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ORIGINAL RESEARCH

# Transformation of nutrients and heavy metals during vermicomposting of the invasive green weed *Salvinia natans* using *Eisenia fetida*

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**Abstract**

*Purpose* Effective vermicomposting of *Salvinia natans* is a good alternative for protecting wetlands damaged by the weed due to the vast potential for use of the product vermicompost in agriculture, but the presence of toxic metals in the weeds may deter the usage.

*Methods* Research was carried out on the physico-chemical and biological characteristics as well as bioavailability and leachability of nutrients and heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during vermicomposting of *S. natans* mixed with cattle manure and sawdust in five different combinations [trial 1 (eight *S. natans*: one cattle manure: one sawdust), trial 2 (seven *S. natans*: two cattle manure: one sawdust), trial 3 (six *S. natans*: three cattle manure: one sawdust), trial 4 (five *S. natans*: four cattle manure: one sawdust) and trial 5 (ten *S. natans*: 0 cattle manure: 0 sawdust)] for 45 days with *Eisenia fetida* earthworm.

*Results* The highest growth of earthworms was in trial 4, having the highest (40 %) cattle manure. Trial 4 also indicated the highest reduction of volatile solids (38.6 %) and soluble BOD (82.3 %). The water-soluble forms of all nutrients were increased significantly. The highly bioavailable water-soluble and DTPA-extractable forms of heavy metals were reduced favourably after the process. The toxicity characteristic leaching procedure (TCLP) test indicated that leachable heavy metals of the vermicomposts were also reduced and were within the threshold limits for agricultural applications.

*Conclusions* *Eisenia fetida* was very effective for reduction of bioavailable and leachable forms of selected heavy metals, and the TCLP test confirmed that the vermicompost was not hazardous for soil applications. The potential of the earthworms to increase the available nutrients, but mitigate the metal toxicity during vermicomposting of *S. natans* will be useful for sustainable land renovation practices.

**Keywords** *Salvinia natans* · Vermicomposting · Nutrients · Heavy metals · Bioavailability · Leachability

## Introduction

The ecology of the wetlands is often damaged by proliferation of aquatic weeds in their water zones. Weeds interfere with the primary production of wetlands and impact severely on the biodiversity by out-competing native plants and degrading the habitat (DEC 2012). The floating water moss *Salvinia natans* is an invasive dominant colonizing aquatic fern species, which prevails over the aquatic ecosystem by its rapid growth and displacement of native plants that provide food and habitat for the native animals and waterfowl (Galka and Szmeja 2012). The growth and spread of *Salvinia natans* may be enormous to the extent of 45 % per day in nutrient-rich water bodies (Blackman 1960). The high viability of spores associated with rapid vegetative propagation is responsible for the spread of the weed producing vast thick mats and, with the impending global warming, the weed will create havoc to water bodies at the expense of submerged exotic plants in

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many parts of the world (Netten et al. 2010). The dense mats have a negative effect on the functioning and biodiversity of the freshwater ecosystems due to hampered photosynthesis, reduced gas exchange at the air–water interface and increased consumption of oxygen by microbial decomposition. The decayed dead weeds increase the nutrient level in the water, leading to the problem of eutrophication (Gupta et al. 2007). *S. natans* also covers vast free water zones of the Loktak Lake (24°25′–24°42′N; 93°46′–93°55′E) of Manipur, northeast India. The lake is the largest freshwater lake of the region and plays an important role in providing regional ecological and economic security by supporting valuable biodiversity or heterogeneity (Trisal and Manihar 2004).

Generation of quality composts through composting or vermicomposting of *S. natans* and subsequent land application of the composts can solve the problems of (1) uncontrolled growth of the weed in the water body and (2) soil infertility, thereby reducing the use of synthetic fertilizers (Singh and Kalamdhad 2013). The pile composting of *S. natans* has been investigated already by the authors (Singh et al. 2015). In case of vermicomposting, the earthworms ingest, grind and digest organic waste into vermicompost with relatively higher concentrations of plant nutrients, microbial population, soil enzymes and humic acids due to the joint action of earthworms and microorganisms (Khwairakpam and Bhargava 2009). Vermicomposting is a bio-oxidative process where the microorganisms, both in the gut of the earthworms and in the feedstock, are responsible for the biochemical degradation of the organic matter while the earthworms fragment the substrate, thereby increasing the surface area exposed to the microorganisms. Hence, the role of the earthworms is to directly or indirectly modify the physical and chemical properties of the substrates (Fornes et al. 2012).

*Salvinia natans* are highly effective in removing water pollutants and heavy metals and there is a possibility of the presence of heavy metals in their biomass. Therefore, the application of the resulting composts has the potential to cause adverse effects on the environment (Dhir and Srivastava 2011; Kumari and Tripathi 2014). High and excessive accumulation of heavy metals in the soil, their uptake by plants, successive accumulation in human tissues and bio-magnifications through the food chain are the main concerns for human health and environment (Wong and Selvam 2006; Iwegbue et al. 2007; Chiroma et al. 2012). The heavy metals exert toxic effects on soil microorganisms, resulting in the change of diversity, population and overall activity of the soil microbial communities (Ashraf and Ali 2007). The bio-available and leachable heavy metals, which are highly toxic due to their mobility, are of major concern to the environment (Liu et al. 2007). Bioavailable forms of heavy metals may be: (1) water

soluble that are the most biologically active and have the highest potential for contaminating the food chain as well as surface and groundwater (Iwegbue et al. 2007); (2) diethylenetriaminepentaacetic acid (DTPA) extractable that are also plant available metals in soil at regular or higher concentrations (Samuel et al. 2013). DTPA is a chelating agent that mimics plant uptake of heavy metals by extracting carbonate-bound and organically bound metal fractions in calcareous soils (Bragato et al. 1998; Fang and Wong 1999; Fuentes et al. 2006; Walter et al. 2006); and (3) leachable that are the mobile forms of the metals associated with the movement of organic and inorganic analytes present in liquid, solid and multi-phasic wastes and determined through toxicity characteristics leaching procedure (TCLP) tests (USEPA 1992).

Earthworms are reported to reduce the possible toxic effects of heavy metals by consuming them during physiological metabolism (Jain et al. 2004; Suthar et al. 2014). Much work has been carried out on the bioavailability of heavy metals during vermicomposting of weeds such as water hyacinth (Singh and Kalamdhad 2013), but none has been reported for *S. natans* weed. A number of earthworm species such as *Eisenia fetida, Eisenia andrei, Eudrilus eugeniae* and *Prionyx excavates* have been studied for their potential to degrade the organic mass, and the epigeic earthworm *E. fetida* was found to be the best species for vermicomposting because of its wide range of tolerance and capability of producing good vermicompost (Edwards 1998; Sinha et al. 2002; Suthar 2007). The objective of the present study was to determine the physico-chemical transformations and assess the bioavailability and leachability of heavy metals of the vermicompost during vermicomposting of *S. natans* blended with cattle manure and sawdust in different combinations.

## Materials and methods

### Feedstock materials

*Salvinia natans* was collected from the Loktak Lake near Thanga Village, Bishnupur District, Manipur, India, and brought to the lakeside by local boatmen. The biomass was then transported to the vermicompost laboratory of the Indian Institute of Technology Guwahati (IITG), India. Cattle manure was obtained from the dairy farm near the IITG campus, North Guwahati, and sawdust was purchased from the nearby saw mill at Amingao, North Guwahati. The *S. natans* biomass was prepared for vermicomposting through cutting/shredding (maximum size restricted to 10 mm to provide better aeration and moisture control) and uniform premixing with cattle manure and sawdust.

## Earthworms

The epigeic earthworm, *Eisenia fetida*, was selected for the experiment as it was reported to be effectively used worldwide for vermicomposting of organic wastes (Edwards 1998). The earthworms were brought from the Central Plantation Crops Research Institute (CPCRI), Indian Council of Agricultural Research, Kahikuchi, Guwahati, Assam. Perspex bin size of 450 × 300 × 450 mm was fabricated for culturing the earthworms in the laboratory. 16 holes (10 mm diameter) were drilled at equal spacing along the longer sides and at the bottom of the bin for aeration and drainage purposes. Before the addition of the culturing media and the earthworms, 10 cm-thick bedding for the earthworms was prepared from partially degraded chopped hay (about 50 mm), cattle manure, banana pulp (chopped about 50 mm) and tree leaves. The bedding was then watered to keep it moist to facilitate breathing of the earthworms. The earthworm species were then added with partially degraded cow dung as culturing media (Singh and Kalamdhad 2013).

