Original Paper

Changes in nutrient and heavy metal content after vermicomposting of water hyacinthbased spent mushroom substrate

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Abstract

Experimental Biology ISSN 2255-9582



Environmental and

The spent biomass of water hyacinth (*Eichhornia crassipes*) after oyster mushroom cultivation is not much suitable for application as soil amendment. As a means to avoid environmental pollution and to decrease waste to landfill, the spent biomass was primarily composted with cow dung and then feed to red wiggler earthworm (*Eisenia fetida*) to transform it into vermicompost. The produced vermicompost showed significant reduction in salt concentration, electrical conductivity, available K concentration, C/N ratio and increase in pH, available N and P. The representative heavy metals (Fe, Cu, Zn, Pb and Cd) detected in the spent biomass of water hyacinth were derived from the initial plant biomass. Among these metals, concentration of Fe increased and that of Zn decreased significantly in the vermicompost, while the other metals did not show significant change. When applied as amendment to control soil for growth of radish plants, the vermicompost treatment resulted in significantly higher shoot and root biomass. Also, the amendment decreased the bioavailability of metals (especially Cd) and their accumulation in radish plants. The metal concentrations in the vermicompost and in the radish plants were found to fall within the permissible limits for compost and the normal range present in plants, respectively, indicating the suitability of the vermicompost for use as organic fertilizer. Utilization of the water hyacinth as substrate for oyster mushroom cultivation and recycling the spent mushroom substrate through vermicomposting could therefore provide food and fertilizer with no waste to landfill, changing the status of the invasive aquatic weed from a prolific pest to a potentially usable product.

Key words: cow dung, *Eichhornia crassipes, Eisenia fetida*, heavy metals, nutrient content, spent mushroom substrate, vermicompost. Abbreviations: BCF, bioconcentration factor; EC, electrical conductivity; OM, organic matter; TC, tota; carbon; TKN, total Kjeldahl notrogen.

Introduction

Water hyacinth (Eichhornia crassipes Mart. Solms.), a plant species native to South America, is a major freshwater weed in most frost-free regions of the world. It has been labelled as the world's most troublesome aquatic weed (Holm et al. 1997; Zhang et al. 2010). Due to the intense rate of proliferation, the weed poses negative impact on biodiversity and on environment, causing great socioeconomic concern (Mailu 2001; Dechassa 2020). On the positive side, water hyacinth has been used as one of the main components of waste water treatment systems because of its ability to bioaccumulate hazardous pollutants and metals (Dos Santos, Lenz 2000; Soltan, Rashed 2003; Tiwari et al. 2007; Magar et al. 2017). Research into utilization of the plant for a variety of beneficial uses and related technologies for mitigating the impact of water hyacinth have been tested for the last few decades (Das, Mukherjee 2007; Ndimele et al. 2011; Mintensnot 2014; Sharma, Chauhan, 2017; Obianuju et al. 2020).

On small scale, the use of the weed especially in conjunction with its manual or mechanical harvesting from waterbodies can be exploited more economically. One such approach includes utilizing biomass of water hyacinth plants as a substrate for cultivation of oyster mushroom (*Pleurotus* spp.; Bandopadhyay Mukhopadhyay, Chatterjee 2009; Bandopadhyay 2013; Bandopadhyay 2020). The weed was observed to allow for a shorter production time and significant increase in the yield of protein and mineral content in mushrooms over that obtained using conventional substrate of paddy straw (Bandopadhyay Mukhopadhyay 2020). Moreover, several heavy metals (Fe, Cu, Zn, Pb and Cd) present in the initial water hyacinth biomass did not accumulate at a toxic level in the mushrooms (Bandopadhyay Mukhopadhyay 2020).

After final harvesting of mushrooms from beds of water hyacinth, the left-over substrate is a bulky waste of byproducts from growing of mushrooms, which needs safe disposal to avoid environmental pollution (Tajbaksh et al. 2008). Several studies have indicated that the spent mushroom substrate possesses sufficient nutrient quality for use as organic manure for crops (Bandopadhyay Mukhopadhyay 2015) or ornamental plants (Ahlawat, Sagar 2007). Therefore, there is a high possibility of using this substrate in its original or modified form. However, due to high salt content in spent mushroom substrate in general as well as because of presence of toxic metals derived from initial water hyacinth biomass, it might not be suitable for application immediately after mushroom harvest (Uzun 2004). This substrate can be composted, weathered, or leached to upgrade its physical and chemical characteristics before further use (Becher, Pakula 2014).

