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Application of rural slaughterhouse waste as an organic fertilizer for pot cultivation of solanaceous vegetables in India

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Abstract

Background: The lack of electricity and water supply in rural abattoirs in developing nations prevents the adoption of waste-processing technologies practiced in economically advanced countries. This research attempts to recycle waste blood and rumen digesta generated in rural slaughterhouses as organic fertilizer, thus promoting sustainable agriculture.

Results: The values of 5-day biochemical oxygen demand, chemical oxygen demand, total Kjeldahl nitrogen, concentrations of oil, grease, total suspended solids, total solids, and total phosphorus characterized blood and rumen digesta as highly polluting wastes. Waste blood and rumen digesta were mixed in 1:1, 2:1, and 3:1 ratios and dried to obtain 'bovine-blood-rumen-digesta-mixture' (BBRDM). The efficacy of the organic fertilizer was compared with diammonium phosphate (DAP) in a pot cultivation of tomato, chili, and brinjal. Five grams of BBRDM (N/P/K = 30.36:1:5.75)/kilogram of soil applied at the second and sixth weeks produced earlier fruiting by 2 weeks and yielded (in terms of total fruit weight) higher by 130% for tomato, by 259% for chili, and by 273% for brinjal in BBRDM (3:1)-cultivated plants compared to DAP. BBRDM-applied soils showed higher C, N, and P concentrations than DAP. High-nitrogen-containing BBRDM mineralized rapidly, and nitrogen and phosphorus were available within 6 days of drying. Although high nitrogen concentration caused toxicity when applied at the time of planting to young plants, BBRDM enhanced the yield and productivity when applied to mature plants after 15 days of plantation. Higher numbers of *Azotobacter*, phosphate-solubilizing bacteria, fungi, and amount of chlorophyll were isolated from soils treated with BBRDM than with DAP. Carbohydrate, protein, and fat contents of the vegetables were comparable to DAP-grown vegetables.

Conclusions: Highly polluting abattoir wastes could be gainfully utilized, promoting a healthy environment around rural slaughterhouses. The application of BBRDM to crops of marginal returns is an attractive proposal.

Keywords: Slaughterhouse, Bovine-blood-rumen-digesta-mixture, Tomato, Chili, Brinjal, Organic fertilizer

Introduction

Globally, blood and rumen contents are the major abattoir wastes. In developing countries, a high proportion of the blood obtained from slaughtered animals ends as waste due to the lack of facilities for drying to produce blood meal. Industrial processing of blood by drum, flash, or spray drying produces good-quality blood meal but requires high capital outlay on heavy equipment as well as central slaughtering and collection of blood. Rumen contents have about 85 g/kg of water content and constitute a disposal problem as their processing involves high investment and operating costs. Methods reported for processing rumen contents include field spreading or landfill, ensiling, drying using gas-fired rotary dryers, fluid-bed dryers, solar dryers, or by pressing to reduce the water content. These methods are not yet feasible in developing countries where slaughtering is mainly done in small and scattered units, which makes collection of large quantities of blood and rumen contents difficult. In addition, electricity and water supply are either lacking or irregular in most developing countries. Therefore, the evolution of acceptable processing

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technologies for abattoir wastes is important (Makinde and Sonaiya 2010).

In a low-cost recycling method for abattoir wastes, a blend of bovine blood and rumen digesta, bovine-blood-rumen-digesta-mixture (BBRDM), has been utilized as a replacement to full-fat soybean meal in broiler chickens' starter and finisher diets (Odunsi et al. 2004). BBRDM was used as a replacement for groundnut cake and fishmeal in the diets of layer chickens (Odunsi 2003). In the present study, the application of BBRDM as a fertilizer and soil conditioner is being attempted for the first time. Generally, solanaceous vegetables require a large quantity of major nutrients like nitrogen (N), phosphorus (P), and potassium (K) for better growth and fruit yield. It is impractical to apply expensive fertilizer inputs for crops of marginal returns, and the rising cost of inorganic fertilizers have made them out of reach of small farmers in India. The application of BBRDM to solanaceous vegetables could be an attractive proposal. This study was conducted in the perspective of Magrahat village, South 24 Parganas district of West Bengal state, India, where the existing practice of random application of local slaughterhouse wastes as fertilizer was endeavored to be scientifically investigated. The plants cultivated in this study were tomato, brinjal, and chili.

