Vermiremediation of paper mill sludge with cow dung and tea waste amendments using epigeic earthworm Eisenia fetida (Savigny)

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Research Article

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Abstract

Vermiremediation of paper mill sludge (PMS) was done by *Eisenia fetida* after adding cow dung (CD) and tea waste (TW). In all, six reactors were prepared: R1 [CD (100%)], R2 [PMS: CD (1:2)], R3 [PMS: CD (1:1)], R4 [PMS: TW: CD (1:1:1)], R5 [PMS: TW: CD (1:2:1)] & R6 [PMS: TW: CD (2:1:1)]. A significant decrease in heavy metals was observed: Cd (37.2–58.2%), Cr (57.0-74.3%), Cu (67.3–79.8%), Ni (74.7–81.9%), Pb (78.8–83.4%) & Zn (71.2–77.4%); while heavy metal concentrations in earthworm tissues ($mg.kg^{-1}$) were recorded as: Cd (2.79–3.24), Cr (23.54–28.76), Cu (9.53–11.56), Ni (3.52–3.99), Pb (8.76–9.43) & Zn (23.12–29.72), after 60 days of study period. The Bioaccumulation factors (BAFs) of *E.fetida* was in the order: Ni > Cd > Cr > Pb > Zn > Cu. R3 obtained the maximum heavy metal removal (74.45%) while that in R4 (72.93%) also emphasized the use of tea waste in the bioremediation process. It was finally observed that cow dung and tea waste amendments favored the vermiremoval of heavy metals from paper mill sludge using *Eisenia fetida*.

1. Introduction

The rapid growth of industrial sector has resulted in increasing industrial solid waste management problems and led to destruction of green space due to excessive land application of agro-industrial solid wastes and open dumping issues. This is mainly due to increasing economy of developing countries like India and demand for adequate infrastructure required for expanding industrialization. The Central Statistics Office, Government of India reported a total of 371,336 operating industries in India that were creating serious issues for the environment (https://unstats.un.org/unsd/envaccounting/londongroup/meeting20/LG20_7_4.pdf). Approximately 30% of 960 million tons solid waste generated in India comes from the industrial sector (Pappu et al., 2007). The inappropriate application of toxic industrial solid wastes has caused adverse impacts on the quality of soil, downgraded the quality of groundwater by percolation of leachate and has also caused air pollution due to greenhouse gas emissions when given certain course of treatments.

The manufacture of paper involves excessive use of water and chemicals such as NaOH, Na_2S, H_2SO_3 and other toxic chemicals that are released in effluents from paper industries. The Annual Report of 2017-18 of Central Pulp & Paper Research Institute (CPPRI) states that there are 859 number of paper mills in India. The annual paper consumption of 13.89 kg/capita in India has resulted in increasing demand for pulp and paper. The consequence has been that the Indian Paper Sector has climbed to 5th position from 15th position in 2004-05 in terms of its production in the entire world (http://cppri.res.in/sites/default/files/annual%20report%2017%20-%2018.pdf). WorldBank, 2007 estimated that paper mill sludge generation is about 4.3% of its new paper production which further get increased to 20–40% for recycled paper produce. Approximately 40–50 kg of dry paper mill sludge is generated from 1 tonne of paper production (Bajpai, 2015). Although this sludge from paper mills can be used as soil amendment due to its chemical characteristics, but the toxicity of heavy metals involved in it poses risks for the safety of environment. The unhygienic removal of Paper mill sludge (PMS) could affect the quality of soil and microflora associated with it. Therefore, there is urgent need is to bioremediate the heavy metals from PMS before its disposal or further use.

Tea is a popular beverage in the entire world. China is the largest producer of tea, followed by India. The annual world production of tea in the year 2018 stood at 5896.65 M.kgs (http://www.teaboard.gov.in/pdf/Website_World_Tea_pdf7894.pdf). Tea waste (TW) has also been increasing due to daily drinking and extraction of tea for ready-to-drink beverages. It was reported in the 23rd session of the Food and Agriculture Organization of United Nations (FAO) Intergovernmental Group (IGG) on Tea that the green tea and black tea production is expected to grow at an annual rate of 7.5% and 2.5% respectively to 3.6 MT and 4.14 MT in 2027 respectively (http://www.fao.org/3/BU642en/bu642en.pdf). Tea waste contains high amount of lignin content that can be harmful for water and soil by its leaching during the rainy season (Abbiramy et al. 2015). Heavy metals might accumulate from the growth till the final processing of tea (Zhong W-S et al. 2015). Zhong W-S et al. 2015 determined heavy metal concentrations in 25 tea samples (including yellow, green, oolong, white, black and jasmine tea) using high-resolution continuum source graphite furnace AAS and reported: Pb (0.48–10.57 $mg.kg^{-1}$), Cd (0.01–0.39 $mg.kg^{-1}$), Cr (0.27–2.45 $mg.kg^{-1}$), Cu (7.73–63.71 $mg.kg^{-1}$) & Ni (2.70-13.41 $mg.kg^{-1}$).

Therefore, an eco-friendly sustainable method needs to be adopted to minimize the hazardous nature of both PMS and TW. Vermistabilization is a low-cost aerobic mechanism used for the breakdown of industrial sludges complex in nature accomplished by the synergetic action of earthworms and micro-organisms to non-toxic and usable forms (Bhat et al. 2017b). This process can be used to degrade the high organic matter of PMS and remediate both PMS and TW. The metal concentrations are influenced by the mineralization of organic content and the bioaccumulation of heavy metals in the body of earthworms (Lv et al., 2016). The combined action chloragocyte cells present in the gut of earthworm *Eisenia fetida* and intestinal microbial community helps in inducing cysteine-rich metal binding protein called metallothioneins which resulted in the reduction of heavy metals from tea coal factory ash (Goswami et al. 2016). Previous researchers have effectively determined the role of metallothionein and phytochelatins mediated removal pathway in gut of earthworms. Therefore, selection of earthworm species is, therefore, a major factor in the assessment of their potential for metal detoxification of different toxic industrial wastes/sludges. Suthar et al. 2014 used *Eisenia fetida* stabilize the wastewater sludge from paper and pulp industry and reported that the increasing accumulation rate in terms of BAF favoured the decrease of heavy metals in the feed mixtures. Yuvaraj et al. 2018a made use of *P. excavates* in the bioremediation of paper mill sludge and significantly reported decrease as: Cd (2.9–27.8%), Cu (0.22–42.3%), Pb (1.3–56.3%) and Cr (0.8–46.2%). Paul et al. employed *E. fetida* and *E. eugeniae* to bioremediate silk processing effluents and sludge (SPES) and reported efficacy of *E.fetida* in reduction of Cd, Cr, Zn and Pb levels by 60–70%. The bioaccumulation of heavy metals in *E.fetida* was in the order: Cu > Zn > Cr > Cd > Fe > Mn; while that in *E.eugeniae* was: Cu > Zn > Mn > Fe > Cr > Cd. Koolivand et al. 2020 reported removal of total petroleum hydrocarbons (TPHs) removal of 81–83% in bioaugmented composting (BC), 31–49% in vermicomposting (VC) and 85–91% in BCVC, thus, indicating the synergistic effect of both bacteria and *E.fetida* in 12 weeks of study. Karwal and Kaushik, 2020 studied the effect of composting (C) and vermicomposting (VC) on the heavy metal levels present in buffalo dung (BD), fly ash (F) and pressmud (PM) during 90 days of research and stated that the feed mixture VC_3 [BP: F = 3:1] was the most efficient in reduction of heavy metals [Cu, Zn, Pb, Cr, Co, Cd and As], where BP = BD + PM. Previous works have also confirmed the bioremediation of industrial sludges such as fermented tannery (Ravindran et al., 2016), municipal solid waste (Soobhany et al., 2015), distillery industry (Suthar and Singh, 2008), etc.