Compared to other composting methods, vermicomposting is a suitable alternative for conversion of organic waste to a compost enriched with nutrients (Dickerson 2001; Ramnarain et al. 2019). Moreover, concentration of toxic metals in vermicompost is significantly lower (Morgan 1999; Pandey et al. 2014). Vermicomposting is a mesophilic biooxidation and stabilization process of organic materials that involves joint action of earthworms and their microorganisms. The optimum temperature for earthworms in vermicomposting is considered to be up to 35 °C, whereas in conventional composting (including thermophilic composting) temperatures may reach up to 70 °C. Certain species of earthworms such as Eisenia fetida, Eisenia andrei, Lumbricus rubellus, Eudrillus eugeniae have previously been used to treat spent mushroom substrate to convert it to nutrient-rich organic fertilizer (Tajbakhsh et al. 2008; Izyan et al. 2009; Prasetya et al. 2013). Of these, E. fetida showed the best results in respect to vermicompost quality considering the accepted standards (Purnawanto et al. 2020). However, earthworms do not feed much on the spent substrate and accept it as diet only after composting with organic supplement as cow dung, creating suitable media for earthworms (Murthy, Manonmani, 2008) and partially decomposing the organic feedstock for fast vermicomposting.

Considering the potential use of vermicomposting technology to improve nutrient quality and reduce heavy metal content, the aim of the present study was to examine recycling of spent water hyacinth biomass from oyster mushroom cultivation into vermicompost, to assess the potential of using the resultant vermicompost as soil amendment for plant growth, and to assess the status of the metal pollutants derived from initial raw material in the system 'water hyacinth biomass - spent water hyacinth substrate - vermicompost - plant'. The objectives were to determine (i) the change in physicochemical properties and concentrations of several heavy metals (Fe, Cu, Zn, Pb and Cd) of spent substrate after composting with cow dung and then vermicomposting with E. fetida, (ii) the growth response of radish plants (Raphanus sativus L.) to vermicompost amendment in soil and (iii) the concentrations of heavy metals in the radish plants.

Materials and methods

Collection of spent water hyacinth biomass

After final harvesting of the mushrooms, the left-over mushroom beds, i.e. the spent mushroom substrate of water hyacinth were collected from the oyster mushroom (*Pleurotus* spp.) cultivation unit and used as primary feedstock for vermicomposting (Bandopadhyay Mukhopadhyay, Chatterjee 2009). The spent beds were shredded into small pieces, mixed thoroughly, spread out, and air dried for two days. The substrate was then collected in an earthen bin with 68 cm diameter and 45 cm depth, with 10 to12 netted holes at the bottom.

Primary composting of spent water hyacinth biomass

Cow dung was added in a 1:4 ratio (weight/weight) to the feedstock of spent water hyacinth biomass in the bin to give a total of 7 kg feed material, and well mixed together with water. The moisture content and temperature of the compost mixture were regularly monitored and maintained between 70 to 80% and 28 to 30 °C, respectively, by sprinkling water and weekly turning for three weeks. Then, the partially decomposed feed material (primary compost) was ready as feed for earthworms.

Vermicompost production

Seventy individuals of red wiggler earthworm (*Eisenia fetida*) were obtained from the Institute of Agriculture, Bisva Bharati University, Santiniketan, West Bengal, India and released on the top layer of primary compost, and bins were then covered with jute sacs to maintain moisture. The temperature and moisture level of the compost were maintained between 26 to 28 °C and 55 to 65%, respectively, for the next seven weeks by sprinkling water at intervals of a few days.

Seven weeks after earthworm release, loose, granular, dark brown coloured vermicompost was prominent on the top layer, when watering was stopped for three to four days.

Vermicompost devoid of worms was collected from the top layer. Earthworms were separated from the bottom layer and maintained as a reserve for further cycles of vermicomposting.

