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Analysis of different nutrient status of liquid bio-fertilizer of different combinations of buffalo dung with gram bran and water hyacinth through vermicomposting by *Eisenia fetida*

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Abstract The animal, agro-wastes and water hyacinth (*Eichhornia crassipes*) are a serious problem for the society and ecosystem. The present study carried out the management of water hyacinth and observation of nutritional status like pH, electrical conductivity (EC), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), C/N ratio, total phosphorus and total calcium (TCa) of liquid bio-fertilizer (vermiwash) before and after vermicomposting of feed materials of different combinations of buffalo dung (BD) with water hyacinth (WH) and gram bran (GB). After vermicomposting of different combinations of BD with WH and agro-wastes, significant decrease in level of pH, EC, TOC and C/N ratio was observed, whereas significant increase in TKN, TK, TAP and TCa level in vermiwash of final vermicompost with respect to initial feed material was observed. The pH of initial mixture in all combinations has tended to basic in nature, while in final vermicompost, it becomes neutral/basic. The significant increase was observed in the level of TKN, TK, TAP in BD + GB + WH (1:2:1) and TCa in BD + GB + WH (1:1:2) vermiwash of final vermicompost of combination, whereas decrease was observed in TOC, C/N ratio, pH and EC in BD + WH (1:1), BD + GB + WH (1:1:1), BD + GB + WH (1:2:1) and BD + GB (1:1), respectively.

Keywords Chemical analysis · Bio-fertilizer · Biological wastes · Vermicomposting · *Eisenia fetida*

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1 Introduction

India produces around 3000 million tones of organic wastes annually. This huge amount of wastes comes from agriculture, urban and industrial sources as well as from domestic activities (Bharadwaj 2010). The animal dung, agro-wastes and water hyacinth (*Eichhornia crassipes*) caused adverse effect on human, animal and environment directly (Nath et al. 2009; Deka et al. 2013). Live stock excreta and exotic weeds as well as agricultural wastes also created problems for the human health and environment, if these are not properly managed (Bhartiya and Singh 2012; Yadav et al. 2013; Gupta 2005; Chauhan and Singh 2012; Yadav et al. 2013; Garg et al. 2011). Water hyacinth is free floating invasive aquatic macrophytes that are known to cause severe damage to the aquatic habitat (Deka et al. 2013). Role of earthworms in the breakdown of organic debris on soil surface and soil turnover process was first highlighted by Darwin in 1881. Agricultural waste, city garbage and kitchen waste have been recycled with vermicomposting along with bioconversion of organic waste material into nutrition-rich vermicompost by earthworm activity (Garg et al. 2005; Suthar 2007; Chauhan 2013). Vermicomposting is a suitable way for conversion of biological wastes into rich nutrient organic vermicompost (Nath et al. 2009; Chauhan and Singh 2013; Mishra et al. 2014). Earthworms act as excellent biological agents for recovery of vermifertilizer, vermiwash and vermiprotein which stabilized the agro-ecosystem, aquaculture and poultry (Dash and Senapati 1986). In India, the exotic epigeic species like *Eudrilus euginae*, *Perionyx excavatus* and *Eisenia fetida* are being used for vermicomposting (Hartenstein et al. 1979; Kale et al. 1982; Ashok 1994; Chauhan 2013). The epigeic earthworm *E. fetida* is a suitable species for management of wastes which are utilized successfully in vermicomposting (Chaudhari and Bhattacharjee 2002; Garg and Kaushik 2003; Gunadi and Edwards 2003). The action of earthworm in the process of vermicomposting of wastes is physical process that includes aeration, mixing and actual grinding, whereas the biochemical process was influenced by microbial decomposition of substrate in the earthworm intestine (Hand et al. 1988). Vermicomposting is a process of bio-oxidation and stabilization of organic wastes and by the joint action of earthworms and microorganisms. Vermiwash is a liquid that is collected after the passage of water through a column of worm action and is very useful as a foliar spray on different crops (Chauhan and Singh 2014). Recently vermiwash production has drawn the attention of commercial vermiculturists, because of its rich organic composition (Ismail 1997; Mishra et al. 2014). Vermiwash has great potential for growth of cereals, vegetables, and ornamental plants because it contains beneficial microorganisms, macro- and micronutrients, enzymes and hormones (Mishra et al. 2014). Adding vermicompost to soil improves the chemical and biological properties of soil and hence improves its fertility.