The changes in heavy metal concentrations of paper mill sludge after addition of cow dung and tea waste and the assessment of bioavailability of heavy metals in earthworm tissues has not been previously monitored. Therefore, to measure the potential of metal bioaccumulation in *E.fetida* and heavy metal concentrations of bioremediated sludge, a separate study was done to stabilize paper mill sludge by epigeic earthworm *Eisenia fetida* using cow dung and tea waste amendments.

2. Materials And Methods

2.1. Earthworms, Paper Mill Sludge, Tea Waste and Cow Dung

The epigeic earthworm Eisenia *fetida* was used for the vermiremediation of wastes. These were collected from the WTP of GNDEC campus. Under accurate laboratory conditions, earthworms were cultured for stock in contaminated-free decomposed biomass. In order to avoid any contamination history, freshly-deposited cocoons were separated so that the second generation earthworms can be used for the experimental work.

The paper mill sludge (PMS) was collected from Hemkunt Paper Mills Ltd., Ladowal, Ludhiana and air-dried for 4 days to remove excess water. Shredding of PMS was done to provide homogeneity for this study. The physico-chemical characteristics of PMS were: pH (7.54 ± 0.08); EC (2.42 ± 0.04 $mS.cm^{-1}$); TOC (31.36 ± 0.55%); TN (0.19 ± 0.02%); TP (0.24 ± 0.15%); TK (0.27 ± 0.20%); C/N ratio (165 ± 0.40). The average values of heavy metals in raw PMS were: Cd(2.23 $mg.kg^{-1}$), Cr (17.44 $mg.kg^{-1}$), Cu (121.86 $mg.kg^{-1}$), Ni (15.55 $mg.kg^{-1}$), Pb (87.49 $mg.kg^{-1}$) & Zn (278.63 $mg.kg^{-1}$).

The tea waste (TW) was collected from the hostel mess of GNDEC campus and air-dried for 4 days before grinding it to powder. The physico-chemical characteristics of TW were: pH (5.27 ± 0.15); EC (2.14 ± 0.07 $mS.cm^{-1}$); TOC (6.78 ± 0.9%); TN (0.18 ± 0.01%); TP (0.73 ± 0.15%); TK (1.73 ± 0.11%); C/N ratio (37 ± 0.67). The average values of heavy metals in raw TW were: Cd(0.093 $mg.kg^{-1}$), Cr (1.41 $mg.kg^{-1}$), Cu (34.67 $mg.kg^{-1}$), Ni (7.21 $mg.kg^{-1}$), Pb (3.45 $mg.kg^{-1}$) & Zn (8.95 $mg.kg^{-1}$).

The bulky material cow dung (CD) accelerates the microbial decomposition process. Fresh pristine un-contaminated CD was brought from a local cow shed and stabilized for 14 days before further use for experimentations. The main characteristics of CD were: pH (8.31 ± 0.20); EC (1.38 ± 0.05 $mS.cm^{-1}$); TOC

(51.56 ± 0.25%); TN (0.98 ± 0.11%); TP (0.44 ± 0.09%); TK (0.56 ± 0.07%); C/N ratio (20.2 ± 0.65). The average values of heavy metals in raw CD were: Cd(0.005 $mg.kg^{-1}$), Cr (0.225 $mg.kg^{-1}$), Cu (0.390 $mg.kg^{-1}$), Ni (0.085 $mg.kg^{-1}$), Pb (0.165 $mg.kg^{-1}$) & Zn (0.280 $mg.kg^{-1}$).

2.2. Vermicomposting reactors and their experimental set-up

For experimental trials, triplicates of six different combinations of PMS, TW and CD were prepared on a dry weight proportions and final mixture was made up to 6 kg. Reactors R1 to R6 were initially degraded in plastic containers of size 20"x 13.5"x 6" for 2 weeks and distilled water was used to maintain appropriate moisture content of about 60–70%. Following combinations were prepared:

Reactor 1 (R1) – CD (100%)

Reactor 2 (R2) – PMS: CD (1:2)

Reactor 3 (R3) – PMS: CD (1:1)

Reactor 4 (R4) – PMS: TW: CD (1:1:1)

Reactor 5 (R5) – PMS: TW: CD (1:2:1)

Reactor 6 (R6) – PMS: TW: CD (2:1:1)

After 2 weeks of substrate softening, 25 mature *E.fetida* earthworms were collected from stock culture and added to each reactor. Reactors were kept in a dark and humid atmosphere and homogenized samples were drawn from them at every 15-day intervals for the heavy metal analysis. After collection, the samples were oven-dried at 60°C and stored decontaminated plastic containers (airtight) for further analysis. The biological parameters of *E.fetida* were observed using methodology given by Negi and Suthar (2013).

2.3. Analytical Procedures

The physico-chemical characteristics of PMS, TW and CD were analyzed by using following standard procedures. Jackson (1955) was followed for determination of pH, EC and TOC (Partial Oxidation Method). Macro Kjedahl method was followed for determination of TN (Humphries, 1956). TP and TK were analyzed as described by Tandon (1993). C: N ratio was determined by division of TOC content with the estimated nitrogen content for respective waste.

The analysis of heavy metals for initial raw wastes and samples drawn from each reactor after every 15th day till the end of study was done by Pedersen and van Gestel (2001). The estimation of heavy metals was done using Atomic Absorption Spectrophotometer (Agilent Technologies Spectra 240FS AA), for which 1g sample was digested with mixture with HNO_3 and $HClO_4$, diluted with deionised water and filtered with Whatman no.42 filter paper.

2.4. Efficiency of heavy metal removal

Sahariah et al. (2015) suggested the following formula to find out the heavy metal removal efficiency ($_r$) of different reactors and draw comparisons between one another:

$_r$ = [(HMC) $_{initial}$ – (HMC) $_{final}$] / [(HMC) $_{initial}$]

where (HMC) $_{initial}$ = Heavy Metal Concentration of initial substrate

and (HMC) $_{final}$ = Heavy Metal Concentration of final bioremediated substrate

2.5. Bioaccumulation Factor (BAF)

Dai et al. (2004) highlighted the below mentioned formula to find out the bioavailability of heavy metals in the tissues of earthworm which is also depicted by a factor known as bioaccumulation factor (BAF):

BAF = [(HMC) $_{earthworm}$] / [(HMC) $_{final}$]

where (HMC) $_{earthworm}$ = Bioavailability of heavy metals in earthworm tissues

If BAF $\leq$ 1, it depicts that the earthworms are only able to absorb heavy metals but are not able to accumulate them. Whereas, if BAF > 1, it shows that the earthworms are able to accumulate heavy metals in their gut.

