Chapter 8. Study on consortia of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* for removal of heavy metals from water under water circulation effects and vermicomposting of the generated plant biomass

8.1. Introduction

Different wetland plants have different growth speed, growth rhythm, root morphology and distribution; hence, it could be deduced that mixed wetlands may have better removal rates because of the temporal and spatial compensation in plant growth, root distribution, and nutrient preference (Kongroy et al., 2012; Turker et al., 2014). Aquatic plant based treatment systems are ecofriendly and cost-effective technologies which can be adopted by developing counties like India for recycling/treatment of waste water, especially contaminated by heavy/toxic metals (Rai et al., 2012). Potential productivity of plants in nutrient enriched wastewaters has lead to its selection for phytoremediation of various industrial effluents and the produced biomass as a feed stock for vermicomposting production to achieve safe disposal of contaminated biomass.

Composting followed by land application represents one of the most economical ways for the treatment and final disposal of plant biomass after phytoremediation of pollutants because it combines material recycling and biomass disposal at the same time (Villasenor et al., 2011). Composting and vermicomposting are the best-known processes for biological stabilization of green waste by transforming them into a safer and more stabilized material (compost), that can be used as a soil conditioner in agricultural applications (Deka et al., 2011 and Gabhane et al., 2012).

The present study is undertaken to remediate metals contaminated water using consortia of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* under mixed cultures and vermicomposting of the generated plant biomass.

8.2. Material and Methods

8.2.1. Plants collection and their acclimatization

The experimental plants *Typha latifolia, Pistia stratiotes* and *Hydrilla Verticellata* were collected from the Gomti River and local ponds of Lucknow city. The plants were washed with running tap water to remove any attached particles. They were acclimatized for 15 days in Hoagland solution (Hoagland and Arnon, 1950) in constructed cemented ponds. After 15 days of acclimatization, fully grown and healthy plants were transferred to the treatment tubs. 200 grams of each plant was put into tubs for their monocultures, covering 80% of surface area of water in the tub. For mixed culture, 100 grams of each plant was put together in the tubs and for the consortium of all three plants 70 grams of each plant was put together in the tub.

8.2.2. Experimental treatment set up and mode of operation

A bench scale microcosm water treatment system, with intermittent flow and circulation, comprising of different parallel treatments of individual and mixed cultures of *Typha latifolia, Pistia stratiotes* and *Hydrilla Verticellata* were set up in the Net House located at field station of Department of Environmental Science, BBAU Lucknow. The experiments were performed in the month of May 2014. Other details of experimental setup are given in Chapter 6 section 6.2.2

8.2.3. Plant cultures and treatment conditions

Mono and mixed cultures of free floating *Typha latifolia, Pistia stratiotes* and submerged *Hydrilla verticellata* were tested for their metal accumulation and removal capacities form multi metal solution of Cu (5ppm); Fe (5ppm); Cd (2.5 ppm) and Cr (2.5 ppm). Different treatments for the experiment were: T1= *Typha latifolia* monoculture with water recirculation at intervals; T2= *Typha latifolia* monoculture without circulation; T3= *Pistia stratiotes* and *Typha latifolia* mixed culture with water recirculation at intervals;

T4 = *Pistia stratiotes* and *Typha latifolia* mixed culture without water recirculation; T5= *Typha latifolia and Hydrilla verticellata* mixed culture with water recirculation; T6= *Typha latifolia and Hydrilla verticellata* mixed culture without water circulation; T7= *Typha latifolia, Pistia stratiotes and Hydrilla verticellata* mixed culture with water recirculation; T8 = *Typha latifolia, Pistia stratiotes and Hydrilla verticellata* mixed culture without water recirculation; T9= Control for water without plants with recirculation at intervals

8.2.4. Metals accumulation, Bioconcentration factor and Translocation factor

Plant samples from each treatment were collected on 5th, 10th, 15th, 20th, 25th and 30th day from the start of the experiment and divided into root and shoot components for further analysis.

Metals analysis: Root and shoot plant samples were oven dried at 90 °C to a constant weight and metals (Cu, Fe, Cd and Cr) in the plant parts were determined in 1 gram (dry weight) of each root and shoot sample after acid digestion of dry samples with an acid mixture (9:4 nitric acid: perchloric acid) at about 100 °C. Metal concentrations were determined by using atomic absorption spectrophotometer (AAS 240 FS, Varian) following standard protocols. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples.

Bioconcentration factor (BCF), expressed as the ratio of metal concentration in plant tissue to that of the water was calculated by:

$$\text{BCF} = \frac{\text{Metal content in plant tissue}}{\text{Initial metal content in water column}}$$

Translocation factor (TF), the ratio of metals in shoot versus root of plants was calculated by the formula of Padmavathiamma and Li (2007).

$$\text{TF} = \frac{\text{Metal content in plant shoot}}{\text{Metal content in plant root}}$$

8.2.5. Vermicomposting of the contaminated plant biomass

For developing the vermicompost, the experiments were conducted in plastic bin of sizes 450 × 300 × 450 mm in the laboratory net house. The reactor was designed for a total weight of 0.5 kg for 45 days of vermicomposting period. Acclimatized 250 earthworms (adult and juvenile) species *Eisena fetida* collected from Biotech Park, Kursi Road Lucknow were randomly picked from the culture and added to each treatment.

