

Reduction of bioavailability and leachability of heavy metals during agitated pile composting of *Salvinia natans* weed of Loktak lake

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Abstract

Purpose Composting of harvested *Salvinia natans* weed of Loktak lake of Manipur, India can protect the precious lake from the unwanted growth of the weeds but there is a need to assess the total amount and bioavailable forms of heavy metals and minerals of the final compost before application as a soil conditioner.

Methods Studies were conducted on the transformations of the physico-chemical parameters and the total amount, bioavailable and leachable forms of heavy metals and minerals during agitated pile composting of *S. natans* with rice husk and cattle manure in five different combinations [trial 1 (5 *S. natans*: 4 cattle manure: 1 rice husk), trial 2 (6 *S. natans*: 3 cattle manure: 1 rice husk), trial 3 (7 *S. natans*: 2 cattle manure: 1 rice husk), trial 4 (8 *S. natans*: 1 cattle manure: 1 rice husk) and trial 5 (10 *S. natans*: 0 cattle manure: 0 rice husk)].

Results The highest temperature (52.2 °C) was monitored in trial 2 having 30 % cattle manure during the process. Highest reduction of moisture content and volatile solid in trial 2 corroborated the temperature profile. Total concentrations of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr), total concentration of nutrients (Na, K, Ca, and Mg) and the water-soluble forms of the nutrients were enhanced significantly. The water-soluble, plant-available and leachable metals decreased favorably with maximum

reduction observed mostly in trial 2. The study also revealed that the total concentrations of Zn (211–254 mg/kg), Ni (310–345 mg/kg) and Pb (805–891 mg/kg) of the final composts were higher than that of Cr (140–207 mg/kg) but the water-soluble forms of Zn (3.6–4.7 % of total Zn), Ni (NIL) and Pb (0.7–1.0 % of total Pb) were lower than that of Cr (13.3–19.3 % of total Cr) indicating that Cr in the composts had higher toxicity potential. The leachable heavy metals were within the threshold limits prescribed for agricultural application.

Conclusions Composting of *S. natans* biomass with appropriate proportion of cattle manure reduced the bioavailable and leachable forms of the heavy metals in the biomass.

Keywords *Salvinia natans* · Composting heavy metals · Bioavailability · DTPA extraction · TCLP test

Introduction

Northeast India has vast natural productive wetlands which support valuable biodiversity or heterogeneity. Loktak lake is one of the important wetlands in the region and plays an important role in providing regional ecological and economic security. The lake is situated in the southern part of the Manipur valley in Bishnupur district (24°25′–24°42′N; 93°46′–93°55′E) and is the largest natural fresh water lake of northeast India. It covers an area of about 287 km² which varies with the maintenance of water level of Ithai barrage across the outlet of the lake (768.5 m above mean sea level). The main characteristic feature of the lake is the presence of floating islands, locally called ‘phumdis’ which are heterogeneous mass of soil, vegetation and organic matter at various stages of decomposition and occurring in

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different sizes. There are also many aquatic species in the clear water zone of the lake and the dominating invasive species, *Salvinia natans* has profusely outgrown and damaged the aquatic ecosystem by its rapid growth and displacement of native plants that provide food and habitat to the native animals and waterfowl.

Proliferation and change in the character of phumdi and the aquatic weeds have led to the problems of eutrophication; water quality deterioration; decline in fish production; reduction of water storage capacity; interfering with the production of agricultural crops; and blockage of channels leading to retardation of natural water flow. These all have resulted in seriously affecting the overall health of the lake (Santosh and Bidan 2002). The authors have demonstrated the potential for effective composting of phumdi biomass (Singh and Kalamdhad 2014c; Singh et al. 2014). The *S. natans* of Loktak lake may also be suitably used for bioprocessing or biomass conversion due to high organic content (Devi et al. 2002; Mande and Lata 2005). Therefore, composting followed by land application can be one of the economical options for the treatment and disposal of the hugely available *S. natans* biomass harvested to control its proliferation in the lake.

Composting of *S. natans* is not reported anywhere and there is scope for investigation in using it as organic manure and as a soil conditioner. But the phumdis and weeds act as a biological sink of the pollutants coming into the lake and there is possible presence in their biomass—heavy metals which have the potential to cause adverse effect on the environment from the application of the resulting composts. High and excessive accumulation of heavy metals in the soil, their uptake by plants, successive accumulation in human tissues, and biomagnifications through the food chain are the main concerns for 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). It is the bioavailable and leachable forms of the heavy metals rather than the total heavy metal concentrations that provide useful information about the risk of toxicity and remobilization of the heavy metals in the environment (Liu et al. 2007). Bioavailable forms of heavy metals may be: (1) water-soluble heavy metals which are the most biologically active heavy metals. They have the highest potential for contaminating the surface and ground water and entering the food chain (Iwegbue et al. 2007); (2) diethylenetriamine penta-acetic acid (DTPA)-extractable heavy metals which are the plant-available metal forms 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 organic-bound metal

fractions of calcareous soils (Walter et al. 2006). Therefore, assessment of DTPA-extractable metal fraction is carried out as a supplemental approach to check the plant availability of heavy metals (Bragato et al. 1998; Fang and Wong 1999; Fuentes et al. 2006); and (3) leachable heavy metals which are the mobile forms of the metals associated with the movement of organic and inorganic analytes present in the liquid, solid and multi-phasic forms of the wastes. The leachable heavy metals are determined through toxicity characteristic leaching procedure (TCLP) tests. If the TCLP extract of the test indicates that the heavy metals are present at concentrations above their regulatory levels after accounting for dilution from other fractions of the extract, then the waste is hazardous (US Environmental Protection Agency 1992).

Much work has been carried out on the bioavailability of heavy metals during composting of water hyacinth and phumdi biomass using different techniques (Singh and Kalamdhad 2012, a, b; Singh et al. 2014). However, no such study has been reported during composting of *S. natans* weed. Therefore, the aim of the study was to determine the variations of total heavy metals and minerals and their bioavailability during the agitated pile composting of *S. natans* amended with cattle manure and rice husk. The study also investigated the leachability of the heavy metals during the composting process.

Materials and methods

Feedstock materials

Salvinia natans was collected from Loktak lake near Thanga village, Bishnupur district, Manipur, India by the local boatmen and brought to the composting shed of Manipur Pollution Control Board, Imphal, Manipur, India. Cattle manure and rice husks were obtained from Central Agriculture University, Imphal, India. The *S. natans* was prepared for composting through cutting/shredding (maximum size restricted to 10 mm to provide better aeration and moisture control) and uniform premixing with cattle manure and rice husk. The initial characteristics of the composting materials and the proportion of the materials of the five trials are shown in Table 1.

Agitated pile composting

Composting materials were formed into trapezoidal piles (length 240 cm, base width 40 cm, top width 10 cm and height not less than 30 cm) with length to base width (L/W) ratio 6 (Singh and Kalamdhad 2012, 2014c). The five different agitated piles containing 150 kg of waste mixture were composted for 30 days and sampling was carried out

Table 1 Initial characteristic of the composting materials

Parameters	<i>Salvinia natans</i>	Cattle manure	Rice husk
Trial 1 (kg)	75	60	15
Trial 2 (kg)	90	45	15
Trial 3 (kg)	115	20	15
Trial 4 (kg)	120	15	15
Trial 5 (kg)	150	0	0
Moisture content (%)	90.5 ± 1.2	86.4 ± 0.9	9.6 ± 0.5
pH	5.6 ± 0.01	6.8 ± 0.05	6.14 ± 0.05
Electrical conductivity (EC) (dS/m)	3.5 ± 0.03	3.7 ± 0.03	1.9 ± 0.02
Volatile solids (VS) (%)	74.8 ± 0.25	72.1 ± 0.22	79.3 ± 0.23
Na (mg/kg dry matter)	6995 ± 35.5	2400 ± 5.5	1925 ± 12.3
K (mg/kg dry matter)	18,920 ± 95.0	987.5 ± 22.5	8250 ± 10.4
Ca (mg/kg dry matter)	9337 ± 90.0	8193 ± 37.2	3885 ± 12.4
Mg (mg/kg dry matter)	7277 ± 55.0	4957 ± 24.5	999 ± 7.8
Zn (mg/kg dry matter)	210 ± 0.50	182.4 ± 1.95	100 ± 2.34
Cu (mg/kg dry matter)	53.5 ± 0.50	57.8 ± 0.8	20 ± 0.45
Mn(mg/kg dry matter)	999 ± 7.0	1128 ± 1.5	550 ± 3.5
Fe (mg/kg dry matter)	6754 ± 32.5	1861 ± 2.8	3350 ± 11.4
Ni (mg/kg dry matter)	265 ± 1.75	266 ± 2.6	140 ± 2.1
Cd (mg/kg dry matter)	53.8 ± 2.2	49.9 ± 1.07	67 ± 0.20
Pb (mg/kg dry matter)	756 ± 0.75	766 ± 0.5	60 ± 0.15
Cr (mg/kg dry matter)	143.2 ± 1.22	124.4 ± 0.5	10 ± 0.08

Mean ± SD (n = 3)

after manual turning on 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30th days. Grab samples were collected from five different locations mainly from the mid and end portions of the piles to make up 1 kg and thoroughly mixed to form a homogenous sample. Collected samples were immediately air dried at 105 °C in oven, ground to pass through 0.2 mm sieve and stored for analysis of the physico-chemical parameters.