## Experimental set up

The experiments were conducted in triplicate at room temperature in locally made curved shaped bamboo containers (reactor) of dimensions 150 mm top radius and 100 mm depth to facilitate better ventilation for the earthworms (Fig. 1). The reactors were designed for 2.5 kg of the substrates and approximately 100 g live weight of earthworms (200 nos. of clitellated and young non-
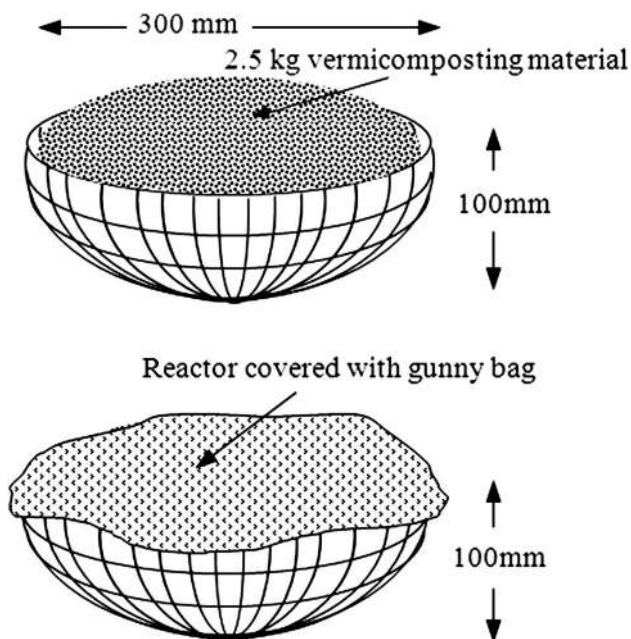


**Fig. 1** Diagram of a bamboo vermireactor (Sonowal et al. 2014)

clitellated *E. fetida*) was randomly picked from the perspex bin culture and introduced gently into the substrates from the top. The earthworm biomass weight was decided considering that earthworms can consume materials approximately half their body weight per day under favourable conditions (Haimi and Huhta 1986). Five trials were run in triplicate with the different mixtures of *S. natans*, cattle manure and sawdust as detailed below:

Trial 1 (T1): *S. natans* (2.0 kg) + cattle manure (0.25 kg) + sawdust (0.25 kg) + *E. fetida* (approx. 0.1 kg)

Trial 2 (T2): *S. natans* (1.75 kg) + cattle manure (0.50 kg) + sawdust (0.25 kg) + *E. fetida* (approx. 0.1 kg)

Trial 3 (T3): *S. natans* (1.50 kg) + cattle manure (0.75 kg) + sawdust (0.25 kg) + *E. fetida* (approx. 0.1 kg)

Trial 4 (T4): *S. natans* (1.25 kg) + cattle manure (1.00 kg) + sawdust (0.25 kg) + *E. fetida* (approx. 0.1 kg)

Trial 5 (T5): *S. natans* (2.5 kg) + cattle manure (0 kg) + sawdust (0 kg) + *E. fetida* (approx. 0.1 kg)

The optimum moisture content is one of the most important requirements for the earthworms throughout the vermicomposting process. Excess moisture content may create anaerobic conditions which may be fatal to the earthworms (Garg and Gupta 2011). All the containers were kept in the dark under identical ambient conditions (room temperature 25 ± 3 °C). The moisture level was maintained at about 50–60 % throughout the study period by periodic sprinkling of adequate quantity of tap (potable) water whenever required. To prevent moisture loss, the reactors were covered with gunny bags. The mixtures were manually turned at every 15th day to provide suitable aeration to the earthworms.

## Sampling and analysis of earthworm biomass and physico-chemical parameters

The earthworms and hatchings were separated by light separation and hand sorting method, and the earthworm biomass was weighed. The cocoons were sorted manually. Wet substrate (free of earthworms, hatchlings and cocoons) were collected from the reactors on day 0 (before inoculation of earthworms) and on the 15th, 30th and 45th day of the vermicomposting period after earthworm separation and stored immediately at 4 °C for biological analysis. Sub-samples were oven dried, ground to pass through a 0.2 mm sieve and stored in plastic bags for further analysis of the physico-chemical parameters.

Each sample was analysed for pH, electrical conductivity (EC) (from 1:10 w/v waste: water extract) and organic matter in terms of volatile solids (VS) (loss on

ignition at 550 °C for 2 h in muffle furnace). Total Kjel-dahl nitrogen (TKN) was analysed using the Kjeldahl method, ammoniacal nitrogen (NH$_4$-N) using KCl extrac-tion, total phosphorous (TP) and available phosphorus (AP) (by acid digest) using the stannous chloride method. The flame photometer (Systronic 128) was used for analysis of sodium (Na), potassium (K) and calcium (Ca) concentra-tion, whereas the atomic absorption spectrometer (Varian Spectra 55B) was used for analysis of magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), nickel (Ni), lead (Pb), cadmium (Cd) and chromium (Cr) con-centrations after digestion of 0.2 g dry sample with 10 mL mixture of 5H$_2$SO$_4$ and 1HClO$_4$ in a block digestion sys-tem (Pelican Equipments Chennai-India) for 2 h at 300 °C (APHA 2005). Water-soluble portions were determined after the extraction of 2.5 g sample with 50 mL of distilled water at room temperature in a shaker at 100 rpm for 2 h (Singh and Kalamdhad 2013).

Soluble bio-chemical oxygen demand (BOD) and sol-uble chemical oxygen demand (COD) were determined from the supernatant of the blended mixture of 10 g wet sample in 100 mL deionized water by the dilution and dichromate method, respectively (APHA 2005). The water-soluble (WS) portions were determined after extraction of 2.5 g sample with 50 mL of distilled water at room tem-perature in a shaker at 100 rpm for 2 h (Singh and Kalamdhad 2013). Diethylenetriaminepentaacetic acid (DTPA) extraction of the metals was carried out by mechanically shaking 4 g ground sample (screened through 0.22 mm sieve) with 40 mL of 0.005 M DTPA, 0.01 M CaCl$_2$ and 0.1 M (triethanolamine) buffered to pH 7.3 at 100 rpm (Guan et al. 2011). The standard Toxicity char-acteristic leaching procedure (TCLP) test was performed to determine the leachable portions (USEPA 1992). 5 g compost sample (size less than 9.5 mm) with 100 mL of acetic acid at pH 4.93 ± 0.05 (pH adjusted with 1 N NaOH) was taken in a 125 mL reagent bottle and kept at room temperature for 18 h in a shaker at 30 ± 2 rpm. The suspensions were centrifuged for 5 min at 10,000 rpm, filtered through Whatman filter paper no. 42 and the filtrate stored in a plastic reagent bottle at 4 °C for analysis of the selected heavy metals.

The results reported are the means of three replicates. Analysis of variance (ANOVA) test using the SPSS soft-ware was carried out for each parameter on all five trials to determine the significance of variations.

## Results and discussion

The initial characteristics of the substrates are shown in Table 1. Cattle manure is rich in soluble BOD (4560 mg/kg) and COD (11,600 mg/kg), indicating good source of easily metabolizable organic matter. The S. natans sample contains 76.8 % VS and is also rich in nutrients. The initial pH of the S. natans, cattle manure and sawdust ranges from 5.7 to 7.0 and is within the range (5.5–8.5), suitable for activity of the earthworms and microorganisms (Yadav and Garg 2011). The S. natans indicated the presence of the following heavy metals in order of their concentrations: Fe > Mn > Pb > Ni > Zn > Cr > Cu > Cd.

### E. fetida biomass

Figure 2a illustrates the changes in the number of earth-worms, hatchings and cocoons. The total number of earthworms (clitellated and young non-clitellated) initially decreased in all trials. The number of earthworms was found to increase on day 45 in T2 (206 ± 1), T3 (235 ± 2) and T4 (242 ± 4) (Fig. 2a). No mortality was indicated in T3 and T4 during the process. The T4 substrate had the highest proportion of cattle manure, containing easily metabolizable organic matter and non-assimilated carbo-hydrates essential for the growth and reproduction of earthworms (Gupta et al. 2007). The growth of the earth-worms is also related to the palatability, nutrient pool and microbial populations of the feed (Flegel and Schrader 2000; Suthar 2009). The high mortality in T5 indicates that S. natans without proper amendments is not suitable for vermicomposting. The cocoon population was first seen in trials during the monitoring of the reactors on day 15 of the process. The highest number of cocoons was also observed on day 30 in T4 (105 ± 5), followed by T3 (90 ± 1). The decrease in the total number of cocoons on day 45 in T2, T3 and T4 was due to the hatching of the cocoons. The production of cocoons in different feed mixtures is also related to the biochemical quality of the feed and the microbial mass and decomposition activities (Flack and Hartenstein 1984; Suthar 2007). Few new hatchings were first observed during monitoring on day 30 in all trials, and the highest number of hatchings was in T4 (203 ± 5) followed by T3 (173 ± 6) on day 45. The highest increase of total biomass was in T4 (302.2 g), followed by T3 (232.0 g) on day 45 (Fig. 2b). In T4, the biomass growth rates from 0 to 15, 15 to 30 and 30 to 45 days were 4.4, 1.7 and 7.4 g/day. In T3, the biomass growth rates from 0 to 15, 15 to 30 and 30 to 45 days were 2.8, 1.0 and 5.0 g/day. The decrease in the biomass growth rate from 15 to 30 days was possibly due to the energy consumed by the adult earthworms for production of cocoons, which other-wise would have been utilized for tissue growth (Chaudhari and Bhattacharjee 2002). The negative peak of the biomass growth rate of the control trial T5 without cattle manure on day 15 was due to the mortality that occurred during the acclimatization period of the earthworms for the substrates. The rise in the biomass growth rate on day 45 was due to