Methods

Characterization of blood and rumen digesta

Bovine blood and rumen digesta were collected from freshly annihilated animals. The following parameters in waste bovine blood were measured: pH by electrometric method, total solids (TS) by gravimetric method (103°C to 105°C), total suspended solids (TSS) by gravimetric method (103°C to 105°C), soluble 5-day biochemical oxygen demand (BOD_5) at 20°C, chemical oxygen demand (COD) by open reflux method, total phosphorus (TP) by vanadomolybdophosphoric acid colorimetric method, total Kjeldahl nitrogen (TKN) by macro-Kjeldahl method, oil and grease by partition gravimetric method, and potassium (K) by flame photometric method. The characterization of rumen digesta was also done by measuring pH, TS, TSS, oil and grease, soluble BOD_5, COD, TKN, TP, and K. Samples were obtained on five separate days and determinations performed in triplicate sets.

Preparation of BBRDM

Fresh blood of cattle was collected in containers immediately following the annihilation of animals. Bovine blood and rumen digesta were weighed in ratios of 1:1, 2:1, and 3:1 in three containers. Containers were placed on a coal-fired earthen stove and boiled for 90 min. The mixture was constantly stirred until the content was substantially free of water. The mass was sun dried for 3 days to obtain BBRDM. The final product was a coarse granular powder and could be easily spread.

N, P, and K analysis of BBRDM

The N, P, and K contents of BBRDM (ratios of 1:1, 2:1, and 3:1) were determined as described in the subsection 'Characterization of blood and rumen digesta.' All experiments were performed thrice in duplicate sets.

N and P mineralization during the storage of BBRDM

Immediately after the preparation of BBRDM (ratios of 1:1, 2:1, and 3:1), available N and P were measured following Subbiah and Asija (1956) and Bray and Kurtz (1945), respectively. Further determinations were made at 5-day intervals for 16 days during the drying of BBRDM. All experiments were performed thrice in duplicate sets.

Cultivation of plants and determination of the dose and application frequency of BBRDM

The plants cultivated in this study were tomato (*Solanum lycopersicum* var. Patharkuchi), brinjal (*Solanum melongena* var. Jhuribegun), and chili (*Capsicum annuum* var. Sonamukhi). Seedlings of approximately the same height, purchased from a local nursery, were planted, one seedling in one pot. Different tubs, (1)filled with soil (of village Magrahat), (2) soil + diammonium phosphate (DAP) $(NH_4)_2HPO_4$ (N/P/K = 18:46:0), (3) soil + BBRDM (composed of one part of blood and one part of rumen digesta, 1:1), (4) soil + BBRDM (composed of two parts of blood and one part of rumen digesta, 2:1),(5) soil + BBRDM (composed of three parts of blood and one part of rumen digesta, 3:1), were used for the plantation of tomato, chili, and brinjal, a total of eight tubs for each of the five treatments following a completely randomized design. In the first series of experiments, BBRDM was applied at different doses (2.5, 5.0, and 10.0 g/kg of soil) at the time of planting. In the second series of experiments, the doses were applied to the plants on second (after an initial 15 days of plantation) and sixth weeks during a 20-week period of cultivation. All experiments were performed thrice in duplicate sets. Plant growth parameters as mentioned in the 'Results' section (Tables 1, 2, and 3) were measured or counted every week to compare plant growth. Pot cultivation was done in the winter seasons (November to March) of 2009 to 2010, 2010 to 2011, and 2011 to 2012. The tubs were kept under a shed and watered every day. Pest and weed control was not necessary during the pot cultivation. The mean maximum temperature was 27.4°C ± 2.2°C, and the mean minimum temperature was 16.4°C ±2.6°C. The fruits, as they matured, were picked toward the end of the cultivation.