2.6. Statistical analysis

The data has been expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's *t*-test using SPSS version 22.0 to find out the statistical significance of reactors with reference to rate of removal of heavy metals. The results reported in this study are at p   0.05 and p   0.001 levels. The relations between interdependent parameters were also evaluated by the use of regression equations.

3. Results And Discussion

3.1. Concentratons of heavy metals in final bioremediated susbstrates

The vermiremediation using *E. fetida* significantly detoxified the substrates in different reactors as depicted by the changes in heavy metal concentrations (Cd, Cr, Cu, Ni, Pb & Zn) in Table 1. The difference among the reactors was statistically significant for Cd (ANOVA; F = 12.67, P   0.0001), Cr (ANOVA; F = 69.36, P   0.0001), Cu (ANOVA; F = 257.47, P   0.0001), Ni (ANOVA; F = 83.56, P   0.0001), Pb (ANOVA; F = 166.03, P   0.0001) and Zn (ANOVA; F = 45.77, P   0.0001). At the end of bioremediation process, Cd ranged from 4.93 ± 0.003 mg.kg^{-1} (R1) to 7.79 ± 0.001 mg.kg^{-1} (R3), Cr ranged from 31.8 ± 0.9 mg.kg^{-1}

(R2) to 57.5 ± 0.6 mg.kg^{-1} (R1), Cu ranged from 27.68 ± 0.43 mg.kg^{-1} (R3) to 38.73 ± 0.48 mg.kg^{-1} (R6), Ni ranged from 2.98 ± 0.001 mg.kg^{-1} (R2) to 3.45 ± 0.024 mg.kg^{-1} (R1), Pb ranged from 12.26 ± 0.3 mg.kg^{-1} (R3) to 13.52 ± 0.5 mg.kg^{-1} (R5) and Zn ranged from 57.54 ± 0.4 mg.kg^{-1} (R6) to 91.60 ± 0.45 mg.kg^{-1} (R3). R3 (PMS: CD = 1:1) was the most feasible reactor in terms of reduction in heavy metal concentrations with average removal efficiency of 74.45%. The heavy metal removal order was: R3 (74.45%) > R2 (72.98%) > R4 (72.93%) > R6 (68.22%) > R1 (67.25%) > R5 (66.63%). Wang et al. (2013a) stated that there are a variety of factors in the vermiremoval process such as pH, equilibrium between absorbing and accumulating forms of metals, metal speciation, chemical-interactions between various ions, physico-chemical characteristics of wastes, etc. among others that work to detoxify the substrates. The efficiency of removal of heavy metals ($_r$) for different metals ranged as: Cd (37.2–58.2%), Cr (57.0-74.3%), Cu (67.3–79.8%), Ni (74.7–81.9%), Pb (78.8–83.4%) and Zn (71.2–77.4%). Removal efficiencies of reactors for individual heavy metals were also obtained: Cd [R3 > R1 > R4 > R2 > R6 > R5], Cr [R4 > R2 > R3 > R5 > R6 > R1], Cu [R3 > R2 > R4 > R5 > R6 > R1], Ni [R3 > R2 > R4 > R6 > R5 > R1], Pb [R3 > R4 > R2 > R6 > R5 > R1] and Zn [R6 > R2 > R5 > R4 > R1 > R3]. Figure 1 shows decrease in concentration of heavy metals in different reactors during the course of this study. The results obtained were significant (P 0.05) and can be correlated with the findings of Suthar et al. (2014) whose study concluded that the increase in proportion of PMS resulted in effective removal of heavy metals, especially Cu and Pb. Yuvaraj et al. (2020) made use of two epigeic earthworm species *E. eugeniae* and *P. excavates* to bio-stabilize textile mill wastewater sludge and reported that the treatment combination (PMS: CD = 1:1) removed heavy metals up to a significant level. Gupta et al. (2007) stabilized water hyacinth using cow dung and significantly observed reduction of Pb (42.7–72.4%), Cd (20.8–58.1%) and Cu (26.9–49.1%) in the final vermicompost. The organic matter breakdown results in release of soluble fractions of heavy metals from substrates (Suthar and Singh, 2008). In this study, the rate of heavy metal removal was of the order: Pb > Ni > Zn > Cu > Cr > Cd. This reduction in heavy metal concentrations is also dependent on the assimilation/ accumulation and excretion of heavy metals through the gut of earthworms. The relationship of reduction of heavy metal concentrations with load of heavy metals in earthworm tissues was also examined by linear regression analysis (Table 3). The reduction of metals and genotoxicity in the final vermistabilized product is indicative of the capability of earthworms and the overall process of vermitechnology in cleaning up of industrial wastes.

Table 1

Parameters	Cd ($mg.kg^{-1}$)	Cr ($mg.kg^{-1}$)	Cu ($mg.kg^{-1}$)	Ni ($mg.kg^{-1}$)	Pb ($mg.kg^{-1}$)	Zn ($mg.kg^{-1}$)
Initial Substrate						
R1 [CD (100%)]	10.53 ± 0.02	133.7 ± 0.4	117.8 ± 0.3	13.66 ± 0.04	63.6 ± 0.3	215.92 ± 0.6
R2 [PMS:CD(1:2)]	14.76 ± 0.04	119.4 ± 0.5	125.6 ± 0.4	15.73 ± 0.08	71.5 ± 0.7	267.56 ± 0.3
R3 [PMS:CD(1:1)]	18.64 ± 0.03	141.8 ± 0.3	137.2 ± 0.6	17.56 ± 0.04	74.2 ± 0.4	317.83 ± 0.6
R4 [PMS:TW:CD(1:1:1)]	16.38 ± 0.02	139.5 ± 0.2	133.4 ± 0.4	16.88 ± 0.06	72.3 ± 0.5	292.67 ± 0.4
R5 [PMS:TW:CD(1:2:1)]	11.97 ± 0.03	134.5 ± 0.3	120.2 ± 0.5	13.98 ± 0.02	65.8 ± 0.4	239.53 ± 0.8
R6 [PMS:TW:CD(2:1:1)]	13.53 ± 0.04	136.8 ± 0.6	122.6 ± 0.3	14.43 ± 0.05	66.5 ± 0.8	255.43 ± 0.5
Final Substrate						
R1 [CD (100%)]	4.93 ± 0.003	57.5 ± 0.6	38.53 ± 0.2	3.45 ± 0.024	13.43 ± 0.4	59.28 ± 0.4
R2 [PMS:CD(1:2)]	7.65 ± 0.002	31.8 ± 0.9	29.13 ± 0.1	2.98 ± 0.001	13.08 ± 0.2	61.85 ± 0.6
R3 [PMS:CD(1:1)]	7.79 ± 0.001	39.4 ± 0.4	27.68 ± 0.4	3.18 ± 0.002	12.26 ± 0.3	91.60 ± 0.4
R4 [PMS:TW:CD(1:1:1)]	7.93 ± 0.005	35.8 ± 0.5	32.49 ± 0.3	3.26 ± 0.003	12.73 ± 0.2	78.54 ± 0.5
R5 [PMS:TW:CD(1:2:1)]	7.51 ± 0.001	48.4 ± 0.9	36.45 ± 0.2	3.34 ± 0.005	13.52 ± 0.5	63.45 ± 0.3
R6 [PMS:TW:CD(2:1:1)]	7.44 ± 0.002	54.8 ± 0.4	38.73 ± 0.4	3.28 ± 0.003	12.48 ± 0.2	57.54 ± 0.4

Changes in heavy metal concentrations in different reactors during bioremediation of substrates (Mean ± SD; n = 3). SD = Standard deviation, mean values indicate that difference between reactors is statistically different (ANOVA; Tukey’s t-test, p 0.05).