The aim of the present work is to access the physicochemical properties of vermiwash obtained from combinations of animal, agro-wastes and water hyacinth before and after vermicomposting with the help of earthworm *E. fetida*.

2 Materials and methods

2.1 Collection of earthworms

The adult cultured earthworm *E. fetida* was used in the experiment for the management of water hyacinth (*Eichornia crassipes*).

2.2 Collection of buffalo dung and gram bran

The buffalo dung was collected from the nearest dairy farm, and gram bran was collected from rural part of Gorakhpur District, Uttar Pradesh, India.

2.3 Collection of water hyacinth (*E. crassipes*)

The water hyacinth (*E. crassipes*) was collected from Ramgarh Lake, Gorakhpur, U.P., India.

2.4 Experimental setup for vermicomposting

The experiment was conducted on cemented earth surface in laboratory. Two kilograms of different combinations of buffalo dung with gram bran and water hyacinth in different ratios were kept on 30 × 30 × 10 cm in bed form at room temperature in dark. Dung was used as control. The vermicomposting beds were turned over manually every 24 h for 10 days in order to eliminate volatile substances. After this, 20 healthy adult earthworms *E. fetida* were inoculated in each bed, and the bed was covered by jute pockets. The vermibed was moistened daily up to 40–45 days for maintaining the moisture. After 7-day interval, the vermibed was turned manually up to 3 weeks. After 60 days, granular, tea-like vermicompost appears on upper surface. Only buffalo dung was used as control. All the experiments were replicated six times.

2.5 Extraction of vermiwash

Vermiwash is an extract of vermicompost and coelomic fluid of earthworms which was obtained from prepared vermicompost of different combinations of buffalo dung with gram bran and water hyacinth. Vermiwash was extracted through vermiwash collecting device which is made up of plastic container. It has capacity of 5 l and a tap at the bottom. The container is filled with broken bricks, about 10 cm thickness, which is followed by sand layer of 2–3 cm thickness at bottom and then filled with 2 kg of vermicompost with heavy population of earthworms; simultaneously, fresh water is added, and a container's tap is opened slightly. The watery yellowish to black extract of vermicompost's vermiwash is obtained. After 1–2 days, the process of extraction has been completed. The different combinations of collected fresh vermiwash were used for chemical analysis.

2.6 Analysis of nutrient status of vermiwash

The pH and EC were determined by using a double distilled water suspension of each waste in the ratio 1:10 (w/v) that had agitated mechanically for 30 min and filtered through Whatman No. 1 filter paper. Total organic carbon (TOC) was measured by the method of Nelson and Sommers (1982). Total Kjeldahl nitrogen (TKN) was determined after digesting the sample with conc. H_2SO_4 and conc. $HClO_4$ (9:1 v/v) according to the method of Bremner and Mulvaney (1982). Total phosphorus was analyzed using the calorimeter method with molybdenum in sulfuric acid (Garg et al. 2006). Total potassium was determined after digesting the sample in diacidic mixture ($HNO_3/HClO_4$ = 4:1 v/v), by flame photometer (Elica, CL 22 days, Hyderabad, India).