The composition for the development of vermicompost in different trials was as follow:

- ❖ Trial 1: *Pistia stratiotes* biomass + earthworms
- ❖ Trial 2: *Typha latifolia* biomass + earthworms
- ❖ Trial 3: *Hydrilla verticellata* biomass + earthworms
- ❖ Trial 4: *Pistia stratiotes* biomass+ *Hydrilla verticellata* biomass + earthworms
- ❖ Trial 5: *Pistia stratiotes* biomass+ *Typha Latifolia* biomass+ earthworms
- ❖ Trail 6: *Pistia stratiotes* biomass+ *Typha Latifolia* + *Hydrilla verticellata* biomass + earthworms
- ❖ Trail 7: CONTROL (*Pistia stratiotes* biomass+ *Typha Latifolia* + *Hydrilla verticellata* biomass without earthworms)

These mixtures were manually turned at every 10th day, in order to provide suitable aeration to the earthworms. All the containers were kept in the dark under identical ambient conditions of NET House DES.

8.2.6. Determination of pH, Nitrate, Nitrite, Phosphate, Total phosphorus, Total organic carbon and % Total organic matter in vermicompost. The details of protocols are given in Chapter 3.

8.2.7. Sequential Extraction of metals: For sequential extraction of metals form the developed vermicompost, the conventional method designed and developed by Tessier et. al., (1979) for heavy metal speciation into five fractions (exchangeable, carbonate, reducible, organically complexed and residual) was employed. The details of the technique are given in Chapter 3.

8.3. Results

8.3.1. Accumulation of Fe, Cu, Cd and Cr from water by selected plant cultures of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* under circulating and no circulating conditions under intermittent circulation

In the present study the concentration of Cu, Fe, Cd and Cr accumulated by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* in roots and shoot under mono and mixed culture with and without effect of circulation are presented in table 8.1 for Cu and Fe and table 8.2 for Cd and Cr.

Table 8.1. Accumulation of Cu and Fe (µg/gm dw) by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* under different culture treatments (n=6±S.D.)

	Cu (µg/gm dw)		Fe (µg/gm dw)	
Treatments	Root	Shoot	Root	Shoot
T1	1708.66±13.05	1736±10.14	1392.65±6.45	1086.17±4.3
T2	1015.33±5.5	1236±10.14	933.25±6.11	842.84±6.11
T3P	2086.36±8.87	2323.8±8.22	1160.13±6.77	1030.26±7.79
T3T	1486.36±8.87	1523.83±8.22	1460.13±6.77	1030.26±7.79
T4P	1026.9±3.55	1047.76±9.54	961.8±9.95	644.23±6.82
T4T	815.33±5.5	936±10.14	633.25±6.11	742.84±6.11
T5H	563.38±7.37	1216.86±6.7	1108.18±9.4	1456.2±7.15
T5T	1385.22±8.76	1436.3±10.37	952.33±6.19	588.2±4.5
T6H	356.71±4.59	922.9±4.4	708.18±2.64	1012.87±4.69
T6T	790.56±5.09	942.96±3.87	644.23±6.82	372.04±4.7
T7P	842.5±10.69	950.06±8.35	671.34±10.92	263.2±10.31
T7H	409.35±9.72	877.3±4.68	932.13±8.76	1256.68±8.86
T7T	1126.9±3.55	1154.43±4.36	1061.8±9.95	644.23±6.82
T8P	517.56±3.8	649.73±7.25	412.34±3.7	162.86±10.4
T8H	222.68±5.87	678.96±5.93	626.8±5.16	856.6±8.86
T8T	627.5±3.58	854.3±4.58	761.8±9.95	544.2±6.8

Considerable differences were observed for metals accumulation by plants under different cultures. Metals accumulation varied from plant to plant and the effect of different consortium of plants on metals accumulation was profound. Cu was maximum accumulated by *Pistia* *stratiotes* roots (2086.36 μg/gm DW) and shoot (2323.8 μg/gm DW) in mixed culture with *Typha latifolia* under the water circulation effects. However, maximum Fe accumulation was observed in *Typha latifolia* roots (1460 μg/gm DW) under intermittent water circulation. Shoot accumulation for Fe was reported to be maximum in *Hydrilla verticellata* (1456.2 μg/gm DW). Overall, it was observed that plants under the mixed culture with intermittent circulation of water accumulated greater metal ions than those under non-circulating effects.

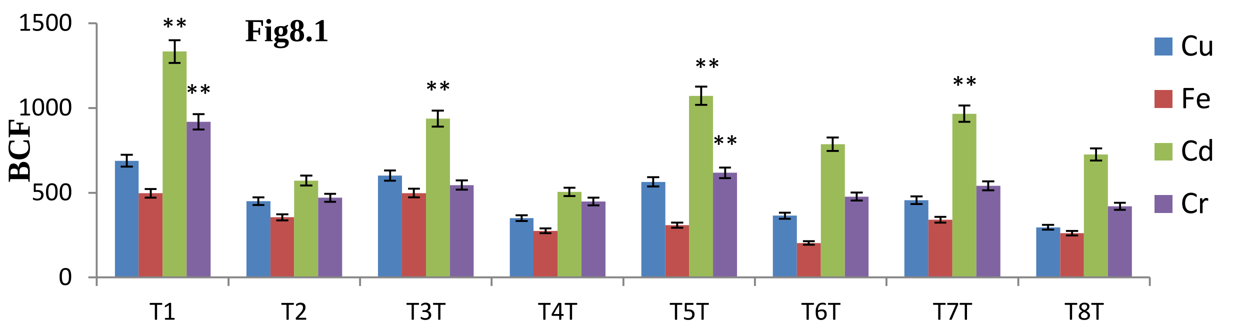
Table 8.2. Accumulation of Cd and Cr (μg/gm dw) by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* under different culture treatments (n=6±S.D.)