Physico-chemical analysis

A digital thermometer was used for measuring temperature during the composting process. Moisture content (MC) was determined from weight loss of the wet compost sample (105 °C for 24 h) using the gravimetric method (BIS 1982). The pH and electrical conductivity (EC) of the compost samples were measured using a pH and a conductivity meter (BIS 1982) on the filtrate (Whatman filter paper no. 42) of 10 g of the sample shaken with 100 mL distilled water in a horizontal shaker for 2 h and kept for ½ h for settling down of the solids. Volatile solids (VS) were determined by the ignition method (550 °C for 2 h in muffle furnace) (BIS 1982). The Flame Photometer (Systronic 128) was used for analysis of sodium (Na), potassium (K) and calcium (Ca) concentrations, whereas 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) concentrations after digestion of 0.2 g dry sample with 10 mL of 5 H₂SO₄ and 1 HClO₄ mixture in block digestion system (Pelican Equipments Chennai-India) for 2 h at 300 °C (Singh and Kalamdhad 2013a, b).

Water-soluble nutrients and heavy metals were determined after extraction of 2.5 g sample with 50 mL of distilled water at room temperature for 2 h in a shaker at 100 rpm (Singh and Kalamdhad 2013b). Diethylene triamine penta-acetic 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 CaCl₂ and 0.1 M (triethanolamine) buffered to pH 7.3 at 100 rpm (Guan et al. 2011). Toxicity characteristic leaching procedure (TCLP) test was performed according to USEPA Method 1311 (US Environmental Protection Agency 1992). Accordingly, 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 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 was stored in a plastic reagent bottle at 4 °C for analysis of



selected heavy metals. All the results reported are the means of three replicates. Repeated measures were treated with analysis of variance (ANOVA) using SPSS software to establish statistical significance.

Results and discussion

Physico-chemical analysis

Figure 1 shows the temperature profile of the five piles during the composting. The temperatures of the piles increased rapidly within 2 days of the composting process. The highest temperature (52.2 °C) was measured on 4th day in trial 2 pile whereas the maximum temperature of trial 1 pile was 49.8 °C on the 6th day. Piles of trials 3 and 4 showed maximum temperatures of 43.2 and 42.3 °C on the 8th day of the composting, respectively. The pile of control trial 5 showed maximum temperature of only 32.2 °C on the 5th day of the process indicating that that pile composting of *S. natans* without addition of cattle manure and rice husk was not feasible. The temperature increased in all the piles due to the release of heat caused by microbial catabolism.

The variation in pH, MC, EC and VSs is shown in Fig. 2. The pH of all the trials increased significantly (ANOVA, $P < 0.05$) during the composting process from 6.2 to 7.5, 6.1 to 7.4, 6.2 to 7.4, 6.2 to 7.1 and 5.6 to 6.8 in trials 1, 2, 3, 4 and 5, respectively (Fig. 2a). The increase in pH can be attributed to ammonification or mineralization of organic nitrogen (Wong et al. 2001) and release of basic salts or degradation (decarboxylation) of organic acids (Cayuela et al. 2008) due to the microbial activity. The pH of trial 4, having highest proportion of cattle manure and least void spaces, increased only after 6 days due to the comparatively higher absorption of CO₂ and formation of organic acids preventing pH rise. In case of control trial 5

without cattle manure and saw dust, the initial pH of *S. natans* was below 6. The pH increased rapidly due to availability of sufficient oxygen from regular turning and the low temperature profile of the pile (below 40 °C) (Smars et al. 2002). The observed pH values were within the optimal range required for the development of bacteria (6.0–7.5) and fungi (5.5–8.0), and the variation in pH is similar with the results of pile composting of water hyacinth (Prasad et al. 2013; Singh and Kalamdhad 2013a, b).

US Composting Council prescribes a maximum limit of 60–80 % MC for the composting materials (TMECC 2002). The composting material should have bare minimum MC for the survival of microorganisms, whereas the rate of oxygen diffusion of the pores decreases when MC is high. Moisture is liberated as water vapor during the composting process and the loss of moisture can be viewed as an index of decomposition rate (Liao et al. 1996). The order of moisture loss in the piles was: trial 2 (30.8 %) > trial 1 (24.9 %) > trial 3 (20.8 %) > trial 4 (16.8 %) > trial 5 (13.1 %) (Fig. 2b). Therefore, trial 2 had the highest decomposition rate which was in agreement with the temperature profile of the piles. The MCs in all the piles were mostly above 60 % during the active phase. ANOVA analysis shows significant variation of MC among the trials ($P < 0.05$).

Figure 2c shows that there was an initial increase in the EC of the *S. natans* composts of all trials due to the release of mineral salts such as phosphates and ammonium ions during the decomposition of organic matter (Fang and Wong 1999). The volatilization of ammonia and precipitation of mineral salts could be the reasons for the subsequent decrease of EC in trials 1, 2, 3 and 4 (Wong et al. 2001). In the final composts, the ECs measured were 3.2, 2.7, 2.8 and 3.1 dS/m in trials 1, 2, 3 and 4, respectively. The final EC (3.7 dS/m) of trial 5 was higher than the initial EC (3.4 dS/m) after 30 days. EC of the compost reflects its degree of salinity (Huang et al. 2004). A

Fig. 1 Temperature profiles of the piles during composting

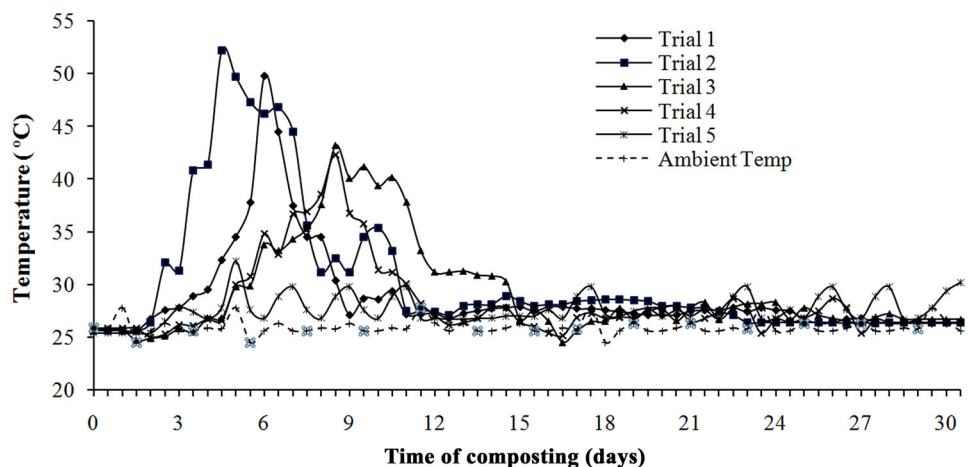
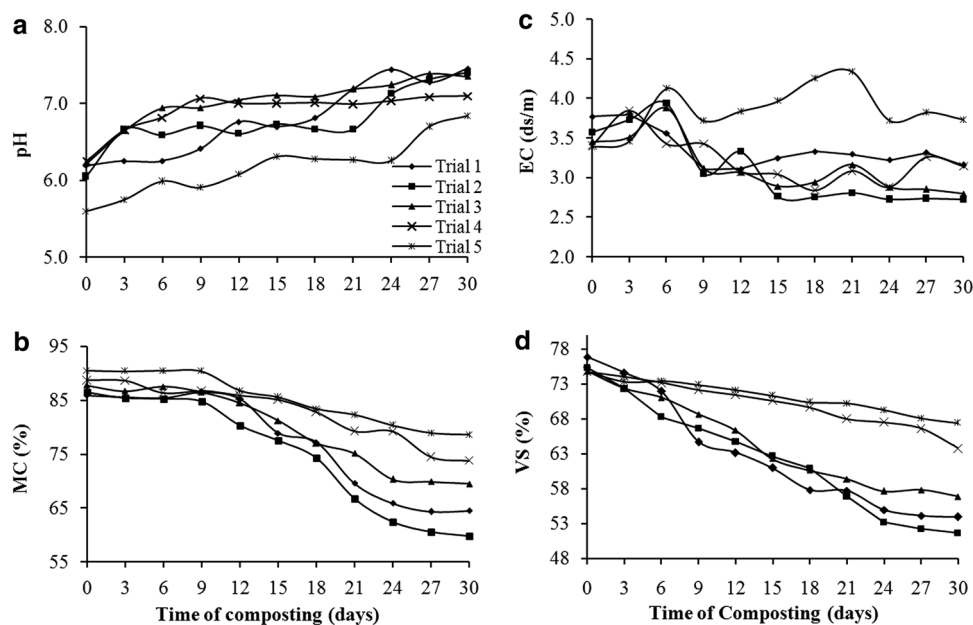


Fig. 2 Temporal variation of pH, electrical conductivity (EC), moisture content (MC) and volatile solids (VS)



common disadvantage of compost as fertilizer is weakening of the water intake ability of plants due to osmotic effect from high salt concentrations (Koivula et al. 2004). EC about 4 dS/m or higher in composts will adversely influence plant growth, e.g., low germination rate, withering, etc. (Chen 1999; Lin 2008; Gao et al. 2010). Therefore, composts with high EC value must be mixed well with soil or other materials with low EC before application for growing crops. The variation of EC among all trials was statistically significant ($P < 0.05$).