Table 1 Comparative analysis of the growth parameters of tomato plants after 12 weeks

Plant growth parameters	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Plant height (cm)	37.05 ± 0.5^{b}	40.5 ± 1^{ab}	42 ± 1.5^{ab}	42.5 ± 0.5^{a}	42 ± 1^{ab}
Number of leaves	10 ± 1^{b}	11 ± 1^{b}	18 ± 1^{a}	20 ± 2^{a}	18 ± 2^{a}
Leaf surface area (cm^2)	125.5 ± 1^{d}	139 ± 2.5^{c}	141 ± 1^{c}	154 ± 2^{a}	150 ± 2^{b}
Stem surface area (cm^2)	0.48 ± 0.05^{d}	0.66 ± 0.02^{c}	1.11 ± 0.04^{b}	1.43 ± 0.02^{a}	0.48 ± 0.02^{d}
Number of buds	22 ± 3^{d}	27 ± 3^{c}	34 ± 4^{b}	38 ± 2^{ab}	40 ± 2^{a}
Number of flowers	15 ± 1^{c}	18 ± 2^{c}	29 ± 1^{b}	28 ± 1^{b}	34 ± 2^{a}
Number of fruits	10 ± 1^{c}	10 ± 1^{c}	14 ± 1^{b}	19 ± 2^{a}	21 ± 3^{a}
Total fruit weight (g)	196.25 ± 3.46^{e}	337.64 ± 4.56^{d}	414.65 ± 3.06^{c}	719.77 ± 4.65^{b}	775.95 ± 4.58^{a}

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in the same row indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture.

Determination of N, P, and organic C content of soil

Available N (Subbiah and Asija 1956), available P (Bray and Kurtz 1945), and organic carbon (C) (Walkely and Black 1934) were measured before and during cultivation in each tub every 2 weeks. All experiments were performed thrice in duplicate sets.

Microbiological analysis of soil

Soil samples of the 2nd, 8th, and 14th weeks (the fertilizers were applied in the second and sixth weeks) were considered for microbiological analysis. Rhizosphere soil was collected at a depth of 3 to 5 cm. Nutrient agar (NA) media were used for enumeration of the total bacterial population. Pikovskaya (PVK) medium was used for the enumeration of phosphate-solubilizing bacteria. Ashbys mannitol (AM) agar medium was used for the selection of *Azotobacter* species (main nitrogen-fixing bacteria) that utilize mannitol and atmospheric nitrogen as sources of C and N, respectively. BG-11 broth was used for the growth of freshwater cyanobacteria. Cooke Rose Bengal (CRB) agar base medium was used for the selective isolation of fungi. For compositions of NA, PVK, AM, BG-11, and CRB media, please see Additional file 1. Test and control soil samples (0.1 g) were taken and mixed with 10 ml of sterile water. Initial abundances of each type of microorganism in all the pots (at day 0) were recorded. Soil sample dilutions were vortexed for 5 min, and 100 μl of each dilution was plated on four types of media (NA, PVK, AM, and CRB) and inoculated in BG-11 liquid broth. NA and PVK plates were observed after incubation for 24 h at 37°C, AM plates after 48-h incubation at 37°C, and fungal plates after 72-h incubation at 25°C. Cyanobacteria were incubated under the light of 50-μmol photons m^2/s intensity, 12 h a day for 4 weeks. Thereafter, the cyanobacterial mass was centrifuged and extracted with methanol, and the absorbance of chlorophyll *a* was measured at 663 nm (Pramanik et al. 2011). All experiments were done thrice in duplicate sets.

Appearance, taste, and food quality analysis

The mature fruits were visually observed and tasted by five individuals. The vegetables obtained from the plants were analyzed for total protein (Lowry's method), total carbohydrate (phenol sulphuric acid method), and fat (Soxhlet method), and the values were compared with vegetables grown with DAP. All experiments were done thrice in duplicate sets.