Application of vermicompost as soil amendment for growth of radish plants

The produced vermicompost was used as soil amendment to determine its effect on the growth of 'Punjab Full Red' radish plants (Raphanus sativus L.). Field experiments were conducted from December to February in sandy loam soil in the garden of the Department of Botany, The University of Burdwan, West Bengal, India, where refuse had been dumped previously. The field was prepared by thorough repeated ploughing up to a satisfactory tilth condition of the sandy loam soil. The experiments were laid out in randomized block design with two treatments in three replications. Plots of 1.5×1.5 m size were laid out for each of the two treatments: control soil (without any amendment) and vermicompost-amended soil [up to 20 cm depth, the top layer of soil was mixed with vermicompost in 3:1 (w/w) proportion]. Seeds were germinated on control soil and vermicompost-amended soil separately. The seedlings were planted on a row hill with inter- and intra-row spacing of 30 cm, giving 25 plants in each plot. Mature root vegetables were harvested after 60 days of seed germination. Sample plants were selected from each treatment plot and growth was estimated by leaf area, shoot biomass (both fresh and dry) and root biomass (both fresh and dry). For each parameter, the result was expressed as mean of 15 plants \pm SD.

Physicochemical analysis

Samples of spent water hyacinth substrate, primary compost and the vermicompost were taken on Day 0, Day 26 and Day 60 of composting, respectively, oven dried at 70 °C for 72 h and homogenized to determine physicochemical parameters (pH, electrical conductivity, total carbon, total Kjeldahl nitrogen, available nitrogen, available phosphorus, and potassium). pH value was determined with a digital pH meter following Gaur (2005). Moisture content of the sample was determined by weight loss on oven-drying at 105 °C for 4 h to reach constant weight. Electrical conductivity (EC) was determined following Gaur (2005) and total soluble salt concentration of the sample suspension (sample/water 1:5) was calculated from EC at 25 °C following Corwin and Yemoto (2017). Total organic carbon (TC) was estimated by the Walkley-Black titration method following standard operating procedure of soil organic carbon (FAO 2019) and expressed as % dry weight. Ash content and organic matter (OM) were calculated from TC following Larney et al. (2005): ash content (g kg⁻¹) = 968.9 – 1.797 TC (g kg⁻¹), and OM (g kg⁻¹) = 31.1 + 1.797TC (g kg⁻¹). Total Kjeldahl nitrogen (TKN) was estimated by Kjeldahl method according to Jackson (1967). Available or mineralizable nitrogen was determined by distillation method according to Subbaiah and Asija (1956). Available phosphorus was estimated following the modified method of Olsen et al. (1954). Available potassium refers to exchangeable K plus water soluble K⁺. Concentration of exchangeable K was determined in a neutral normal ammonium acetate extract of the respective samples using a flame photometer according to Rao and Reddy (2005). Concentrations of OM and macronutrients (N, P and K) were expressed as % of dry weight.

To compare the nutrient quality of the experimental vermicompost with commercial vermicompost, the above physicochemical parameters were also measured in a commercial vermicompost sample obtained from the Rural Technology Centre of The University of Burdwan, West Bengal, India. The raw materials used for this vermicompost were common lignocellulosic waste of paddy straw, leaf litter, garden clippings, vegetable waste and water hyacinth biomass.

Determination of concentration of heavy metals

The concentrations (mg kg⁻¹ dry weight) of iron (Fe), copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd) as representatives of potentially toxic metals were measured in spent water hyacinth substrate, vermicompost and in radish plants, using an Atomic Absorption Spectrophotometer

(Perkin Elmer 5100 PC AAS at Bose Institute, Kolkata) after acid digestion of the dried samples (Suthar 2009). The coefficient of accumulation or bioconcentration factor (BCF) for individual metals was calculated as the ratio of concentration of the metal in the plant to that in the soil.

Statistical analysis

All samples were analyzed in triplicates and the results were presented as mean ± SD. The experimental data were analyzed using descriptive statistics and also subjected to one-way ANOVA to ascertain any significant difference ($p \le 0.01$ for physicochemical parameters and at $p \le 0.05$ for heavy metal concentration and plant growth parameters) between the initial and final product.

Results

The volume of spent water hyacinth substrate was reduced by more than 58% during primary composting and vermicomposting (from 0.17 to 0.10 m³). Results of analysis of physicochemical parameters (pH, EC, moisture, C, N, P and K) of spent water hyacinth substrate, primary compost and vermicompost are presented in Table 1. pH value increased in primary compost to neutral range in vermicompost. In contrast, the EC of spent water hyacinth substrate (4.2 mS cm⁻¹) decreased significantly in vermicompost (0.8 mS cm⁻¹). Total salt content also decreased. Moisture content of the vermicompost was reduced to 41% at harvesting. Organic matter content of spent water hyacinth substrate decreased although not significantly, during its bioconversion to primary compost and vermicompost, with a corresponding increase in ash content. Mineralizable N of spent water hyacinth substrate increased significantly during vermicomposting. Available P increased significantly in the primary compost but not in the vermicompost. Available K concentration decreased significantly as the water-soluble K⁺ increased in the vermicompost. The nutrient profile of the commercial vermicompost was comparable with that of experimentally produced vermicompost, except for available N and K content, which showed significant difference.