**Table 1** Initial characterization of *S. natans* composting materials (mean ± SD, *n* = 3)

| Parameters | Feedstock materials | | |
|---|---|---|---|
| | *S. natans* | Cattle manure | Sawdust |
| MC (%) | 90.5 ± 1.2 | 86.4 ± 0.9 | 10.0 ± 0.14 |
| pH | 5.71 ± 0.01 | 7.04 ± 0.05 | 6.1 ± 0.05 |
| EC (dS/m) | 3.5 ± 0.03 | 3.7 ± 0.03 | 0.8 ± 0.02 |
| VS (g/kg dry matter) | 768 ± 1.8 | 720 ± 1.5 | 794 ± 2.1 |
| TKN (%) | 2.2 ± 0.05 | 1.5 ± 0.02 | 0.4 ± 0.03 |
| TP (mg/kg dry matter) | 2100 ± 40 | 3200 ± 25 | 205 ± 1.90 |
| Total mineral nutrients (mg/kg dry matter) | | | |
| Na | 6995 ± 35.5 | 2800 ± 5.5 | 1097 ± 12.3 |
| K | 18,920 ± 95.0 | 988 ± 22.5 | 698 ± 10.4 |
| Ca | 9337 ± 90.0 | 5892 ± 17.2 | 2420 ± 12.4 |
| Mg | 7277 ± 55.0 | 6957 ± 24.5 | 3020 ± 2.34 |
| Water-soluble mineral nutrients (mg/kg dry matter) | | | |
| Na | 1545 ± 21.5 | 702 ± 7.5 | 297 ± 5.3 |
| K | 3532 ± 16.4 | 290 ± 12.5 | 198 ± 6.4 |
| Ca | 3255 ± 22.5 | 2493 ± 31.2 | 420 ± 12.4 |
| Mg | 2058 ± 19.1 | 954 ± 11.5 | 820 ± 2.34 |
| Total heavy metals (mg/kg dry matter) | | | |
| Zn | 210 ± 0.50 | 160 ± 2.0 | 107 ± 2.3 |
| Cu | 53.5 ± 0.50 | 47.8 ± 0.8 | 20.5 ± 1.0 |
| Mn | 999 ± 7.0 | 527 ± 1.5 | 143 ± 3.5 |
| Fe | 6754 ± 32.5 | 1861 ± 12.8 | 2749 ± 13.3 |
| Ni | 265 ± 1.75 | 236 ± 2.6 | 279 ± 1.5 |
| Cd | 53.8 ± 2.2 | 47.9 ± 1.1 | 58.1 ± 0.5 |
| Pb | 756 ± 10.5 | 747.4 ± 7.5 | 769.0 ± 5.0 |
| Cr | 143 ± 1.22 | 124 ± 4.5 | 80.2 ± 0.20 |
| Water-soluble heavy metals (mg/kg dry matter) | | | |
| Zn | 14.7 ± 0.05 | 6.4 ± 0.05 | 2.1 ± 0.03 |
| Cu | 8.0 ± 0.15 | 3.8 ± 0.8 | 0.9 ± 0.02 |
| Mn | 55.5 ± 1.5 | 21.4 ± 0.9 | 5.2 ± 0.20 |
| Fe | 268 ± 2.5 | 140 ± 12.8 | 124 ± 2.30 |
| Ni | ND | ND | ND |
| Cd | ND | ND | ND |
| Pb | 9.9 ± 0.15 | ND | ND |
| Cr | 34.5 ± 0.05 | 6.4 ± 0.05 | 2.1 ± 0.16 |
| DTPA-extractable heavy metals (mg/kg dry matter) | | | |
| Zn | 94.5 ± 0.5 | 46.4 ± 1.5 | 10.8 ± 0.70 |
| Cu | 9.8 ± 0.1 | 6.8 ± 0.6 | 1.7 ± 0.20 |
| Mn | 220 ± 5.5 | 227 ± 1.5 | 40.4 ± 0.90 |
| Fe | 314 ± 14.5 | 375 ± 12.5 | 8.2 ± 0.40 |
| Ni | 17.4 ± 0.1 | 0.1 ± 0.01 | 0.08 ± 0.03 |
| Cd | ND | ND | ND |
| Pb | 31.4 ± 0.5 | ND | ND |
| Cr | 50.4 ± 0.2 | 12.4 ± 0.2 | 0.6 ± 0.02 |
| TCLP-extractable heavy metals (mg/kg dry matter) | | | |
| Zn | 129.1 ± 0.6 | 32.4 ± 1.5 | 15.5 ± 1.50 |
| Cu | 23.1 ± 0.6 | 8.8 ± 0.6 | 2.1 ± 0.10 |
| Mn | 291 ± 3.5 | 450 ± 5.5 | 43.9 ± 1.30 |
| Fe | 474 ± 9.5 | 320 ± 12.5 | 13.0 ± 0.20 |

**Table 1** continued

| Parameters | Feedstock materials | | |
|---|---|---|---|
| | S. natans | Cattle manure | Sawdust |
| Ni | 205 ± 1.5 | 1.9 ± 0.04 | 0.9 ± 0.05 |
| Cd | 2.40 ± 0.05 | 1.4 ± 0.05 | 7.9 ± 0.10 |
| Pb | 64.7 ± 0.2 | 2.8 ± 0.4 | 0.9 ± 0.04 |
| Cr | 66.6 ± 0.3 | 14.5 ± 0.05 | 4.0 ± 0.15 |
| Soluble BOD (mg/kg wet mass) | 3678 ± 30 | 4560 ± 30 | 1550 ± 7.00 |

*ND* not detected, *SD* standard deviation, *n* no. of observations



**Fig. 2 a** Variations of the number of earthworms (e), cocoons (c) and hatchings (h) **b** Variation of total biomass (b) and biomass growth rate (r)
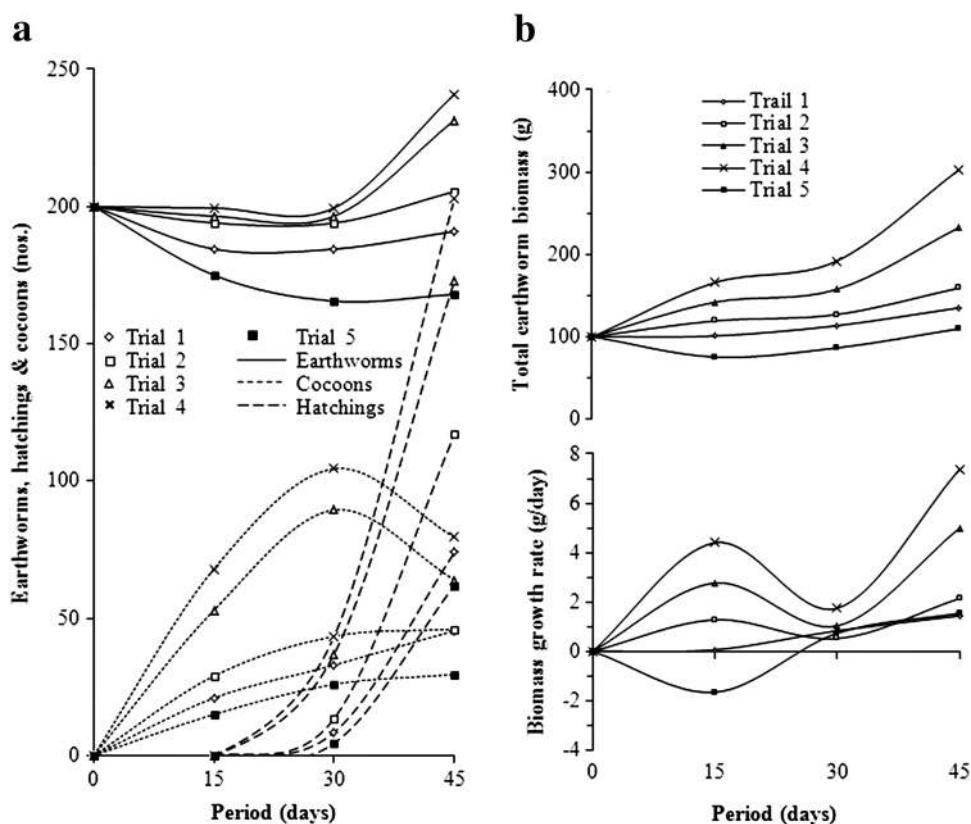
the hatchings and their rapid growth. The average total biomass growth rate after the vermicomposting process was highest in T4 (4.5 g/day) followed by T3 (2.9 g/day). Therefore, the growth and fecundity were dependent on the cattle manure proportions of the substrates. The rate of growth of the earthworms and their increase in population as well as fecundity are indicators of the suitability for vermicomposting of the substrates (Chaudhari and Bhattacharjee 2002). ANOVA test indicates that the variations of the number of earthworms ($F = 7.2$, $P = 0.0 < 0.01$), cocoons ($F = 10.5$, $P = 0.00 < 0.01$) and hatchings ($F = 3.0$, $P = 0.04 < 0.05$) and the total biomass ($F = 8.8$, $P = 0.0 < 0.01$) are significant for the five trials.