Table 2 Comparative analysis of the growth parameters of brinjal plants after 12 weeks

Plant growth parameters	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Plant height (cm)	22.5 ± 0.5^{b}	36 ± 1^{a}	32.5 ± 1.5^{a}	32 ± 0.5^{a}	32.5 ± 0.5^{a}
Number of leaves	11 ± 1^{bc}	13 ± 2^{ab}	10 ± 1^{b}	15 ± 1^{a}	13 ± 1^{ac}
Leaf surface area (cm^2)	136 ± 1.01^{c}	135 ± 1.06^{c}	163 ± 2.02^{a}	153 ± 2.25^{b}	164 ± 2^{a}
Stem surface area (cm^2)	0.36 ± 0.02^{c}	0.35 ± 0.01^{c}	0.63 ± 0.01^{a}	0.53 ± 0.01^{b}	0.64 ± 0.02^{a}
Number of buds	6 ± 1.58^{c}	7 ± 1.56^{c}	8 ± 1.58^{bc}	10 ± 2.07^{ab}	13 ± 2.35^{a}
Number of flowers	3 ± 1.21^{c}	4 ± 1.2^{c}	6 ± 1.32^{bc}	8 ± 1.56^{ab}	11 ± 2.11^{a}
Number of fruits	1 ± 0.84^{d}	2 ± 1.67^{cd}	4 ± 1.19^{bc}	5 ± 1.23^{ab}	7 ± 1.58^{a}
Total fruit weight (g)	44.87 ± 2.99^{e}	149.3 ± 1.58^{d}	326.37 ± 5.6^{c}	420.61 ± 6.2^{b}	556.18 ± 7.89^{a}

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in the same row indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture.

Table 3 Comparative analysis of the growth parameters of chili plants after 12 weeks

Plant growth parameters	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Plant height (cm)	32 ± 2^{b}	29 ± 2^{c}	42 ± 2^{a}	41.5 ± 1^{a}	42.5 ± 1^{a}
Number of leaves	108 ± 4^{e}	129 ± 3^{d}	150 ± 6^{c}	155 ± 3^{b}	180 ± 4^{a}
Leaf surface area (cm^2)	12.7 ± 0.7^{b}	11.4 ± 1.2^{d}	11.9 ± 1.5^{c}	13.8 ± 1.3^{a}	14 ± 1.5^{a}
Stem surface area (cm^2)	0.41 ± 0.02^{d}	0.43 ± 0.02^{d}	1.74 ± 0.02^{a}	1.15 ± 0.01^{b}	1.09 ± 0.01^{c}
Number of buds	12 ± 1.68^{e}	28 ± 2.03^{c}	25 ± 1.99^{d}	32 ± 2.5^{b}	46 ± 3.78^{a}
Number of flowers	10 ± 2.07^{e}	22 ± 1.98^{c}	17 ± 1.63^{d}	28 ± 2.02^{b}	39 ± 2.95^{a}
Number of fruits	7 ± 1.46^{e}	18 ± 1.56^{c}	10 ± 1.45^{d}	25 ± 2.14^{b}	36 ± 2.88^{a}
Total fruit weight (g)	6.79 ± 1.39^{e}	28.11 ± 2.03^{c}	13.39 ± 2.15^{d}	64.39 ± 3.78^{b}	100.81 ± 4.62^{a}

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in the same row indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture.

Statistical methods

Analysis of variance (ANOVA) for one-way classified data on plant growth parameters was performed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). Differences were significant at 0.05 level. Tukey's test was performed as *post hoc* analysis of one-way ANOVA for pairwise comparison. Arithmetic mean of the microbial numbers and mass (in the case of cyanobacteria) was determined considering the values at the 2nd, 8th, and 14th weeks. Standard deviations of the means were calculated. Tukey's test was done as described before.