Table 2 presents the concentrations of several heavy metals (Fe, Cu, Zn, Pb and Cd) in the initial water hyacinth biomass before cultivation of mushrooms (Bandopadhyay Mukhopadhyay 2020), in the spent water hyacinth substrate after mushroom harvest, in the cow dung used for primary composting, in the final product of vermicomposting and in the control soil used for radish cultivation. The metals initially were derived from the water hyacinth biomass, the initial substrate for mushroom cultivation, and were accumulated in vermicompost after primary composting and vermicomposting. The concentration of metals in vermicompost was found to be in the following descending order: Fe > Zn > Pb > Cu > Cd. The concentration of Zn decreased significantly, Fe increased significantly while that of Cu, Pb and Cd did not show significant change in

Table 1. Nutrient (physicochemical) qualities of the spent water hyacinth substrate, primary compost with cow dung, vermicompost, commercial vermicompost and control soil. Results are mean \pm SD. Values with different letters in the same row indicate significant difference at $p \le 0.01$. FW, fresh weight; DW, dry weight

Parameter (unit)	Spent water hyacinth substrate	Primary compost	Vermicompost	Commercial vermicompost	Control soil
pH	5.7 ± 0.4 a	7.9 ± 1.1 b	$6.9 \pm 0.7 \text{ a}$	6.2 ± 0.8 a	7.5
Moisture (% FW)	$81.0 \pm 4.5 \text{ a}$	86.6 ± 3.8 a	$41.0\pm1.2~\mathrm{b}$	$44.0\pm4.5~\mathrm{b}$	32 ± 4
Ash (% DW)	75.2 ± 1.4 a	82.5 ±2.3 a	84.0 ± 3.0 a	81.3 ± 2.3 a	89.9 ± 1.1
Electrical conductivity (mS cm ⁻¹)	$4.2 \pm 0.1 \text{ a}$	$1.1 \pm 0.4 \text{ b}$	$0.8\pm0.2~b$	$1.1 \pm 0.2 \text{ b}$	0.50 ± 0.04
Salt concentration (% DW)	1.3 ± 0.2 a	$0.35\pm0.04~b$	$0.25\pm0.04~b$	$0.35\pm0.03~b$	0.16 ± 0.04
Total carbon (% DW)	12.1 ± 1.4 a	8.0 ± 3.3 a	$7.2 \pm 3.0 \text{ a}$	8.7 ± 2.3 a	3.9 ± 1.1
Organic matter (% DW)	24.8 ±1.4 a	17.5 ± 3.3 a	16.0 ± 3.0 a	18.7 ± 2.3 a	10.1 ± 1.1
Total N (% DW)	1.1 ± 0.1a	1.50 ± 0.15 a	2.1 ± 0.17 a	2.00 ± 0.07 a	0.10 ± 0.01
Available N (% DW)	0.17 ± 0.01 a	0.13 ± 0.04 a	$0.37\pm0.03~b$	0.17 ± 0.20 a	0.07 ± 0.01
Available P (% DW)	0.27 ± 0.01 a	$0.70\pm0.08~b$	0.36 ± 0.03 a	0.310 ± 0.008 a	0.12 ± 0.05
Exchangeable K (% DW)	$1.50\pm0.08~\mathrm{a}$	1.6 ± 0.1a	$1.10\pm0.12~\mathrm{b}$	1.80 ± 0.14 a	0.1 ± 0.3
Soluble K ⁺ (% DW)	$0.03\pm0.001a$	$0.150\pm0.002~b$	$0.13\pm0.01~b$	$0.16\pm0.02~b$	0.010 ± 0.01

the vermicompost from the spent water hyacinth substrate. The accumulation (as concentration in the vermicompost divided by that in initial spent water hyacinth substrate) of the metals in the vermicompost was found in the order of Fe > Cu > Pb > Zn > Cd. For comparison, Table 2 also presents the concentrations of these metals in the control soil used for radish cultivation, the permissible limits of heavy metals in compost and safe limits of concentrations for agricultural soil (Indian standard).

