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Effect of Composting and Vermicomposting on Recycling of Three Aquatic Weeds Treated with Rock Phosphate on P-dynamics, Phosphatase Activity and Biomass P

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ABSTRACT

In this experiment, three aquatic weeds viz., *Lemna*, *Vallisneria* and water hyacinth were mixed with two doses to rock phosphate (RP) i.e., P_0 – control and P_1 – 200 mg RP per kilogram of waste and these treatments were used for both compost and vermicompost preparation. Results suggested that vermicomposting was faster technique which produced organic amendment comparatively more nutrient enriched than traditional composts. Vermicomposting not only increased phosphorus content of organic substrates, but also increased phosphatase activities and microbial properties, which are responsible for solubilization of insoluble phosphorus. Addition of RP to aquatic weeds significantly ($P \leq 0.05$) increased total phosphorus (TP) content of final product. Both composting and vermicomposting increased microbial biomass P (MBP). In both the cases, addition of RP increased MBP content in final product, these along with enhanced phosphatase activity was possibly responsible for higher TP content in these treatments.

Keywords: aquatic weeds, microbial fatty acids assay, microbial biomass P, phosphatase activities, recycling techniques, rock phosphate

Abbreviations: C, carbon; **$CHCl_3$**, chloroform; **$HClO_4$**, perchloric acid; **HNO_3**, nitric acid; **H_2SO_4**, sulphuric acid; **MBC**, microbial biomass carbon; **MBP**, microbial biomass phosphorus; **N**, nitrogen; **P**, phosphorus; **PSB**, phosphate-solubilizing bacteria; **RP**, rock phosphate; **SPA**, acid phosphatase activity; **TP**, total phosphorus; **TOC**, total organic carbon; **TKN**, total Kjeldahl nitrogen

INTRODUCTION

Aquatic weeds are those unabated plants which grow and complete their life cycle in water and cause harm to aquatic ecosystem as well as to related eco-environment. Removal and proper disposal of aquatic weeds are very important to maintain water bodies. *Lemna*, *Vallisneria* and water hyacinth (*Eichhornia crassipes*) are three major aquatic weeds in tropical countries. The use of decayed tissues of unwanted plants to provide nutrients for crops is a crude but effective way of exploiting weeds and is a simpler technique than any other alternatives available. Therefore, composting technique could be employed to recycle different aquatic weeds (Pramanik *et al*. 2007; Pramanik 2010a). Composting is microorganism-mediated controlled oxidative decomposition of organic substrates through successive mesophilic and thermophilic phases whereas vermicomposting is the non-thermophilic oxidative decomposition of organic substrates by microorganisms in presence of earthworms.

After nitrogen (N), phosphorus (P) is the second most limiting nutrient for crop production in most of the arable soils (Ochwoh *et al*. 2005) and crop yields are often adversely affected by low availability of P in soil. Natural rock phosphates (RP) have been recognized as a valuable alternative source for P fertilizer especially due to increasing cost of soluble phosphatic fertilizers like single and triple super phosphates. Different methods like partial acidulation (Biswas and Narayanasamy 1998), thermal alteration (Reddy *et al*. 2000), blending with water soluble fertilizers (Xiong *et al*. 1996) and preparation of RP enriched compost (Biswas and Narayanasamy 2006) were adopted to improve P availability from low-grade RP. In this experiment, three different aquatic weeds were mixed with RP and allowed to decompose through composting and vermicomposting. Both composting and vermicomposting are microorganism-mediated process and microorganisms assimilate nutrient elements from organic substrates during decomposition. Due to their short life-cycle, death of microorganisms leads to the release of those nutrients in ionic form within very short duration of time (Schlegel 1995). That's why, microorganisms are considered as source and sink of plant nutrients and microbial biomass of organic amendment could be used as an indicator for quality of compost or vermicompost i.e., its potential to release nutrients to the soil. During this experiment, RP was used to improve phosphorus content of final stabilized products and effect of this P-enrichment was studied on several P-solubilizing factors during compost or vermicompost preparation. The objectives of this experiment were to 1) compare the suitability of composting and vermicomposting for stabilization of different aquatic weeds, 2) compare the effect of these techniques on different P-solubilizing factors such as organic C content, C/P ratio, acid and alkaline phosphatase activities, microbial respiration and microbial biomass and 3) evaluate the effect of RP addition on TP dynamics and on some biochemical properties during composting and vermicomposting of different aquatic weeds.