Results and discussion

Characterization of waste blood and rumen digesta

The average pH of blood was 8.0 ± 0.1, the BOD_5 was 69,558 ± 880 mg/l, the COD was 268,571 ± 3194.5 mg/l, and TKN was 12,414 ± 721.3 mg/l. The concentrations of oil and grease, TSS, TS, TP, and K were 27.3 ± 1.5, 433,192 ± 2,476, 824,762 ± 4,752, 236 ± 17, and 3,933 ± 21 mg/l, respectively. The N, P, and K content of rumen digesta was determined as N/P/K = 945.0 ± 30:1,817.33 ± 11.24:400 ± 14 mg/kg. The average pH of rumen digesta was 8.0 ± 0.1, soluble BOD_5 was 120 ± 70 mg/l, the COD was 3,440 ± 27 mg/l, TS was 57,400 ± 437 mg/l, TSS was 42,000 ± 527 mg/l, while oil and grease was 77,100 ± 657 mg/l. Error ranges indicate one standard deviation from the mean (n = 15). These parameters characterized the slaughterhouse refuse as highly polluting wastes.

Characterization of BBRDM

The N, P, and K, content of BBRDM was 49,440.64 ± 236.25:1,628.75 ± 322.25:9,367.94 ± 1,132.06 mg/kg. The N/P/K ratio was approximately 30.36:1:5.75. Error ranges indicate one standard deviation from the mean (n = 6).

Plant growth and yield of vegetables

Results of the experiments done as described in the subsection 'Cultivation of plants and determination of the dose and application frequency of BBRDM' in the 'Methods' section showed that all plants treated with BBRDM (3:1) died when 2.5 g BBRDM/kg of soil was added at the time of planting. When fertilized with 5.0 g BBRDM/kg of soil at the time of planting, all plants treated with BBRDM (3:1) and half of the plants treated with BBRDM (2:1) died. When fertilized with 10 g BBRDM/kg of soil at the time of planting, all plants treated with the three preparations of BBRDM died. On the other hand, none of the plants died when BBRDM was applied at the second and sixth weeks, and 5.0 g of BBRDM/kg soil produced the best results compared to 2.5 g as well as 10.0 g BBRDM/kg of soil in terms of plant growth parameters and yields of fruits. Results are shown in Tables 1, 2, and 3. Error ranges in the tables indicate one standard deviation from the mean (n = 24). Table 1 shows the results of the effect of BBRDM on the growth of tomato plants.

Tukey's test shows that the highest yield in terms of total fruit weight was obtained when BBRDM (3:1) was applied. In terms of the number of fruits, BBRDM (3:1) and BBRDM (2:1) produced more yields than the other treatments, though the difference between BBRDM (3:1) and BBRDM (2:1) is not statistically significant. The onset of flowering and fruiting was observed to be 2 weeks earlier than DAP. Chalkos et al. (2010) observed in pot studies that *Mentha spicata* compost added at 4% to 8% (*w*/*w*) proved to be a very promising soil amendment. Results obtained in pot studies showed that anaerobically fermented pig slurry could be a suitable alternative to the use of mineral fertilizer for tomato cultivation (Kouřimská et al. 2009). Experiments carried out in pots revealed that the yield of tomato and critical nutritional parameters showed significant increase upon the application of biowaste from the tobacco industry (Chaturvedi et al. 2008).

Table 2 shows the results of the effect of different soil conditioners on the growth of brinjal plants. The highest yield, as recorded by total fruit weight, was attained in soils treated with BBRDM (3:1). In terms of the number of fruits, BBRDM (3:1) and BBRDM (2:1) produced higher yields than the other applications, though the difference between BBRDM (3:1) and BBRDM (2:1) is not statistically significant nor is the difference between BBRDM (2:1) and BBRDM (1:1). The onset of flowering and fruiting was observed to be 2 weeks earlier than in plants treated with inorganic fertilizer. Górecki and Górecki (2010) reported that sheep wool could serve as a valuable and environmentally friendly fertilizer for brinjal cultivation in pot studies.

Table 3 shows the results of the effect of BBRDM on the growth of chili plants. Tukey's test shows that the highest yields in terms of the number of fruits and total fruit weight were obtained when BBRDM (3:1) was applied. The onset of flowering and fruiting was observed to be 2 weeks earlier than in plants treated with DAP. Damke et al. (1988) and Surlekov and Rankov (1989) noted the enhancement of height and number of branches and leaves of chili plants upon the application of farmyard manure with inorganic supplements.

