive plant parts	Variables	F_{cal}	F_{crit}
Pb	2016	Roots	Between species	49.5514	6.9443
			Between seasons	20.1636	
		Edible parts	Between species	69.0481	
			Between seasons	28.7903	
	2017	Roots	Between species	55.0981	
			Between seasons	28.0854	
		Edible parts	Between species	73.5346	
			Between seasons	36.9883	
Cd	2016	Roots	Between species	132.9815	
			Between seasons	28.9794	
		Edible parts	Between species	399.5879	
			Between seasons	124.3323	
	2017	Roots	Between species	609.2857	
			Between seasons	170.1475	
		Edible parts	Between species	89.4437	
			Between seasons	34.2301	
Cr	2016	Roots	Between species	50.9196	
			Between seasons	4.7924	
		Edible parts	Between species	65.5419	
			Between seasons	8.4271	
	2017	Roots	Between species	99.7378	
			Between seasons	13.5087	
		Edible parts	Between species	83.8178	
			Between seasons	11.7295	
Hg	2016	Roots	Between species	18.5525	
			Between seasons	1.9626	
		Edible parts	Between species	11.5026	
			Between seasons	17.8114	
	2017	Roots	Between species	30.1544	
			Between seasons	6.4684	
		Edible parts	Between species	4.5817	
			Between seasons	3.0633	

Note: Cd, cadmium; Cr, chromium; Hg, mercury; Pb, lead

Analysis of Variance of Heavy Metals in the Roots and Edible Plant Parts of the Selected Vegetables Between Seasons and Species for 2016 and 2017

The ANOVA results exhibit significant variations of Cd, Cr, Pb, and Hg in the roots and edible parts between species and seasons, with a few exceptions (Table 1). In 2016, it was noticed that the variation of Cr and Hg in the roots between seasons is insignificant. A similar trend was observed for the seasonal variation of Hg in the roots

Table 2 Correlation between heavy metal concentration in soil and heavy metal concentrations in roots and edible parts of three selected vegetables during 2016 and 2017

Heavy metals	Vegetable species	Combination	2016		2017	
			r value	*p* value	*r* value	*p* value
Pb	*L. siceraria*	Soil × roots	− 0.9996	$p < 0.01$	− 0.9801	$p < 0.01$
		Soil × edible parts	− 0.9908	$p < 0.01$	− 0.9487	$p < 0.01$
	A. esculentus	Soil × roots	− 0.9680	$p < 0.01$	− 0.9955	$p < 0.01$
		Soil × edible parts	− 0.9781	$p < 0.01$	− 0.9783	$p < 0.01$
	Z. mays	Soil × roots	− 0.9986	$p < 0.01$	− 0.9966	$p < 0.01$
		Soil × edible parts	− 0.9999	$p < 0.01$	− 0.9984	$p < 0.01$
Cd	*L. siceraria*	Soil × roots	− 0.9391	$p < 0.01$	− 0.9961	$p < 0.01$
		Soil × edible parts	− 0.9698	$p < 0.01$	− 0.9987	$p < 0.01$
	A. esculentus	Soil × roots	− 0.7455	$p < 0.01$	− 0.9982	$p < 0.01$
		Soil × edible parts	− 0.9030	$p < 0.01$	− 0.9900	$p < 0.01$
	Z. mays	Soil × roots	− 0.9502	$p < 0.01$	− 0.9894	$p < 0.01$
		Soil × edible parts	− 0.8998	$p < 0.01$	− 0.9896	$p < 0.01$
Cr	*L. siceraria*	Soil × roots	− 0.9900	$p < 0.01$	− 0.9902	$p < 0.01$
		Soil × edible parts	− 0.9885	$p < 0.01$	− 0.9311	$p < 0.01$
	A. esculentus	Soil × roots	− 0.9829	$p < 0.01$	− 0.9710	$p < 0.01$
		Soil × edible parts	− 0.9092	$p < 0.01$	− 0.8218	$p < 0.01$
	Z. mays	Soil × roots	− 0.7741	$p < 0.01$	− 0.9997	$p < 0.01$
		Soil × edible parts	− 0.7412	$p < 0.01$	− 0.9992	$p < 0.01$
Hg	*L. siceraria*	Soil × roots	− 0.2674	NS	− 0.9977	$p < 0.01$
		Soil × edible parts	− 0.9394	$p < 0.01$	− 0.9818	$p < 0.01$
	A. esculentus	Soil × roots	− 0.9869	$p < 0.01$	− 0.9547	$p < 0.01$
		Soil × edible parts	− 0.9909	$p < 0.01$	− 0.9932	$p < 0.01$
	Z. mays	Soil × roots	− 0.9332	$p < 0.01$	− 0.8466	$p < 0.01$
		Soil × edible parts	− 0.9970	$p < 0.01$	− 0.8906	$p < 0.01$