The VS content is an indicator of organic matter present in the compost and decreases during the composting process due to degradation of the organic components by the microorganisms and loss of carbon in the form of CO_2 . In the present study, the highest reduction of VS (31.4 %) was observed in trial 2 and the order of reduction was trial 2 (31.4 %) > trial 1 (29.7 %) > trial 3 (24.1 %) > trial 4 (14.7 %) > trial 5 (9.9 %) (Fig. 2d). The reductions in the concentration of the VS in all the five trials were significant ($P < 0.05$).

The changes in concentrations of nutrients (total and water-soluble forms) and heavy metals (total and water-soluble forms) during the composting process are shown in Tables 2 and 3, respectively. The total concentrations of nutrients (Na, K, Ca and Mg) and heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) increased due to weight loss of the dry matter from the decomposition of the organic matter resulting in release of CO_2 and the subsequent mineralization during the composting process (Singh and Kalamdhad 2013a). The order of total nutrients and heavy metal concentrations of *S. natans* compost was as follows:

Nutrients: $\text{K} > \text{Ca} > \text{Mg} > \text{Na}$

Heavy metals: $\text{Fe} > \text{Mn} > \text{Pb} > \text{Ni} > \text{Zn} > \text{Cr} > \text{Cu} > \text{Cd}$

In case of phumdi biomass composting, the order of metal concentrations of phumdi compost was $\text{Fe} > \text{Pb} > \text{Mn} > \text{Zn} > \text{Ni} > \text{Cr} > \text{Cd} > \text{Cu}$ (Singh et al. 2014). Increase of concentration of trace elements in soils due to prolonged application of composts may cause toxicity to plants, animals and humans. Therefore, bioavailability studies of the heavy metal contents of the *S. natans* composts are extremely important before applying the compost to the agriculture field.

Bioavailability of nutrients and heavy metals

Water-soluble nutrients and heavy metals

The water-soluble nutrients of the final composts of all trials increased in the range of about 10.9–26.4 % for Na, 14.8–55.1 % for K, 14.2–40.1 % for Ca and 17.4–66.2 % for Mg (Table 2). Lowest enhancement in water solubility of K, Ca and Mg was in trial 5 which contained only *S. natans* biomass. Similar results were also reported during the agitated pile composting of phumdi biomass (Singh et al. 2014). The increase in concentration of water-soluble nutrients was also due to weight loss of the dry matter during the decomposition and mineralization of the organic matter (Singh and Kalamdhad 2012). The variation in concentration of water-soluble nutrients among all trials was significant ($P < 0.05$).

Water-soluble fraction of heavy metals are the most easily bioavailable fraction and belongs to the most toxic constituents of composts (Singh and Kalamdhad 2013b, c, 2014a). The concentration of the water-soluble forms of heavy metals (Zn, Cu, Mn, Fe Pb and Cr) reduced significantly ($P < 0.05$) in all trials during the composting of

Table 2 Variation of total and water-soluble nutrients concentration during the composting process

Days	Nutrients concentration (mg/kg dry matter)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	<i>Total Na</i>				
0	4825 ± 65	4820 ± 95	4990 ± 90	5817 ± 27	6175 ± 35
6	5525 ± 85	5165 ± 55	5785 ± 55	6160 ± 20	6375 ± 35
12	7175 ± 35	6365 ± 55	6580 ± 20	6685 ± 75	6570 ± 40
18	7850 ± 30	7180 ± 60	6755 ± 5	6675 ± 205	6965 ± 155
24	7985 ± 95	8210 ± 85	7240 ± 70	7115 ± 15	7280 ± 70
30	8350 ± 70	8440 ± 70	7460 ± 50	7505 ± 25	7510 ± 5
	<i>Water-soluble Na</i>				
0	1632 ± 14	1739 ± 7	1725 ± 3	1535 ± 3	1545 ± 21
6	1800 ± 9	1747 ± 3	1816 ± 3	1515 ± 2	1528 ± 4
12	1814 ± 3	1809 ± 3	1852 ± 5	1607 ± 2	1634 ± 11
18	1906 ± 3	1987 ± 2	1893 ± 6	1748 ± 3	1652 ± 3
24	1948 ± 3	2160 ± 4	1947 ± 1	1753 ± 2	1704 ± 3
30	1953 ± 2	2198 ± 9	2087 ± 1	1792 ± 5	1718 ± 3
	<i>Total Ca</i>				
0	7385 ± 50	7718 ± 53	8130 ± 25	8400 ± 25	8920 ± 103
6	8204 ± 55	7903 ± 08	8670 ± 50	8833 ± 13	9668 ± 58
12	8340 ± 80	8638 ± 18	9848 ± 28	9063 ± 18	9693 ± 13
18	8635 ± 05	9070 ± 15	9861 ± 25	9176 ± 05	9863 ± 23
24	9763 ± 43	10,103 ± 68	9778 ± 43	9460 ± 50	10,283 ± 48
30	10,895 ± 75	11,550 ± 03	10,533 ± 88	10,710 ± 95	10,293 ± 13
	<i>Water-soluble Ca</i>				
0	2874 ± 08	2666 ± 24	3097 ± 05	3179 ± 05	3262 ± 22
6	3115 ± 09	2958 ± 04	2875 ± 03	3237 ± 13	3301 ± 07
12	3255 ± 07	3080 ± 02	3362 ± 12	3448 ± 04	3277 ± 13
18	3252 ± 10	3339 ± 07	3755 ± 21	3422 ± 16	3568 ± 14
24	3529 ± 41	3632 ± 10	3943 ± 07	3676 ± 32	3617 ± 05
30	3679 ± 29	3736 ± 14	3968 ± 00	3677 ± 07	3635 ± 07
	<i>Total K</i>				
0	15,780 ± 45	18,135 ± 90	17,190 ± 85	18,601 ± 15	18,825 ± 95
6	16,333 ± 107	19,340 ± 18	18,125 ± 15	18,935 ± 20	18,606 ± 38
12	17,523 ± 18	19,344 ± 112	19,713 ± 152	19,405 ± 10	18,900 ± 40
18	18,415 ± 30	21,168 ± 63	19,148 ± 77	19,673 ± 38	19,255 ± 25
24	19,503 ± 28	22,430 ± 0	19,943 ± 3	19,930 ± 28	19,745 ± 10
30	20,108 ± 48	23,548 ± 18	21,085 ± 20	20,725 ± 56	20,150 ± 14
	<i>Water-soluble K</i>				
0	2860 ± 10	3044 ± 22	3336 ± 38	3392 ± 16	3536 ± 6
6	3144 ± 6	3334 ± 38	3572 ± 6	3421 ± 3	3615 ± 7
12	3154 ± 22	3572 ± 6	3580 ± 4	3697 ± 9	3508 ± 64
18	3471 ± 41	3722 ± 12	3852 ± 10	3920 ± 48	3751 ± 13
24	4364 ± 12	3937 ± 39	4370 ± 6	3903 ± 51	3900 ± 6
30	4437 ± 11	4381 ± 9	4542 ± 8	4036 ± 12	4133 ± 23
	<i>Total Mg</i>				
0	6445 ± 05	6848 ± 128	6520 ± 90	6958 ± 57	7215 ± 55
6	6682 ± 99	7450 ± 140	7046 ± 90	7530 ± 115	7361 ± 35
12	6983 ± 98	7720 ± 110	7250 ± 92	7631 ± 170	7459 ± 15
18	8403 ± 72	7884 ± 54	7661 ± 23	7796 ± 20	7580 ± 25
24	8530 ± 70	8208 ± 238	7802 ± 139	8026 ± 34	7795 ± 25
30	8502 ± 64	9214 ± 167	7851 ± 184	8064 ± 41	7888 ± 48
	<i>Water-soluble Mg</i>				
0	1878 ± 36	1770 ± 20	1914 ± 60	1659 ± 29	2063 ± 19
6	1832 ± 10	1853 ± 09	2070 ± 03	17,780 ± 03	2069 ± 05
12	2127 ± 15	1827 ± 02	2433 ± 30	1851 ± 03	2249 ± 24
18	2433 ± 05	2361 ± 08	2414 ± 15	1874 ± 08	2353 ± 00
24	2550 ± 18	2751 ± 22	2553 ± 20	2102 ± 35	2226 ± 23
30	2713 ± 25	2942 ± 28	2741 ± 11	2248 ± 00	2476 ± 32

Mean ± SD (*n* = 3) (ANOVA: *P* < 0.05)

Table 3 Variation of total and water-soluble heavy metal concentrations during the composting process