**Physico-chemical transformations**

The pH in all trials increased after the vermicomposting process (Fig. 3a). The pH of T1, T2, T3, T4 and T5 increased from 6.13, 6.40, 6.50, 6.56 and 6.00 to 7.11, 7.25, 7.43, 7.61 and 7.02 on day 45, respectively. The pH of T1, T2 and T4 initially decreased to 6.10, 6.30 and 6.48 on day 15, respectively. The initial decrease of pH in some of the trials could be attributed to the production of organic acid by the associated microbial decomposition during the vermicomposting process, which lowers the pH (Suthar 2007). Different authors have different views about the variation of pH values during composting. The

**Fig. 3** **a** Variation of pH.
**b** Variation of electrical
conductivity (EC). **c** Variation
of volatile solids (VS).
**d** Variation of soluble BOD
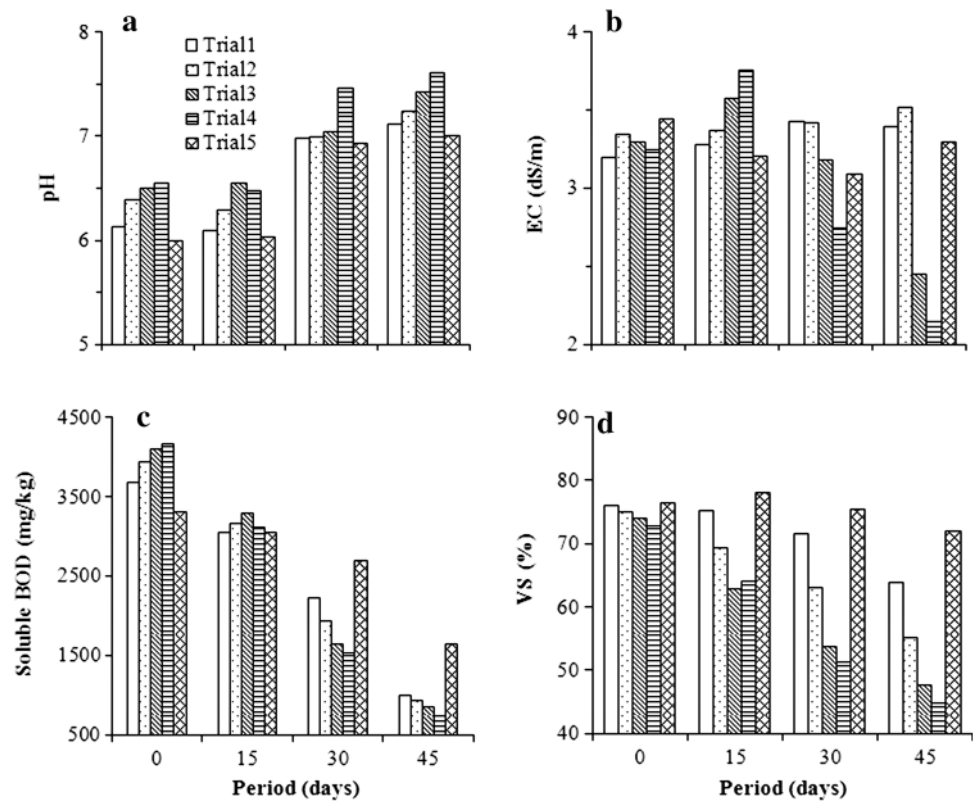


decomposition of organic matter leads to the formation of ammonium ($NH_4^+$) ions which increases the pH value (Pramanik et al. 2007). The inconsistency in the findings is because different substrates result in the production of different intermediate species and different wastes showing different pH behaviours (Gupta and Garg 2008). The gradual increase in pH during vermicomposting was corroborated by Datar et al. (1997), Hait and Tare (2010), Kaur et al. (2010) and Singh and Kalamdhad (2013). Earthworms selectively increased the populations of catabolically more active microbes (Aira et al. 2007) and, in the process, the degradation of short chain fatty acids and precipitation of calcium carbonate might have increased the pH during the process (Tognetti et al. 2005). It must be borne in mind that the amorphous calcium carbonate in bio-minerals fulfils the functions of skeletal growth and maintenance in higher organisms which is not relevant in the case of earthworms. The secretion of calcium carbonate appears to be related to pH regulation, with calcium carbonate precipitating when $HCO_3^-$ ions are in excess of those required to buffer tissue fluid pH (Versteegh et al., 2014). Also, the excess organic nitrogen that was not entailed by microbes and released as ammonia might have dissolved in the moisture of the substrates and increased the pH of the vermicompost (Vig et al. 2011). The variation of pH amongst the trials was highly significant ($F = 34.5$, $P = 0.0 < 0.01$).

The EC of T1 and T2 increased from 3.20 and 3.35 to 3.44 and 3.52 dS/m on day 45, respectively (Fig. 3b). The increase in EC was due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium and potassium. (Lim et al. 2014). Some of these ions are essential for plant growth ($K^+$, $Ca^{2+}$, $Mg^{2+}$, etc.), while others are often undesirable ($Na^+$, $NH_4^+$, heavy metal ions, etc.). The EC of T5 decreased from 3.45 to 3.30 dS/m during the process, indicating the slow mineralization process of the T5 feedstock. The EC of T3 and T4 after an initial increase from 3.30 and 3.25 dS/m to 3.58 and 3.76 dS/m decreased to 2.45 and 2.15 dS/m, respectively. The precipitation of mineral salts could be the reason for the final decrease in EC and it corroborated with the increased pH. The change in the availability of cations or anions with pH may also affect metal mobility and availability through competitive sorption and complexation reactions (Sizmur and Hodson 2009). EC reflects the salinity of the composting product and its suitability for plant growth. High EC in the final product is undesirable, because it will inhibit plant rooting and also reduce the transportation of water and nutrients to the plants (Fang and Wong 1999; Chiang et al. 2007). ANOVA test shows no significant difference in the EC amongst all trials ($F = 1.4$, $P = 0.27 > 0.05$). However, the decrease in EC on day 45 for all trials was significant ($F = 3.2$, $P = 0.04 < 0.05$).

The VS concentration is an indicator of organic matter content and decreased during the vermicomposting process (Fig. 3c). The metabolic activity of the earthworms resulting in assimilation of carbohydrates/other polysaccharides from the substrates and the microbial respiration of the active microorganisms thriving in the microclimatic conditions promoted by the earthworms degraded the organic components, resulting in the loss of carbon in the form of $CO_2$ and subsequent mineralization (Vig et al. 2011). The loss of VS in the trials was in the order T4 (38.6 %) > T3 (35.6 %) > T2 (26.5 %) > T1 (16.0 %) > T5 (5.8 %). T4 had the highest proportion of cattle manure containing fungal stains and greater population of other microbes such as bacteria, protozoa, nematodes, fungi and actinomycetes that played an important role in organic matter decomposition by providing extracellular enzymes in the reactors (Suthar et al., 2014). The results were consistent with the findings during vermicomposting of water hyacinth (Singh and Kalamdhad 2013). The reduction trend in VS was also in agreement with the growth profile of *E. fetida* (Fig. 2). ANOVA analysis shows significant difference in the reduction of VS amongst all trials ($F = 16.3$, $P = 0.00 < 0.05$).

Soluble BOD analysis is a measure of the readily degradable organic matter of the compost mixture and is an important parameter in the analysis of the stability of the compost, as the readily bioavailable compounds are subjected to rapid aerobic microbial degradation. Oxygen consumption will continue if soluble BOD is high, indicating unstable compost. During land applications of such composts for crops, the biological processes can continue and strip nutrients from soil (Wang et al. 2004). All trials showed reduced soluble BOD after the process. The order of soluble BOD reduction was T4 (82.3 %) > T3 (79.1 %) > T2 (76.1 %) > T1 (72.7 %) > T5 (66.7 %).

## Nutrients

The TKN increased in all trials during the vermicomposting process from 1.95, 1.88, 1.81, 1.74 and 2.20 % to 2.41, 2.43, 2.49, 2.63 and 2.66 % in T1, T2, T3, T4 and T5, respectively (Table 2). The highest increase was in T4 (51.0 %), followed by trial 3 (37.3 %). The concentration of TP gradually increased due to the mineralization and mobilization of phosphorous by bacterial and faecal phosphatase activity of the earthworms (Khwairakpam and Bhargava 2009). TP of T1, T2, T3, T4 and T5 increased from 2.02, 2.13, 2.24, 2.35 and 2.10 g/kg to 3.20, 3.55, 4.00, 4.38 and 2.91 g/kg, and the highest increase was in T4 (86.2 %) followed by T3 (78.3 %).

The nutrients (Na, K, Ca and Mg) are needed in minimal amounts for earthworm metabolism. K, Ca and Mg are also essential for plant growth, but Na in high concentration is often undesirable. Na is, however, considered as a functional plant nutrient by some authors (Subbarao et al. 2003). Ca and Na in the form of oxides or hydroxides or carbonates in composts when applied to soil may counteract soil acidification, making soil nutrients more available to plants. The total minerals in all trials increased after the process on day 45 (Table 2). The highest increase of Na (84.6 %), K (48.9 %), Ca (65.4 %) and Mg (34.2 %) was in T4, followed by T3. The result was in agreement with the highest growth of earthworms and highest reduction of VS in T4. The microflora present in the gut of earthworms, the associated microbial activity during the vermicomposting enhancing mineralization and the net loss in dry mass increased the concentration of the nutrients of the vermicompost (Suthar 2007; Hait and Tare 2010). The variations of total nutrients during the composting process were significant amongst all trials (TKN: $F = 13.1$, $P = 0.0 < 0.01$; TP: $F = 14.7$, $P = 0.0 < 0.01$; Na: $F = 5.3$, $P = 0.004 < 0.05$; K: $F = 238$, $P = 0.00 < 0.01$; Ca: $F = 3.2$, $P = 0.04 < 0.05$; Mg: $F = 3.5$, $P = 0.04 < 0.05$).

The water-soluble (WS) nutrients are the most available forms for the plants and they increased during the process also due to net loss of dry mass. The feeding action of the earthworms and the presence of a large number of microorganisms and enzymes during vermicomposting transformed the insoluble plant nutrients into more soluble and available forms, thereby increasing their concentrations in the vermicomposts (Aira et al. 2007; Liu et al. 2012). When the organic substrates pass through the gut of the worm, some fraction of organic minerals are converted into more available species of nutrients (i.e. exchangeable forms) due to the action of endogenic and/or exogenic enzymes (Suthar 2010). The highest increase of WS Na (77.5 %), WS K (66.9 %), WS Ca (64.0 %) and WS Mg (72.7 %) was also in T4. In evaluating the contents of nutrients as well as undesirable plant salts, it is to be borne in mind that compost is primarily an organic soil conditioner and the use of pure compost is not recommended (CCME 2005). The variations of water-soluble nutrients during the composting process were significant amongst all trials for Na: $F = 6.4$, $P = 0.00 < 0.01$; K: $F = 61$, $P = 0.00 < 0.01$; Mg: $F = 7.4$, $P = 0.00 < 0.01$. The water-soluble nutrients of all trials increased significantly on day 45.