Note: 1. Cd, cadmium; Cr, chromium; Hg, mercury; Pb, lead

2. $p < 0.01$, significant; NS, not significant

in 2017. Additionally, in the year 2017, the variation of Hg in the edible parts between species and seasons was found to be insignificant.

Correlation Between Heavy Metal Concentrations in Soil, Roots, and Edible Parts of the Three Selected Food Crop Species

With only one exception (*L. siceraria*$_{\text{Soil} \times \text{roots}}$), significant negative correlation coefficient values are observed between the HMs in soil and vegetables (Table 2), representing efficient uptake of the metals from the underlying soil substratum. This can be avoided by sprinkling 5% lime [$Ca(OH)_2$] on the soil surface, thereby increasing the soil pH and preventing the transference of metals from the soil to the vegetables.

Discussion

The importance of a balanced diet has been emphasized since time immemorial. However, it has become more relevant presently when the entire humanity is grappling with the COVID-19 (coronavirus disease 2019) pandemic [40], and plant-based foods are popular among populations worldwide because of their rich nutrient, antioxidant, phytochemical, and metabolite (viz., vitamins, phytoenzymes) content [41]. In addition, they help neutralize the acidity caused by some substances aiding in digestion [42]. The increasing population worldwide has escalated the use of pesticides (herbicides, insecticides, etc.) and inorganic fertilizers in farming practices, which has impacted the health benefits and quality of the farm produce, along with the surrounding environment [43]. During their growth and development, the plants derive water and essential elements (including micro- and macronutrients) through the soil, and the contaminating HMs present in the rhizosphere soil are also taken up by the plant root systems together with these nutrients. These toxic HMs bioaccumulate in the vegetative parts and translocate from belowground biomass (roots) to aboveground biomass (stems and leaves) and eventually to the edible reproductive parts (fruits) [44]. Upon consumption of such HM-contaminated vegetables, various psychological and clinical symptoms are seen in both animals and humans that may ultimately lead to acute and/or chronic metal-induced toxicity [45].

Moreover, HM toxicity adversely impacts plant health, retards plant growth, and reduces crop yield, as reviewed extensively by Sathya et al. [7]. The study site, EKW, serves as one of the major food baskets of Kolkata and adjoining areas. The selected vegetables used in the present study are usually popular among people of all ranks of the society and hence consumed by this sizeable metropolitan population. It is the right of every individual to get access to safe food products to ensure their good health and well-being. Therefore, monitoring toxic substances in food crops, including HMs, is crucial because these toxicants tend to biomagnify in the food chain, causing severe threats to millions of people. The present work would help provide risk assessment data for the end-users in the current geographical locale, and such types of studies need to be carried out regularly to get an overall status of HM contamination in the environment and plants.

Based on previous works on EKW, it has been noticed that the four selected HMs, viz., Pb, Cd, Cr, and Hg, investigated in the present study are abundant in this unique Ramsar site [46–48]. The concentrations of the selected HMs in the ambient soil samples were found to be maximum in premonsoon and minimum in monsoon. The minimum value of HMs during monsoon may be attributed to two factors, namely (a) washing of the topsoil due to surface run-off caused by heavy precipitation, and (b) mixing of the study site soil with the soils from relatively uncontaminated areas around through run-offs.