Days	Heavy metal concentrations (mg/kg dry matter)									
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
<i>Total Zn</i>										
0	192.0 ± 1.0	159.5 ± 1.0	196.8 ± 2.3	203.0 ± 1.5	210.0 ± 0.5	51.0 ± 1.0	63.5 ± 7.0	52.8 ± 1.3	47.3 ± 1.8	53.0 ± 0.5
6	195.5 ± 3.0	167.3 ± 1.8	194.0 ± 1.5	206.0 ± 2.0	211.8 ± 5.8	58.0 ± 1.0	76.5 ± 0.5	54.3 ± 1.3	55.5 ± 1.0	54.3 ± 1.3
12	208.5 ± 2.0	190.3 ± 2.8	206.0 ± 1.5	211.8 ± 2.3	232.5 ± 1.5	62.0 ± 0.5	79.5 ± 1.0	70.5 ± 1.5	63.3 ± 0.8	63.3 ± 0.8
18	209.8 ± 1.3	195.1 ± 1.9	218.3 ± 2.3	216.8 ± 2.3	247.5 ± 1.0	67.8 ± 1.3	87.8 ± 1.8	72.5 ± 1.5	77.5 ± 1.5	77.3 ± 1.3
24	218.5 ± 2.5	207.5 ± 2.0	234.8 ± 0.8	219.8 ± 0.8	243.3 ± 2.3	85.0 ± 1.0	87.3 ± 3.8	83.0 ± 6.0	76.5 ± 1.0	79.5 ± 1.0
30	218.3 ± 1.3	211.3 ± 0.8	234.3 ± 2.3	225.5 ± 1.3	254.3 ± 1.3	88.5 ± 1.0	92.3 ± 1.8	86.8 ± 0.8	85.5 ± 1.5	82.8 ± 0.8
<i>Water-soluble Zn</i>										
0	10.5 ± 0.08	10.3 ± 0.11	13.5 ± 0.05	13.5 ± 0.1	14.4 ± 0.07	7.6 ± 0.1	6.4 ± 0.0	7.6 ± 0.1	7.7 ± 0.1	7.9 ± 0.1
6	10.3 ± 0.08	9.9 ± 0.01	12.3 ± 0.08	13.1 ± 0.1	13.7 ± 0.02	6.3 ± 0.1	6.3 ± 0.1	7.0 ± 0.0	7.8 ± 0.1	7.4 ± 0.1
12	10.4 ± 0.06	9.2 ± 0.14	12.4 ± 0.04	12.2 ± 0.0	13.3 ± 0.09	5.6 ± 0.1	5.2 ± 0.2	6.4 ± 0.1	7.3 ± 0.1	7.0 ± 0.3
18	9.6 ± 0.05	9.2 ± 0.14	11.6 ± 0.07	11.8 ± 0.1	12.1 ± 0.08	5.5 ± 0.1	4.4 ± 0.0	5.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.0
24	8.7 ± 0.11	8.3 ± 0.08	11.1 ± 0.08	11.1 ± 0.1	11.7 ± 0.06	4.8 ± 0.0	4.4 ± 0.0	5.1 ± 0.0	5.7 ± 0.1	6.3 ± 0.0
30	7.8 ± 0.06	7.8 ± 0.04	11.0 ± 0.05	10.3 ± 0.1	11.4 ± 0.05	4.8 ± 0.1	4.3 ± 0.0	4.7 ± 0.1	5.6 ± 0.1	6.3 ± 0.0
<i>Total Mn</i>										
0	804 ± 2.0	1076 ± 20.8	1167 ± 11.5	1165 ± 7.8	1000 ± 7.0	6786 ± 25	5736 ± 0.3	6652 ± 26.8	7559 ± 51.5	6755 ± 32.3
6	839 ± 6.3	1322 ± 12.3	1319 ± 1.8	1308 ± 7.3	1038 ± 5.5	6679 ± 13.3	5929 ± 18.5	6894 ± 12.3	7236 ± 29.8	7110 ± 2.8
12	932 ± 4.3	1433 ± 5.0	1318 ± 12.3	1234 ± 10.5	1112 ± 8.8	7410 ± 33	6078 ± 1.3	7335 ± 09.0	7962 ± 15.0	7455 ± 23.3
18	868 ± 10.0	1498 ± 4.5	1383 ± 2.8	1425 ± 15.5	1191 ± 3.0	7745 ± 5.8	6635 ± 7.5	7441 ± 00.3	8449 ± 06.8	7545 ± 27.8
24	959 ± 1.3	1553 ± 7.8	1375 ± 16.8	1465 ± 8.5	1255 ± 0.3	8416 ± 9.3	7280 ± 17.5	7712 ± 01.5	8532 ± 19.0	7625 ± 12.5
30	1028 ± 12	1609 ± 1.8	1584 ± 9.8	1466 ± 15.5	1222 ± 4.3	8608 ± 2.8	7905 ± 3.0	8120 ± 09.3	9294 ± 32.0	7678 ± 4.3
<i>Water-soluble Mn</i>										
0	51.9 ± 1.5	56.4 ± 0.1	56.1 ± 0.3	61.0 ± 0.8	55.4 ± 1.7	249.6 ± 0.10	257.9 ± 0.4	266.9 ± 2.1	231.1 ± 6.2	267.4 ± 2.9
6	51.1 ± 0.1	44.2 ± 1.1	55.6 ± 0.3	58.9 ± 0.8	54.8 ± 0.4	243.8 ± 18.7	229.1 ± 4.0	249.8 ± 3.0	207.1 ± 0.2	248.0 ± 1.1
12	48.4 ± 0.2	40.3 ± 0.1	53.0 ± 0.1	58.1 ± 0.1	49.1 ± 0.2	176.3 ± 0.95	189.0 ± 3.9	260.6 ± 0.3	200.7 ± 5.9	249.3 ± 2.7
18	43.2 ± 0.5	36.7 ± 0.1	51.5 ± 0.3	53.0 ± 0.6	53.5 ± 0.2	183.8 ± 1.30	146.1 ± 1.0	186.6 ± 0.3	185.3 ± 1.0	235.3 ± 1.8
24	43.7 ± 0.1	36.2 ± 0.2	51.2 ± 0.2	51.0 ± 0.7	45.1 ± 3.1	152.3 ± 0.90	142.2 ± 0.1	182.5 ± 0.2	165.5 ± 2.7	222.3 ± 0.5
30	37.4 ± 0.5	34.2 ± 0.3	47.0 ± 1.1	52.3 ± 0.7	44.8 ± 0.1	137.5 ± 0.20	126.1 ± 1.0	172.0 ± 1.1	143.9 ± 1.0	205.4 ± 0.9
<i>Total Ni</i>										
0	265.5 ± 9.5	261.3 ± 0.3	280.8 ± 9.8	289.3 ± 1.3	265.8 ± 1.8	766.5 ± 2.0	746.0 ± 2.5	717.3 ± 2.3	739.0 ± 2.0	756.3 ± 0.8
6	268.3 ± 1.3	265.8 ± 1.3	275.0 ± 3.0	303.5 ± 1.5	274.3 ± 13.3	765.3 ± 3.3	763.0 ± 2.5	767.5 ± 4.5	730.5 ± 3.0	754.3 ± 1.3
12	314.1 ± 8.4	288.8 ± 1.8	275.5 ± 7.5	312.3 ± 1.8	289.3 ± 1.3	792.8 ± 4.3	707.8 ± 9.8	779.5 ± 4.0	757.0 ± 1.5	801.3 ± 2.8
18	321.5 ± 4.5	308.3 ± 1.3	307.3 ± 1.3	336.3 ± 7.3	375.8 ± 7.3	789.3 ± 4.8	799.5 ± 1.0	791.8 ± 2.8	776.0 ± 2.0	807.3 ± 1.8
24	344.0 ± 1.5	326.8 ± 1.3	302.3 ± 1.8	346.3 ± 2.8	302.5 ± 6.0	837.0 ± 3.5	839.0 ± 2.0	802.8 ± 1.8	804.0 ± 3.5	834.0 ± 2.0