Table 2

Heavy metal concentrations in earthworm tissues						
Reactors	**Cd ($mg.kg^{-1}$)**	**Cr ($mg.kg^{-1}$)**	**Cu ($mg.kg^{-1}$)**	**Ni ($mg.kg^{-1}$)**	**Pb ($mg.kg^{-1}$)**	**Zn ($mg.kg^{-1}$)**
R1 [CD (100%)]	2.92 ± 0.01	23.54 ± 0.4	9.75 ± 0.2	3.76 ± 0.03	9.43 ± 0.02	25.78 ± 0.8
R2 [PMS:CD(1:2)]	2.79 ± 0.02	24.77 ± 0.8	10.68 ± 0.8	3.81 ± 0.02	8.96 ± 0.02	27.35 ± 0.5
R3 [PMS:CD(1:1)]	3.24 ± 0.04	28.76 ± 0.7	11.25 ± 0.3	3.99 ± 0.05	8.92 ± 0.04	28.81 ± 0.4
R4 [PMS:TW:CD(1:1:1)]	3.08 ± 0.05	27.58 ± 0.3	11.56 ± 0.3	3.92 ± 0.05	8.76 ± 0.01	29.72 ± 0.4
R5 [PMS:TW:CD(1:2:1)]	2.94 ± 0.03	24.32 ± 0.4	9.72 ± 0.5	3.61 ± 0.03	8.83 ± 0.03	23.12 ± 0.3
R6 [PMS:TW:CD(2:1:1)]	2.86 ± 0.01	23.92 ± 0.5	9.53 ± 0.6	3.52 ± 0.08	8.79 ± 0.04	26.53 ± 0.5
Bioaccumulation Factors (BAFs) for metals						
Reactors	**BAF_{Cd}**	**BAF_{Cr}**	**BAF_{Cu}**	**BAF_{Ni}**	**BAF_{Pb}**	**BAF_{Zn}**
R1 [CD (100%)]	0.27 ± 0.002	0.17 ± 0.004	0.083 ± 0.001	0.27 ± 0.001	0.15 ± 0.001	0.119 ± 0.002
R2 [PMS:CD(1:2)]	0.19 ± 0.001	0.21 ± 0.003	0.085 ± 0.002	0.24 ± 0.003	0.12 ± 0.001	0.102 ± 0.003
R3 [PMS:CD(1:1)]	0.17 ± 0.001	0.20 ± 0.001	0.081 ± 0.004	0.22 ± 0.004	0.12 ± 0.005	0.090 ± 0.001
R4 [PMS:TW:CD(1:1:1)]	0.18 ± 0.004	0.19 ± 0.005	0.086 ± 0.003	0.23 ± 0.001	0.12 ± 0.004	0.101 ± 0.002
R5 [PMS:TW:CD(1:2:1)]	0.24 ± 0.006	0.18 ± 0.003	0.080 ± 0.006	0.25 ± 0.003	0.13 ± 0.002	0.096 ± 0.004
R6 [PMS:TW:CD(2:1:1)]	0.21 ± 0.003	0.17 ± 0.004	0.077 ± 0.005	0.24 ± 0.005	0.13 ± 0.003	0.103 ± 0.006

Bioavailability of heavy metal in tissues of earthworms in different reactors and bioaccumulation factor (BAF) (Mean ± SD, n = 3). SD = Standard deviation, mean values indicate that difference between reactors is statistically different (ANOVA; Tukey's t-test, p   0.05).

Table 3

Linear regression analysis	R^2	P-value
Loss of heavy metals and load of heavy metals in earthworm tissue		
$Cd_{removal} = -24.7642 + 10.7204\ Cd_{earthworm}$	0.58	**
$Cr_{removal} = -36.4782 + 4.9504\ Cr_{earthworm}$	0.92	***
$Cu_{removal} = -38.0901 + 12.5193\ Cu_{earthworm}$	0.84	***
$Ni_{removal} = -17.0926 + 7.7534\ Ni_{earthworm}$	0.66	**
$Pb_{removal} = +139.4968 – 9.3235\ Pb_{earthworm}$	0.25	**
$Zn_{removal} = -30.7809 + 8.4394\ Zn_{earthworm}$	0.59	**
Loss of heavy metals and BAF		
$Cd_{removal} = +17.3067 – 48.6351\ Cd_{BAF}$	0.67	**
$Cr_{removal} = 13.46 + 408.25\ Cr_{BAF}$	0.36	**
$Cu_{removal} = -18.9142 + 1356.25\ Cu_{BAF}$	0.15	**
$Ni_{removal} = 33.6876 – 89.2247\ Ni_{BAF}$	0.81	***
$Pb_{removal} = 100.2446 – 344.2439\ Pb_{BAF}$	0.76	***
$Zn_{removal} = 396.9294 – 1972.0071\ Zn_{BAF}$	0.55	**

The relationship of loss of heavy metal concentrations with both bioavailability of heavy metals in earthworm tissues and BAF, obtained by linear regression analysis.

**Significant (P 0.05).

***Significant (P 0.001).

On the contrary, increase in heavy metal concentrations were also reported by previous studies. Vig et al. (2011) used *Eisenia fetida* to bioremediate tannery sludge, but, reported 2.6–13.4% and 4.6–17.3% increase in concentrations of Mn and Zn respectively. This increase was due to increase in loss of carbon content due to mineralization. Li et al. (2009) studied the transition changes in Cu and Zn contents by passing pig manure through the gut of earthworms and observed 1.2–3.4 folds increase in Cu content and 1.3–2.5 folds increase in Zn in the end-product. The study signified this increase to the affinity of Cu and Zn to Fe and Mn oxides, which may not have significantly changed after transit through earthworm gut. Bioavailability of metals, therefore, depend upon different mechanisms such as bioaccumulation (accumulation of heavy metals in inoculated worms) (Suthar and Singh, 2008), leaching of heavy metals from sludge during bioremediation and adsorption of heavy metals on waste surface fractions (Wang et al., 2013a).