Soil amendment with vermicompost resulted in significantly higher shoot and root biomass of radish plants in comparison to those for plants grown in control soil (Table 3). Table 4 presents the heavy metal concentrations in radish plants grown in vermicompost-amended soil and in control soil. Compared with the control soil, the concentrations of Cd, Zn and Pb in the radish plants of vermicompost-amended soil were significantly lower, whereas the concentration of Fe was higher. The bioaccumulation of these metals as indicated by the BCF showed the opposite trend. Fe showed highest and Cd showed the lowest bioaccumulation from vermicompostamended soil. However, in control plants, the highest accumulation was observed for Cd and the lowest for Fe (Table 4). Zn, Pb and Cu showed an intermediate position of bioaccumulation for both types of soil. Excepting Pb, the concentration of all other metals in the amended soil plants was within the standard permissible limit and within the normal range generally present in plants (Table 4).

Discussion

The biomass of aquatic weed, water hyacinth *Eichhornia crassipes* reduced in weight and volume due to loss in organic matter, during use as substrate for mushroom cultivation by biodegradation with mushroom fungi (*Pleurotus* spp.; Suthar 2006; Sangwan et al. 2008). After mushroom harvest the spent mushroom beds were regarded as organic waste. In order to determine the potential of reusing this organic waste, it was analyzed for important physical (pH, EC, moisture) and chemical characteristics (C, N, P, K). The acidic pH, high EC value and total salt concentration of spent water hyacinth substrate necessitated its aging and

Table 2. Concentrations (mg kg–1 DW) of heavy metals in initial water hyacinth biomass before mushroom cultivation, spent water hyacinth substrate after mushroom cultivation, vermicompost, cow dung, control soil and the maximum permissible levels. Results are mean \pm SD; Values with different letters in the same row indicate significant difference at $p \le 0.05$

Metal	Water	Spent water	Cow dung	Vermicompost	Control soil	Permissible levels	
	hyacinth biomass	hyacinth substrate				For compost (Saha et al. 2013)	For agricultural soil (Indian Standards; Awashthi 2000)
Fe	441 ± 104 a	414 ± 276 a	1352 ± 201 b	1656 ± 211 b	$1780\pm15~\mathrm{b}$	20 000 - 550 000	-
Cu	7.2 ± 1.6 a	5.1 ± 4.6 a	13.0 ± 3.6 b	8.3 ± 2.1 a	$12.3\pm2.0~\mathrm{b}$	135 - 270	340
Zn	52.2 ± 6.2 e	59.1 ± 5.5 e	36.2 ± 5.6 b	45.4 ± 3.2 b	56 ± 6 a	300 - 600	750
Pb	8.6 ± 1.5 a	7.5 ± 3.9 a	9.4 ± 3.2 a	11.0 ± 1.2 a	$18 \pm 4 \text{ b}$	250 - 500	160
Cd	3.7 ± 1.8 a	2.3 ± 1.2 a	$1.2 \pm 1.0 \text{ b}$	1.7 ± 0.6 a	1.6 ± 0.5 a	3 - 6	0.8

Growth medium	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area (cm ²)	Root fresh weight (g)	Root dry weight (g)
Control soil	74 ± 14 a	$4.1 \pm 0.1 \text{ c}$	157 ± 33 d	29.3 ± 10 e	$1.5 \pm 0.3 \text{ g}$
Soil + vermicompost	118 ± 32 b	7.9 ± 3.2 c	187 ± 30 d	93 ± 18 f	3.4 ± 0.8 g

Table 3. Effect of application of vermicompost as amendment to control soil on the growth of radish plants (*Raphanus sativus*). Results are mean \pm SD. Values with different letters in the same column indicate that results are significantly different at $p \le 0.05$

processing before it could be used as suitable organic manure.

Water hyacinth has been well studied as an aquatic plant that can accumulate high amounts of heavy metals and dissolved ions (Thapa et al. 2016; Priya, Selvan 2017) in tissue and as a main component of integrated systems of waste water treatment (Dhole, Dixit 2009). In an attempt to reduce heavy metal concentration in spent water hyacinth substrate after mushroom cultivation, vermitechnology was exploited for a period of 7 to10 weeks. Before inoculation of earthworms for vermicomposting, spent water hyacinth substrate was composted primarily with cow dung, which accelerated mineralization of nutrients as well as microbial biodegradation, thus creating suitable media for vermiculture (Murthy, Manonmani 2008), as earthworms could not consume the complex organic substrate directly.