Table 3 continued

Days	Heavy metal concentrations (mg/kg dry matter)									
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
30	344.5 ± 4.0	333.0 ± 2.5	316.8 ± 0.3	345.3 ± 0.8	309.8 ± 3.8	890.5 ± 3.5	844.0 ± 3.5	805.3 ± 1.3	805.0 ± 0.5	848.3 ± 3.8
	<i>Water-soluble Ni</i>									
0	–	–	–	–	–	8.9 ± 0.36	10.8 ± 0.40	8.6 ± 0.3	10.5 ± 0.1	9.7 ± 0.1
6	–	–	–	–	–	8.5 ± 0.09	10.3 ± 0.10	8.5 ± 0.0	9.9 ± 0.2	8.8 ± 0.1
12	–	–	–	–	–	7.7 ± 0.05	8.6 ± 0.10	7.8 ± 0.0	9.1 ± 0.6	9.2 ± 0.0
18	–	–	–	–	–	7.5 ± 0.16	8.9 ± 0.00	7.7 ± 0.0	9.3 ± 0.2	8.6 ± 0.1
24	–	–	–	–	–	7.7 ± 0.04	7.4 ± 0.20	6.0 ± 0.0	8.3 ± 0.1	9.0 ± 0.1
30	–	–	–	–	–	6.4 ± 0.10	6.5 ± 0.30	5.5 ± 0.2	8.3 ± 0.1	8.3 ± 0.0
	<i>Total Cr</i>									
0	47.8 ± 1.3	47.5 ± 1.8	42.8 ± 1.8	49.5 ± 1.0	53.8 ± 2.3	96.9 ± 0.9	89 ± 1.5	82.5 ± 1.0	109.3 ± 2.2	143.3 ± 1.2
6	52.0 ± 0.5	43.5 ± 1.5	47.5 ± 1.5	52.0 ± 0.5	52.3 ± 1.8	109.0 ± 2.0	96.8 ± 0.7	89.7 ± 1.5	122.3 ± 1.2	104.5 ± 2.5
12	56.0 ± 1.0	55.8 ± 0.2	51.0 ± 0.5	58.5 ± 0.5	58.5 ± 1.0	131.9 ± 1.2	108.8 ± 1.8	94.5 ± 2.0	137.8 ± 0.3	164.4 ± 2.4
18	57.8 ± 1.3	57.0 ± 0.5	54.0 ± 2.8	59.0 ± 0.5	59.5 ± 1.0	126.6 ± 4.5	122.5 ± 0.5	124.3 ± 3.2	164.5 ± 7.5	132.3 ± 4.8
24	61.5 ± 0.5	59.0 ± 0.5	58.5 ± 1.0	62.3 ± 1.3	58.8 ± 0.3	159.5 ± 2.5	132.8 ± 4.3	133.6 ± 0.6	185.8 ± 1.8	194.0 ± 1.5
30	63.8 ± 0.3	61.5 ± 0.5	58.0 ± 1.0	62.0 ± 1.5	63.0 ± 1.0	167.0 ± 8.9	142.0 ± 1.5	139.8 ± 2.3	189.5 ± 2.5	206.8 ± 0.8
	<i>Water-soluble Cd</i>									
0	–	–	–	–	–	29.4 ± 0.40	34.5 ± 0.17	34.4 ± 0.07	32.4 ± 1.04	33.7 ± 0.04
6	–	–	–	–	–	28.0 ± 0.12	30.3 ± 0.16	33.0 ± 0.59	30.7 ± 0.11	31.6 ± 0.33
12	–	–	–	–	–	25.7 ± 0.69	28.4 ± 0.06	28.9 ± 0.31	30.2 ± 0.05	31.9 ± 1.00
18	–	–	–	–	–	24.5 ± 0.17	26.8 ± 0.33	30.4 ± 0.25	28.8 ± 0.40	30.5 ± 0.17
24	–	–	–	–	–	25.6 ± 0.13	25.4 ± 0.11	27.8 ± 0.06	27.3 ± 0.10	29.5 ± 0.14
30	–	–	–	–	–	22.2 ± 0.07	24.7 ± 0.10	27.1 ± 0.25	27.2 ± 0.19	28.9 ± 0.30

–Not detected (mean ± SD, $n = 3$, $P < 0.05$)

Table 4 Variation of DTPA-extractable metals during the composting

Days	DTPA-extractable metal concentrations (mg/kg dry matter)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
<i>Zn</i>					
0	83.4 ± 0.3	79.4 ± 0.3	88.8 ± 0.1	88.8 ± 0.1	92.5 ± 0.4
6	75.6 ± 0.3	80.5 ± 0.4	86.9 ± 0.3	88.3 ± 0.1	91.5 ± 0.4
12	74.7 ± 0.1	72.6 ± 0.3	81.6 ± 0.2	83.9 ± 1.5	88.8 ± 0.2
18	68.7 ± 0.1	68.9 ± 0.1	79.6 ± 0.3	81.1 ± 1.6	84.6 ± 0.1
24	64.6 ± 0.3	64.3 ± 0.4	78.2 ± 0.5	77.8 ± 0.1	82.2 ± 0.5
30	63.4 ± 0.1	59.4 ± 0.5	72.4 ± 0.2	72.4 ± 0.2	82.0 ± 0.2
<i>Mn</i>					
0	181.7 ± 0.4	179.7 ± 0.3	185.6 ± 1.1	191.0 ± 0.2	192.4 ± 0.1
6	171.9 ± 0.5	174.4 ± 1.2	171.3 ± 0.9	177.1 ± 1.3	187.6 ± 2.1
12	164.4 ± 1.1	166.9 ± 0.5	172.6 ± 0.5	175.3 ± 0.9	178.7 ± 0.1
18	151.8 ± 0.5	157.9 ± 0.4	169.5 ± 0.4	171.9 ± 0.8	175.3 ± 1.0
24	144.6 ± 4.2	148.5 ± 0.5	163.8 ± 0.4	170.8 ± 0.3	175.3 ± 0.9
30	144.1 ± 0.3	139.3 ± 0.2	161.8 ± 0.4	170.4 ± 0.6	171.9 ± 0.8
<i>Ni</i>					
0	18.7 ± 0.1	17.9 ± 0.0	17.6 ± 0.2	17.1 ± 0.0	17.4 ± 0.1
6	17.7 ± 0.2	17.6 ± 0.2	17.3 ± 0.1	16.9 ± 0.0	17.1 ± 0.0
12	16.9 ± 0.1	15.7 ± 0.0	16.8 ± 0.1	16.3 ± 0.0	16.6 ± 0.0
18	16.8 ± 0.0	14.8 ± 0.0	16.3 ± 0.0	15.8 ± 0.0	16.5 ± 0.1
24	15.9 ± 0.0	14.7 ± 0.1	15.8 ± 0.0	15.2 ± 0.1	16.5 ± 0.0
30	15.4 ± 0.2	13.8 ± 0.1	15.1 ± 0.2	14.5 ± 0.0	16.3 ± 0.1
<i>Cd</i>					
0	–	–	–	–	–
6	–	–	–	–	–
12	–	–	–	–	–
18	–	–	–	–	–
24	–	–	–	–	–
30	–	–	–	–	–
Days	DTPA-extractable metal concentrations (mg/kg dry matter)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
<i>Cu</i>					
0	7.8 ± 0.0	8.8 ± 0.1	9.6 ± 0.1	9.5 ± 0.1	9.7 ± 0.1
6	7.5 ± 0.1	8.5 ± 0.0	9.4 ± 0.1	9.6 ± 0.0	9.0 ± 0.0
12	7.2 ± 0.1	7.7 ± 0.0	8.8 ± 0.0	9.3 ± 0.1	8.7 ± 0.0
18	6.2 ± 0.1	6.2 ± 0.0	8.6 ± 0.3	8.7 ± 0.1	8.5 ± 0.0
24	6.1 ± 0.1	6.2 ± 0.0	7.7 ± 0.1	8.4 ± 0.0	8.4 ± 0.0
30	5.6 ± 0.2	5.9 ± 0.0	7.7 ± 0.2	8.0 ± 0.0	8.4 ± 0.0
<i>Fe</i>					
0	281.2 ± 2.3	290.9 ± 0.8	293.3 ± 1.0	300.8 ± 0.4	314.1 ± 2.6
6	263.2 ± 6.9	289.7 ± 0.3	263.6 ± 1.5	294.4 ± 1.1	301.8 ± 0.7
12	223.0 ± 0.5	264.5 ± 1.0	253.2 ± 0.1	262.8 ± 0.5	286.0 ± 2.6
18	236.0 ± 4.5	212.9 ± 0.6	231.3 ± 0.2	223.3 ± 0.2	262.9 ± 0.4
24	200.7 ± 0.5	185.0 ± 1.5	210.6 ± 0.3	211.6 ± 0.9	251.8 ± 0.6
30	188.8 ± 4.3	175.9 ± 1.5	202.1 ± 0.3	205.6 ± 0.3	241.0 ± 2.3
<i>Pb</i>					
0	29.5 ± 1.4	27.2 ± 0.0	28.9 ± 0.2	28.7 ± 0.2	31.4 ± 0.1

Table 4 continued

Days	DTPA-extractable metal concentrations (mg/kg dry matter)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
6	26.7 ± 0.4	25.2 ± 0.4	27.3 ± 0.4	28.1 ± 0.1	31.2 ± 2.0
12	25.4 ± 0.5	24.7 ± 0.1	25.4 ± 1.2	24.3 ± 0.2	25.6 ± 1.7
18	24.1 ± 0.5	20.7 ± 0.5	22.8 ± 0.3	21.8 ± 0.6	25.6 ± 2.4
24	22.4 ± 0.8	18.4 ± 0.2	21.8 ± 0.3	22.5 ± 0.2	26.7 ± 0.5
30	22.0 ± 0.2	17.4 ± 0.2	21.8 ± 0.2	21.9 ± 0.7	25.9 ± 0.1
	<i>Cr</i>				
0	45.9 ± 0.7	52.6 ± 0.5	56.0 ± 0.5	47.8 ± 1.4	50.4 ± 0.2
6	42.3 ± 0.4	47.4 ± 0.7	49.4 ± 0.8	44.5 ± 0.8	50.5 ± 0.5
12	38.8 ± 0.1	37.6 ± 1.1	36.3 ± 0.1	46.1 ± 0.7	46.7 ± 0.0
18	26.5 ± 0.1	32.3 ± 0.8	31.9 ± 0.3	38.9 ± 0.0	49.0 ± 0.2
24	24.4 ± 0.0	23.4 ± 0.3	31.9 ± 0.2	36.6 ± 0.1	46.8 ± 0.1
30	22.1 ± 0.5	23.2 ± 0.0	29.4 ± 0.5	32.73 ± 0.58	41.7 ± 0.1