	Cd (μg/gm dw)		Cr (μg/gm dw)	
Treatments	Root	Shoot	Root	Shoot
T1	1696.6±6.65	1636±10.14	951.98±1.22	1345.44±1.4
T2	693.33±5.85	736±10.14	442.11±7.05	754.32±3.54
T3P	1984.9±9.81	2060.2±4.06	1142.1±9.32	1121.66±5.11
T3T	984.95±9.81	1360.2±4.06	642.1±9.32	721.66±5.11
T4P	1250.3±6.25	1065.8±10.04	766.7±8.1	585.43±7.65
T4T	626.6±55.98	636±10.14	342.11±7.05	554.32±3.54
T5H	722.53±6.27	1422.6±7.32	845.94±3.44	1217.22±4.54
T5T	1407.07±6.04	1272.09±4.86	851.2±6.63	692.03±6.15
T6H	623.2±6.2	1025.96±4.55	615.94±3.44	823.5±5.12
T6T	1105.85±2.67	861.8±9.95	577.8±3.9	615.03±3.84
T7P	918.82±8.4	984.94±8.26	573.76±8.84	561.76±6.64
T7H	523.06±5.46	970.3±7.64	671.72±7.4	1049.3±7.53
T7T	1250.3±6.25	1165.86±10.04	766.7±8.16	585.43±7.65
T8P	628.48±3.55	714.943±3.05	457.1±8.18	555.1±3.89
T8H	419.73±7.67	721.63±5.56	571.72±7.46	877.3±5.59
T8T	850.33±6.25	965.86±10.04	566.7±8.16	485.43±7.65

Cd was observed to be highest accumulated by *Pistia stratiotes* roots (1984.9 μg/gm DW) and shoot (2060.2 μg/gm DW) under water circulation effects. It was followed by to be greater accumulated by *Typha latifolia* in both root and shoot parts (1696.6 μg/gm DW) and (1636.0 μg/gm DW), respectively. In case of Cr, maximum accumulation was observed in shoot of *Typha latifolia* (1345.44 μg/gm DW) followed by shoot *Hydrilla verticillata* (1217.22 μg/gm DW) in mixed cultures under water circulation.

8.3.2. Bioconcentration factor (BCF) and Translocation factor (TF) of *Typha latifolia* under different cultures and water circulation effects

The BCF and TF of *Typha latifolia* under different treatment conditions have been presented in figures 8.1 and 8.2, respectively. Significant differences were observed for BCF of *Typha latifolia* under different plant combinations. BCF around 1400 for Cd was observed to be highest for *T. latifolia* in monoculture under intermittent water circulation effect. It was followed by 900 for Cr. Lowest BCF was observed for Fe and Cr for *Typha latifolia* under different cultures.



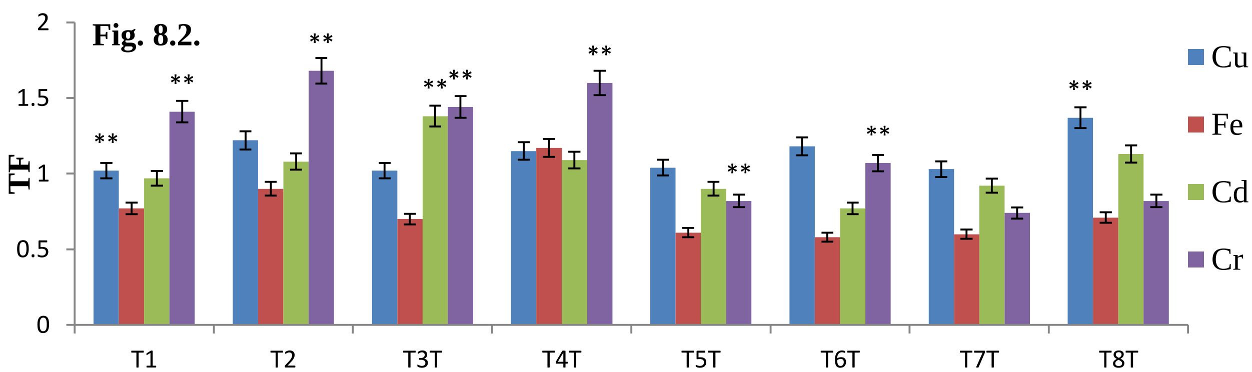


Fig. 8.1 and 8.2. BCF and TF of *Typha latifolia* for Cu, Fe, Cd and Cr in different treatment. **represents significant results.

The translocation factor of *Typha latifolia* for metals is presented in figure 8.2. Highest TF was observed for Cr followed by Cd and Cu under various plant cultures in different treatments. Fe and observed to be least translocated in all the treatments.

8.3.3. Changes in the levels of pH, nitrate, nitrite phosphate and total phosphorus of the developed vermicompost on different days

The vermicomposting of the contaminated plant biomass under different trails was very effective in enhancing the nutritional status of developed vermicompost towards the end of the experiment. Except for control treatment significant changes in the observed chemical characteristics were seen at the maturity of compost. The changes in the pH of the plant biomass subjected to vermicomposting are presented in Fig.8.3. Significant differences were observed for pH changes among trails on different days ($p<0.05$). Except for the control treatment, pH of all trails increased during the vermicomposting period. Highest pH (>8) was observed for trails 1 and 4. The nitrate content also showed significant differences among all trails, except control. Highest nitrate content was observed in trail 6 and 4 towards the end.