We have found that all three selected vegetable species take up HMs selectively from the ambient soil, mainly during the monsoon season. The BF values show seasonal variation, with the highest value obtained during monsoon, followed by postmonsoon and premonsoon, respectively. A similar trend in TF values was observed with the exception of Hg for *Z. mays* in 2016 and *L. siceraria* in 2017, with lower values during monsoon than postmonsoon. It was observed that Pb maximally translocated from the belowground biomass to the edible parts of the plants irrespective of the species in the study area, whereas the translocation of Hg was found to be minimum. The species *Z. mays* showed maximum TF values for Pb for both the years through all seasons. However, a clear trend in TF for Cd, Cr, and Hg was not observed in *Z. mays* and *A. esculentus*. *L. siceraria* showed minimum translocation among the selected vegetable species. Compared to HM bioaccumulation, translocation of HMs from the roots into the aboveground plant tissues might be less due to the sequestration of metal ions inside the root vacuoles of the plants where HMs are fixed along with other essential elements [49]. Moreover, TF is, in turn, dependent on the pattern of HM bioaccumulation. In addition, bioaccumulation of metals in plant species varies from metal to metal and from species to species [50]. Furthermore, it has been documented by many researchers that there exists a wide variation in HM accumulation and translocation/distribution in vegetable species, which is regulated by the uptake capacity of the species, their specific parts, plant genotypes, metal types, environmental factors, edaphic factors (soil pH, soil temperature, etc.), root exudates, metal bioavailability, and anthropogenic activities [32–34, 51–55]. It can also be stated that although the amount of Pb present in the site soil was relatively less, the bioaccumulated Pb in food crops was more compared to Cd. This might either be attributed to the selective nature of Pb accumulation by these food crop species or most likely could be due to the atmospheric pollution fallout of Pb, its precipitation on the plant surfaces, and subsequent diffusion into the edible parts [56]. Since the present study area (i.e., Dhapa within EKW) is located near the megacity of Kolkata, and the busy roadway of the Eastern Metropolitan Bypass or EM Bypass (a 32-km-long major road on the east side of Kolkata) runs parallel to this site, it is pretty natural that vehicular emission of Pb from alkyl-leaded petrol or fuel with tetraethyl lead additive coupled with industrial emission of Pb from the bordering leather processing industries [57] causes Pb to concentrate in the ambient atmosphere and its deposition on the plant surfaces through atmospheric pollution fallout. In general, the monsoon seems to be highly vulnerable in terms of bioaccumulation of HMs as both BF and TF were highest in this season irrespective of the food crop species; hence there is a demand for careful monitoring along with proper blanching (soaking in warm water), washing, and cooking before consuming these vegetables.

The study site Dhapa has been used since 1879 to cultivate vegetables and has been a selected dumping ground for Kolkata city's MSWs. Being an MSW dumping ground, it is very fertile. The sewage water generated from in and around Kolkata flows through the channels and is directed to fish ponds to culture endemic fish species and is also used for agriculture purposes. The livelihoods of many local people (around 20,000 families) depend on EKW for their sustenance by cultivating cereals (rice), vegetables, and fishes [58, 59]. In addition to the ongoing use of domestic sewage/wastewater, illegal construction, cremation, solid waste incineration, plastic recycling, and leather processing units in and around the wetlands are serious threats to this peri-urban facility, along with the HM toxicants from some of these point sources, which eventually find their way into this unique natural ecosystem. Since several toxic HMs are found in the Dhapa soil, it is regarded as metal-infested land. However, unlike other metal-impacted abandoned sites, it is very fertile because apart from the MSWs, wastewater from nearby sewage canals also reaches the soil, thereby boosting vegetation and crop cultivation. Besides the organic and inorganic contaminants, the soil also contains several essential nutrients, such as nitrates and phosphates, which promote plant growth. Therefore, the local farmers have chosen this land for growing vegetables. Owing to its proximity to the busy township of Kolkata, the produce (vegetables) from Dhapa farmland is often sold near the roadside by the local vendors; this is a common sight along the EM Bypass. Hence the farm produce reaches many households, as people stop their vehicles and purchase the products from these sellers because they are farm fresh. Upon reaching the local markets, these vegetables will be eventually bought and consumed by the populace of Kolkata.

The study results have shed light on the presence of toxic HMs in the vegetables cultivated at the study site, even though they are at lower concentrations in the reproductive parts

Table 3 Comparison of the selected heavy metal content in soil and edible parts of dominant food crop species of East Kolkata Wetlands with the World Health Organization's recommended permissible values