–Not detected (mean ± SD, $n = 3$, $P < 0.05$)

S. natans (Table 3). The order of reduction of water-soluble heavy metals in the trials was as below:

Zn: T1 (25.4 %) > T2 (24.7 %) > T4 (23.7 %) > T5 (20.8 %) > T3 (18.4 %)

Cu: T3 (38.4 %) > T1 (36.4 %) > T2 (32.6 %) > T4 (27.7 %) > T5 (19.8 %)

Mn: T2 (39.5 %) > T1 (27.9 %) > T5 (19.1 %) > T3 (16.0 %) > T4 (14.3 %)

Fe: T2 (51.1 %) > T1 (44.9 %) > T4 (37.7 %) > T3 (35.6 %) > T5 (23.3 %)

Pb: T2 (39.7 %) > T3 (35.7 %) > T1 (28.0 %) > T4 (20.8 %) > T5 (14.1 %)

Cr: T2 (28.3 %) > T1 (24.4 %) > T3 (21.3 %) > T4 (16.0 %) > T5 (14.2 %)

Water-soluble Ni and Cd were not detected in the composts similar to the results of phumdi biomass compost (Singh et al. 2014). Reduction of water-soluble forms of Zn, Cu, Mn, Fe, Pb and Cr during the process may be attributed to the binding of the metals with the –OH and –COOH groups enriched by cattle manure. These groups and the newly formed humus increased the binding sites and combined with metals (released during mineralization of organic biomass) to form insoluble and immobile complexes (Guan et al. 2011; Singh and Kalamdhad 2013a, 2014a). The increase in concentration of water-soluble Fe was reported during phumdi biomass composting without cattle manure (Singh et al. 2014) but in the present study the concentration of water-soluble Fe was reduced in all the trials. The reduction of water-soluble fraction of heavy metals was also reported by other researchers during solid waste composting (Castaldi et al. 2005; Singh and Kalamdhad 2013b, c).

The solubility of the nutrients and heavy metals depends on the solubility equilibria for which the initial concentration of the ions, pH, complex formations, etc. are important (Larson et al. 1973; Benefield et al. 1982; Wang et al. 2009). The pH of the *S. natans* composts was in the range where the alkali or alkaline earth metals (i.e., Na⁺, K⁺, Ca²⁺, Mg²⁺, etc) were soluble. The possible reason for the decrease in water-soluble trace metals was the formation of complexes with the heavy metal ions. Composting results in humification of the organic matter with the formation of humus substances rich in humic acid-like organic carbons having increased aromatic characteristics; oxygen and nitrogen concentrations; functional groups; etc., depending on the nature and composition of the initial organic matter. The humic substances are low in fulvic acid-like organic carbon and water-extractable organic carbon (Roletto et al. 1985; Senesi 1989). Structurally, humic substances are 20–30 % aliphatic and 20–40 % aromatic. 20 % of hydrogen is bound to oxygen as carboxyl (–COOH) and phenolic hydroxyl (–OH) groups and the rest bound directly to carbon (Young 2010). The interaction of macronutrients (i.e., alkali or alkaline earth metals) and heavy metals with the humic substances is a complex phenomenon with differing views. The alkali or alkaline earth metals are held weakly by exchangeable hydrated ions, or by electrostatic forces by charged humus carboxyl groups. In case of heavy metals, a metal chelate complex is formed and two or more coordinate positions of the metal ion are occupied by donor groups of a single ligand to form an internal ring structure (Stevenson 1994; Tan 2010). The divalent and trivalent metals formed highly insoluble complexes, i.e., multidentate or multinuclear chelate sites with the functional groups of humus and are dominated by

Table 5 Variation of leachable concentrations of heavy metals during the composting process

Days	Leachable heavy metal concentrations (mg/kg dry matter)									
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	<i>Zn</i>									
0	124.6 ± 0.1	123.4 ± 0.1	141.0 ± 0.7	137.6 ± 0.1	129.1 ± 0.6	20.5 ± 0.2	24.2 ± 0.1	24.4 ± 0.2	24.7 ± 0.3	23.1 ± 0.6
6	121.0 ± 0.7	119.0 ± 0.1	136.5 ± 0.2	133.8 ± 0.1	128.0 ± 2.9	20.1 ± 0.0	22.3 ± 0.1	22.1 ± 0.1	24.2 ± 0.1	22.2 ± 0.1
12	114.6 ± 0.6	118.3 ± 0.8	133.4 ± 0.5	130.6 ± 0.3	122.8 ± 0.3	19.2 ± 0.1	19.5 ± 0.0	19.6 ± 0.1	23.3 ± 0.1	22.1 ± 0.1
18	103.5 ± 0.4	104.5 ± 0.2	134.9 ± 0.4	124.2 ± 0.1	124.2 ± 0.0	19.0 ± 0.1	18.3 ± 0.1	18.5 ± 0.1	20.2 ± 0.1	19.1 ± 0.2
24	100.5 ± 0.2	97.7 ± 0.1	132.3 ± 0.1	122.3 ± 0.2	122.0 ± 0.3	17.2 ± 0.1	17.1 ± 0.1	18.2 ± 0.2	19.2 ± 0.1	19.0 ± 0.1
30	100.5 ± 0.2	97.2 ± 0.2	126.7 ± 0.1	118.4 ± 0.0	121.3 ± 0.3	15.8 ± 0.2	15.8 ± 0.1	17.7 ± 0.1	18.6 ± 0.1	18.5 ± 0.1
	<i>Mn</i>									
0	305.1 ± 3.8	298.6 ± 5.5	289.2 ± 1.6	290.9 ± 9.0	291.0 ± 2.3	467.6 ± 1.4	494.1 ± 2.9	407.3 ± 0.2	478.9 ± 1.0	473.8 ± 9.5
6	282.7 ± 0.2	278.0 ± 1.6	286.0 ± 4.6	298.3 ± 9.7	284.4 ± 1.4	415.2 ± 0.2	464.4 ± 0.1	396.4 ± 0.6	462.6 ± 2.1	477.1 ± 2.1
12	276.8 ± 2.0	271.7 ± 0.9	271.8 ± 0.5	288.3 ± 9.3	274.6 ± 2.7	397.5 ± 0.2	407.9 ± 1.1	363.8 ± 1.3	442.7 ± 0.0	451.3 ± 0.5
18	266.1 ± 3.7	261.9 ± 0.2	261.6 ± 4.5	273.4 ± 4.7	247.0 ± 7.3	378.2 ± 0.9	353.4 ± 0.3	351.8 ± 0.5	393.2 ± 0.2	429.3 ± 0.1
24	259.0 ± 0.3	247.8 ± 3.0	247.8 ± 9.1	266.9 ± 5.7	265.4 ± 1.7	326.8 ± 0.3	347.2 ± 0.1	305.3 ± 0.2	391.7 ± 0.6	411.1 ± 2.0
30	248.4 ± 0.3	242.2 ± 4.1	235.0 ± 9.5	248.0 ± 8.5	262.6 ± 1.2	305.4 ± 1.1	337.3 ± 0.4	284.8 ± 0.3	364.0 ± 0.7	385.5 ± 1.1
	<i>Ni</i>									
0	175.0 ± 0.3	197.0 ± 0.5	209.1 ± 1.1	201.2 ± 1.0	205.6 ± 0.7	56.6 ± 0.4	63.0 ± 0.1	56.5 ± 1.0	65.2 ± 0.1	64.7 ± 0.2
6	165.0 ± 0.7	193.2 ± 0.5	193.3 ± 0.3	192.9 ± 0.5	202.3 ± 0.0	53.3 ± 0.5	59.6 ± 0.5	55.5 ± 0.2	66.2 ± 0.0	62.7 ± 0.4
12	153.0 ± 0.3	172.9 ± 0.3	183.1 ± 0.1	184.5 ± 0.3	201.7 ± 0.5	49.1 ± 0.7	54.4 ± 0.2	53.4 ± 0.1	56.9 ± 0.7	63.0 ± 0.1
18	144.4 ± 0.1	163.5 ± 0.4	163.2 ± 0.5	182.1 ± 0.0	192.7 ± 0.4	48.6 ± 2.3	51.1 ± 0.3	45.2 ± 0.3	51.4 ± 5.1	62.2 ± 0.1
24	136.9 ± 0.4	145.8 ± 1.1	155.3 ± 0.4	168.9 ± 0.1	182.1 ± 0.1	43.1 ± 0.1	48.6 ± 1.1	46.4 ± 0.1	54.5 ± 0.0	59.6 ± 0.1
30	130.3 ± 0.4	137.6 ± 0.1	152.5 ± 0.0	169.3 ± 0.4	177.5 ± 0.1	37.3 ± 0.0	41.2 ± 0.9	41.7 ± 0.1	50.5 ± 0.2	57.0 ± 0.7
	<i>Cd</i>									
0	1.51 ± 0.05	2.24 ± 0.02	2.51 ± 0.05	2.35 ± 0.03	2.40 ± 0.02	68.6 ± 4.3	77.9 ± 1.0	58.9 ± 0.4	61.2 ± 0.4	66.6 ± 0.3
6	1.25 ± 0.03	1.71 ± 0.05	2.43 ± 0.05	2.29 ± 0.03	2.42 ± 0.02	62.8 ± 1.5	54.3 ± 0.7	54.0 ± 1.1	58.1 ± 0.8	58.8 ± 1.4
12	1.11 ± 0.05	1.38 ± 0.04	2.29 ± 0.03	2.18 ± 0.04	2.33 ± 0.01	54.6 ± 0.7	37.9 ± 0.8	50.0 ± 2.8	53.3 ± 0.1	57.8 ± 1.0
18	0.89 ± 0.03	1.17 ± 0.01	2.10 ± 0.02	2.14 ± 0.04	2.34 ± 0.02	46.8 ± 0.4	42.0 ± 0.7	49.0 ± 0.2	53.2 ± 0.3	52.7 ± 1.2
24	0.75 ± 0.03	1.31 ± 0.07	1.78 ± 0.04	1.90 ± 0.04	2.16 ± 0.02	45.6 ± 0.7	31.3 ± 1.0	54.2 ± 1.3	44.4 ± 0.2	52.8 ± 0.6
30	0.77 ± 0.01	1.11 ± 0.03	1.60 ± 0.04	1.77 ± 0.01	1.92 ± 0.02	45.6 ± 0.7	31.2 ± 0.2	37.1 ± 0.6	42.6 ± 0.3	51.3 ± 1.0