Table 1 Nutrient content in initial feed mixture and final vermiwash obtained from prepared vermicompost of the different combinations of buffalo dung with gram bran and water hyacinth through vermicomposting by earthworm *E. fetida*

Combinations	Parameters							
	TOC (g/kg)	TKN (g/kg)	C/N ratio	TK (g/kg)	TP (g/kg)	pH	EC (ds/m)	TCa (g/kg)
Initial feed mixture								
WH[a]	242.00 ± 6.49	7.57 ± 0.51	32.60 ± 2.01	8.53 ± 0.31	4.75 ± 0.31	7.55 ± 0.26	1.58 ± 0.16	0.74 ± 0.04
GB[a]	364.50 ± 8.45	9.02 ± 0.72	42.36 ± 4.96	9.48 ± 0.41	5.87 ± 0.30	8.98 ± 0.15	1.60 ± 0.10	0.87 ± 0.03
BD	556.67 ± 9.51	6.17 ± 0.55	93.85 ± 8.13	7.14 ± 0.51	4.73 ± 0.18	8.48 ± 0.07	2.74 ± 0.21	1.52 ± 0.08
BD + GB (1:1)	401.17 ± 7.55	7.87 ± 0.53	52.14 ± 3.27	8.57 ± 0.54	4.06 ± 0.26	8.67 ± 0.09	2.17 ± 0.17	1.21 ± 0.01
BD + WH (1:1)	350.50 ± 8.83	6.86 ± 0.58	53.13 ± 4.94	7.59 ± 0.74	4.66 ± 0.37	8.51 ± 0.10	2.12 ± 0.13	1.11 ± 0.02
BD + GB + WH (1:1:1)	381.00 ± 7.90	7.82 ± 0.87	50.03 ± 3.75	8.44 ± 0.68	4.91 ± 0.26	8.4 ± 0.11	1.94 ± 0.17	1.03 ± 0.01
BD + GB + WH (1:2:1)	438.67 ± 7.92	9.37 ± 0.60	47.74 ± 3.00	9.58 ± 0.55	5.56 ± 0.16	8.68 ± 0.10	2.21 ± 0.13	1.18 ± 0.09
BD + GB + WH (1:1:2)	420.67 ± 5.35	9.10 ± 0.51	46.90 ± 2.52	9.78 ± 0.60	5.75 ± 0.17	8.52 ± 0.04	2.19 ± 0.18	1.15 ± 0.05
BD + GB + WH (2:1:1)	422.00 ± 5.54	8.53 ± 0.80	52.15 ± 5.58	9.56 ± 0.61	5.60 ± 0.14	8.19 ± 0.07	2.40 ± 0.18	1.41 ± 0.05
Vermiwash of final vermicompost								
BD	253.58 ± 5.55*	10.65 ± 0.38*	23.89 ± 0.53*	7.49 ± 0.07*	6.18 ± 0.11*	7.16 ± 0.05*	1.55 ± 0.05*	3.20 ± 0.13*
BD + GB (1:1)	289.02 ± 5.89*	21.38 ± 0.32*	13.53 ± 0.36*	8.70 ± 0.09*	7.57 ± 0.16*	7.20 ± 0.06*	1.20 ± 0.05*	4.74 ± 0.14*
BD + WH (1:1)	233.17 ± 6.52*	17.37 ± 0.35*	13.46 ± 0.50*	8.52 ± 0.11*	5.88 ± 0.17*	6.97 ± 0.04*	1.29 ± 0.05*	4.91 ± 0.15*
BD + GB + WH (1:1:1)	250.49 ± 5.38*	23.38 ± 0.25*	10.72 ± 0.28*	9.04 ± 0.14*	6.53 ± 0.12*	7.46 ± 0.08*	1.37 ± 0.10*	5.57 ± 0.11*
BD + GB + WH (1:2:1)	314.56 ± 5.80*	27.46 ± 0.32*	11.46 ± 0.22*	9.60 ± 0.09*	7.96 ± 0.10*	6.71 ± 0.10*	1.43 ± 0.07*	6.02 ± 0.12*
BD + GB + WH (1:1:2)	299.29 ± 5.98*	25.34 ± 0.30*	11.82 ± 0.31*	9.39 ± 0.10*	6.87 ± 0.13*	7.48 ± 0.04*	1.51 ± 0.06*	6.22 ± 0.21*
BD + GB + WH (2:1:1)	302.10 ± 5.46*	25.43 ± 0.33*	11.89 ± 0.29*	9.33 ± 0.11*	6.67 ± 0.16*	7.68 ± 0.07*	1.38 ± 0.09*	3.93 ± 0.20*