Sl. no.	Selected heavy metals	Selected heavy metal concentration in the soil sample (mg/kg)	Selected food crop species	Selected heavy metal concentration in edible parts (mg/kg)	Reference concentration of heavy metals in the soil sample (mg/kg) [60, 61]	Permissible concentration of heavy metals in vegetables (mg/kg) [60, 61]	Adverse effects of heavy metal toxicity on human health [7]
1	Pb	12.02–45.22	*L. siceraria*	23.59–35.45	10	0.1–0.3	•Mental retardation in children •Developmental delay •Fatal infant encephalopathy •Congenital paralysis •Sensorineural hearing loss •Epilepsy •CNS, liver, kidney, and GI tract damage
			A. esculentus	13.51–29.67			
			Z. mays	31.78–42.33			
2	Cd	1.69–7.68	*L. siceraria*	5.88–13.98	< 0.3	0.05–0.2	•Cancer •Bone defects •Renal dysfunction •Bronchitis •Blood pressure problem
			A. esculentus	3.42–9.64			
			Z. mays	11.93–18.43			
3	Cr	298.76–443.85	*L. siceraria*	2.04–7.63	100	2.3	•Irritability •Fatigue •Damage to CNS
			A. esculentus	1.86–5.56			
			Z. mays	9.83–17.21			
4	Hg	0.98–1.81	*L. siceraria*	0.88–2.04	< 0.3	0.01–0.03	•CNS damage •Tremors •Gingivitis •Psychological changes •Acrodynia* •Spontaneous abortion •Protoplasm poisoning
			A. esculentus	0.00–1.04			
			Z. mays	0.45–3.52			

Note: 1. Cd, cadmium; Cr, chromium; Hg, mercury; Pb, lead

2. CNS, central nervous system; GI, gastrointestinal

3. Each value is the mean of 10 samples

4. *A rare disorder in infants and young children caused due to chronic Hg poisoning

than in the roots and soil. Nevertheless, still, it is not advisable to eat fruits/vegetables grown in a metal-contaminated land. Since cultivation is vital for the sustenance of livelihood for many farmers, it would be impossible to stop them from using the plot to grow and sell the produce on the roadside/local markets. Therefore, the need of the hour is to remediate this metal-impacted soil by co-cultivating with some metal-accumulating perennial grassy species. This will lessen the adverse impact of toxic HMs on humans as these perennial grasses will eventually accumulate more metals than the cultivable food crop species; therefore, fewer metals would be taken up by the food crops. Thus, this can be a practical roadmap to ecorestore the soil of EKW, the designated Ramsar site of West Bengal, Kolkata.

As evident from Table 3, the selected HM content in both the soil samples and the edible parts is higher than the recommended levels by the World Health Organization (WHO)/Food and Agriculture Organization (FAO) [60–63]. This requires immediate remedial measures and constant environmental monitoring for the ecorestoration of EKW as this wetland system is a unique natural ecosystem that provides not only various non-consumptive services, including biodiversity preservation, biopurification, and Kolkata's natural drainage system, but also consumptive services, such as endemic fishes, fruits, and vegetables. The edible vegetables with HM toxicants within their body tissues can cause adverse effects in humans like central nervous system (CNS) damage, respiratory disorders, renal dysfunction, cancer, bone defects, and psychological problems [7].

Conclusions

As stated here, we can conclude some of the key findings from the present research work.

1. All three selected (commonly cultivated) vegetable species of EKW, namely *L. siceraria*, *A. esculentus*, and *Z. mays*, selectively uptake toxic HMs from the surrounding soil. The order of HM bioaccumulation in the roots and edible parts of three food crop species is Pb > Cd > Cr > Hg.
2. The biologically available HMs in the ambient soil followed the sequence Cr > Pb > Cd > Hg, which is not in sync with the accumulated HMs in the roots and edible parts of the vegetable species.
3. Cr content was found to be maximum in soil samples, followed by roots and edible parts in all plant species. The soil samples contained less Pb and Cd than the roots and edible parts of plants irrespective of selected species, proving that there are more anthropogenic sources of these two HMs other than HM contamination in the soil. The concentration of Hg in soil samples was more only in *A. esculentus* than in the other two vegetable species.
4. Moreover, HM concentration (in soil, roots, and edible parts) shows striking seasonal variations with maximum accumulation occurring in monsoon, followed by postmonsoon and premonsoon. Monsoon poses a significant threat in terms of bioaccumulation and translocation of HMs since both BF and TF were highest in this season regardless of the vegetable species, with some exceptions, i.e., TF values of Hg in 2016 for *Z. mays* and 2017 for *L. siceraria* when the monsoon values were less than postmonsoon. With the selected plant species being part of the daily diet of the local population of the district of Kolkata and adjoining districts of the state of West Bengal (due to their nutrient content, pleasant taste, affordability, and availability), it is therefore important to monitor the HM content in the vegetative and reproductive plant parts from the human