(Mean ± SD, n = 3, P < 0.05)

double or triple bonds to the mix of carboxyl and phenolic hydroxyl groups (Young 2010). Cations with two positive charges such as Cu^{2+} can only be replaced by another transitional metal ion that has two positive charges. Chelation of toxic heavy metals such as mercury (Hg), lead (Pb) and cadmium (Cd) also forms organo-metal complexes which are less available for plant uptake. The stability of a metal chelate complex is determined by such factors as the number of atoms that form a bond with the metal ion, the number of rings that are formed, the nature and concentrations of the metal ions, and pH. The order of decreasing ability of metal ions to form chelating complexes with humic acids is $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Fe}^{2+} > \text{Mn}^{2+}$ (Stevenson 1994). Whereas, Tipping and Hurley (1992) evaluated the binding strength of the metals with humus in the increasing order $\text{VO}^{2+} > \text{Cu}^{2+} > \text{Pb}^{2+} > \text{Zn}^{2+} = \text{Ni}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+} > \text{Mn}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+}$.

Diethylene triamine penta-acetic acid (DTPA) extraction of heavy metals

The variation of DTPA-extractable heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during the composting process is shown in Table 4. It has been considered that DTPA-extractable metals are potentially bioavailable for plant uptake (Chiang et al. 2007; Singh and Kalamdhad 2013b, 2014b). DTPA-extractable heavy metals were reduced significantly ($P < 0.05$) in all trials during the composting process. The order of DTPA reduction was as below:

Zn: T2 (25.3 %) > T1 (24.1 %) > T4 (18.5 %) > T3 (18.4 %) > T5 (11.3 %)
 Cu: T2 (32.8 %) > T1 (28.8 %) > T3 (20.8 %) > T4 (17.2 %) > T5 (13.7 %)
 Mn: T2 (22.5 %) > T1 (20.7 %) > T3 (12.9 %) > T4 (10.8 %) > T5 (10.7 %)
 Fe: T2 (39.5 %) > T1 (32.8 %) > T4 (31.6 %) > T3 (31.1 %) > T5 (23.3 %)
 Ni: T2 (22.9 %) > T1 (17.7 %) > T4 (15.2 %) > T3 (14.5 %) > T5 (6.2 %)
 Pb: T2 (36.1 %) > T1 (25.5 %) > T3 (24.9 %) > T4 (23.8 %) > T5 (17.5 %)
 Cr: T2 (56.0 %) > T1 (51.9 %) > T3 (47.4 %) > T4 (31.5 %) > T5 (17.2 %)

DTPA-extractable Cd was not detected in the *S. natans* composts of all trials. Highest reduction of DTPA-extractable heavy metals in trial 2 is consistent with the results of phumdi biomass and water hyacinth composting (Singh et al. 2014; Singh and Kalamdhad 2013a, 2014b) and is due to higher degradation and higher humus formation. The reduction of DTPA-extractable heavy metals in all trials can be attributed to the degraded organic matter

forming complex compounds with heavy metals (Fang and Wong 1999; Singh and Kalamdhad 2013a, b). The bioavailable heavy metals renovated into a more stable form during composting process (Castaldi et al. 2005; Singh and Kalamdhad 2013a) due to the increase in pH, metal biosorption by the microbial biomass or metal complexation with the newly formed humic substances (Castaldi et al. 2006; Cai et al. 2007; Singh and Kalamdhad 2014a). The pH is considered as one master factor controlling ion exchange, reduction/oxidation, adsorption and complexation reactions (Walter et al. 2006). Cations are adsorbed on organic matter at high pH. The effect of organic matter amendments on heavy metal solubility also depends greatly upon the degree of humification of the organic matter and subsequent effect upon soil pH (Gupta and Sinha 2007). The variations of DTPA-extractable metals were all statistically significant for the trials ($P < 0.05$). A noticeable result of the study was that the total concentrations of Zn (211.3–254.3 mg/kg); Ni (309.8–345.3 mg/kg); and Pb (805.0–890.5 mg/kg) of the final composts were higher than that of Cr (139.8–206.8 mg/kg) but the water-soluble forms of Zn [7.8–11.4 mg/kg (3.6–4.7 % of total Zn)]; Ni (NIL) and Pb [5.5–8.3 mg/kg (0.7–1.0 % of total Pb)] were lower than Cr [22.2–28.9 mg/kg (13.3–19.3 % of total Cr)] indicating that Cr was more bioavailable and had higher toxicity potential.

Toxicity characteristic leaching procedure (TCLP) test for heavy metals

The changes in leachability of Zn, Cu, Mn, Fe, Ni, Cd, Pb and Cr during the composting process are given in Table 5. Leachability of heavy metals was reduced in all trials during the composting of *S. natans* in the range 6.1–21.2 % for Zn; 19.7–35.0 % for Cu; 10.2–18.9 % for Mn; 11.1–34.7 % for Fe; 13.7–30.1 % for Ni; 11.9–34.5 % for Pb; 20.0–50.5 % for Cd; and 22.9–60.0 % for Cr. The reduction of leachable fraction of heavy metals was also reported during phumdi biomass composting (Singh et al. 2014) and the results were also consistent with the findings of other researchers (Chiang et al. 2007; Singh and Kalamdhad 2014a, b). The threshold limit for heavy metals' contamination is: Cd—20 mg/kg, Cr—100 mg/kg and Pb—100 mg/kg (US EPA method 1311, US Environmental Protection Agency 1992). Therefore, the results of TCLP test confirmed that the heavy metal concentrations in all trials were under the threshold limits for compost use for agriculture purposes. The reduction of leachability of all selected heavy metals during the composting process may also be attributed to the metals forming complexes with humic substances (Singh and Kalamdhad 2013b, 2014a). The pH had also an important role in the reduction of the

leachable concentration of the metals. The pH controls the solubility equilibria or complexation by soluble and surface legends and the increasing pH within the optimum range resulted in reducing the solubility and bioavailability of heavy metals (Cambier and Charlatchka 1999). ANOVA analysis showed significant differences in the leachable concentration of heavy metals (Zn, Cu, Fe, Mn, Fe, Ni, Pb, Cd and Cr) of all trials ($P < 0.05$).

Conclusion

Composting of *S. natans* biomass with the addition of optimum amount of cattle manure and rice husk enhanced the microbial degradation as well as the reduction of the bioavailability of heavy metals of the composts. Highest and lowest reductions of MC (30.8 and 13.1 %) and VS (31.4 and 9.9 %) were observed in trial 2 and trial 5, respectively. The highest and lowest reduction of water-soluble, plant-available and leachable metals was also mostly observed in trial 2 and trial 5, respectively. The pH of the trials was enhanced significantly and reached neutral. The total concentrations of Zn, Ni and Pb of the composts were higher than that of total Cr; but the concentrations of the water-soluble Cr of the composts were comparatively higher than that of water-soluble Zn, Ni and Pb, indicating higher toxicity of Cr. The leachable concentrations of heavy metals of the composts were within the prescribed limits. Transformation of *S. natans* biomass into compost may help in the protection of Loktak lake and at the same time decrease the use of chemical fertilizers.