3.2. Bioavailability of heavy metals in tissues of earthworms and BAFs

The reduction of heavy metal concentrations in different reactors clearly suggests the role and bioaccumulating ability of *E. fetida* in the bioconversion process. The concentrations of heavy metals in tissues of earthworms were statistically significant (P   0.05) for Cd (ANOVA; F = 128.61, P < 0.0001), Cr (ANOVA; F = 177.26, P < 0.0001), Cu (ANOVA; F = 132.37, P < 0.05), Ni (ANOVA; F = 229.68, P   0.0001), Pb (ANOVA; F = 323.24, P < 0.0001) and Zn (ANOVA; F = 221.24, P < 0.0001). The concentrations of heavy metals in tissues of earthworms ranged as: Cd [2.79 ± 0.02 (R2) to 3.24 ± 0.04 $mg.kg^{-1}$ (R3)], Cr [23.54 ± 0.4 (R1) to 28.76 ± 0.8 $mg.kg^{-1}$ (R3)], Cu [9.53 ± 0.6 (R6) to 11.56 ± 0.3 $mg.kg^{-1}$ (R4)], Ni [3.52 ± 0.08 (R6) to 3.99 ± 0.05 $mg.kg^{-1}$ (R3)], Pb [8.76 ± 0.01 (R4) to 9.43 ± 0.02 $mg.kg^{-1}$ (R1)] and Zn [23.12 ± 0.3 (R5) to 29.72 ± 0.4 $mg.kg^{-1}$ (R4)], as shown in Table 2. The difference among the reactors for concentration values of heavy metals in tissues of earthworms may have been due to changes in physico-chemical characteristics of substrates. The bioaccumulation of heavy metals in the tissues of earthworms for different reactors can be arranged in descending order as: Cd [R3 > R4 > R5 > R1 > R6 > R2], Cr [R3 > R4 > R2 > R5 > R6 > R1], Cu [R4 > R3 > R2 > R1 > R5 > R6], Ni [R3 > R4 > R2 > R1 > R5 > R6], Pb [R1 > R2 > R3 > R5 > R6 > R4] and Zn [R4 > R3 > R2 > R6 > R1 > R5]. Heavy metals such as Co, Cu, Cd and Zn have high affinity to metal binding protein called metallothionein, which renders them biologically inactive (Samal et al., 2019). The rate of accumulation of heavy metals in tissues of earthworms largely depends on the metal pollution level of the environment into which the earthworms are fed. If the metal pollution level would be high, then earthworms would not be able to accumulate the heavy metals in their gut and will excrete them out at a faster rate. This is solely done to maintain equilibrium conditions in their physiological metabolism. The defence mechanism of *Eisenia* species is prolific at cellular levels, as a result of which they are strong bioaccumulators of toxic metals (Suleiman et al., 2017) and by which the bioremediation of metals can be achieved sustainably through vermicomposting. The bioaccumulation of heavy metals in the tissues of earthworms has also been in well documented in published literature (Suthar and Singh, 2008; Azizi et al., 2013; Srivastava et al., 2005).

The bioaccumulation factors (BAFs) of heavy metals are shown in Table 2. The values of BAF obtained were statistically significant for Cd (ANOVA; F = 154.98, P < 0.0001), Cr (ANOVA; F = 34.21, P < 0.0001), Cu (ANOVA; F = 325.54, P < 0.05), Ni (ANOVA; F = 255.78, P   0.0001), Pb (ANOVA; F = 128.87, P < 0.0001) and Zn (ANOVA; F = 344.79, P < 0.0001). The BAF for different heavy metals ranged as: Cd [0.17 ± 0.001 (R3) to 0.27 ± 0.002 $mg.kg^{-1}$ (R1)], Cr [0.17 ± 0.004 (R1, R6) to 0.21 ± 0.003 $mg.kg^{-1}$ (R2)], Cu [0.077 ± 0.001 (R6) to 0.086 ± 0.004 $mg.kg^{-1}$ (R4)], Ni [0.22 ± 0.004 (R3) to 0.27 ± 0.001 $mg.kg^{-1}$ (R1)], Pb [0.12 ± 0.001 (R2) to 0.15 ± 0.001 $mg.kg^{-1}$ (R1)] and Zn [0.090 ± 0.001 (R3) to 0.119 ± 0.002 $mg.kg^{-1}$ (R1)]. The BAF for heavy metals can be arranged in the following order: Ni > Cd > Cr > Pb > Zn > Cu. The relationship of reduction of heavy metal concentrations with bioaccumulation factors (BAFs) was also determined by linear regression analysis (Table 3). The results obtained are similar to that obtained by Suthar et al. (2014), thus, support his hypothesis which suggested that leaching is the main mechanism of loss of heavy metals in this study rather than absorption by worms. Previous studies have also used BAF in tissues of earthworms for evaluation of toxicity in bioremediation of substrates. (Mountouris et al., 2002; Wang et al., 2013a; Yuvaraj et al., 2018a).

3.3. Changes in pH, EC, TOC, TN, TP, TK level and C/N ratio during vermiremediation of substrate

Vermistabilization of PMS and TW resulted in increase of nutrient contents beneficial for the soil and growth of plants. The difference among the reactors was statistically significant: pH(ANOVA; F = 13.43, P 0.0001), EC (ANOVA; F = 263.56, P 0.0001), TOC (ANOVA; F = 344.71 P 0.0001), TN (ANOVA; F = 113.89, P 0.0001), TP (ANOVA; F = 166.26, P 0.0001), TK (ANOVA; F = 49.54, P 0.0001) and C/N ratio (ANOVA; F = 534.75, P 0.0001). The physico-chemical characteristics of both initial and final substrate are given in Table 4 and ranged as: pH[7.32 ± 0.50 (R1) to 7.65 ± 0.50 (R6)], EC [3.79 ± 0.20 (R1) to 5.25 ± 0.10 (R6)], TOC [38.79 ± 0.75 (R3) to 42.63 ± 0.50 (R6)], TN [1.24 ± 0.03 (R6) to 1.87 ± 0.01 (R3)], TP [0.68 ± 0.01 (R6) to 1.08 ± 0.01 (R3)], TK [0.28 ± 0.01 (R2) to 0.49 ± 0.02 (R6)] and C/N ratio [20.74 ± 0.90 (R3) to 34.37 ± 0.33 (R6)]. Previous researchers have also documented the enrichment of nutrients by the process of vermistabilization (Yuvaraj et al., 2020; Kaur et al., 2010). Formation of organic acids and other intermediate metabolic products in the gut of earthworms with the help of mucus and enzymic activities would have accelerated the degradation of organic matter (TOC) and resulted in simultaneous decrease of pH and increase of EC (Badhwar et al., 2020; Lim et al., 2011). Maximum reduction of TOC content and increase of earthworm population in R3 (PMS: CD = 1:1) indicates the efficacy of good vermistabilization process. The earthworms have the ability to eliminate the nitrogenous substances from the substrate which would have resulted increase of TN content (Suthar et al., 2017). The increase in TP content is due to the ability of earthworms to convert the insoluble phosphorus to soluble phosphorus in the process of vermitechnology (Fu et al., 2015). High TK content in R5 is attributed to the TK content of tea waste. C/N ratio of all the reactor substrates decreased significantly, which indicates efficient recovery of wastes and promotes the sustainable cycle of transformation of waste to resource.