Each value is the mean ± SE of six replicates

BD buffalo dung, *GB* gram bran, *WH* water hyacinth

* Student's *t* test was applied to determine the significant ($p < 0.05$) difference in between different physicochemical parameters of vermiwash of initial feed mixture and final vermicompost. Product momentum correlation shows that significant positive and negative coefficients ($p < 0.05$) were observed between different physicochemical parameters of vermiwash of different combinations of wastes

[a] The final vermiwash of GB and WH was not obtained because it is not used as feed material alone

2.7 Statistical analysis

The data have been expressed as mean ± SE of six replicates. Student's *t* test was applied to determine the significant ($p < 0.05$) difference in between different physicochemical parameters of vermiwash of initial feed mixture and final vermicompost's vermiwash. Product momentum correlation was applied to show the significant relation between different physicochemical parameters of vermiwash of different combinations of wastes by correlation coefficient ($p < 0.05$; Table 1).

3 Results

The major changes were observed in physicochemical property of vermiwash of final vermicompost with respect to initial feed mixture. After vermicomposting, the significant decrease in level of pH, EC, TOC and C/N ratio was observed, whereas significant increases in level of TKN, TK, TAP and TCa in vermiwash of final vermicompost with respect to vermiwash of initial feed material were observed (Figs. 1, 2, 3, 4, 5, 6). The initial feed mixture of BD, GB and WH and its different combinations show basic pH, i.e., 7.55 ± 0.26–8.98 ± 0.15, whereas after vermicomposting, there was significant decrease in pH range 6.71 ± 0.10–7.68 ± 0.07. The highest pH 8.98 ± 0.15 in initial feed mixture of GB and significantly decreased pH 6.71 ± 0.10 were recorded in vermiwash of final vermicompost observed from combinations of BD + GB + WH (1:2:1). Similarly, significant decrease EC was noticed in vermiwash compared to initial feed material. The highest EC (2.74 ± 0.21 ds/m) was noticed in BD, and after vermicomposting, the lowest EC (1.55 ± 0.05 ds/m) was observed in the combination of BD due to composition of feed material. The significant decrease in TOC (233.17 ± 6.52 g/kg) was observed in BD + WH (1:1) after vermicomposting. The C/N ratio was also used as an index for maturity of organic waste degradation. In our study, it has been found that the C/N ratio is decreased in feed materials of all the combinations compared to initial feed mixture. The C/N ratio of initial feed mixture was ranged from 32.6 ± 2.01–93.85 ± 8.13, while it was ranged from 10.72 ± 0.28–23.89 ± 0.53 in vermiwash of final vermicompost. The

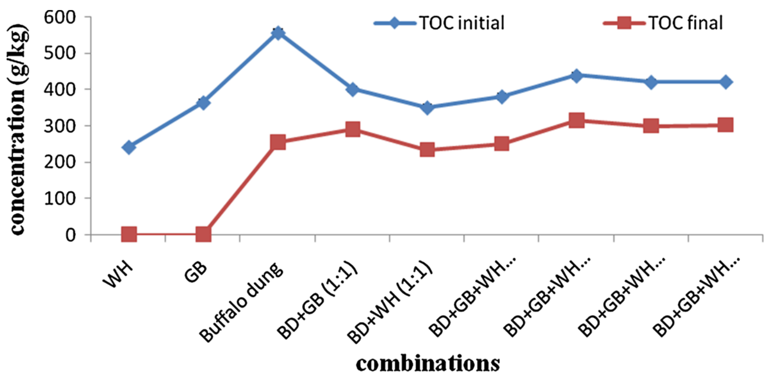

Fig. 1 Total organic carbon (TOC) concentration in vermiwash of different combinations of buffalo dung with gram bran and water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