MATERIALS AND METHODS

Substrates used

This study was conducted in polythene-lined earthen pots. Three different aquatic weeds viz., lemna (*Lemna major* and *Lemna minor* were in comparable proportion), vallisneria (*Vallisneria gracilis*) and water hyacinth (*Eichhornia crassipes*) were used as food for *Eudrilus eugeniae*. These aquatic weeds were precomposted for 7 days with sufficient water and the resultant substrates were mixed with 10% cow manure and then used for compost or

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vermicompost preparation. Fresh cow manure was air-dried before using to remove its ammonia, which is toxic to the earthworms. In this experiment, initial organic substrates referred to the mixture of 7 days precomposted aquatic weeds and cow manure.

Rock phosphate (RP), having available P-content 27%, was used in this experiment.

Experimental design

All three aquatic weeds were decomposed in excess water for 3 days to make it more palatable to earthworms and 1.5 kg (with 55-60% moisture content) of different aquatic weeds viz., *Lemna* (S_1), *Vallisneria* (S_2) and water hyacinth (S_3) were taken in suitable size (5 L capacity) earthen pots. Cow manure was air-dried and 150 g (10% w/ w of applied aquatic weeds) was mixed with each treatment to provide an initial favourable environment to earthworms. Four sets were prepared for each waste material, and out of those two were used for composting and others for vermicomposting. Again in these two pots of each set, RP was added in two levels (P_0 - control and P_1 - 200 mg RP per kg of organic wastes). Twenty-five (almost equal weight, mean weight 3.82 ± 0.15 g) matured *E. eugeniae* was inoculated in organic wastes taken for vermicomposting, whereas no earthworms were added to the composting treatments. In vermicomposting treatments, water was sprinkled twice a day to maintain the moisture content at about 60 to 68% during the whole process. Total phosphorus (TP) content was estimated in these samples after every 15 days and total organic carbon (TOC), total Kjeldahl nitrogen (TKN), microbial biomass C and P, population of phosphate solubilizing bacteria (PSB) by dilution plate method and by bioluminescence assay were estimated in initial and final sample.

Total concentration of organic carbon, nitrogen and phosphorus

Total organic carbon (TOC) of the vermicompost was estimated using the standard dichromate oxidation method of Nelson and Sommers (1982). Total Kjeldahl nitrogen (TKN) was estimated after digesting the sample with concentrated H_2SO_4 (1:20, w/v) followed by alkaline distillation (Bremner and Mulvaney 1982). Total phosphorus (TP) content was analyzed from the wet digest [tri-acid (HNO_3–H_2SO_4–$HClO_4$) mixture was used for digestion] of vermicompost (Jackson 1973). TP was estimated by observing the absorbance using ammonium molybdate in hydrochloric acid.

Acid and alkaline phosphatase activities

Vermicompost was incubated in a test tube with 4 ml of modified universal buffer (MUB) and 1 ml of 50 mM sodium *p*-nitrophenyl phosphate at 37°C for 30 min and one drop of toluene was added to stop the microbial growth. Buffers having pH 6.5 (Tabatabai and Bremner 1969) and pH 11.0 (Tabatabai 1982) were used for the determination of acid and alkaline phosphatase activities, respectively. Four millilitres of 0.5 M sodium hydroxide solution was then added to stop the reaction. After a further 20 min at room temperature, the concentration of *p*-nitrophenol was determined by reading the absorbance at 420 nm and compared them with serially diluted solution of 100 ppm p-nitrophenol standards.

Microbial respiration and microbial biomass

Microbial activity was measured by estimating evolved CO_2 after introducing 1 ml of 1% glucose solution as substrate (Anderson 1982). Microbial biomass C (MBC) and P (MBP) was analyzed by the chloroform fumigation-extraction method (Vance *et al.* 1987). Both fumigated and without fumigated samples were extracted with K_2SO_4 solution and this extract was used for measuring microbial biomass. MBC in this extract was determined by simple dichromate oxidation method.