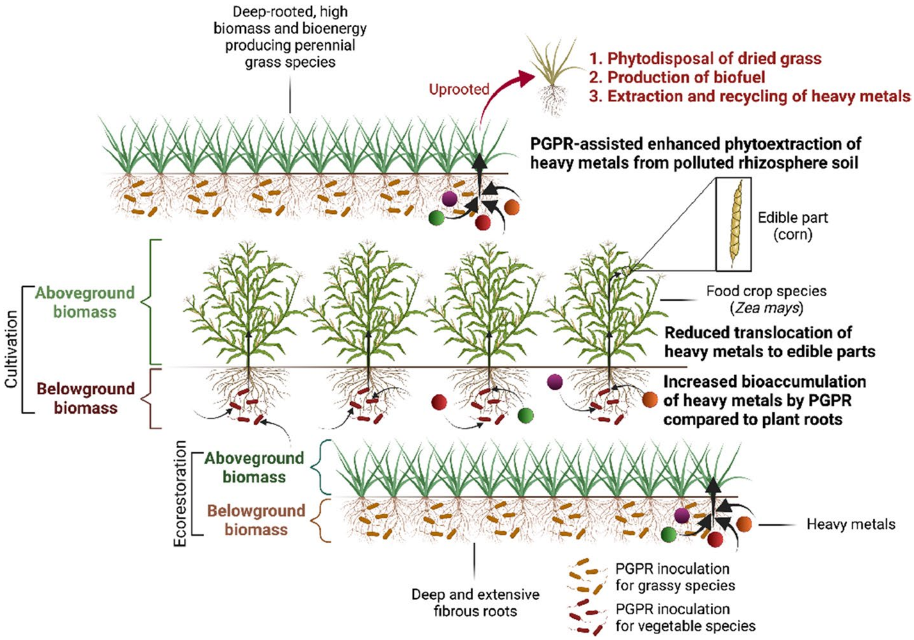

Fig. 10 Multi-metallotolerant plant growth-promoting rhizobacteria (PGPR)-assisted co-cultivation of selected vegetable species with low-metal-accumulating, high-biomass-yielding, deep-rooted, bioenergy-producing perennial grass species for ecorestoration of metal-impacted, municipal solid waste dumping site of Kolkata (Dhapa) in East Kolkata Wetlands. Among the three food crop species under study, only one species (i.e., *Z. mays*) has been chosen for ease of representation. The use of two separate categories of PGPR strains, one for inoculating the food crop species and the other for inoculating the grassy species, is shown. It is to be noted that the graphic is not drawn to scale (created with BioRender.com)

health perspective. It should be noted that the vegetable species with less TF should be selected for sale and cooking purposes as it indicates lower bioavailability of the HMs to the edible parts.

To summarize, the overall result suggests proper environmental monitoring and ecorestoration of the study site, along with a few proposed preventive measures, such as careful washing and cooking while consuming these vegetables.

Future Perspectives

In keeping with the recent trend of conservative development in and around EKW, the authors view co-cultivating with low-metal-accumulating, deep-rooted, high-biomass-yielding, bioenergy-producing perennial grass species and selective PGPR strains can assist in HM phytoremediation [36, 64–66]. Therefore, this combinatorial approach will make the cultivable produce from the selected study site safe for human consumption besides restoring the soil ecosystem of this metal-impacted site from the perils of HM toxicity, as illustrated in detail in Fig. 10. For example, Kans grass (*Saccharum spontaneum*) [67–70], tall perennial grass with spreading rhizomatous roots native to the Indian subcontinent, can