Table 4

Parameters	pH	EC ($mS.cm^{-1}$)	TOC (%)	TN (%)	TP (%)	TK (%)	C/N ratio
Initial Substrate							
R1 [CD (100%)]	8.13 ± 0.50	1.48 ± 0.05	43.26 ± 0.60	1.17 ± 0.01	0.62 ± 0.02	0.29 ± 0.01	36.97 ± 0.20
R2 [PMS:CD(1:2)]	8.46 ± 0.30	1.67 ± 0.05	44.85 ± 0.25	1.28 ± 0.03	0.58 ± 0.01	0.17 ± 0.01	35.03 ± 0.55
R3 [PMS:CD(1:1)]	8.63 ± 0.50	1.96 ± 0.10	46.65 ± 0.45	1.41 ± 0.01	0.65 ± 0.04	0.15 ± 0.01	33.08 ± 0.30
R4 [PMS:TW:CD(1:1:1)]	8.75 ± 0.50	2.08 ± 0.05	47.90 ± 0.20	1.33 ± 0.01	0.51 ± 0.03	0.21 ± 0.03	36.01 ± 0.64
R5 [PMS:TW:CD(1:2:1)]	8.83 ± 0.10	2.19 ± 0.15	48.72 ± 0.15	0.99 ± 0.02	0.43 ± 0.01	0.25 ± 0.01	49.21 ± 0.36
R6 [PMS:TW:CD(2:1:1)]	8.92 ± 0.20	2.27 ± 0.10	49.51 ± 0.10	0.94 ± 0.01	0.39 ± 0.02	0.31 ± 0.02	52.67 ± 0.27
Final Substrate							
R1 [CD (100%)]	7.32 ± 0.50	3.79 ± 0.20	39.96 ± 0.40	1.65 ± 0.02	0.82 ± 0.02	0.41 ± 0.03	24.21 ± 0.35
R2 [PMS:CD(1:2)]	7.42 ± 0.20	4.23 ± 0.15	39.12 ± 0.55	1.79 ± 0.03	0.95 ± 0.02	0.28 ± 0.01	21.85 ± 0.60
R3 [PMS:CD(1:1)]	7.48 ± 0.40	4.38 ± 0.05	38.79 ± 0.75	1.87 ± 0.01	1.08 ± 0.01	0.34 ± 0.01	20.74 ± 0.90
R4 [PMS:TW:CD(1:1:1)]	7.53 ± 0.30	4.65 ± 0.10	39.43 ± 0.30	1.68 ± 0.05	0.89 ± 0.04	0.30 ± 0.04	23.47 ± 0.45
R5 [PMS:TW:CD(1:2:1)]	7.62 ± 0.50	5.12 ± 0.05	40.84 ± 0.15	1.38 ± 0.02	0.75 ± 0.02	0.49 ± 0.01	29.59 ± 0.25
R6 [PMS:TW:CD(2:1:1)]	7.65 ± 0.50	5.25 ± 0.10	42.63 ± 0.50	1.24 ± 0.03	0.68 ± 0.01	0.46 ± 0.02	34.37 ± 0.33

Changes in physico-chemical parameters of reactors during bioremediation process (Mean ± SD, n = 3). SD = Standard deviation, mean values indicate that difference between reactors is statistically different (ANOVA; Tukey’s t-test, p 0.05).

3.4. Survival of earthworms, biomass changes, cocoon production and mortality rate

Earthworms are known to improve the quality of soil by adding organically bound nutrients (C, N, P, K, S, etc.) and detoxify heavy metals in the soil environment. Bioremediation occurs due to unique bioaccumulation mechanism of earthworms (Maity et al., 2009) and their strong metabolic system where diverse intestinal microflora, enzymic activities and chloragocyte cells function accordingly in the remediation of industrial sludges (Srivastava et al., 2005). The changes in the biomass of earthworms, reproduction rate and rate of mortality can be directly correlated with the feed materials (Table 5). The difference in the biomass of earthworms was statistically significant (P 0.05) in different reactors and was in the order: R3 (62.0%) > R2 (59.8%) > R4 (55.9%) > R5 (52.8%) > R1 (51.3%) > R6 (50.3%). Maximum mortality rate (19%) and minimum reproduction rate (6.1 ± 0.2 cocoon.worm^{-1}) was seen in R6 [PMS: TW: CD (2:1:1)] which clearly indicates the unsuitability of higher proportions of TW in feed mixtures, although lesser proportions can be used for the degradation of substrates as seen in R5 and R4. Conversely, least mortality rate (2%) and maximum reproduction rate (7.8 ± 0.1 cocoon.worm^{-}1) was seen in R3 [PMS: CD (1:1)] which showed the best earthworm survival rate among all the reactors and indicates its enhanced applicability in vermiremoval process. The other important factors in the rise of earthworm population in vermistabilized waste substrates include cocoon viability, hatchling success and the survival rate of juveniles, etc. The production of cocoons was highest in R3 followed by R4, R2, R5, R1 and R6 (Table 5). Suthar et al. (2014) stated that the production of cocoons depend upon various factors such as the toxicity of wastes, palatability of food, quality of waste mixtures and the overall environmental conditions required for the successful bioremediation of industrial sludges. The rate of cocoon production (cocoon.worm^{-1}) and juvenile production (juvenile.worm^{-1}) also determines the overall reproduction rate of earthworms in the process of vermitechnology.

Table 5

Reactors	Individual biomass of earthworms(g)		Biomass change (%)	Cocoon Production (n)	Reproduction rate [a]	Mortality rate (%)
	Initial	Final				
R1 [CD (100%)]	9.56 ± 0.14	19.63 ± 0.85	+ 51.3	42.00 ± 1.00	6.8 ± 0.3	12
R2 [PMS:CD(1:2)]	9.14 ± 0.44	22.78 ± 0.55	+ 59.8	49.00 ± 2.00	7.2 ± 0.1	6
R3 [PMS:CD(1:1)]	9.22 ± 0.57	24.26 ± 0.36	+ 62.0	63.00 ± 1.50	7.8 ± 0.2	2
R4 [PMS:TW:CD(1:1:1)]	9.41 ± 0.19	21.35 ± 0.25	+ 55.9	51.50 ± 1.50	7.0 ± 0.1	6
R5 [PMS:TW:CD(1:2:1)]	9.05 ± 0.28	19.18 ± 0.15	+ 52.8	44.00 ± 3.00	6.4 ± 0.3	13
R6 [PMS:TW:CD(2:1:1)]	9.32 ± 0.09	18.76 ± 0.28	+ 50.3	40.50 ± 1.50	6.1 ± 0.2	19

Biological properties of earthworms *E.fetida* in different reactors during bioremediation process (Mean ± SD, n = 3). SD = Standard deviation, mean values indicate that difference between reactors is statistically different (ANOVA; Tukey’s t-test, p 0.05).