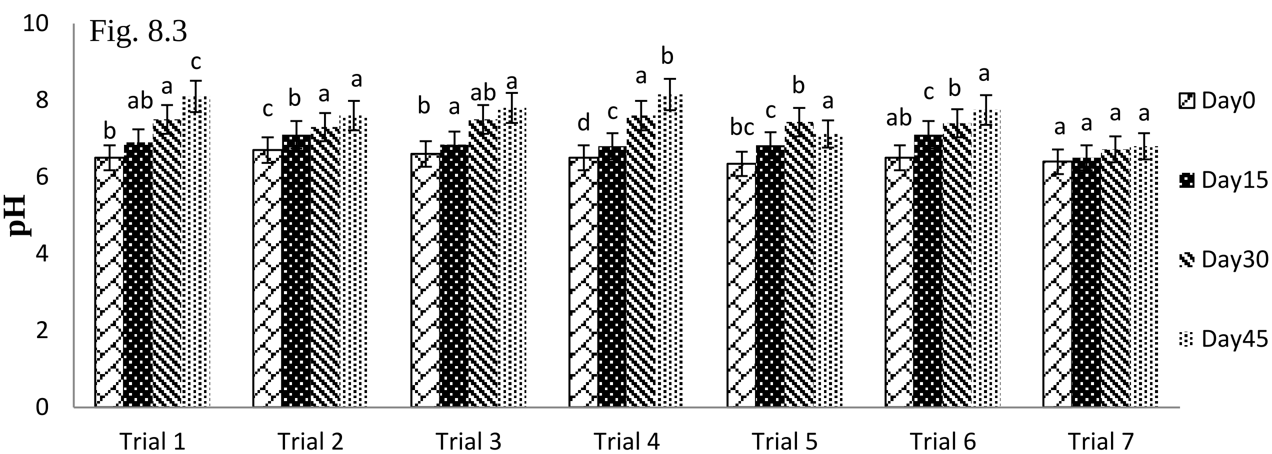


Fig. 8.3. Changes in pH of different plant biomass trails during vermicomposting

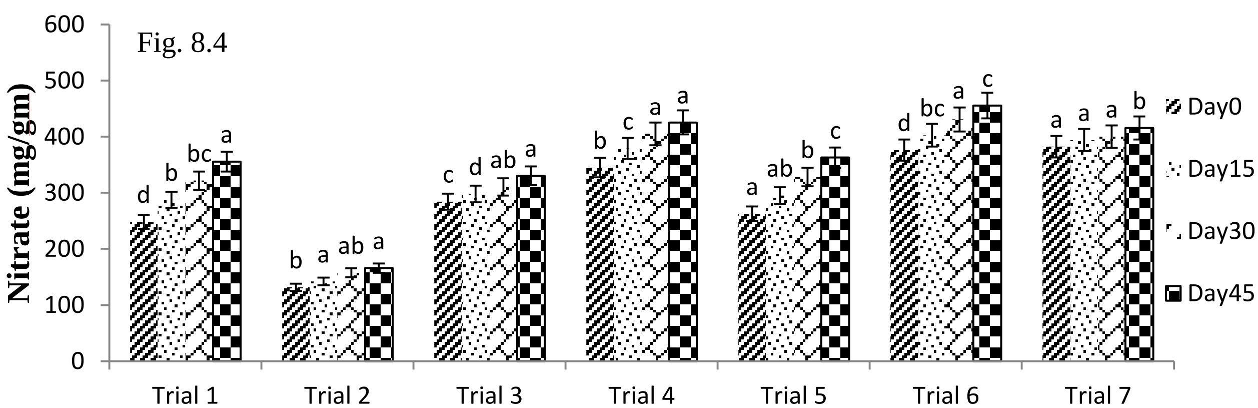


Fig. 8.4. Changes in nitrate content of different plant biomass trails during vermicomposting

The changes in the levels of nitrite, phosphate and total phosphorus of different trails are depicted in figures 8.5, 8.6 and 8.7, respectively. Nitrite content increased with time of composting. Highest increase was observed in trail 6 and 4. Similarly, phosphate content also increased in different trails. Significant differences were observed for total phosphorus concentrations among different days of vermicomposting. Total phosphorus was also reported highest in trail 6.

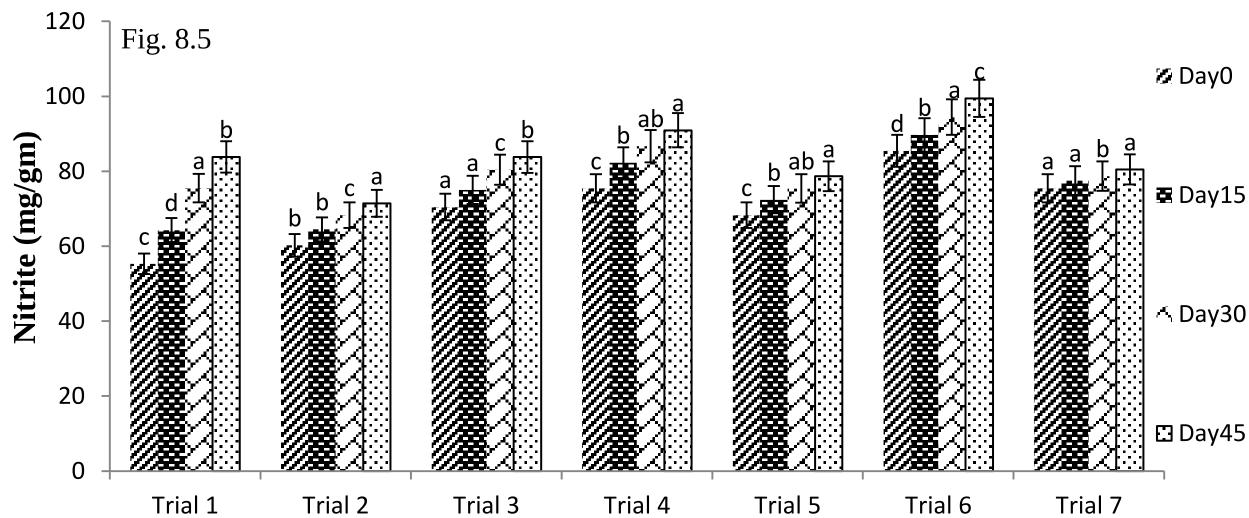


Fig. 8.5. Changes in nitrite content of different plant biomass trails during vermicomposting