For microbial biomass P determination, samples were extracted with 0.5 M sodium bicarbonate solution (pH 8.5) immediately after fumigation with $CHCl_3$. Inorganic P (P_i) in aliquots of that extracts was estimated by the ammonium molybdate - ascorbic acid method described by Murphy and Riley (1962). A spike of potassium dihydrogen phosphate equivalent to 25 mg P g^{-1} soil was used to correct for P_i fixation during the sodium bicarbonate extraction. Biomass P (Bp) was calculated by Bp = EP/0.38, where EP is the difference between Pi extracted from fumigated and non-fumigated sample (Brookes *et al.* 1982).

Statistical analysis

In this experiment, data were analysed by two-way ANOVA considering different components of decomposing substrates (aquatic weeds and RP) as one factor and composting and vermicomposting as another factor. Then data with significant difference were analysed for post-hoc study using SPSS 14.0 software.

RESULTS AND DISCUSSION

Total organic carbon and total Kjeldahl nitrogen

During composting, organic substrates are decomposed by the action of both mesophilic and thermophilic microorganisms. While in case of vermicomposting, microorganisms act on the organic matter under non-thermophilic condition in presence of earthworms. Both composting and vermicomposting reduced the dry weight and C/N ratio of organic substrates (**Table 1**). The extent of decrease in TOC content during both compost and vermicompost preparation was dependent on the chemical nature of wastes. Stabilization of TOC during decomposition was considered as an index of maturity of the organic substrates, i.e., completion of the decomposition process. In case of vermicomposting, TOC content and C/N ratio of organic substrates became stable after 75-90 days while it took 135-150 days during composting. Data revealed that rate of mineralization (decomposition of organic matter) was comparatively higher during vermicomposting, though the final TOC content of composts and vermicomposts of same wastes were statistically at par. Both composting and vermicomposting were effective for recycling different aquatic weeds. During these processes, organic mass of the decomposing substrates was lost as CO_2 and moisture was evaporated due to mineralization (Viel *et al.* 1987). Faster loss of TOC due to vermicomposting may be attributed to the presence of earthworms. Due to greater biomass, earthworms assimilate more organic carbon during their growth which in turn leads to the faster TOC loss from organic substrates. Comparatively faster reduction in TOC after vermicomposting might be due to higher activity of microorganisms in presence of earthworms, which in turn leads to the faster mineralization of organic matter. Irrespective of the treatments, the trend of TOC content in final product of different wastes was *Vallisneria* ≈ water hyacinth > *Lemna*. During these processes, addition of RP did not show any specific effect on TOC content of final stabilized product. This decrease in TOC content was inversely proportional to the relative increase in TKN content of organic substrates. Increase in relative concentration of nitrogen leads to decrease in C/ N ratio of these wastes. After both composting and vermicomposting, C/ N ratio of all the aquatic weeds came within the range of 14 - 21 (**Table 1**). Except for vermicompost prepared from S_1P_1, RP addition increased TKN content in all the treatments after both of these processes. Results indicated that for the same treatment, vermicomposts recorded comparatively higher TKN content than composts. However, the highest TKN content was registered in vermicompost of S_1 and it was followed by TKN content S_2 and S_3. The increase in TKN content in vermicompost might also be attributed to the secretion of several nitrogenous substrates (protein, glycoprotein etc.) with their body fluid in decomposing substrates (Morris 2005; Khwairakpam and Bhurgava 2010). Pramanik *et al.* (2007) also revealed that population of free-living N-fixing bacteria was increased due to vermicomposting. These bacteria might be responsible for increasing N-content through fixing atmospheric nitrogen. Dry mass of organic substrates was lost more rapidly due to vermicomposting. Nitrogen mass balance calculation was based on the dry mass of organic amendment and it was expressed as

Table 1 Total concentration of organic carbon and nitrogen in rock phosphate amended composts and vermicomposts of different aquatic weeds.

	Organic carbon (mg g^{-1})		**Total nitrogen (mg g^{-1})**		**C/ N ratio**	
	P_0	**P_1**	**P_0**	**P_1**	**P_0**	**P_1**
Lemna	412.2 ± 22.5		4.49 ± 0.29		91.8	
Compost	258.7 ± 7.1	257.1 ± 5.1	15.36 ± 0.49	15.56 ± 0.51	16.8	16.5
Vermicompost	251.5 ± 4.6	254.7 ± 4.0	17.16 ± 0.60	17.03 ± 0.56	14.6	14.9
Vallisneria	497.9 ± 19.7		4.70 ± 0.32		105.9	
Compost	267.9 ± 4.0	266.5 ± 5.1	13.85 ± 0.41	14.17 ± 0.58	19.3	18.8
Vermicompost	257.4 ± 4.5	255.0 ± 4.7	15.94 ± 0.48	16.28 ± 0.44	16.1	15.8
Water hyacinth	445.8 ± 26.6		4.02 ± 0.31		110.9	
Compost	262.0 ± 4.4	263.8 ± 4.5	12.09 ± 0.39	12.44 ± 0.42	20.3	21.2
Vermicompost	248.1 ± 4.1	250.6 ± 3.6	13.40 ± 0.45	13.79 ± 0.47	18.5	18.2