[a](cocoon $worm^{-1}$)

4. Conclusion

The reduction in heavy metal concentrations in final substrates indicates suitability of vermiremoval process and bioaccumulation ability of *E.fetida*. BAFs of different reactors clearly depict the successful transition of heavy metals from substrates to tissues of earthworms. The cocoon production along with change in biomass determines the suitability of PMS and TW as feed materials. *Eisenia fetida* showed good growth patterns and waste combination PMS: TW: CD (1:1:1) also proved to be useful for reduction of hazardous wastes like PMS. TW should be used in equal or lesser proportions of PMS or CD for effective vermiremediation of wastes.

Declarations

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Ethical Approval

All applicable international, national, and /or institutional guidelines for the care and use of animals were followed.

Consent to Participate

The authors mutually give their consent to participate in peer review process of this journal.

Consent to Publish

The authors give their consent to publish this work in Environmental Science and Pollution Research.

Authors Contributions

Vinay Kumar Badhwar : Formal analysis; Investigation; Methodology; Project administration; Validation; Visualization; Roles/Writing - original draft, review & editing

Sukhwinderpal Singh: Project administration; Resources; Software; Supervision; Validation

Balihar Singh: Project administration; Validation, visualization, review

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Competing Interests

The authors declare on mutual understanding that there is no conflict of interest, whatsoever, in this work.

Availability of data and materials

Refer to Section 2.1, 2.3, 2.4 and 2.6.

References

1. Abbiramy, K.S., Ross, P.R., Paramanandham, J., 2015. Degradation of Tea Factory Waste by Mushroom Cultivation and Vermicomposting. J Environ. Science & Engg., 57:126-130.
2. Azizi, A.B., Lim, M.P.M., Noor, N.A., 2013. Vermiremoval of heavy metal in sewage sludge by utilizing *Lumbricyus rubellus*. Ecotoxicol.Environ.Saf., 90: 13–20.
3. Badhwar, V.K., Singh, C., 2022. Vermicomposting of textile mill sludge employing Eisenia fetida: Role of cow dung and tea waste amendments. Environmental Science and Pollution Research, 29:19823–19834. https://doi.org/10.1007/s11356-021-17185-z.
4. Bajpai, P., 2015. Management of Pulp and Paper Mill Waste. Springer, New York.
5. Bhat, S.A., Singh, J., Vig, A.P., 2016b. Genotoxicity reduction in bagasse waste of sugar industry by earthworm technology. Springerplus 5, 1186.

6. Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Nahmani, J., Lavelle, P., 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. Soil Biol. Biochem. 36–98.
7. Fu, X., Huang, K., Cui, G., Chen, X., Li, F., Zhang, X., Li, F., 2015. Dynamics of bacterial and eukaryotic community associated with stability during vermicomposting of pelletized dewatered sludge. Int. Biodeterior. Biodegrad. 104, 452–459. https://doi. org/10.1016/j.ibiod.2015.07.015.
8. Goswami, L., Pratihar, S., Dasgupta, S., Bhattacharyya, P., Mudoi, P., Bora, J., Bhattacharya, S.S., Kim, K.H., 2016. Exploring metal Bioremediation and accumulation potential during vermicomposting of Tea factory coal ash: sequential extraction and fluorescence probe analysis. Sci. Rep. 6, 30402. https://doi.org/10.1038/srep30402.
9. Gupta, R., Mutiyar, P.K., Rawat, N.K., Saini, M.S., Garg, V.K., 2007. Development of a water hyacinth based vermireactor using an epigeic earthworm *Eisenia fetida*. Bioresour. Technol. 98: 2605–2610.
10. http://cppri.res.in/sites/default/files/annual%20report%2017%20-%2018.pdf
11. https://unstats.un.org/unsd/envaccounting/londongroup/meeting20/LG20_7_4.pdf
12. http://www.fao.org/3/BU642en/bu642en.pdf
13. http://www.teaboard.gov.in/pdf/Website_World_Tea_pdf7894.pdf
14. Humphries EC., 1956. Mineral components and ash analysis. Modern Methods of Plant Analysis. Springer, 468-502.
15. Jackson, M.L., 1955. Soil Chemical Analysis. Constable & Co., Ltd., London.
16. Karwal, K., Kaushik, A., 2020. Co-composting and vermicomposting of coal fly-ash with press mud: Changes in nutrients, micro-nutrients and enzyme activities. Environmental Technology & Innovation 18, 100708. https://doi.org/10.1016/j.eti.2020.100708.
17. Kaur, A., Singh, J., Vig, A.P., Dhaliwal, S.S., Rup, P.J., 2010. Cocomposting with and without *Eisenia fetida* for conversion of toxic paper mill sludge to a soil conditioner. Bioresour. Technol., 101: 8192 8198.
18. Koolivand, A., Saeedi, R., Coulon, F., Kumar, V., Villaseñor, J., Asghari, F., Hesampoor, F., 2020. Bioremediation of petroleum hydrocarbons by vermicomposting process bioaugmentated with indigenous bacterial consortium isolated from petroleum oily sludge. Ecotoxicology and Environmental Safety 198, 110645. https://doi.org/10.1016/j.ecoenv.2020.110645.
19. Li, L., Wu, J., Tian, G., Xu, Z., 2009. Effect of the transit through the gut of earthworm (*Eisenia fetida*) on fractionation of Cu and Zn in pig manure, J. Hazard. Mater., 167: 634–640.
20. Lim, P.N., Wu, T.Y., Sim, E.Y.S., Lim, S.L., 2011. The potential of reuse of soyabean husk as feedstock of *Eudrilus eugeniae* in vermicomposting. J.Sci.FoodAgric., 91: 2637–2642.
21. Lv, B., Xing, M., Yang, J., 2016. Speciation and transformation of heavy metals during vermicomposting of animal manure. Bioresour. Technol., 209, 397–401.
22. Maity, S., Bhattacharya, S., Chaudhury, S., 2009. Metallothionein response in earthworms *Lampito mauritii* (Kinberg) exposed to fly ash. Chemosphere 77 (3): 319-324.

http://www.sciencedirect.com/science/article/pii/S0045653509008066.