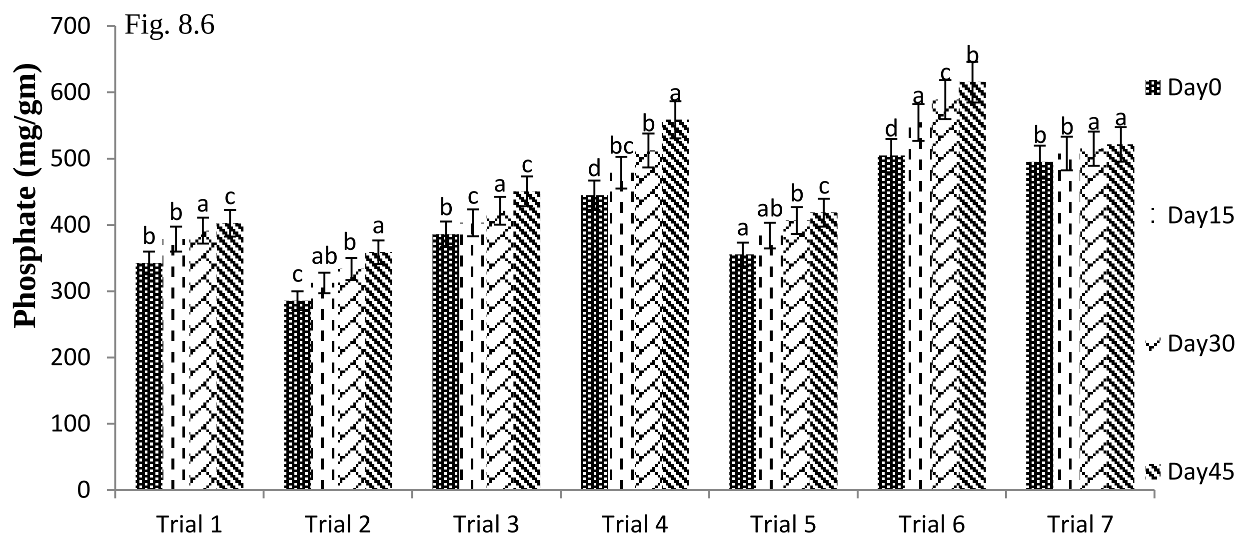


Fig. 8.6. Changes in phosphate content of different plant biomass trails during vermicomposting

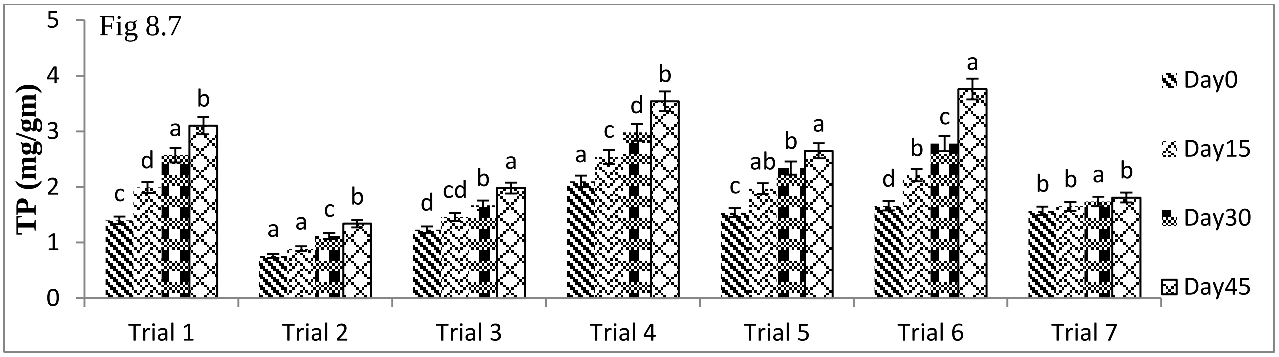
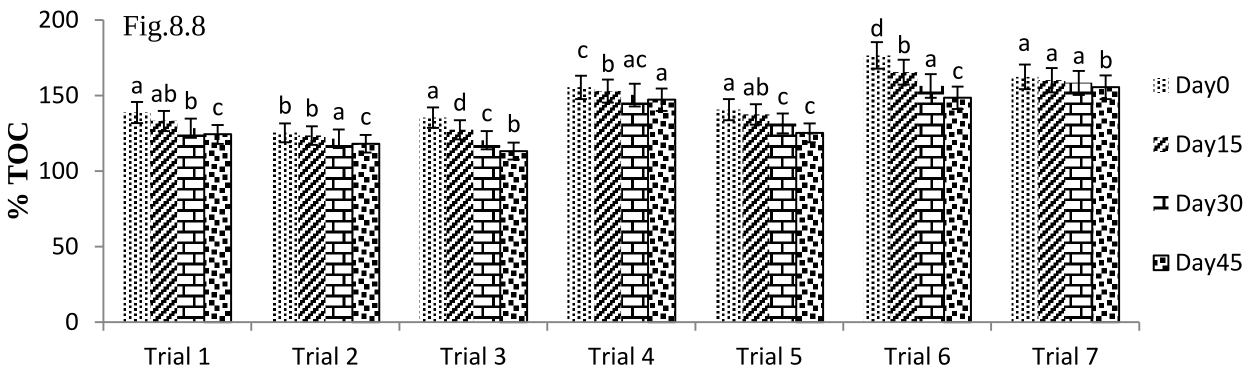


Fig. 8.7. Changes in total phosphorus of different plant biomass trails during vermicomposting

The changes in the % organic carbon and organic matter of different vermicomposting trails of plant biomass are represented in figures 8.8 and 8.9, respectively. The organic carbon and organic matter content of all trails showed significant differences between different days of composting ($p<0.05$). A decrease in both organic carbon and organic matter content was observed for all trails.