## Total heavy metals concentration

The variation in total heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) and their water-soluble (WS) and DTPA-extractable portions during the vermicomposting are shown at Table 3. The heavy metals are important ingredients for plants, animals and humans, but their higher content is known to have toxic effects. The total concentration of all selected heavy metals in the final vermicompost increased

**Table 2** Variation of the total and water-soluble nutrients (Na, K, Ca and Mg) during *S. natans* vermicomposting

| Days | Nutrients concentration | | | | |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| **TKN (g/kg dry matter)** | | | | | |
| 0 | 1.95 ± 0.05 | 1.88 ± 0.02 | 1.81 ± 0.01 | 1.74 ± 0.02 | 2.20 ± 0.01 |
| 15 | 2.09 ± 0.02 | 2.04 ± 0.05 | 2.05 ± 0.04 | 2.02 ± 0.03 | 2.25 ± 0.03 |
| 30 | 2.17 ± 0.03 | 2.14 ± 0.01 | 2.10 ± 0.02 | 2.19 ± 0.02 | 2.41 ± 0.01 |
| 45 | 2.41 ± 0.01 | 2.43 ± 0.03 | 2.49 ± 0.02 | 2.63 ± 0.04 | 2.66 ± 0.04 |
| **Total P (g/kg dry matter)** | | | | | |
| 0 | 2.02 ± 0.01 | 2.13 ± 0.02 | 2.24 ± 0.01 | 2.35 ± 0.05 | 2.10 ± 0.05 |
| 15 | 2.26 ± 0.01 | 2.40 ± 0.01 | 2.75 ± 0.03 | 2.81 ± 0.03 | 2.33 ± 0.05 |
| 30 | 3.00 ± 0.05 | 3.36 ± 0.02 | 3.79 ± 0.03 | 3.91 ± 0.04 | 2.58 ± 0.04 |
| 45 | 3.20 ± 0.06 | 3.55 ± 0.03 | 4.00 ± 0.01 | 4.38 ± 0.04 | 2.91 ± 0.01 |
| **Total Na (mg/kg dry matter)** | | | | | |
| 0 | 5915 ± 65 | 5325 ± 35 | 5185 ± 55 | 4728 ± 15 | 6995 ± 20 |
| 15 | 6371 ± 31 | 5974 ± 44 | 6712 ± 28 | 6775 ± 41 | 7343 ± 45 |
| 30 | 7194 ± 30 | 6895 ± 28 | 7524 ± 20 | 7874 ± 30 | 7902 ± 21 |
| 45 | 8204 ± 48 | 7977 ± 29 | 8018 ± 46 | 8730 ± 28 | 8466 ± 29 |
| **Total K (mg/kg dry matter)** | | | | | |
| 0 | 15,305 ± 115 | 13,511 ± 114 | 11,681 ± 112 | 9925 ± 91 | 18,920 ± 101 |
| 15 | 16,001 ± 112 | 14,281 ± 110 | 13,518 ± 110 | 11,556 ± 94 | 19,422 ± 103 |
| 30 | 16,995 ± 102 | 15,640 ± 103 | 15,021 ± 102 | 14,010 ± 92 | 20,848 ± 110 |
| 45 | 19,302 ± 101 | 18,190 ± 102 | 16,456 ± 101 | 14,778 ± 98 | 22,314 ± 108 |
| **Water-soluble Na (mg/kg dry matter)** | | | | | |
| 0 | 1342 ± 21 | 1251 ± 22 | 1168 ± 32 | 1083 ± 34 | 1545 ± 28 |
| 15 | 1366 ± 19 | 1311 ± 27 | 1294 ± 29 | 1184 ± 19 | 1607 ± 24 |
| 30 | 1544 ± 25 | 1483 ± 24 | 1687 ± 28 | 1561 ± 37 | 1718 ± 32 |
| 45 | 1745 ± 27 | 1789 ± 26 | 1834 ± 32 | 1922 ± 34 | 1898 ± 37 |
| **Water-soluble K (mg/kg dry matter)** | | | | | |
| 0 | 2874 ± 42 | 2550 ± 44 | 2226 ± 38 | 1902 ± 46 | 3532 ± 47 |
| 15 | 2991 ± 41 | 2830 ± 43 | 2546 ± 39 | 2264 ± 43 | 3643 ± 46 |
| 30 | 3402 ± 43 | 3187 ± 58 | 2789 ± 41 | 2879 ± 42 | 3737 ± 45 |
| 45 | 3587 ± 47 | 3348 ± 49 | 3182 ± 48 | 3172 ± 41 | 3989 ± 42 |
| **Total Ca (mg/kg dry matter)** | | | | | |
| 0 | 8301 ± 55 | 7956 ± 78 | 7609 ± 87 | 7267 ± 68 | 9337 ± 98 |
| 15 | 8469 ± 45 | 8703 ± 67 | 8225 ± 65 | 8634 ± 56 | 9750 ± 68 |
| 30 | 9096 ± 87 | 9299 ± 86 | 9058 ± 78 | 11,028 ± 87 | 10,135 ± 98 |
| 45 | 10,140 ± 87 | 10,045 ± 98 | 10,882 ± 96 | 12,020 ± 90 | 10,474 ± 102 |
| **Total Mg (mg/kg dry matter)** | | | | | |
| 0 | 6819 ± 20 | 6787 ± 25 | 6756 ± 35 | 6723 ± 20 | 7277 ± 36 |
| 15 | 7414 ± 17 | 6927 ± 27 | 7121 ± 55 | 7832 ± 48 | 7608 ± 15 |
| 30 | 7898 ± 26 | 7387 ± 43 | 8687 ± 35 | 8539 ± 93 | 8199 ± 34 |
| 45 | 8177 ± 73 | 8769 ± 45 | 8844 ± 57 | 9025 ± 36 | 8775 ± 69 |
| **Water-soluble Ca (mg/kg dry matter)** | | | | | |
| 0 | 3103 ± 24 | 2950 ± 26 | 2801 ± 25 | 2616 ± 19 | 3221 ± 25 |
| 15 | 3346 ± 21 | 3457 ± 34 | 3398 ± 42 | 3146 ± 34 | 3262 ± 27 |
| 30 | 3403 ± 25 | 3750 ± 32 | 3501 ± 40 | 3819 ± 32 | 3523 ± 28 |
| 45 | 3736 ± 32 | 3959 ± 31 | 4134 ± 38 | 4290 ± 31 | 3742 ± 42 |
| **Water-soluble Mg (mg/kg dry matter)** | | | | | |
| 0 | 1824 ± 31 | 1713 ± 34 | 1603 ± 41 | 1493 ± 31 | 2041 ± 36 |
| 15 | 2164 ± 32 | 2045 ± 32 | 2125 ± 40 | 1930 ± 36 | 2127 ± 43 |
| 30 | 2335 ± 34 | 2318 ± 36 | 2374 ± 36 | 2324 ± 32 | 2607 ± 44 |
| 45 | 2501 ± 37 | 2657 ± 47 | 2482 ± 49 | 2578 ± 58 | 2746 ± 56 |

Mean ± SD, $n = 3$, $P < 0.05$

[Zn (14.9–19.7 %), Cu (8.7–19.7 %), Mn (14.8–23.2 %), Fe (11.8–21.9 %), Cr (15.9–31.3 %), Ni (10.5–19.2 %), Pb (2.1–22.9 %) and Cd (3.4–13.7 %)] due to reduction in the weight and volume of the *S. natans* biomass (Vig et al. 2011). Increase in heavy metals' concentration in the vermicompost of different wastes was also reported by Kaushik and Garg (2003). Even though net increase in all heavy metals was indicated in all trials, Table 4 shows that there were downward trends in T2, T3 and T4 for Cr, Ni, Cd and Pb on day 45. There were also downward trends for Cu and Fe in T3 and T4 on day 45. The reason for the downward trend might be the bio-accumulation of heavy metals in the earthworm tissues (Suthar et al. 2014) The variation in Zn ($F = 82$, $P = 0.0 < 0.01$), Cu ($F = 39$, $P = 0.0 < 0.01$), Mn ($F = 76$, $P = 0.0 < 0.01$), Fe ($F = 194$, $P = 0.0 < 0.01$), Ni ($F = 7.5$, $P = 0.0 < 0.01$), Pb ($F = 6.1$, $P = 0.0 < 0.01$), Cd ($F = 9.3$, $P = 0.0 < 0.01$) and Cr ($F = 3.8$, $P = 0.02 < 0.05$) concentrations in different trials were significant. The order of total metal concentration of the vermicompost was Fe > Mn > Pb > Ni > Zn > Cr > Cd > Cu.