Introduction of Eisenia fetida in the primary compost gradually changed the physical and chemical qualities of the feedstock and transformed it into finely divided, black granular vermicast, vermicompost. Vermicompost from cow dung has been reported to have high nutrient content as well as enzymatic and microbial activity (Pramanik et al. 2007). The physicochemical characteristics of the spent water hyacinth substrate was found to differ in the end product of primary composting and vermicomposting. The increase in pH after primary composting might be due to increased ammonification in the mixture of cow dung and spent water hyacinth substrate, which then decreased gradually by rapid nitrification and humic acid formation in the final vermicast (Kaur et al. 2010; Vig et al. 2011). This led to shift of pH towards neutral, as also observed by Pramanik et al. (2007). Several studies have found that most species of earthworms prefer a pH of about 7.0, although *Eisenia fetida* can grow well in substrate with a wide range of pH (Chauhan, Singh 2013).

The increase in ash content and the decrease in total carbon or organic matter of vermicompost, which indicate mineralization and decomposition of the substrate (Mortada et al. 2020), were not significant, as the initial feedstock was an already decomposed spent substrate. High EC is equivalent to high salinity and is undesirable for plant growth (Uzun 2004), because it inhibits plant rooting and/or reduces transport of mineral solution to the plants. Organic fertilizers with low EC release the mineral salts slowly, at a level that is sufficient for plant growth (Ansari, Sukhraj 2010). In the present study, EC of the initial feedstock of spent water hyacinth substrate was above the threshold value (4 mS cm⁻¹), but after composting and vermicomposting, the EC value and total salt concentration decreased to a levels that were favourable for plant growth (Ansari, Rajpersaud 2012). EC decreased due to decomposition of organic matter and precipitation of mineral salts in substrate leading to NH₄⁺ formation, which resulted in increase in pH (Pramanik et al. 2007).

Significant increase in mineralizable N concentration of vermicompost of spent water hyacinth substrate was supported by earlier findings on vermicomposting (Tajbakhsh et al. 2008; Vig et al. 2011). It was reported that total nitrogen concentration increased more in cow dung vermicompost than in vermicompost without cow dung (Pramanik et al. 2007). Microorganisms in cow dung slurry convert organic nitrogen to ammonium and nitrate, which are highly available forms for plants.

Higher total N and lower organic carbon concentration

Table 4. Concentrations (mg kg⁻¹ DW) of heavy metals in radish plants grown on control soil and on vermicompost-amended soil, bioconcentration factor (BCF) for metals, and maximum permissible level by Indian Standard in plants, by FAO/WHO in food and their normal (Hammed et al. 2017), and phytotoxic levels in plants (Chaney et al. 1983). Results are mean \pm SD. Values with different letters in the same row indicate significant difference ($p \le 0.05$).

Metal	In plants in control soil	In plants on vermicompost- amended soil	BCF for control soil vs. plants	BCF for vermicompost amended soil vs. plants	Indian Standard in plants (Awashthi, 2000)	In food (FAO/ WHO 2019)	Level in plant	
							Normal	Phytotoxic
Fe	885 ± 73 a	958 ± 20 b	0.49	0.77	-	425	400 - 500	_
Cu	7.9 ± 2.1 a	6.1 ± 2.6 a	0.64	0.54	30	40	3 - 20	25 - 40
Zn	$46.4 \pm 5.2 \text{ a}$	$34.2\pm4.7~\mathrm{b}$	0.82	0.64	50	60	20-100	500 - 1500
Pb	11.7 ± 1.2 a	8.2 ± 0.6 b	0.65	0.50	2.5	5	0.50-30	-
Cd	1.5 ± 0.3 a	1.1 ± 0.6 b	0.90	0.67	1.5	0.3	<2.4	5 - 700

and therefore, lower C/N ration of vermicompost indicated greater decomposition of spent water hyacinth substrate. A C/N ratio equal to or lower than 15 indicates a high agronomic value of compost (Suthar 2009). Earthworms were reported to selectively increase the populations of catabolically more active microorganisms and trigger enzymatic activity, which in turn accelerates decomposition (Aira et al. 2007).

Significant increase in the available P concentration in the primary compost might be due to solubilization of insoluble P with acid produced by microbial activity during organic matter decomposition, and the presence of large microbial community in the gut of earthworms might play an important role in increasing P concentration in vermicompost (Sharma, 2003). Cow manure appears to be the most viable option to increase P concentration in soil (Almeida et al. 2019) and, therefore it is used in promoting soil fertility (Swain et al. 2012).