Table 4 SWOT analysis of the present study

Sl. no.	Strengths	Weaknesses	Opportunities	Threats
1	The study determines the seasonal variation of HM concentrations (ppm per dry weight) in the selected vegetable species and the relative translocation of toxic HMs in the edible plant parts	The ecorestorative strategy needs to be standardized through in situ pilot-scale field studies and interaction with responsible authorities (institutional support)	The co-cultivation of selected vegetable species with low-metal-accumulating grassy species and PGPR as biofertilizers will give us safe food products	The overall result of this study might affect the livelihood of the farmers and vegetable vendors as people will stop purchasing these farm produce once they become aware of the toxicity of the vegetables they are consuming
2	Analysis of HM content in the selected vegetable species provides us with the knowledge and awareness of how safe is the food crops that the people of Kolkata are consuming from this farmland	It is also necessary to conduct field studies for regular monitoring of the growing food crop species for HM content using AAS in the study area of multi-metallotolerant PGPR-assisted co-cultivation where selected vegetable species and low-metal-accumulating, high-biomass-yielding, deep-rooted, bioenergy-producing perennial grassy species will be grown [79]	Moreover, the use of PGPR as biofertilizers will not only help in stimulating the crop yield but also help in generating alternative livelihoods among the local populace	In addition, since Dhapa (in EKW) is a major food basket as well as a MSW dumping ground of the city, continued waste disposal in this area will result in unsafe farm produce that might affect the health and well-being of the people of Kolkata (due to the lack of alternative waste disposal site). So, both waste disposal and farming are equally crucial for the proper functioning of the city and the sustenance of its population

AAS, atomic absorption spectroscopy; EKW, East Kolkata Wetlands; HM, heavy metal; MSW, municipal solid waste; PGPR, plant growth-promoting rhizobacteria be used that is capable of ethanol and biogas production [71, 72]. Alternatively, ecological restoration of the metal-infested cultivable/fertile land with hyperaccumulator bioindicator species (plants with a remarkable capacity of accumulating particular HMs/metalloids within their body tissues that are hundred to thousand folds higher than other usual species of plants) [73–75], as listed in [76], can be done. Hyperaccumulating biodiesel/bioenergy crops with oil-rich seeds (e.g., leguminous plants like *Pongamia pinnata* and *Brassica juncea*) [77, 78] can also be applied for ecorestoration of the study site. It is noteworthy that ecorestorative studies on EKW have not been previously reported, neither PGPR-assisted ecorestorative strategies been earlier proposed by any research groups.

A SWOT analysis has also been provided in Table 4 that mentions the strengths, gap areas, opportunities, and threats of the present study to implement the ecorestorative or remedial measures.

In context to phytoremediation, it is well appreciated that natural hyperaccumulator plant species are capable of phytoextracting (meaning uptake, accumulate, and transport) high concentrations of HMs; however, their slow rates of growth and limited biomass-yielding potential retard the overall rate of HM ion removal. Moreover, it is worth mentioning here that incorporating chelating substances to enhance metal bioavailability and subsequent plant-aided removal incurs additional costs and introduces new health hazards. Therefore, instead of using hyperaccumulator plants, the recently emerging phytomanagement strategy emphasizes the application of easily cultivable, low-metal-accumulating, deep-rooted, high-biomass-yielding, and biofuel/bioenergy-producing perennial grass species.

Perennial crops, including grasses and herbs, exhibit better phytoremediation traits as they show (a) fast growth, (b) high biomass yield, (c) deep and extensive fibrous root systems, (d) familiar agronomic strategies, (e) tolerance to soil contaminants, (f) greater root biomass and root surface area, and (g) tolerance to physical stresses, such as drought, soil acidity, and cold temperatures. Their inherent potential to withstand, extract, and/or stabilize HMs and their ability to yield high biomass for biofuel/bioenergy, fibers, and other commercial value-added products have generated considerable interest among managers for their implementation in several economic phytomanagement programs. Finally, besides reducing and mitigating the risks posed by the HMs, such biofuel-producing perennial grasses will provide an alternative livelihood option for the poor or low-income population of this region by opening new job markets for their products and contribute substantially to the overall economic development of the community/locality, along with meeting their energy demands through bioenergy generation [36].

For avoiding complexity, only one representative illustration has been shown for *Z. mays*, and the same co-cultivation procedure can be implemented for other food crop species as well (see Fig. 10). Targeted application of multiple HM-resistant and HM-bioaccumulating *Halomonas* and non-*Halomonas* PGPR strains (isolated from the rhizosphere of a true mangrove plant (*Avicennia marina*) of central Indian Sundarbans, West Bengal, India) [37, unpublished data] in the roots or rhizosphere soils of these routinely cultivated, dietary food crops using a biotechnological approach will facilitate plant health and nutrition besides bioremediation of toxic HMs and limiting metal uptake in food crops. This is because the perennial grass species will otherwise compete with the food crop species for available soil nutrients, negatively affecting their growth vigor, whereas the low-metal-accumulator, high-biomass-yielding, bioenergy-producing grass species will phytoextract HMs from the metal-infested ambient soil of the Dhapa site, which is otherwise used as a dumping ground of MSWs from in and around the metropolitan city of Kolkata. In contrast, targeted application of multiple HM-resistant PGPR strains (viz., *Bacillus anthracis* strain MHR2