### Water-soluble heavy metals

Evaluation of water-soluble heavy metals is necessary in the final vermicompost before agronomic application, because these metal fractions are biologically dynamic and have the highest potential of contaminating the food chain (Iwegbue et al. 2007; Hait and Tare 2012). Water solubility of metals was reduced in the range of about 13.5–27.5 % for Zn, 6.6–35.1 % for Cu, 9.7–36.3 % for Mn, 3.3–37.8 % for Fe, 23.6–80.0 % for Pb and 23.2–50.6 % for Cr during the vermicomposting process (Table 4). The order of water-soluble heavy metals after vermicomposting was: Fe > Mn > Cr > Zn > Cu > Pb. Water-soluble concentrations of Ni and Cd were not detected during the vermicomposting of *S. natans*. ANOVA test indicated that the variation of water-soluble heavy metals was found to be statistically different in all the trials during the vermicomposting process (Zn: $F = 172$, $P = 0.0 < 0.01$; Cu: $F = 119$, $P = 0.0 < 0.01$; Mn: $F = 77$, $P = 0.0 < 0.01$; Fe: $F = 44$, $P = 0.0 < 0.01$; Cr: $F = 544$, $P = 0.0 < 0.01$; Pb: $F = 54$, $P = 0.0 < 0.01$).

Considerable reductions in concentrations of water-soluble Cu, Zn and Cr during vermicomposting of sewage sludge were reported by Hait and Tare (2012). The pH and microbial activity of the gut of the earthworm were enhanced during the passage of the partially digested organic matter. Consequently, the possibilities of binding of metals to the more soluble ions and carbonates increased in the ingested material. According to Goswami et al. (2013), earthworms can bio-accumulate heavy metal ions by forming organo-metallic complexes in their intestines,

which reduces the solubility of the heavy metals. Therefore, the bioaccumulation of water-soluble fraction of metals by *E. fetida* and the formation of organometallic complexes decreased the concentration of water-soluble form of heavy metals (Suthar 2009; Singh and Kalamdhad 2013). Earthworms decrease the mobility and bioavailability of heavy metals during vermicomposting through two major types of cellular adaptations: binding of metals to nuclear proteins forming inclusion nuclear bodies; and the cytoplasmic process involving synthesis of a specific metal binding protein, metallothionein, within the chloragogenous tissue (Cherian and Nordberg 1983; Hait and Tare 2012).

### Plant-available heavy metals

The toxicity of heavy metals in the final product is dependent on the metal mobility in free form, the route of uptake mechanism and the bioavailability if it is accumulated in plants (Vig et al. 2011). DTPA is a chelating agent that mimics plant uptake of heavy metals and generally, metal fraction extracted with DTPA can be considered as potentially available for plants (Chiang et al. 2007). The DTPA extractability of metals was reduced in the range of about 8.3–31.0 % for Zn, 11.8–39.2 % for Cu, 7.0–18.2 % for Mn, 18.4–37.2 % for Fe, 35.9–82.7 % for Pb, 43.0–78.9 % for Ni and 17.4–61.9 % for Cr during the vermicomposting process. The order of DTPA-extractable heavy metal concentrations of the vermicompost was: Fe > Mn > Zn > Cr > Pb > Ni > Cu. The DTPA-extractable Cd was not detected during the vermicomposting of *S. natans*. The conversion of highly toxic Cr(VI) to nontoxic form Cr(III) through the metabolic process in the mitochondrial and cytoplasmic fractions was reported during vermicomposting with *E. fetida* (Jain et al. 2004). The reduction of DTPA-extractable metals during vermicomposting has also been reported by other researchers (Suthar 2009; Singh and Kalamdhad 2013). The interaction of the humic acid with metal is one of the main factors affecting the partitioning of heavy metals during the process (Hait and Tare 2012). The DTPA-extractable heavy metals were found to be statistically different (Zn: $F = 47$, $P = 0.0 < 0.01$; Cu: $F = 47$, $P = 0.0 < 0.01$; Mn: $F = 6.5$, $P = 0.0 < 0.01$; Fe: $F = 45$, $P = 0.0 < 0.01$; Cr: $F = 106$, $P = 0.0 < 0.01$; Pb: $F = 272$, $P = 0.0 < 0.01$) amongst all the trials during the vermicomposting process.

### Leachable heavy metals

The TCLP test is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid and multiphasic wastes. The leachable fractions of the

**Table 3** Variation of total and water-soluble heavy metals during *S. natans* vermicomposting

Heavy metals concentration (mg/kg dry matter)

| Days | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
|---|---|---|---|---|---|
| **Total Zn** | | | | | |
| 0 | 195 ± 3.5 | 190 ± 2.5 | 185 ± 3.3 | 180 ± 1.5 | 210 ± 2.5 |
| 15 | 197 ± 2.9 | 198 ± 2.1 | 189 ± 2.5 | 180 ± 2.9 | 211 ± 3.1 |
| 30 | 222 ± 3.1 | 225 ± 2.8 | 211 ± 2.5 | 204 ± 2.1 | 227 ± 2.1 |
| 45 | 226 ± 2.5 | 227 ± 3.1 | 216 ± 2.1 | 206 ± 3.1 | 242 ± 2.1 |
| **Water-soluble Zn** | | | | | |
| 0 | 13.8 ± 0.05 | 13.3 ± 0.05 | 11.9 ± 0.20 | 11.0 ± 0.05 | 14.4 ± 0.10 |
| 15 | 12.9 ± 0.10 | 12.3 ± 0.05 | 11.3 ± 0.05 | 10.5 ± 0.05 | 13.7 ± 0.10 |
| 30 | 12.7 ± 0.05 | 11.2 ± 0.10 | 10.3 ± 0.05 | 9.0 ± 0.05 | 13.2 ± 0.15 |
| 45 | 11.8 ± 0.05 | 11.0 ± 0.10 | 9.5 ± 0.05 | 7.9 ± 0.05 | 12.5 ± 0.15 |
| **Total Mn** | | | | | |
| 0 | 866 ± 8.0 | 819 ± 2.5 | 772 ± 7.1 | 725 ± 8.7 | 999 ± 8.2 |
| 15 | 997 ± 9.3 | 824 ± 3.0 | 748 ± 7.4 | 736 ± 9.7 | 1090 ± 4.8 |
| 30 | 1008 ± 9.5 | 944 ± 3.2 | 891 ± 7.9 | 790 ± 8.2 | 1131 ± 8.3 |
| 45 | 1010 ± 9.3 | 973 ± 5.5 | 906 ± 7.4 | 893 ± 8.0 | 1147 ± 8.6 |
| **Water-soluble Mn** | | | | | |
| 0 | 47.1 ± 0.10 | 43.7 ± 0.55 | 40.2 ± 0.42 | 36.8 ± 0.25 | 55.5 ± 0.30 |
| 15 | 41.4 ± 0.18 | 40.3 ± 0.45 | 37.9 ± 0.30 | 29.4 ± 0.38 | 52.7 ± 0.27 |
| 30 | 43.6 ± 0.15 | 34.5 ± 0.81 | 27.7 ± 0.86 | 27.6 ± 0.46 | 53.6 ± 0.35 |
| 45 | 39.6 ± 0.15 | 32.3 ± 0.28 | 25.7 ± 0.58 | 23.5 ± 0.59 | 50.1 ± 0.40 |
| **Total Ni** | | | | | |
| 0 | 264 ± 6.0 | 260 ± 2.8 | 257 ± 1.8 | 253 ± 2.3 | 265 ± 2.5 |
| 15 | 273 ± 6.5 | 272 ± 6.8 | 260 ± 2.4 | 263 ± 1.7 | 280 ± 3.5 |
| 30 | 308 ± 4.1 | 310 ± 5.3 | 308 ± 2.5 | 300 ± 2.3 | 303 ± 3.3 |
| 45 | 309 ± 1.1 | 297 ± 3.5 | 285 ± 1.2 | 279 ± 1.1 | 316 ± 5.8 |
| **Water-soluble Ni** | | | | | |
| 0 | – | – | – | – | |
| 15 | – | – | – | – | |
| 30 | – | – | – | – | |
| 45 | – | – | – | – | |
| **Total Cd** | | | | | |
| 0 | 56.8 ± 0.13 | 57.8 ± 0.10 | 59.0 ± 0.15 | 61.2 ± 0.16 | 62.3 ± 0.13 |
| 15 | 64.3 ± 0.23 | 56.6 ± 0.15 | 53.9 ± 0.10 | 53.7 ± 0.08 | 66.3 ± 0.18 |
| 30 | 62.8 ± 0.10 | 67.8 ± 0.10 | 69.0 ± 0.05 | 71.2 ± 0.06 | 62.3 ± 0.13 |
| 45 | 70.3 ± 0.13 | 66.6 ± 0.05 | 63.9 ± 0.05 | 63.7 ± 0.08 | 66.3 ± 0.08 |