() Parenthesis indicates percent increase or decrease over initial values of substrates

P_0 – control and P_1 – 200 mg rock phosphate per kg of organic substrates

Table 2 Nitrogen balance calculation data for composting and vermicomposting.

	Initial organic substrates			**Mineralized substrates**		
	Dry weight (g)	**Nitrogen (%)**	**Total Nitrogen (g)**	**Dry weight (g)**	**Nitrogen (%)**	**Total Nitrogen (g)**
S_1W_0	1074	0.449	4.822	290	1.536	4.454
S_1W_1	1074	0.449	4.822	257	1.716	4.410
S_2W_0	1014	0.470	4.766	307	1.385	4.252
S_2W_1	1014	0.470	4.766	251	1.594	4.001
S_3W_0	958	0.402	3.851	297	1.209	3.591
S_3W_1	958	0.402	3.851	259	1.340	3.471

S_1 - *Lemna*, S_2 – *Vallisneria* and S_3 – Water hyacinth; W_0: Traditional composting and W_1: Vermicomposting

the nitrogen content in total dry mass of organic substrates (**Table 2**). Analysis revealed that total nitrogen content in the whole dry mass of organic wastes was decreased due to mineralization and extent of decrease in nitrogen mass was comparatively higher in case of vermicomposting than traditional composting. Therefore, it could be concluded that nitrogen concentration was relatively increased after both composting and vermicomposting, but nitrogen mass balance revealed that total nitrogen content in whole dry mass was reduced due to nitrogen loss during mineralization. More reduction in this nitrogen dry mass also indicated comparatively higher rate of mineralization during vermicomposting.

Dynamics of total phosphorus (TP)

Both composting and vermicomposting increased TP content of aquatic weeds (**Fig. 1**). During these processes, C/P ratio of organic substrates decreased steadily and during completion, it became stable within the range of 27–32 and 23–26, respectively. Application of RP to initial wastes significantly ($P \leq 0.05$) increased concentration of TP, which in turn further decreased C/P ratio of final products (24–28 and 20–23, respectively). Like C/N ratio, narrower C/P ratio of organic manure facilitates P-mineralization (or P solubilization) after application to soil. During these processes, TP content of organic substrates increased steadily, though the rate of increase of TP content was comparatively higher during vermicomposting as compared to traditional composting. Microorganisms produce several organic acids during organic matter decomposition. Kumari *et al.* (2008) revealed that citric and oxalic were the main organic acids which were responsible for RP solubilization, though maleic and formic acids were also present in compost. Among these acids, citric acid had shown the highest P-solubilizing ability. Kaviraj and Sharma (2003) concluded that organic acids generated by microorganisms during mineralization might be the prime mechanism for insoluble P solubilization. High humic acid content and comparatively higher microbial activity in vermicompost was probably responsible for greater solubilization of RP which in turn leads to the higher P-content and lower C/P ratio in these treatments. Presence of low molecular weight organic acids and high molecular weight humic substrates in high proportion might be attributed to the highest TP content in RP treated vermicomposts. Organic substrates recorded sharp increase in TP content only after 15 days in presence of earthworms. However, TP content of organic substrates was stabilized after 60 days of vermicomposting and remained almost constant till the completion of the process. The results of this experiment were supported by the findings of Bayon and Milleret (2009). Irrespective of decomposition process, RP treated organic substrates registered higher rate of increase in TP content as compared to corresponding P_0 treatments. Among three aquatic weeds, S_1 recorded the highest TP content after both composting and vermicomposting. When RP was added to the initial organic substrates, TP content increased up to 90–105 days of composting and thereafter it gradually became constant. However, in case of vermicomposting, TP content in RP treated wastes increased till the end of the process.