Table 5 Heavy metal resistivity and plant growth-promoting traits of Kans grass rhizobacteria isolated from fly ash ponds of Mejia Thermal Power Station, West Bengal, India

Isolated multi-metallo-tolerant PGPR strains	GenBank accession no.	HM resistivity				Pi solubilization (mg l^{-1}) in 4 d	PSI	IAA (μg ml^{-1}) in 120 min	Siderophore units (%) in 72 h	HCN (ppm)	NH_3 (μmol ml^{-1}) in 72 h	Bioassay with *Brassica juncea* (BF and TF)	Reference
		Pb	Cd	Cr	Hg								
Bacillus anthracis strain MHR2	KT238975.1	++++	++	+++	+	700	2.86	26.71	90	19.5	0.5	0.700 and 0.210	80
Staphylococcus sp. strain MHR3	KT238976.1	++++	++	+++	+	600	2.31	19.04	60	35.2	6.0	0.678 and 0.186	
Bacillus sp. strain MHR4	KT238977.1	++++	++	+++	+	640	2.40	13.75	85	10.6	6.2	0.669 and 0.180	

Note: 1. HCN, hydrogen cyanide; HM, heavy metal; IAA, indole-3-acetic acid; NH_3, ammonia; PGPR, plant growth-promoting rhizobacteria; Pi, inorganic phosphate; PSI, phosphate solubilization index

2. ++++, +++, ++, and + denote very high, high, moderate, and low HM resistivity, respectively

3. The mean values of triplicate data are given

4. HM bioaccumulation (for all four HMs) needs to be studied in these PGPR strains to see the level of HM concentration in the cell biomass in ppm per dry weight

5. Pot assays need to be done with the selective food crop species

6. Pb bioaccumulation increased in the *B. juncea* roots in the presence of individual PGPR strain or PGPR consortia. This might not be the case in the case of the selected vegetable species

7. Cd, Cr, and Hg bioaccumulation need to be assessed in the selected plant species in the presence or absence of PGPR strains in pot trials

8. Bioaccumulation factor (BF) and translocation factor (TF) for control were 0.501 and 0.120, respectively

(KT238975.1), *Staphylococcus* sp. strain MHR3 (KT238976.1), and *Bacillus* sp. strain MHR4 (KT238977.1)) isolated from the rhizosphere of Kans grass growing in the abandoned fly ash ponds of Mejia Thermal Power Station (MTPS), West Bengal, India (Table 5) [80], in the roots/rhizosphere soils of these grassy species (mentioned above) will facilitate enhanced HM uptake in these low-metal-accumulating, high-biomass-yielding species. It is already established in the field that the concentration of HM(s) is usually more in the belowground biomass and less in the aerial parts (and least in the fruits and seeds) [81]. However, the fruits or vegetables from these metal-incorporated food crops must be carefully soaked and washed in water before cooking and/or consumption. From the phytomanagement/phytodisposal perspective, the aboveground biomass and belowground biomass of the perennial grass species must be disposed of properly or used strategically for biodiesel extraction, following the possibility of HM recovery and recycling to be used in other industries.

Acknowledgements The authors are thankful to Techno India University, West Bengal, for providing the necessary instruments, equipment, and reagents for carrying out the entire research work. The authors are grateful to the RCC Institute of Information Technology (RCCIIT) staff and management for extending all possible support in conducting the present study.

Author Contribution Sangita Agarwal, Pritam Mukherjee, and Abhijit Mitra have conceived the idea, designed experiments, and done the field samplings. Pritam Mukherjee and Prosenjit Pramanick prepared the materials, while Sangita Agarwal and Pritam Mukherjee carried out the experiments. Sangita Agarwal, Pritam Mukherjee, and Abhijit Mitra have analyzed and interpreted the data. Pritam Mukherjee and Prosenjit Pramanick have made all the graphs and tables and have done the statistical analyses. Sangita Agarwal, Pritam Mukherjee, and Abhijit Mitra wrote the paper. All authors have read and approved the final manuscript.

Data Availability The authors will provide the primary data if and whenever required.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Competing Interests The authors declare no competing interests.

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