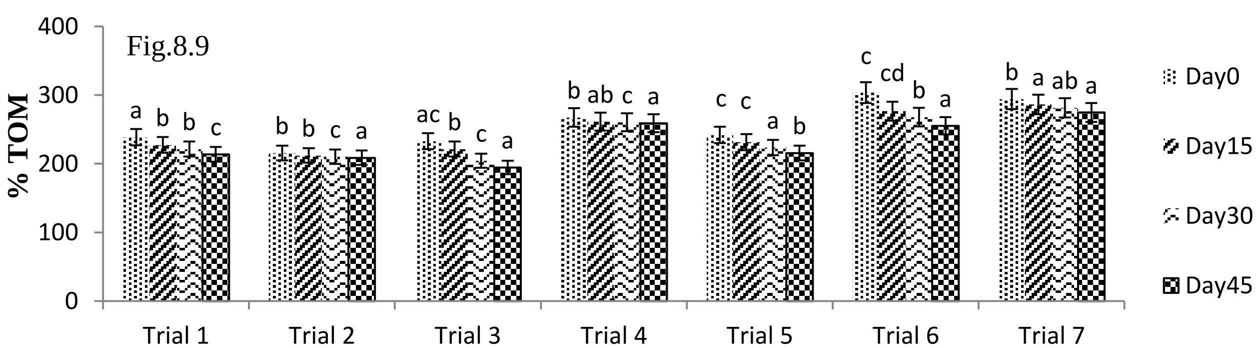


Fig. 8.8 and 8.9. Changes in % organic carbon and organic matter of different plant biomass trails during vermicomposting

8.3.4. Heavy metal speciation during vermicomposting of plant biomass.

Table 8.3, 8.4, 8.5 and 8.6 illustrates the concentration of Cu, Fe, Cd and Cr as different chemical species in selected trails during the composting process. Except for control trail, significant differences were observed for among the Exchangeable, Carbonate, Reducible, Organically Bound and Residual forms of metals among different trails ($p<0.05$). Cu was converted into organically bound and residual fractions in different trails. Among trails 1, 4 and 6 greater residual fractions of Cu were observed in the final metal content after vermicomposting. The initial exchangeable and carbonate fractions got significantly reduced and the final content of metals was found to be containing residual and organically bound fractions. Similarly, Fe was also converted into less mobile fractions like reducible and residual in the final developed compost. Trail 4 and 6 were observed for greater Fe contents in case of reducible and residual fractions.