| Days | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
|---|---|---|---|---|---|
| **Total Cu** | | | | | |
| 0 | 49.6 ± 1.10 | 49.1 ± 0.15 | 48.5 ± 0.51 | 47.9 ± 1.04 | 53.5 ± 0.51 |
| 15 | 51.3 ± 0.40 | 50.6 ± 0.40 | 48.8 ± 0.53 | 47.4 ± 1.15 | 54.2 ± 0.40 |
| 30 | 58.1 ± 0.38 | 56.9 ± 0.41 | 55.1 ± 0.58 | 54.2 ± 1.22 | 59.5 ± 0.35 |
| 45 | 59.4 ± 1.15 | 58.2 ± 0.51 | 54.0 ± 0.67 | 52.1 ± 0.63 | 61.3 ± 0.35 |
| **Water-soluble Cu** | | | | | |
| 0 | 6.9 ± 0.05 | 6.5 ± 0.05 | 6.0 ± 0.05 | 5.6 ± 0.01 | 8.0 ± 0.05 |
| 15 | 6.4 ± 0.02 | 5.4 ± 0.04 | 5.1 ± 0.02 | 4.7 ± 0.04 | 7.7 ± 0.05 |
| 30 | 5.5 ± 0.02 | 4.8 ± 0.04 | 4.6 ± 0.04 | 4.1 ± 0.02 | 7.6 ± 0.06 |
| 45 | 5.1 ± 0.01 | 4.5 ± 0.05 | 4.2 ± 0.05 | 3.6 ± 0.05 | 7.5 ± 0.02 |
| **Total Fe** | | | | | |
| 0 | 5867 ± 43.5 | 5377 ± 43.8 | 4891 ± 46.8 | 4399 ± 33.5 | 6762 ± 43.5 |
| 15 | 6101 ± 45.4 | 5422 ± 41.0 | 5082 ± 56.5 | 4413 ± 32.2 | 6962 ± 43.8 |
| 30 | 6740 ± 46.5 | 5999 ± 49.5 | 5688 ± 40.2 | 5218 ± 30.2 | 7151 ± 24.4 |
| 45 | 7152 ± 43.5 | 6140 ± 50.5 | 5575 ± 10.5 | 4903 ± 35.1 | 7557 ± 49.5 |
| **Water-soluble Fe** | | | | | |
| 0 | 241 ± 2.0 | 226 ± 1.2 | 215 ± 2.5 | 202 ± 2.0 | 268 ± 2.5 |
| 15 | 228 ± 1.4 | 187 ± 1.3 | 206 ± 1.1 | 184 ± 0.9 | 265 ± 1.3 |
| 30 | 195 ± 1.2 | 172 ± 1.3 | 153 ± 1.7 | 136 ± 1.7 | 261 ± 2.5 |
| 45 | 189 ± 1.1 | 151 ± 1.5 | 138 ± 1.3 | 126 ± 1.4 | 259 ± 1.9 |
| **Total Pb** | | | | | |
| 0 | 756 ± 2.0 | 756 ± 2.5 | 755 ± 2.1 | 754 ± 2.5 | 756 ± 3.1 |
| 15 | 789 ± 3.0 | 771 ± 1.6 | 769 ± 1.6 | 767 ± 2.6 | 798 ± 4.3 |
| 30 | 896 ± 2.3 | 830 ± 1.0 | 803 ± 3.4 | 804 ± 2.5 | 856 ± 2.3 |
| 45 | 929 ± 2.5 | 807 ± 4.9 | 771 ± 1.5 | 771 ± 1.2 | 870 ± 2.8 |
| **Water-soluble Pb** | | | | | |
| 0 | 7.92 ± 0.11 | 6.93 ± 0.11 | 5.94 ± 0.11 | 4.95 ± 0.12 | 9.90 ± 0.11 |
| 15 | 7.69 ± 0.12 | 5.18 ± 0.28 | 4.76 ± 0.45 | 2.55 ± 0.46 | 9.07 ± 0.28 |
| 30 | 4.99 ± 0.28 | 3.88 ± 0.10 | 3.04 ± 0.11 | 1.40 ± 0.61 | 8.05 ± 0.20 |
| 45 | 2.93 ± 0.26 | 2.01 ± 0.21 | 1.25 ± 0.15 | 0.99 ± 0.29 | 7.56 ± 0.33 |
| **Total Cr** | | | | | |
| 0 | 135 ± 1.5 | 133 ± 2.1 | 131 ± 1.5 | 129 ± 1.9 | 143 ± 1.5 |
| 15 | 134 ± 1.6 | 139 ± 1.9 | 136 ± 1.5 | 133 ± 1.2 | 149 ± 2.5 |
| 30 | 163 ± 2.5 | 165 ± 1.9 | 168 ± 3.2 | 170 ± 2.8 | 169 ± 2.5 |
| 45 | 177 ± 1.3 | 157 ± 1.1 | 152 ± 1.8 | 150 ± 1.6 | 172 ± 2.2 |

**Table 3** continued

| Days | Heavy metals concentration (mg/kg dry matter) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water-soluble Cd | | | | | Water-soluble Cr | | | | |
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 0 | – | – | – | – | – | 28.5 ± 0.1 | 25.6 ± 0.1 | 22.8 ± 0.2 | 20.0 ± 0.1 | 34.5 ± 0.2 |
| 15 | – | – | – | – | – | 23.2 ± 0.1 | 20.4 ± 0.6 | 16.4 ± 0.2 | 14.6 ± 0.3 | 28.3 ± 0.3 |
| 30 | – | – | – | – | – | 20.2 ± 0.1 | 17.8 ± 0.1 | 14.1 ± 0.3 | 11.0 ± 0.2 | 27.7 ± 0.4 |
| 45 | – | – | – | – | – | 18.4 ± 0.8 | 16.7 ± 0.1 | 13.1 ± 0.2 | 9.9 ± 0.3 | 26.5 ± 0.8 |

– Not detected, mean ± SD, $n = 3$

heavy metals are shown at Table 4. The leachability of heavy metals was reduced in the range of about 9.8–33.5 % for Zn, 15.1–35.3 % for Cu, 10.8–24.3 % for Mn, 12.2–32.6 % for Fe, 15.7–52.7 % for Ni, 23.0–69.0 % for Pb, 39.2–98.0 for Cd and 23.5–63.2 % for Cr during the vermicomposting process. The order of leachable heavy metals' content of the vermicompost was: Fe > Mn > Ni > Zn > Cr > Pb > Cu > Cd. The variations in the leachability of heavy metals amongst all trials were significant during the process (Zn: $F = 241$, $P = 0.0 < 0.01$; Cu: $F = 174$, $P = 0.0 < 0.01$; Mn: $F = 9.6$, $P = 0.0 < 0.01$; Fe: $F = 41$, $P = 0.0 < 0.01$; Cr: $F = 161$, $P = 0.0 < 0.01$; Pb: $F = 58$, $P = 0.0 < 0.01$).

The accumulation of metals in the earthworm tissues might be the reason for reduction of leachable concentration of metals during vermicomposting of *S. natans*. Similar results were also reported by Jain et al. (2004) and Singh and Kalamdhad (2013). The evidence of cutaneous absorption of metals by earthworms was reported by Suthar (2009). The leachability of metals might have also reduced due to increase in pH and complexity of metal humic substances. An increase in the pH of vermicompost resulted in an increase of surface negative charge, which increased cationic adsorption, formation of metal hydroxyl species that have a greater affinity for adsorption sites than the metal cations, and precipitation of metal as metal hydroxides (Maity et al. 2008). Another reason for the reduction of leachable metal concentration during vermicomposting was the affinity of the metals with the functional groups –OH or –COO– of humic substances of the vermicompost (Kang et al. 2011). The threshold limits for heavy metal contamination are 20 mg/kg for Cd, 100 mg/kg for Cr and 100 mg/kg for Pb (US EPA method 1311, 1992). Therefore, the results of the TCLP test confirmed that the heavy metals' concentrations in all trials were under the threshold limits for compost use for agriculture purposes.

## Conclusion

The addition of appropriate cattle manure in the feed mixture enhanced the growth and production of the earthworms. Reduction of organic matter was related to the amount of cattle manure addition, as indicated by the highest reduction of VS in trial T4 (38.6 %), followed by trial T3 (35.6 %). The performance of trial T4 was the best in terms of the highest reduction of VS, highest increase of the nutrients and the highest reduction of most of the bioavailable forms of heavy metals. With the appropriate proportion of cattle manure, *E. fetida* was effective in the reduction of water-soluble, DTPA-extractable and