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References

- Ashraf R, Ali TA (2007) Effect of heavy metals on soil microbial community and mung seed germination. *Pak J Bot* 39:629–636
- Benefield LD, Judkins JF, Weand BL (1982) Process chemistry for water and waste water treatment. Prentice-Hall Inc., Englewood Cliffs
- BIS (1982) Methods for analysis of solid wastes (excluding industrial solid wastes). Indian Standards Institution, New Delhi
- Bragato G, Leita L, Figliolia A, Nobili M (1998) Effects of sewage sludge pre-treatment on microbial biomass and bioavailability of heavy metals. *Soil Till Res* 46:129–134
- Cai QY, Mo C, Wu QT, Zeng QY, Katsoyiannis A (2007) Concentration and speciation of heavy metals in six different sewage sludge-composts. *J Hazard Mater* 147:1063–1072
- Cambier P, Charlatchka R (1999) Influence of reducing conditions on the mobility of divalent trace metals in soils. In: Selim HM, Iskandar IK (eds) Fate and transport of heavy metals in the vadose zone. Lewis Publishers, Boca Raton
- Castaldi P, Santona L, Melis P (2005) Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere* 60:365–371
- Castaldi P, Santona L, Melis P (2006) Evolution of heavy metals mobility during municipal solid waste composting. *Fresenius Environ Bull* 15:1133–1140
- Cayuela ML, Mondini C, Sánchez-Monedero MA, Roig A (2008) Chemical properties and hydrolytic enzyme activities for the characterisation of two phase olive mill wastes composting. *Bioresour Technol* 99:4255–4262
- Chen JH (1999) Characteristic and applications of domestic animal wastes. Animal waste compost products quality and treatment alternatives manual. Soil Survey and Testing Center, Department of Soil and Environmental Science, National Chung Hsing University, Taiwan, pp 15–22
- Chiang KY, Huang HJ, Chang CN (2007) Enhancement of heavy metal stabilization by different amendments during sewage sludge composting process. *J Environ Eng Manag* 17:249–256
- Chiroma TM, Ebewele RO, Hymore FK (2012) Levels of heavy metals (Cu, Zn, Pb, Fe and Cr) in bushgreen and roselle irrigated with treated and untreated urban sewage water. *Int Res J Environ Sci* 1:50–55
- Devi V, Chanakya HN, de Alwis AAP, Deepa GB, Modak J (2002) Management of non-point inorganic and organic pollution of water bodies using decentralized installation of bioreactors. In: Proceedings on Symposium on Conservation, Restoration and Management of Aquatic Eco-System, Lake 2002, Session 2 Paper 5. Centre for Ecological Sciences, Indian Institute of Sciences, Bangalore
- Fang M, Wong JWC (1999) Effects of lime amendment on availability of heavy metals and maturation in sewage sludge composting. *Environ Pollut* 106:83–89
- Fuentes A, Llorens M, Saez J, Aguilar MI, Marin ABP, Ortuno JF, Meseguer VF (2006) Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. *Environ Pollut* 143:355–360
- Gao M, Liang F, Yub A, Li B, Yang L (2010) Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere* 78:614–619
- Guan TX, He HB, Zhang XD, Bai Z (2011) Cu fractions, mobility and bioavailability in soil–wheat system after Cu-enriched livestock manure applications. *Chemosphere* 82:215–222
- Gupta AK, Sinha S (2007) Phytoextraction capacity of the plants growing on tannery sludge dumping sites. *Bioresour Technol* 98:1788–1794
- Huang GF, Wong JWC, We QT, Nagar BB (2004) Effect of C/N on composting of pig manure with sawdust. *Waste Manag* 24:805–813
- Iwegbue CMA, Emuh FN, Isirimah NO, Egun AC (2007) Fractionation, characterization and speciation of heavy metals in composts and compost-amended soils. *Afr J Biotechnol* 6:067–078
- Koivula N, Raikkonen T, Urpilainen S, Ranta J, Hanninen K (2004) Ash in composting of source-separated catering waste. *Bioresour Technol* 93:291–299



- Larson ET, Sollo FW, Mcgurk FF (1973) Complexes affecting the solubility of calcium carbonate in water. Research Report No. 68, Illinois State Water Survey, University of Illinois, Urbana
- Liao PH, Jones L, Lau AK, Walkemeyer S, Egan B, Holbek N (1996) Composting of fish wastes in a full-scale in-vessel system. *Bioresour Technol* 59:163–168
- Lin C (2008) A negative-pressure aeration system for composting food wastes. *Bioresour Technol* 99:7651–7656
- Liu Y, Ma L, Li Y, Zheng L (2007) Evolution of heavy metal speciation during the aerobic composting process of sewage sludge. *Chemosphere* 67:1025–1032
- Mande S, Lata K (2005) Potential of Converting Phumdi Waste of Loktak Lake into Briquettes for Fodder and Fuel Use. In: Mathur SM, Mathur AN, Trivedi RK, Bhat YC, Mohonot P (eds) *Aquatic weeds: problems, control and management*. Himanshu, New Delhi, pp 100–108
- Prasad R, Singh J, Kalamdhad AS (2013) Assessment of nutrients and stability parameters during composting of water hyacinth mixed with cattle manure and sawdust. *Res J Chem Sci* 3:1–4
- Roletto E, Barberis R, Consiglio M, Jodice R (1985) Chemical parameters for evaluating compost maturity. *Biocycle* 26:46–48
- Samuel P, Ingmar P, Boubie G, Daniel L (2013) Trivalent chromium removal from aqueous solution using raw natural mixed clay from Burkina Faso. *Int Res J Environ Sci* 2:30–37
- Santosh S, Bidan C (2002) Distribution of aquatic vegetation in Loktak lake. In: *Proceedings of Management of Phumdis in Loktak lake, Manipur, India*
- Senesi N (1989) Composted materials as organic fertilisers. *Sci Total Environ* 81(82):521–542
- Singh J, Kalamdhad AS (2012) Concentration and speciation of heavy metals during water hyacinth composting. *Bioresour Technol* 124:169–179
- Singh J, Kalamdhad AS (2013a) Bioavailability and leachability of heavy metals during water hyacinth composting. *Chem Speciat Bioavailab* 25:1–14
- Singh J, Kalamdhad AS (2013b) Assessment of bioavailability and leachability of heavy metals during rotary drum composting of green waste (water hyacinth). *Ecol Eng* 52:59–69
- Singh J, Kalamdhad AS (2013c) Reduction of bioavailability and leachability of heavy metals during vermicomposting of water hyacinth (*Eichhornia crassipes*). *Environ Sci Pollut Res* 20:8974–8985
- Singh J, Kalamdhad AS (2014a) Effect of lime on speciation of heavy metals during agitated pile composting of water hyacinth. *Front Environ Sci Eng* 10:10–22
- Singh J, Kalamdhad AS (2014b) Effects of natural zeolite on speciation of heavy metals during agitated pile composting of water hyacinth. *Int J Recycl Org Waste Agricult* 3:1–17
- Singh WR, Kalamdhad AS (2014c) Potential for composting of green phumdi biomass of Loktak lake. *Ecol Eng* 67:119–126
- Singh WR, Pankaj S, Singh J, Kalamdhad AS (2014) Evaluation of bioavailability of heavy metals and nutrients during agitated pile composting of green Phumdi. *Res J Chem Environ* 18:1–8
- Smars S, Gustafsson L, Beck-Friis B, Jönsson H (2002) Improvement of the composting time for household waste during an initial low pH phase by mesophilic temperature control. *Bioresour Technol* 84:237–241
- Stevenson FJ (1994) *Humus chemistry*. John Wiley Sons, New York
- Tan KH (2010) *Principles of soil chemistry*, 4th edn. CRC Press, Boca Raton
- Tipping E, Hurley MA (1992) A unifying model of cation binding by humic substances. *Geochim Cosmochim Acta* 56:3627–3641
- TMECC (2002) *Test methods for the examination of composting and compost*. US Composting Council, Bethesda
- US Environmental Protection Agency (1992) Method 1311—toxicity characteristic leaching procedure (TCLP) In: SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. US Environment Protection Agency. <http://www.epa.gov/solidwaste/hazard/testmethods/sw846/index.htm>. Accessed 21 Jan 2014
- Walter I, Martinez F, Cala V (2006) Heavy metal speciation and phytotoxic effects of three representative sewage sludge for agricultural uses. *Environ Pollut* 139:507–514
- Wang SG, Sun XF, Gong WX, Ma Y (2009) Biosorption of metals on to granular sludge. In: Wang LK, Chen JP, Huang YT, Shammas NK (eds) *Heavy metals in the environment*. Taylor and Francis Group/CRC Press, Boca Raton, pp 201–223
- Wong JWC, Selvam A (2006) Speciation of heavy metals during co-composting of sewage sludge with lime. *Chemosphere* 63:980–986
- Wong JWC, Mak KF, Chan NW, Lam A, Fang M, Zhou LX, Wu QT, Liao X (2001) Co-composting of soya bean residues and leaves in Hongkong. *Bioresour Technol* 76:99–106
- Young SD (2010) Chemistry of Heavy Metals and Metalloids in Soils. In: Alloway BJ (ed) *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability*. Springer, Dordrecht, pp 51–97