Table 8.3. Speciation of Cu (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.72±0.02^{a}	0.97±0.03^{ab}	2.1±0.051^{c}	2.54±0.03^{b}	5.56±0.05^{c}
	Final Conc.	0.31±0.01^{d}	1.32±0.05^{c}	1.93±0.13^{ab}	4.43±0.05^{b}	7.33±0.05^{a}
Trail 2	Initial Conc.	0.72±0.02^{c}	0.94±0.02^{c}	1.12±0.01^{b}	2.2±0.02^{ab}	5.48±0.04^{a}
	Final Conc.	0.68±0.02^{d}	1.12±0.05^{a}	1.21±0.01^{a}	1.67±0.03^{b}	6.1±0.1^{c}
Trail 3	Initial Conc.	1.76±0.27^{c}	0.68±0.01^{c}	1.21±0.03^{d}	1.98±0.05^{b}	4.87±0.03^{a}
	Final Conc.	0.32±0.01^{a}	1.12±0.06^{a}	2.34±0.1^{b}	2.98±0.07^{b}	7.98±0.1^{c}
Trail 4	Initial Conc.	1.45±0.11^{c}	1.87±0.04^{b}	2.45±0.07^{bc}	4.3±0.04^{a}	6.88±0.06^{a}
	Final Conc.	1.12±0.06^{e}	2.98±0.11^{d}	5.78±0.12^{a}	6.11±0.05^{c}	9.12±0.11^{b}
Trail 5	Initial Conc.	1.89±0.08d	1.56±0.1b^{c}	2.11±0.03^{c}	2.76±0.01^{b}	4.45±0.04^{a}
	Final Conc.	1.12±0.05^{a}	2.34±0.13^{a}	3.31±0.05^{a}	5.72±0.02$_{b}$	6.74±0.05^{b}
Trail 6	Initial Conc.	2.11±0.12^{a}	2.87±0.11^{b}	3.65±0.06^{b}	5.78±0.03^{c}	8.21±0.04^{a}
	Final Conc.	0.87±0.02^{c}	3.89±0.21^{d}	5.87±0.11^{b}	7.98±0.07^{a}	12.56±0.12^{c}
Trail 7	Initial Conc.	1.32±0.02^{b}	1.62±0.15^{c}	2.05±0.03^{a}	3.83±0.04^{a}	7.62±0.04^{ac}
	Final Conc.	0.92±0.01^{a}	1.68±0.12^{a}	3.18±0.04^{a}	3.11±0.05^{a}	6.12±0.03^{b}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.4. Speciation of Fe (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.51±0.01^{b}	0.82±0.02^{c}	1.87±0.1^{d}	2.54±0.2^{a}	4.53±0.15^{a}
	Final Conc.	0.23±0.02^{c}	1.17±0.04^{d}	2.29±0.12^{ab}	3.56±0.23^{b}	6.44±0.17^{a}
Trail 2	Initial Conc.	0.81±0.02^{e}	1.15±0.03^{b}	1.55±0.1^{c}	1.84±0.12^{d}	6.29±0.16^{a}
	Final Conc.	1.14±0.024^{a}	1.54±0.02^{ab}	1.33±0.04^{b}	2.11±0.1^{c}	6.85±0.13^{d}
Trail 3	Initial Conc.	1.81±0.021^{b}	0.73±0.01^{c}	1.43±0.04^{a}	2.15±0.1^{ab}	5.28±0.1^{a}
	Final Conc.	0.21±0.01^{d}	1.33±0.04^{e}	2.35±0.13^{bc}	3.11±0.21^{b}	8.24±0.21^{a}
Trail 4	Initial Conc.	2.19±0.1^{a}	2.33±0.12^{ab}	4.8±0.12^{c}	5.8±0.23^{b}	10.5±0.24^{c}
	Final Conc.	1.31±0.34^{bc}	3.45±0.11^{a}	6.72±0.15^{a}	5.92±0.25^{a}	11.51±0.3^{b}
Trail 5	Initial Conc.	1.72±0.12^{e}	1.34±0.12^{c}	1.43±0.11^{d}	1.94±0.1^{ab}	3.32±0.12^{a}
	Final Conc.	1.42±0.05^{cd}	1.94±0.21^{d}	2.31±0.12^{b}	3.42±0.14^{c}	4.17±0.15^{a}
Trail 6	Initial Conc.	1.63±0.04^{c}	1.82±0.14^{a}	3.21±0.11^{a}	4.55±0.16^{b}	7.33±0.14^{a}
	Final Conc.	0.84±0.01^{d}	1.08±0.05^{cd}	3.05±0.12^{c}	6.48±0.11^{b}	12.43±0.11^{a}
Trail 7	Initial Conc.	1.98±0.1^{a}	2.26±0.13^{a}	4.75±0.13^{a}	5.88±0.12^{b}	8.95±0.11^{a}
	Final Conc.	2.75±0.14^{a}	2.36±0.12^{a}	3.45±0.12^{a}	5.01±0.11^{a}	7.88±0.13^{b}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.5. Speciation of Cd (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.21±0.01^{d}	0.35±0.01^{c}	1.12±0.04^{bc}	2.01±0.04^{c}	3.23±0.06^{a}
	Final Conc.	0.08±0.01^{b}	0.54±0.02^{b}	1.04±0.03^{c}	3.51±0.07^{c}	5.14±0.1^{a}
Trail 2	Initial Conc.	0.42±0.02^{a}	0.76±0.022^{a}	1.21±0.04^{c}	1.02±0.02^{b}	5.92±0.1^{d}
	Final Conc.	0.69±0.02^{c}	2.48±0.04^{cd}	2.67±0.1^{b}	3.61±0.05^{b}	5.35±0.07^{a}
Trail 3	Initial Conc.	0.32±0.02^{a}	0.43±0.03^{b}	1.37±0.05^{d}	2.85±0.01^{c}	4.78±0.04^{e}
	Final Conc.	0.16±0.01^{d}	1.33±0.042^{c}	3.55±0.11^{b}	5.74±0.1^{ab}	6.37±0.05^{a}
Trail 4	Initial Conc.	1.09±0.03^{c}	1.33±0.032^{c}	3.86±0.1^{b}	4.58±0.06^{b}	6.5±0.07^{a}
	Final Conc.	0.65±0.01^{d}	2.25±0.023^{b}	4.12±0.07^{c}	6.88±0.12^{a}	8.76±0.06^{ac}
Trail 5	Initial Conc.	0.53±0.01^{c}	0.76±0.02^{c}	1.06±0.03^{c}	2.14±0.03^{b}	2.2±0.01^{a}
	Final Conc.	0.2±0.03^{c}	1.32±0.05^{b}	1.98±0.05^{b}	2.64±0.04^{ab}	3.43±0.02^{a}
Trail 6	Initial Conc.	1.13±0.05^{b}	1.43±0.03^{b}	2.27±0.08^{c}	3.21±0.05^{d}	5.23±0.03^{a}
	Final Conc.	0.41±0.03^{e}	0.78±0.01^{d}	2.5±0.05^{b}	8.98±0.13^{c}	9.13±0.05^{a}
Trail 7	Initial Conc.	0.87±0.02^{a}	1.06±0.03^{a}	2.15±0.03^{b}	4.91±0.03^{b}	5.95±0.06^{a}
	Final Conc.	1.05±0.04^{a}	0.89±0.02^{a}	3.49±0.1^{b}	3.01±0.06^{c}	5.58±0.05^{a}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Chemical speciation of Cd (mg/kg) during vermicomposting of plant biomass is presented in table 8.5. Cd fractions were also found to be organically bound and reducible in nature in the final compost. Highest reducible fractions of Cd were reported in trails 6, 4 and 3. Organically bound fractions of Cd were also observed highest in the same trails.