Acid and alkaline phosphatase activities

Acid and alkaline phosphatase activities of organic substrates increased after both compost and vermicompost preparation (**Fig. 2**). Results suggested that acid phosphatase activity (SPA) of vermicomposts was comparatively higher than that of composts. Higher enzyme activities in vermicomposts might be attributed to the ability of earthworm to produce several enzymes. Guts of epigeic earthworms secrete several hydrolytic enzymes including phosphatases (Pramanik 2010b). During passage through the earthworm guts, these enzymes mix with organic substrates and come out with ejected materials. But when RP was added with wastes, composts recorded higher SPA as compared to vermicomposts. Pang and Kolenko (1986) proposed that activities of phosphatase enzymes were adversely affected by phosphate ion concentration. Therefore, higher phosphate ion, released due to enhanced solubilization of RP in presence of earthworm, possibly suppressed phosphatase activity in RP treated vermicomposts as compared to P_0-vermicomposts. However, vermicompost prepared from S_2 registered the highest SPA and it was followed by that of S_3 and S_1. Irrespective of treatments, vermicomposts registered significantly ($P \leq 0.05$) higher alkaline phosphatase activity (APA) than that of composts. The highest APA was observed in vermicomposts prepared from S_3. In P_0 treatments, APA of vermicomposts was comparatively higher than that of composts, while in case of RP treatments, the values did not differ significantly (**Fig. 2**). Though these enzymes are very thermo-sensitive, the activities of these enzymes were protected in composts or vermicomposts. Atiyeh *et al.* (2000) proposed that enzymes probably remain bound with humic substrates, which protect the active sites of enzymes. Pramanik *et al.* (2007) also found high positive correlation

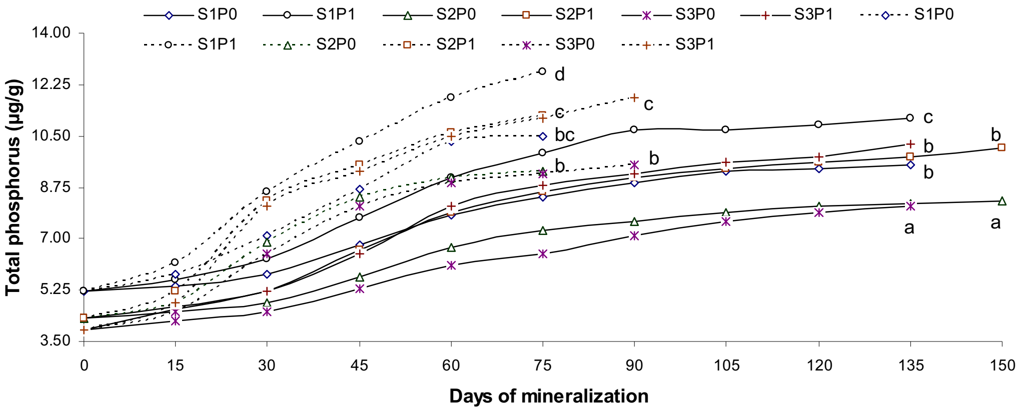

Fig. 1 Phosphorus dynamics (mg g^{-1}) during traditional composting (solid line) and vermicomposting (dotted line) of organic wastes. S_1 – *Lemna*, S_2 – *Vallisneria*, S_3 – water hyacinth; P_0 – control, P_1 – 200 mg rock phosphate/kg of organic substrates.