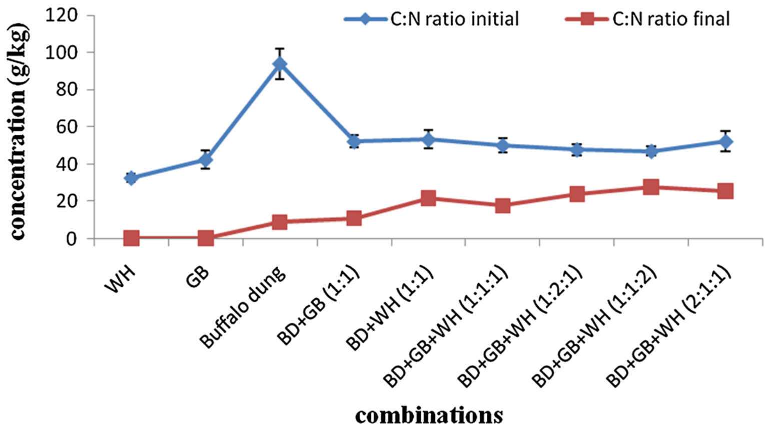

Fig. 2 C/N ratio in vermiwash of different combinations of buffalo dung with gram bran and water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

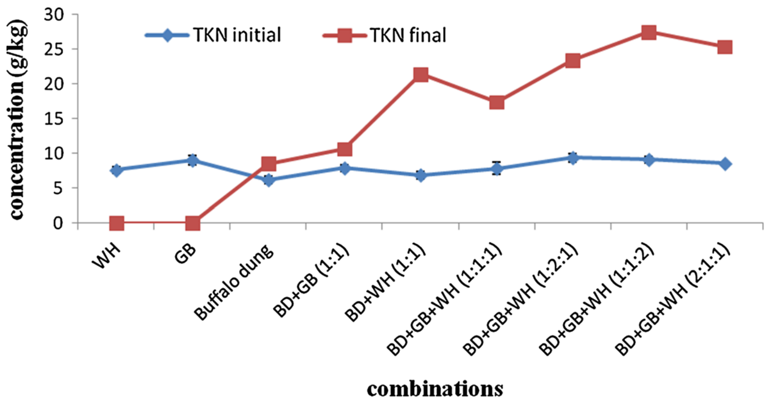

Fig. 3 Total Kjeldahl nitrogen (TKN) concentration in vermiwash of different combinations of buffalo dung with gram bran and water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

maximum increased level of TKN (27.46 ± 0.32 g/kg) was observed in the combination of BD + GB + WH (1:2:1). Phosphorus content was slightly higher in vermiwash of final vermicompost obtained from all feed materials of different combinations of wastes. In vermiwash, the TP ranges were from 5.88 ± 0.17 g/kg in BD + WH (1:1) to 7.96 ± 0.10 g/kg in BD + GB + WH (1:2:1). The phosphorus (TP) potassium (TK) concentration was significantly increased in final vermiwash of vermicompost of all combinations of feed materials in comparison with initial feed material. The highest increased level of TK 9.60 ± 0.09 g/kg was observed in vermiwash of final vermicompost of BD + GB + WH (1:2:1), whereas TP in BD + GB + WH (1:1:2) 7.96 ± 0.10 g/kg. The maximum TCa 6.22 ± 0.21 g/kg was recorded in vermiwash of final vermicompost of combination of BD + GB + WH (1:1:2). Product momentum correlation shows that