Table 8.6. Speciation of Cr (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initail Conc.	$0.17±0.01^{b}$	$0.22±0.01^{b}$	$0.92±0.03^{b}$	$1.61±0.03^{c}$	$2.39±0.02^{ac}$
	Final Conc.	$0.1±0.001^{c}$	$0.38±0.01^{c}$	$0.84±0.02^{b}$	$2.61±0.05^{a}$	$4.4±0.05^{a}$
Trail 2	Initail Conc.	$0.23±0.01^{b}$	$0.62±0.02^{cd}$	$1.01±0.03^{d}$	$1.32±0.03^{c}$	$3.52±0.04^{a}$
	Final Conc.	$0.45±0.02^{e}$	$1.83±0.04^{de}$	$2.03±0.05^{c}$	$2.71±0.06^{b}$	$4.21±0.08^{ab}$
Trail 3	Initail Conc.	$0.21±0.02^{c}$	$0.31±0.01^{c}$	$1.07±0.03^{b}$	$1.85±0.04^{ab}$	$3.28±0.02^{a}$
	Final Conc.	$0.06±0.01^{d}$	$1.56±0.03^{c}$	$2.95±0.05^{ab}$	$4.24±0.05^{b}$	$5.98±0.04^{a}$
Trail 4	Initail Conc.	$0.93±0.03^{c}$	$1.57±0.03^{d}$	$4.39±0.06^{ab}$	$4.89±0.06^{b}$	$5.35±0.05^{a}$
	Final Conc.	$0.15±0.01^{d}$	$3.03±0.06^{cd}$	$3.87±0.04^{b}$	$5.28±0.07^{c}$	$9.26±0.1^{a}$
Trail 5	Initail Conc.	$0.41±0.02^{ab}$	$0.53±0.03^{a}$	$0.94±0.02^{c}$	$1.83±0.02^{b}$	$2.42±0.03^{c}$
	Final Conc.	$0.23±0.02^{a}$	$1.12±0.06^{c}$	$1.45±0.03^{b}$	$2.04±0.03^{d}$	$3.73±0.03^{bc}$
Trail 6	Initail Conc.	$1.38±0.05^{b}$	$1.72±0.04^{c}$	$2.79±0.05^{ac}$	$3.79±0.05^{a}$	$6.44±0.05^{a}$
	Final Conc.	$0.33±0.01^{d}$	$0.18±0.01^{b}$	$1.05±0.02^{c}$	$7.65±0.06^{a}$	$12.87±0.14^{a}$
Trail 7	Initail Conc.	$0.77±0.02^{c}$	$1.42±0.02^{bc}$	$3.95±0.05^{b}$	$4.18±0.05^{a}$	$5.32±0.04^{ab}$
	Final Conc.	$0.65±0.03^{a}$	$1.79±0.03^{b}$	$2.73±0.01^{b}$	$3.81±0.03^{c}$	$4.88±0.05^{a}$

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.6 represents the chemical speciation of Cr during the initial and final stages of vermicomposting of contaminated biomass of selected plants. Significant differences were reported for all fractions of Cr in selected trails during the final compost analysis ($p<0.05$). The highest reducible fractions in case of Cr were observed for trails 2 and 6. Reducible fractions in the final compost were also dominant in Cr speciation after organically bound fractions. Trails 2 and 3 had increased reducible fractions of Cr in the final compost. Final concentration of Exchangeable fractions of Cr was found to be lesser than the initial contents in all the trails of composting.

8.4. Discussion

Data obtained from the present research showed that *T. latifolia* performed better in accumulation of metals from monoculture than other cultures, under the same operational conditions and over the same length of time. This indicates that removal efficiencies differ according to the plant species in the treatment system; therefore, the selection of plant species is a key component to the success of CWs designed for wastewater treatment (Turker, 2014). This study revealed a similar allocation patterns of metals in the emergent macrophyte species as the most wetland plants having higher concentrations of metals in their belowground biomass than in their shoot tissues (Mays and Edwards 2007; Vymazal et al. 2007). Mixed consortia of plants resulted in significant variations in metals accumulation.

pH is a parameter which greatly affects the composting process. The pH values during the vermicomposting were within the optimal range for the development of bacteria 6.0–7.5 and fungi 5.5–8.0 (Amir et al., 2005). The pH value of a solution strongly influences the speciation and bio-sorption availability of the heavy metals (Wang et al., 2009). During the composting process, carbon dioxide is emitted from the composting mass as a metabolic end product. Thus the total organic carbon content of the composting mass decreases as composting proceeds (Singh et al., 2009). Similar results pertaining to the current study were observed for different trails of composting. Humic substances have a capacity to interact with metal ions and the ability to buffer pH and to act as a potential source of nutrients for plants (Amir et al., 2005; Singh et al., 2009). The increase in the nitrate, nitrite, phosphate and total phosphorus content of the final developed compost adds to the nutritional value of compost.

Chemical speciation of metals is the process of identifying different species or forms in a material (Cai et al., 2007). The determination of total heavy metal content does not provide useful information about the threat of bioavailability, which depends on their chemical forms. Chemical speciation of heavy metals during composting is a useful technique for determining the chemical forms in which these metals are present (Hsu and Lo, 2001). The reduction in the exchangeable and carbonate fractions of selected metals by vermicomposting was effective in reducing the toxicity of developed compost towards the end of experiment. Earthworms are capable of reducing possible toxic effects of heavy metals by utilizing them for physiological metabolism (Jain et al., 2004).

8.5. Conclusions

From the current study it is concluded that using multiple plants in consortia for water treatment is effective in removing the metals. The metals containing biomass of plants used for production of vermicompost showed enhanced nutritional qualities at the termination of composting process. Metal availability was also reduced to a large extent. Increase in the pH during the vermicomposting was most important factor for reducing bioavailability of heavy metals. Therefore, it is concluded that vermicomposting of metal treated plant biomass mixed with cattle manure by using *Eisenia fetida* was very effective for enhancing the nutritional status of the developed vermicompost and reducing most bioavailable fractions (exchangeable and carbonate) and enhancing inert fraction (residual) of heavy metals.