**Table 4** Variation of DTPA-extractable and TCLP-extractable heavy metals during *S. natans* vermicomposting

| Days | Heavy metal concentration (mg/kg dry matter) | | | | |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| **DTPA Zn** | | | | | |
| 0 | 91.1 ± 1.02 | 89.6 ± 1.34 | 78.9 ± 1.18 | 78.7 ± 0.68 | 94.6 ± 0.98 |
| 15 | 87.8 ± 1.11 | 88.2 ± 1.15 | 74.3 ± 1.21 | 74.6 ± 0.27 | 92.2 ± 0.81 |
| 30 | 83.2 ± 1.24 | 83.2 ± 1.13 | 69.4 ± 1.48 | 73.8 ± 0.78 | 91.8 ± 0.74 |
| 45 | 80.6 ± 1.59 | 73.5 ± 2.26 | 57.5 ± 1.37 | 54.3 ± 1.17 | 86.7 ± 0.48 |
| **TCLP Zn** | | | | | |
| 0 | 108 ± 1.1 | 99 ± 0.7 | 89 ± 0.2 | 81 ± 1.0 | 129 ± 1.0 |
| 15 | 103 ± 0.1 | 90 ± 0.4 | 74 ± 0.3 | 74 ± 0.2 | 125 ± 0.5 |
| 30 | 94 ± 1.5 | 84 ± 0.2 | 72 ± 0.4 | 72 ± 0.3 | 121 ± 0.5 |
| 45 | 88 ± 1.8 | 73 ± 0.4 | 65 ± 1.5 | 54 ± 3.6 | 116 ± 1.2 |
| **DTPA Mn** | | | | | |
| 0 | 203 ± 1.5 | 203 ± 1.1 | 204 ± 1.0 | 204 ± 0.5 | 220 ± 1.0 |
| 15 | 180 ± 1.3 | 205 ± 1.2 | 209 ± 1.5 | 210 ± 0.6 | 216 ± 0.5 |
| 30 | 181 ± 1.5 | 200 ± 1.4 | 189 ± 1.5 | 195 ± 1.0 | 209 ± 0.7 |
| 45 | 183 ± 1.6 | 202 ± 1.6 | 200 ± 1.0 | 203 ± 1.0 | 180 ± 0.5 |
| **TCLP Mn** | | | | | |
| 0 | 282 ± 1.1 | 298 ± 1.0 | 314 ± 1.5 | 330 ± 1.4 | 291 ± 1.1 |
| 15 | 273 ± 1.8 | 293 ± 1.6 | 307 ± 1.7 | 324 ± 1.6 | 287 ± 1.6 |
| 30 | 246 ± 1.1 | 275 ± 1.2 | 256 ± 1.8 | 277 ± 1.2 | 269 ± 1.1 |
| 45 | 231 ± 1.4 | 246 ± 1.3 | 238 ± 1.6 | 250 ± 1.9 | 259 ± 1.3 |
| **DTPA Ni** | | | | | |
| 0 | 14.3 ± 0.05 | 13.4 ± 0.05 | 12.3 ± 0.05 | 11.3 ± 0.15 | 17.6 ± 0.08 |
| 15 | 13.4 ± 0.15 | 12.5 ± 0.05 | 11.4 ± 0.05 | 10.4 ± 0.17 | 16.7 ± 0.07 |
| 30 | 13.0 ± 0.15 | 10.1 ± 0.07 | 9.4 ± 0.06 | 9.4 ± 0.16 | 16.5 ± 0.08 |
| 45 | 6.0 ± 0.05 | 3.5 ± 0.08 | 2.6 ± 0.07 | 3.0 ± 0.14 | 10.0 ± 0.05 |
| **TCLP Ni** | | | | | |
| 0 | 164 ± 2.0 | 144 ± 1.5 | 124 ± 1.5 | 103 ± 2.0 | 205 ± 2.5 |
| 15 | 156 ± 1.9 | 126 ± 1.2 | 113 ± 1.5 | 89 ± 1.3 | 200 ± 1.1 |
| 30 | 150 ± 1.2 | 120 ± 1.1 | 94 ± 1.7 | 79 ± 1.3 | 186 ± 2.1 |
| 45 | 135 ± 1.9 | 96 ± 1.9 | 60 ± 1.6 | 49 ± 1.4 | 173 ± 1.8 |
| **DTPA Cd** | | | | | |
| 0 | – | – | – | – | – |
| 15 | – | – | – | – | – |
| 30 | – | – | – | – | – |
| 45 | – | – | – | – | – |
| **DTPA Cu** | | | | | |
| 0 | 8.7 ± 0.02 | 8.4 ± 0.02 | 8.1 ± 0.01 | 7.8 ± 0.04 | 9.8 ± 0.05 |
| 15 | 8.5 ± 0.04 | 7.8 ± 0.02 | 7.3 ± 0.06 | 7.1 ± 0.04 | 9.4 ± 0.01 |
| 30 | 7.1 ± 0.03 | 7.3 ± 0.02 | 6.7 ± 0.04 | 6.6 ± 0.04 | 9.0 ± 0.04 |
| 45 | 6.9 ± 0.04 | 6.5 ± 0.09 | 5.5 ± 0.02 | 4.7 ± 0.05 | 8.6 ± 0.05 |
| **TCLP Cu** | | | | | |
| 0 | 19.6 ± 0.05 | 18.1 ± 0.01 | 16.7 ± 0.05 | 15.3 ± 0.02 | 23.1 ± 0.05 |
| 15 | 17.1 ± 0.04 | 16.4 ± 0.05 | 14.7 ± 0.05 | 13.5 ± 0.05 | 22.4 ± 0.05 |
| 30 | 17.0 ± 0.04 | 15.2 ± 0.02 | 12.2 ± 0.05 | 12.2 ± 0.02 | 21.5 ± 0.13 |
| 45 | 16.5 ± 0.04 | 14.2 ± 0.05 | 11.2 ± 0.02 | 9.9 ± 0.15 | 19.6 ± 0.14 |
| **DTPA Fe** | | | | | |
| 0 | 241 ± 2.0 | 226 ± 1.2 | 215 ± 2.5 | 202 ± 2.0 | 268 ± 2.5 |
| 15 | 228 ± 1.4 | 187 ± 0.3 | 206 ± 1.1 | 184 ± 0.9 | 265 ± 1.3 |
| 30 | 195 ± 0.2 | 172 ± 1.3 | 153 ± 1.7 | 136 ± 1.7 | 261 ± 2.5 |
| 45 | 189 ± 0.1 | 151 ± 1.5 | 138 ± 1.3 | 126 ± 1.4 | 259 ± 1.9 |
| **TCLP Fe** | | | | | |
| 0 | 413 ± 2.5 | 396 ± 0.6 | 382 ± 2.5 | 366 ± 2.0 | 474 ± 1.4 |
| 15 | 394 ± 3.6 | 367 ± 1.3 | 271 ± 1.9 | 318 ± 1.7 | 452 ± 1.5 |
| 30 | 383 ± 2.9 | 374 ± 1.9 | 283 ± 1.3 | 357 ± 1.9 | 444 ± 1.0 |
| 45 | 321 ± 2.8 | 305 ± 2.8 | 265 ± 2.5 | 247 ± 1.3 | 416 ± 1.4 |
| **DTPA Pb** | | | | | |
| 0 | 25.1 ± 0.05 | 21.9 ± 0.06 | 18.8 ± 0.06 | 15.7 ± 0.06 | 31.4 ± 0.05 |
| 15 | 21.5 ± 0.08 | 17.4 ± 0.08 | 14.4 ± 0.06 | 11.3 ± 0.06 | 28.6 ± 0.09 |
| 30 | 20.3 ± 0.06 | 17.0 ± 0.05 | 13.6 ± 0.05 | 8.2 ± 0.05 | 27.2 ± 0.04 |
| 45 | 9.9 ± 0.09 | 7.4 ± 0.06 | 3.9 ± 0.05 | 2.7 ± 0.05 | 20.1 ± 0.05 |
| **TCLP Pb** | | | | | |
| 0 | 52.1 ± 0.75 | 45.9 ± 0.80 | 39.8 ± 0.81 | 33.6 ± 0.87 | 62.7 ± 0.78 |
| 15 | 44.5 ± 0.78 | 45.6 ± 0.90 | 37.6 ± 0.84 | 43.8 ± 0.89 | 65 ± 0.75 |
| 30 | 38.3 ± 0.80 | 33.9 ± 0.98 | 23.4 ± 0.89 | 27.6 ± 0.87 | 56.9 ± 0.76 |
| 45 | 32.6 ± 0.69 | 28.0 ± 0.90 | 15.6 ± 0.90 | 10.4 ± 0.65 | 48.3 ± 0.85 |
| **DTPA Cr** | | | | | |
| 0 | 41.2 ± 0.2 | 37.5 ± 1.1 | 33.4 ± 0.4 | 30.0 ± 1.1 | 50.5 ± 0.5 |
| 15 | 39.4 ± 0.8 | 35.1 ± 0.8 | 27.0 ± 0.1 | 28.9 ± 1.5 | 48.5 ± 0.7 |
| 30 | 36.4 ± 1.5 | 30.0 ± 0.9 | 21.5 ± 0.8 | 16.9 ± 0.5 | 44.3 ± 0.9 |
| 45 | 31.7 ± 1.9 | 25.5 ± 1.7 | 19.4 ± 0.8 | 11.4 ± 0.8 | 41.7 ± 0.1 |

**Table 4** continued

| Days | Heavy metal concentration (mg/kg dry matter) | | | | |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| | TCLP Cd | | | | |
| 0 | 2.9 ± 0.01 | 2.8 ± 0.02 | 2.7 ± 0.01 | 2.6 ± 0.02 | 2.4 ± 0.01 |
| 15 | 2.2 ± 0.04 | 2.1 ± 0.01 | 1.3 ± 0.02 | 1.1 ± 0.03 | 2.3 ± 0.01 |
| 30 | 1.4 ± 0.04 | 1.1 ± 0.01 | 0.8 ± 0.07 | 0.3 ± 0.03 | 1.7 ± 0.02 |
| 45 | 1.3 ± 0.01 | 1.0 ± 0.01 | 0.4 ± 0.02 | 0.1 ± 0.05 | 1.5 ± 0.02 |
| | TCLP Cr | | | | |
| 0 | 55.0 ± 0.1 | 49.6 ± 0.4 | 44.5 ± 0.3 | 39.4 ± 0.2 | 66.4 ± 0.3 |
| 15 | 50.3 ± 0.7 | 44.1 ± 1.8 | 39.4 ± 1.4 | 29.7 ± 0.3 | 65.4 ± 1.2 |
| 30 | 46.5 ± 1.6 | 33.8 ± 1.4 | 28.0 ± 0.4 | 16.5 ± 0.4 | 58.0 ± 1.3 |
| 45 | 39.1 ± 1.4 | 29.1 ± 0.9 | 25.5 ± 0.2 | 14.5 ± 0.4 | 50.8 ± 1.7 |

– Not detected, mean ± SD, n = 3

leachable heavy metals (Zn, Cu, Mn, Fe, Pb and Cr). The leachable concentration of heavy metals in all the trials was under the threshold limits. Ni and Cd were not detected in the water extract. The change in the order of concentrations of the bioavailable and leachable forms of heavy metals compared with the order of the total metal concentrations clearly indicated that the toxicology was dependent only on the bioavailable and leachable forms rather than the total metal concentrations.

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