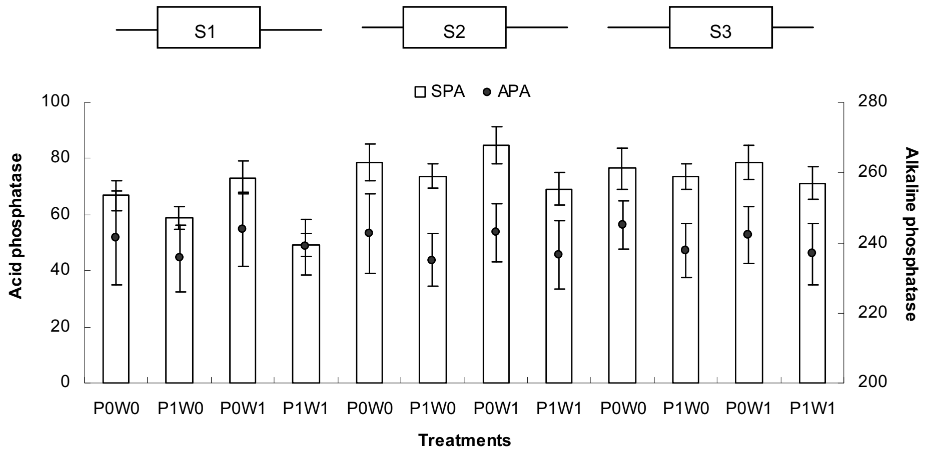

Fig. 2 Acid and alkaline phosphatase activities (µg PNP g^{-1} h^{-1}) in compost and vermicompost prepared from different aquatic weeds and rock phosphate mixture. S_1 – *Lemna*, S_2 – *Vallisneria*, S_3 – water hyacinth; P_0 – control, P_1 – 200 mg rock phosphate/kg of organic substrates; W_0 – composting, W_1 – vermicomposting.

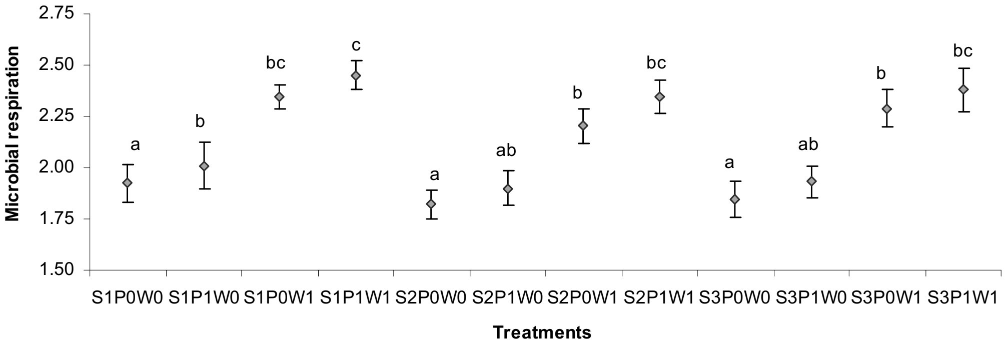

Fig. 3 Microbial respiration (µg CO_2 - C g^{-1} d^{-1}) in composts and vermicomposts of three aquatic weeds as affected the addition of rock phosphate (RP). S_1 – *Lemna*, S_2 – *Vallisneria*, S_3 – water hyacinth; P_0 – control, P_1 – 200 mg rock phosphate/kg of organic substrates; W_0 – composting, W_1 – vermicomposting. Different letters of the alphabet adjacent to dots indicate statistically significant differences at $P < 0.05$ according to Duncan's Multiple Range test.

between phosphatase activity and humic acids content of vermicomposts. Data suggested that alkaline phosphatase activity in each treatment was comparatively higher than acid phosphatase and this might be attributed to the neutral pH of both composts and vermicomposts.

Microbial respiration and microbial biomass

Composting and vermicomposting significantly ($P \leq 0.05$) increased substrate-induced microbial respiration (SMR) of different aquatic weeds (**Fig. 3**). In case of same treatment, SMR of vermicomposts was significantly ($P \leq 0.05$) higher than that of traditional composts. Bhardwaj (1999) also found the higher microbial activity in vermicomposts as compared to traditional composts. Frachhia *et al.* (2006) revealed that different organic substrates and method of composting greatly influenced bacterial community structure. Results of this experiment indicated that method of decomposition had significant effect on microbial activity of three different aquatic weeds. Addition of RP with initial organic wastes enhanced microbial activity after both composting and vermicomposting, though the effect varied depending on nature of aquatic weeds. The highest SMR was registered in vermicompost prepared from S_1 and RP mixture and it was followed by S_3 and S_2, respectively.

Both composting and vermicomposting significantly ($P \leq 0.05$) increased microbial biomass C (MBC) and biomass P (MBP) content of wastes (**Fig. 4**). Data suggested that MBC and MBP of vermicomposts were significantly ($P \leq 0.05$) higher than that of traditional composts. Addition of