23. Mountouris, J.E., Norey, C.G., Morgan, A.J., Kay, J., 2002. Bioconcentrations of heavy metals in aquatic environment: the importance of bioavailability. Mar. Pollut. Bull., 44: 1136–1141.
24. Negi, R., Suthar, S., 2013. Vermistabilization of paper mill wastewater sludge using *Eisenia fetida*. Bioresour. Technol. 128, 193–198.
25. Pappu, A., Saxena, M., Asolekar, S.R., 2007. Solid wastes generation in India and their recycling potential in building materials. Build. Environ., 42: 2311–2320.
26. Paul, S., Das, S., Raul, P., Bhattacharya, S.S., 2018. Vermi-sanitization of toxic silk industry waste employing *Eisenia fetida* and *Eudrilus eugeniae*: substrate compatibility, nutrient enrichment and metal accumulation dynamics. Bioresour. Technol. 266: 267-274.
27. Pedersen, M.B., van Gestel, C.A.M., 2001. Toxicity of copper to collembolan *Folsomia fimeteria* in relation to the age of soil contamination.Ecotoxicol.Environ.Saf49, 54–59.
28. Ravindran, B., Wong, J.W.C., Selvam, A., Sekaran, G., 2016. Influence of microbial diversity and plant growth hormones in compost and vermicompost from fermented tannery waste. Bioresour. Technol. 217, 200–204.
29. Sahariah, B., Goswami, L., Kim, K.H., Bhattacharyya, P., Bhattacharyya, S.S., 2015. Metal remediation and biodegradation potential of earthworm species on municipal solid waste: A parallel analysis between *Metaphire posthuma* and *Eisenia fetida*. Bioresour. Technol. 180, 230–236.
30. Samal, K., Mohan, A.R., Chaudhary, N., Moulick, S., 2019. Application of vermitechnology in waste management: A review on mechanism and performance. J. Environ. Chem. Eng. 7, 103392. https://doi.org/10.1016/j.jece.2019.103392.
31. Soobhany, N., Mohee, R., Garg, V.N., 2015. Recovery of nutrient from Municipal Solid Waste by composting and vermicomposting using earthworm *Eudrilus eugeniae*. J. Environ. Chem. Eng. 3, 2931–2942.
32. Srivastava, R., Kumar, D., Gupta, S.K., 2005. Bioremediation of municipal sludge by vermitechnology and toxicity assessment by *Allium cepa*. Bioresour. Technol. 96, 1867–1871.
33. Suleiman, H., Rorat, A., Grobelak, A., Grosser, A., Milczarek M., Płytycz B., Kacprzak M., Vandenbulcke F., 2017. Determination of the performance of vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses in three earthworm species: *Eisenia fetida*, *Eisenia andrei* and *Dendrobaena veneta.* Bioresour. Technol. 241, 103–112.
34. Suthar, S., Singh, S., 2008. Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. Sci. Total Environ. 394, 237–244.
35. Suthar, S., Sajwan, P., Kumar, K., 2014. Bioremediation of heavy metals in wastewater sludge from paper and pulp industry using earthworm *Eisenia fetida*. Ecotoxicol. Environ. Saf. 109: 177-184.
36. Suthar, S., Pandey, B., Gusain, R., Gaur, R.Z., Kumar, K., 2017. Nutrient changes and biodynamics of *Eisenia fetida* during vermicomposting of water lettuce (*Pistia* sp.) biomass: a noxious weed of aquatic system. Environ. Sci. Pollut. Control Ser. 24, 199–207. https://doi.org/10.1007/s11356-016-7770-2.

37. Tandon, H.Z., 1993. Methods of Analysis of Soils, Plant, Water and Fertilizers. Fertilizer Development and Consultation Organization, New Delhi.
38. Vig, A.P., Singh, J., Wani, S.H., Dhaliwal, S.S., 2011. Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). Bioresour. Technol., 102: 7941–7945.
39. Wang, L., Zhang, Y., Lian, J., Chao, J., Gao, Y., Yang, F., Zhang, L., 2013a. Impact of fly ash and phophatic rock on metal stabilization and bioavailability during sewage sludge vermicomposting. Bioresour. Technol., 136: 281–287.
40. World Bank, 2007. Environmental, Health, and Safety Guidelines for Pulp and Paper Mills. Draft Technical Document. Environment and Social Development Department, International Finance Corporation, Washington, DC.
41. Yuvaraj, A., Karmegam, N., Thangaraj, R., 2018a. Vermistabilization of paper mill sludge by an epigeic earthworm *Perionyx excavatus*: Mitigation strategies for sustainable environmental management. Ecol. Eng., 120: 187–197. https://doi.org/10.1016/j.ecoleng.2018.06.008.
42. Yuvaraj A, Karmegam N, Tripathi S, Kannan S, Thangaraj R., 2020. Environment-friendly management of textile mill wastewater sludge using epigeic earthworms: Bioaccumulation of heavy metals and metallothionein production. J Environ Manage., 254 (2020) 109813. https://doi.org/10.1016/j.jenvman.2019.109813.
43. Zhong W-S, et al., 2015. Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry, Journal of Food and Drug Analysis. http://dx.doi.org/10.1016/j.jfda.2015.04.010.

Figures

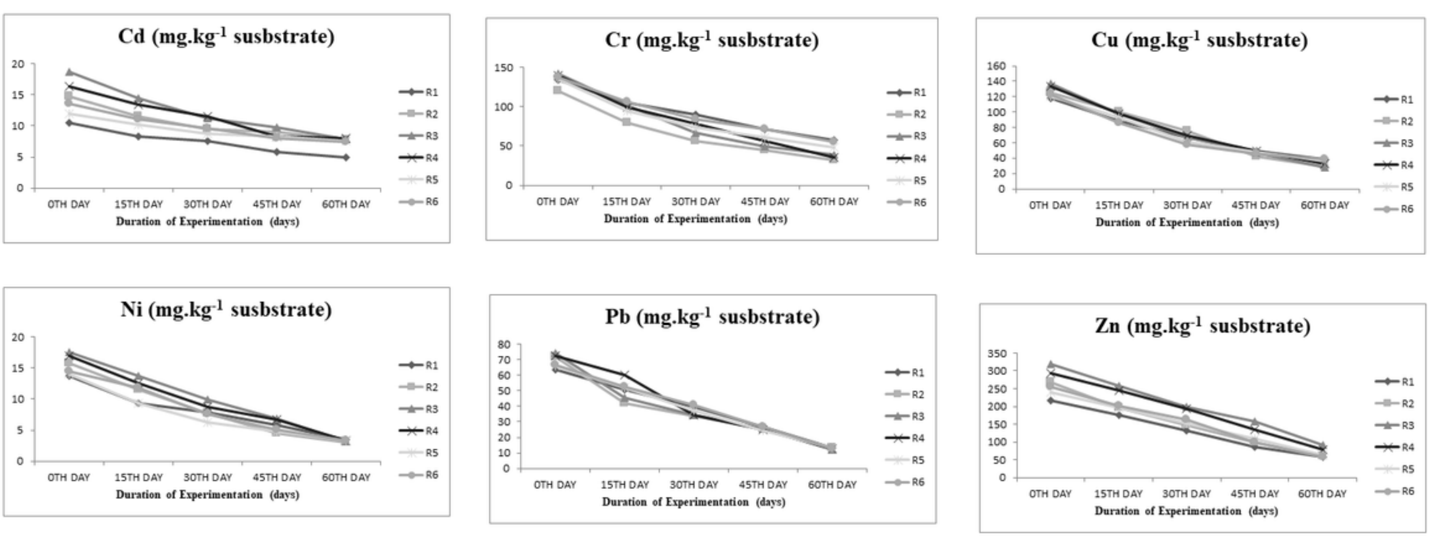

Figure 1

Vermiremediation of substrates due to loss of heavy metals in reactors (R1, R2, R3, R4, R5, R6).