Potassium concentration of spent water hyacinth substrate decreased significantly in the vermicompost, but the water-soluble K^+ concentration increased four times, indicating probable leaching of K (Uzun 2004; Sangwan et al. 2008), which in turn resulted in low salt concentration and EC in vermicompoast. Similar trends were also observed in a previous study on vermicomposting of spent mushroom substrate (Tajbakhsh et al. 2008).

When compared with commercial vermicompost, vermicompost produced from spent water hyacinth substrate had significantly higher concentration of N and P. Decrease in mineralizable N was observed after 77 days of vermicomposting of coffee pulp (Orozco et al. 1996), similar to that in the commercial vermicompost sample in the present study. Lower EC, salt, and K concentration in vermicompost were supportive of good plant growth. The other physicochemical properties of experimentally produced vermicompost were very much comparable to commercial vermicompost.

Mineralization and loss of organic matter as CO₂ in the compost of spent water hyacinth substrate and cow dung caused an increase in concentration of nutrients as well as heavy metals like Fe in the vermicompost. The trend of increasing heavy metal concentration in different types of waste after vermicomposting has been reported in other studies (Tajbakhsh et al. 2008; Singh et al. 2010; Vig et al 2011). Considering allowed compost application rates in cropland that corresponds to safe limits of concentration of heavy metals in soil, the maximal permissible concentrations of Cd, Pb, Cu and Zn in compost were calculated to be 0.8, 160, 340 and 750 mg kg⁻¹, respectively (Saha et al. 2013). The safe limits of concentration of heavy metals for agricultural soil are 3 to 6 mg kg⁻¹ for Cd, 250 to 500 mg kg $^{-1}$ for Pb, 135 to 270 mg kg $^{-1}$ for Cu and 300 to 600 mg kg⁻¹ for Zn (Indian Standards; Awashthi 2000). The typical range of iron concentration in soil is from 0.2 to 55% (20 000 to 550 000 mg kg⁻¹) (Bodek et al. 1988). In the present study, the concentration of heavy metals

except Cd in the vermicompost were found to be within the acceptable range of compost for use as organic manure in agriculture. It was also observed that the concentration of heavy metals tended to decrease as the compost become older, which can be explained by formation of complex aggregates with the humic acids and the polymerized organic fractions of compost (Kulikowska et al. 2015). The significant reduction in Zn concentration in vermicompost under some environmental conditions might be attributed to its affinity for organometallic complex formation with the functional groups -OH or -COO- of humus of the vermicompost (Kang et al. 2011) and its absorption and bioaccumulation by earthworm (E. fetida) tissues (Singh, Kalamdhad 2012b; Singh, Kalamdhad 2013c). Reduction in concentration of water-soluble metals like Zn and Cu due to vermicomposting has also been reported (Hait, Tare 2012). Iron oxides adsorb many metals and coprecipitate with many elements like Zn, Pb, Cu (EPA 2003) and may be the major controlling factor in the distribution of the metals in a non-reducing environment.

The toxicity of heavy metals in vermicompost is not only associated with their total concentration, but also on their mobility and bioavailability to plants (Vig et al. 2011). Hence, vermicompost prepared from spent water hyacinth substrate was tested as soil amendment for growth of radish plants to assess any change in bioaccumulation with respect to unamended control soil. Radish is mainly used as root vegetable, but its tender leaves are also consumed as a vegetable. Therefore, in the present study, heavy metal concentration was measured in the whole radish plant, not separately in roots or shoots. Bioaccumulation in plants grown in vermicompost-amended soil was found to be the highest for Fe followed by Cd, Zn, Cu and Pb. In contrast, Cd showed highest accumulation followed by Zn, Pb, Cu and Fe in plants in control soil. This indicated that amendment of control soil with vermicompost caused significant decrease in accumulation of Cd, Zn and Pb in the radish plants. Bioavailability of heavy metals to plants is related to their mobility in soil, which is governed mostly by the soil pH and organic matter. Metals in general are likely to exist in free cationic forms, which are less prone to sorption, and more soluble at low pH than in alkaline conditions (Bradl 2004; Gentili et al. 2018). The metals become less bioavailable when they form barely soluble phosphates and carbonates (Olaniran et al. 2013). Previous studies showed that vermicomposting of organic waste improved the content of microbial matter with chelating elements and accelerated stabilization of humic substances (Gupta, Garg 2008; Suthar 2009; Hait, Tare 2012). Organic matter (natural multiligand complexing system) is a much more effective sorbent of polyvalent cations (Cd, Zn, Pb, Cu) and therefore, complexation with organic matter often reduces their bioavailability (Violante et al. 2010). Besides organic matter, increased P concentration of vermicompost had significant effect on decreasing the heavy metal accumulation, as phosphate can form complexes with Cd

and Zn, decreasing their availability to plants (Mignardi et al. 2012; Yen et al. 2021).