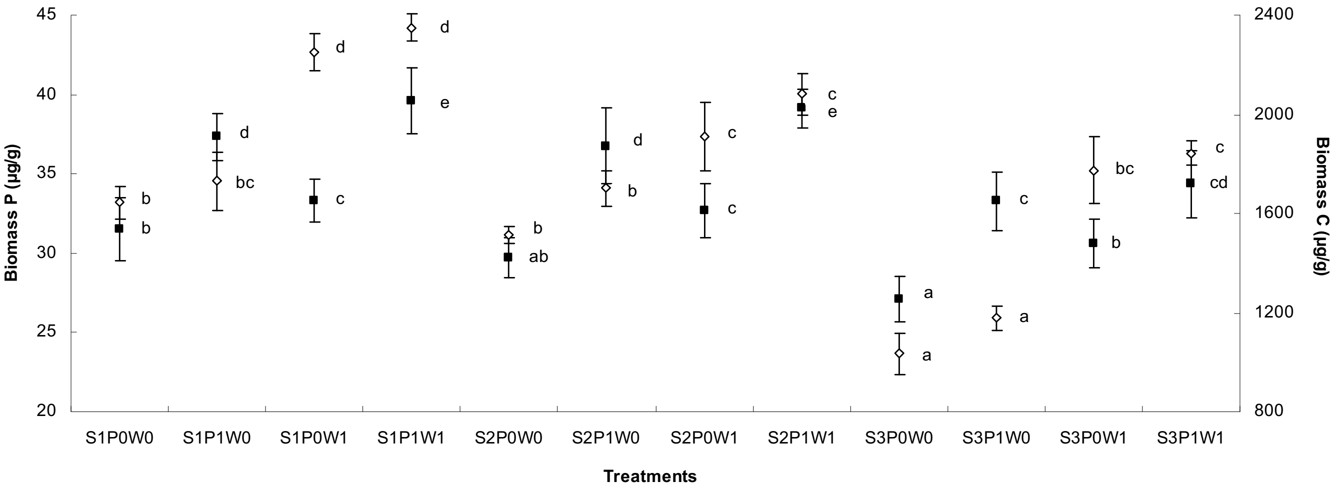

Fig. 4 Microbial biomass C and P of composts and vermicomposts of three aquatic weeds as affected by the addition of rock phosphate (RP). S_1 – *Lemna*, S_2 – *Vallisneria*, S_3 – water hyacinth; P_0 – control, P_1 – 200 mg rock phosphate/kg of organic substrates; W_0 – composting, W_1 – vermicomposting. Different letters of the alphabet adjacent to dots indicate statistically significant differences at $P < 0.05$ according to Duncan's Multiple Range test.

RP to aquatic weeds significantly ($P \leq 0.05$) increased microbial biomass of both composts and vermicomposts. Among different organic substrates, the highest microbial biomass was recorded in S_1 followed by S_2 and S_3 respectively. However, MBC and MBP of vermicomposts, prepared from S_2P_1 and S_3P_1, were statistically at par. The addition of RP increased microbial biomass P in organic amendment. Vermicomposting recorded a comparatively lower value of biomass C/P ratio than that of composts. Alteration in microbial biomass C/N and C/P ratios suggested that vermicomposting not only increased microbial biomass and microbial activity, but possibly also influenced microbial community structure of decomposing substrates. Release of immobilized nutrients may have a stimulating effect on microbial cells and also activate dormant microbes (Zhang *et al.* 2000). Earthworms selectively consume organic matter with high concentration of microorganisms (Hendriksen 1990), but not all the ingested microorganisms are killed during passage through earthworm intestine (Edwards and Fletcher 1988; Hendriksen 1990). Increase in metabolic quotient (qCO_2) in these treatments also confirmed the fact that microorganisms proliferate under the favourable condition of earthworm guts. Metabolic quotient (qCO_2) was determined as the ratio of substrate-induced microbial respiration to microbial biomass C. enhanced qCO_2 of earthworm acted substrates suggested rejuvenation of the microbial community, as qCO_2 of 'young' microorganisms are frequently greater than that of 'aged' ones (Zhang *et al.* 2000).

CONCLUSION

Vermicomposting yielded comparatively better plant amendment from aquatic weeds through much faster process. Therefore, aquatic weeds could be recycled most effectively by vermicomposting and Vallisneria gave the best quality vermicompost as compared to other aquatic weeds. Vermicomposting not only increased phosphorus content of organic substrates, but also increased phosphatase activities and microbial properties, which are responsible for solubilization of insoluble phosphorus. RP could be mixed with aquatic weeds prior to vermicomposting to increase total phosphorus content of vermicompost. Addition of RP increased microbial biomass P content and increased microbial activity during vermicomposting.

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