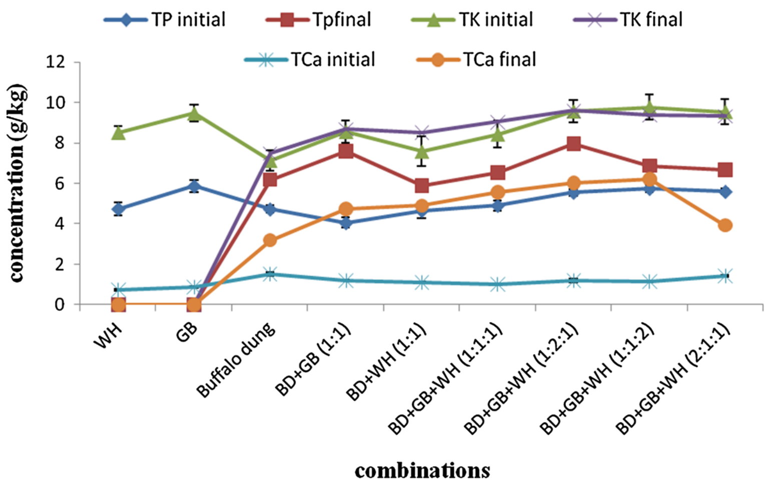

Fig. 4 TP (total phosphorus), TK (total potassium), TCa (total calcium) concentration in vermiwash of different combinations of buffalo dung with gram bran and water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

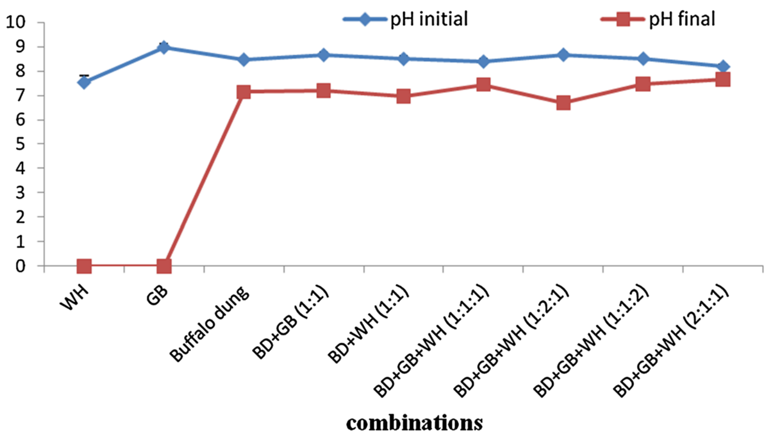

Fig. 5 pH concentration in vermiwash of different combinations of buffalo dung with gram bran and water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

significant positive and negative coefficients ($p < 0.05$) were observed between different physicochemical parameters of vermiwash of different combinations of wastes (Table 2).

4 Discussion

After vermicomposting, the significant decrease in level of pH, EC, TOC and C/N ratio was observed, whereas significant increase in level of TKN, TK, TP and TCa in vermiwash of final vermicompost with respect to vermiwash of initial feed material was observed,

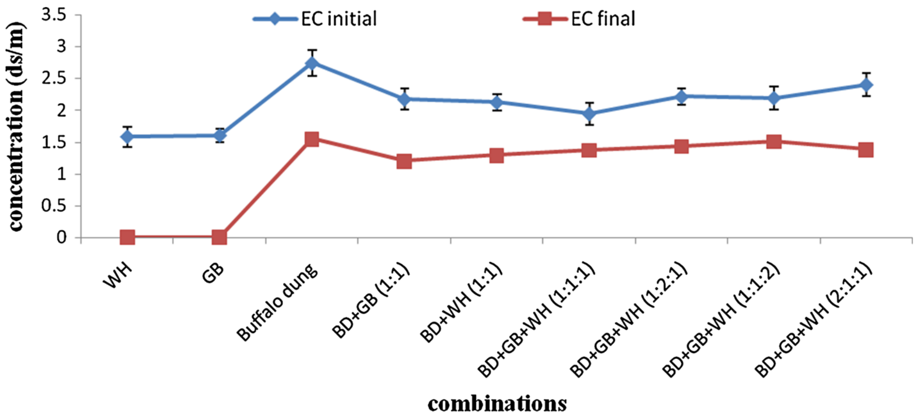

Fig. 6 Electrical conductivity (EC) concentration in vermiwash of different combinations of buffalo dung with gram bran water hyacinth. Initial = initial feed mixture before vermicomposting, final = final vermiwash after vermicomposting