Heavy metals can occur naturally in soil from paedogenetic processes of weathering of parent materials. Metals like Cu, Zn, Pb and Cd are among the important chemical species (including metal complexes) common in soils, which are considered to be potentially toxic above certain concentration limits (Pollak, Favoino 2004). The non-essential elements Pb and Cd have no known physiological role in plants. Iron, although essential for plant growth, is highly reactive and considered environmentally significant because of its interaction with metals that are toxic. Consequently, plants tightly control Fe homeostasis (Morissey, Guerinot 2010). Iron availability is governed by the soil redox potential and pH. In soils that are aerobic or have higher pH, Fe readily oxidized to insoluble ferric oxides, while at lower pH ferric Fe (Fe³⁺) is reduced (Fe²⁺) to become more available for uptake by roots. On the other hand, Cd has the opposite bioavailability profile compared to Fe (Nikanishi et al. 2006). Studies have shown that most soil Cd is adsorbed to organic matter or oxyhydroxides of Fe. In iron-rich soil, iron reduction in certain conditions causes Cd immobilization, thereby reducing soil Cd mobility (Muehe et al. 2013). It has been shown that 80 to 90% of Pb is dissolved as Pb2+ at pH 5 to 6, and at pH above 6 its availability to plant is hindered (Raskin, Ensley 2000). These interactive behaviours of metals and lower pH, higher organic matter, and P concentration of the vermicompost in contrast to control soil might have provided suitable conditions for more complexation and formation of insoluble forms of Cd (Muehe et al. 2013) and of Zn or Pb, thus decreasing their availability for uptake by radish plant roots. Similar results were reported by previous studies, which showed that the application of organic amendment could decrease the availability of Cd and thus, its accumulation in radish plants (Alam et al. 2020; Yen et al. 2021). Iron deficiency due to decreased bioavailability was shown to lead to uptake of other metals (Cd, Zn, Cu, Pb) that are potentially toxic (Morissey, Guerinot 2010). This supported the bioaccumulation trend in the plants on control soil in the present study.

The concentrations of the heavy metals in plants on vermicompost amended soil were within the normal range generally found in plants (Hammed et al. 2017) and within FAO/WHO limits, excepting Pb. Considering the FAO/ WHO limits for potentially toxic elements (Pb and Cd), daily consumption of an average amount of 100 to 150 g vegetable grown on such vermicompost-amended soil would impose no health risk.

The study provided insight into the feasibility of recycling spent mushroom substrate of water hyacinth through composting and vermicomposting. The primary compost of spent water hyacinth and cow dung, which becomes nutrient enriched, but its higher pH, phosphorus and heavy metal concentration would not be favourable for overall growth of plants. Further composting with *Eisenia* fetida converted it into a better-quality manure with more favourable concentrations of major macronutrients (N, P, K), enabling its use for agricultural soil to improve overall growth and vigour of plants. The heavy metals of the spent substrate, which were derived from the initial water hyacinth biomass, mostly did not decrease in concentration after composting and vermicomposting, but their bioavailability to plants was lower when applied to soil for plant growth. Mixing of weed material or wastes rich in organic matter to the initial feedstock of spent water hyacinth would be better than using it as the sole feed material for vermicomposting. Hence, water hyacinth after harvesting from waste water (primary waste after clearing of water bodies) can be used as a substrate for cultivation of oyster mushroom and the spent water hyacinth biomass can then be used as a component of feedstock mixture for vermicomposting.

Establishment of integrated mushroom cultivation and vermicomposting units in the vicinity of waste water treatment sites with huge growth of water hyacinth can recycle the weed plants in an eco-friendly way to produce nutritious mushroom as well as nutrient-rich organic manure and increase farm output with no waste to the landfill.

Acknowledgements

Author thankfully acknowledges the financial assistance under "Women Scientists Scheme for Societal Programmes" of Department of Science and Technology, New Delhi, India.

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Received 10 July 2022; received in revised form 9 January 2023; accepted 5 March 2023