Table 2 Product momentum correlation matrix within different physicochemical parameters/nutrients

Parameters	TOC	TKN	C/N ratio	TK	TP	pH	EC	TCa
TOC	1	0.725	−0.378	0.681	0.818	0.063	0.124	0.323
TKN		1	−0.897	0.991	0.643	0.163	−0.160	0.716
C/N			1	−0.911	−0.393	−0.161	0.437	−0.749
TK				1	0.574	0.132	−0.134	0.745
TP					1	−0.304	−0.171	0.462
pH						1	0.085	−0.189
EC							1	−0.054
TCa								1

because the vermicomposting increases the population of beneficial soil microflora, destroys the pathogen of soil and converts organic wastes into valuable products (Suthar 2007; Chauhan 2013). During vermicomposting, important nutrients of feed materials are converted into much soluble nutrients through earthworm action which are now easily usable for plants (Payal et al. 2006). Earthworms play an important role in stabilization of inorganic plant nutrients to organic form and increase the soil fertility. Nath et al. (2009) reported that the significant change in physicochemical tenure of feed materials was observed after vermicomposting by earthworm *E. fetida.*

There was a significant change in pH. The pH decreased from alkaline to acidic or neutral, and when the pH becomes acidic was attributed to mineralization of nitrogen into nitrate/nitrites and phosphorous into orthophosphate (Nath et al. 2009; Chauhan and Singh 2013). Bioconversion of the organic materials into intermediate species of organic acids was important for these results (Ndegwa et al. 2000). Production of CO_2 and organic acid by microbial decomposition during vermicomposting showed the lower pH of feed material (Heartenstien and Heartenstien 1981; Haimi and Hutha 1986; Elvira et al. 1998). The decrease in pH could be due to production of CO_2, ammonia, NO_3^- and organic acids during vermicomposting process (Yadav et al. 2010). Decrease in pH level may play an important role in nitrogen retention as this element is lost as volatile ammonia at high pH values (Heartenstien and Heartenstien 1981). The EC of initial feed material was higher than of vermiwash that decreased significantly after vermicomposting. The EC and pH of feed material could be a limiting factor for survival and growth of earthworm *E. fetida* reported by Gunadi and Edwards (2003). The increased EC values might have been due to the loss of weight of organic matter and release of different mineral salts in available forms (Wong et al. 1997; Kaviraj and Sharma 2003; Chauhan and Singh 2012).

The increased amount of TOC was recorded in initial feed mixture that drastically declined after vermicomposting. A large fraction of TOC was lost as CO_2 during the biological activity of earthworm. In the process of vermicomposting, microbial degradation and feeding of organic matter by the earthworm modify the status of material, which emphasizes the carbon losses from the raw material through microbial respiration in the form of CO_2 and by mineralization of organic matter (Viel et al. 1987). It is reported that the huge amount of organic substances in the initial substrate was lost as CO_2 between 20 and 43 % as TOC by the end of vermicomposting (Elvira et al. 1998; Chauhan and Singh 2012). The body fluid (mucus) and excreta of earthworm promote the microbial growth in vermicomposting (Kale et al. 1982; Elvira et al. 1998; Suthar 2007). The present findings reinforce the earlier findings that up to 49 % carbon loss is possible during the vermicomposting process as a result of CO_2 loss due to respiratory activity (Khwairakpam and Bhargava 2009).

Total Kjeldahl nitrogen was increased in final products of all the combinations because of the mineralization of organic matter. It is already reported by the Hand et al. (1988) that *E. fetida* in cow dung slurry increased the nitrate-nitrogen content. The increasing value of nitrogen is directly related to the loss of organic carbon and mineralization of organic matter by conversion process (Garg and Gupta 2011). The loss of dry mass as well as water loss by evaporation during mineralization of organic matter (Viel et al. 1987) might have determined the relative increase in nitrogen. Tripathi and Bharadwaj (2004) reported the addition of nitrogen in the form of mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes from earthworms. These nitrogen-rich substances were not originally present in feed and might contribute to additional nitrogen (Garg et al. 2006). Atiyeh et al. (2000) reported that by enhancing nitrogen mineralization, earthworm has great impact on nitrogen transformation in manure so that nitrogen retained in the nitrate form.

Carbon/nitrogen ratio is one of the most widely used indices for maturity of organic waste decreased with time in all the experiments due to the decrease in TOC and increase in TKN. Senesi (1989) observed that decline of C/N ratio <20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes. The C/N ratio of the substrate reflects the organic waste mineralization and stabilization during the process of decomposition (Suthar et al. 2008). The loss of carbon as CO_2 through microbial respiration and addition of nitrogenous excretory material between the C/N ratios of the substrate were mostly used as indicators of vermicompost maturation (Kale 1998; Gupta and Garg 2007; Suthar et al. 2008; Nath et al. 2009).

Total potassium content was increased in vermiwash of all the combinations in comparison with initial feed mixture. Kaviraj and Sharma (2003) observed that level of TK was increased 10 % by *E. fetida* and 5 % by *L. mauritii* during vermicomposting. Benitez et al. (1999) observed that the leacheats collected during vermicomposting process need higher K concentration. Suthar (2007) suggested that earthworm processed waste material contains higher concentration of exchangeable potassium, and it may be due to enhanced microbial activity during the vermicomposting process, which consequently enhanced the rate of mineralization.

The phosphorous content was greater in final vermiwash in comparison with initial feed mixture. Maximum increase was observed in the combination of BD + GB + WH (1:2:1) ratio during the process of vermicomposting. Increase in TP content is probably through mineralization and mobilization of phosphorous by bacterial and fecal phosphate activity of earthworms (Edwards and Lofty 1972; Satchell and Martin 1984). Mansell et al. (1981) reported the increase in phosphorous content by the breakdown of the plant material by earthworm. The feed mixture with earthworm shows faster increase in TP than the feed mixture without earthworms. It should be the efficiency of earthworm in mineralization of TP in the feed mixture. Lee (1992) described that the flow of organic residue through the gut of earthworm to plant and the release of phosphorous in available form is performed partly by earthworm gut.

Total calcium concentration was increased in vermiwash in all the combinations compared to initial feed material. The highest calcium content obtained in 1:1:2 ratio in the combination of BD + GB + WH may be due to synergetic ratio of WH. Orozco et al. (1996) reported an increase in TCa and a decrease in TK after ingestion of coffee pulp waste by earthworms. TCa concentration was not significantly increased in vermicompost of paper mill sludge (Elvira et al. 1996). Calcium metabolism is primarily responsible for enhanced content of inorganic calcium by worm cast associated with gut process. However, the similar pattern of calcium enhancement is well documented in the available literature (Heartenstien and Heartenstien 1981; Garg et al. 2006; Chauhan and Singh 2012, 2013).

5 Conclusion

It is clear from the result that the different combinations of buffalo dung with gram bran and water hyacinth (*E. crassipus*) can be converted into rich organic liquid bio-fertilizer through vermicomposting with help of *E. fetida*. Vermicomposting resulted in the decrease in level of pH, EC, TOC and C/N ratio, but increase in TKN, TAP, TCa, and TK level. The value of C/N ratio, below 20, for the vermicompost suggests the satisfactory degree of maturity. The vermiwash obtained from the combination of BD + GB + WH (1:2:1) has maximum rich organic nutrients. The foliar spray of liquid bio-fertilizer may enhance the growth of plants as well suppress the pest infestation. The liquid bio-fertilizer is less expensive, non-hazards to environment and animal health and eco-friendly.

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