The induction of early flowering is probably due to better nutritional status of the plants. Increased production of leaves might have helped to elaborate more photosynthates and induce flowering, thus effecting to early initiation of the flower bud. The results are in conformity with the report for tomato (Sharma and Mahendra 1963) as well as for chili (Revanappa et al. 1998; Patil and Biradar 2001). The increase in leaf area may be attributed to more number of leaves per plant. Increased plant height may be due to the increased uptake of primary nutrients, which might have enhanced cell division and cell elongation. Yield is the manifestation of morphological, physiological, biochemical, and growth parameters and is considered to be the result of efficient trapping and conversion of solar energy. This might have helped in producing a higher amount of carbohydrates which might have translocated from the source (leaf) to the reproductive parts (sink), resulting in more number of fruits and fruit weight. Yield attributes are closely associated with growth components like plant height, number of leaves, and leaf area per plant (Jagadeesha 2008). Higher number of fruits and fruit weight obtained in plants grown with BBRDM may be due to the increased growth components of the three crops. Similar findings were also reported in brinjal by Amburani and Manivannan (2002).

Soil nutrient status during cultivation

The fertilizers were added at the start of the cultivation (after 2 weeks) and after 6 weeks. Figure 1 shows the results of the chemical analysis of soil samples during the entire period of cultivation.

In general, BBRDM showed higher C, N, and P concentrations than DAP. With the progression of time, N was rapidly utilized by the plants, while the utilization of P was lower than that of N. Vegetable crops require an adequate and continuous supply of N for proper growth and productivity. The presence of N in plant tissue is known to increase its vegetative growth and chlorophyll formation. Nitrogen is the chief constituent of protein that leads to cell enlargement, cell division, and ultimately increased plant growth. Phosphorus is known to increase root growth, seed formation, and flower development.

In our experiments, N and P are obtained from BBRDM. Even if a proportion of soil nitrogen is fixed from the air, this is not sufficient to maintain agricultural productivity and requires external addition of fertilizers. In our experiments, the yields obtained from plants without added fertilizers were significantly lower than the yields obtained upon the addition of DAP or BBRDM. Approximately two and four times more yields of tomato were attained upon the addition of DAP and BBRDM (3:1), respectively. The addition of DAP and BBRDM (3:1) improved the yields of brinjal by about 4 and 15 times, respectively. Similarly, the application of DAP and BBRDM (3:1) improved the yields of chili by 3 and 12 times, respectively. BBRDM promoted the growth of nitrogen-fixing bacteria that may have contributed to the soil N content. However, the distinction between direct N derived from BBRDM and that gained from nitrogen-fixing bacteria was not possible in this study and can be objectives of future fundamental studies on soil chemistry.

In general, solanaceous vegetables require more amounts of nitrogen compared to phosphorus. For tomato, to produce 1 metric ton of fresh fruit, plants need to absorb, on average, 2.5 to 3 kg N and 0.2 to 0.3 kg P. Brinjals need 3 to 3.5 kg N and 0.2 to 0.3 kg P, while chilies need 3 to 3.5 kg N and 0.8 to 1 kg P (Hegde 1997). From Figure 1a,b,c, it is observed that the phosphorus content of soils treated with BBRDM progressively increases, indicating accumulation. In our experiments, phosphorus mineralization using soil microbes continued following the application, and due to the low uptake, accretion occurred. Mohammadi et al. (2009) observed that the mineralization of organic P may release P into the soil solution, contributing to the observed high water-soluble P content with a high-P-manured soil. Gaskell et al. (2006) also noted that soil phosphorus can rapidly build up to high levels when composts and other organic amendments are used.

One important characteristic of an agricultural waste product to be used as an organic fertilizer is the potentially available N (Cavaleri et al. 2004). Nitrogen content in BBRDM (5.5%, *w*/*w*) was similar to cotton meal (8.21%) and castor oil (7.54%) and higher than traditional waste products used as fertilizers like sugarcane bagasse (0.24%), wood ash (0.51%), and bovine manure (0.77%) (Lima et al. 2011). The majority of the N present in the soil is found in the organic form, which is not available for plant absorption. To be taken up by the

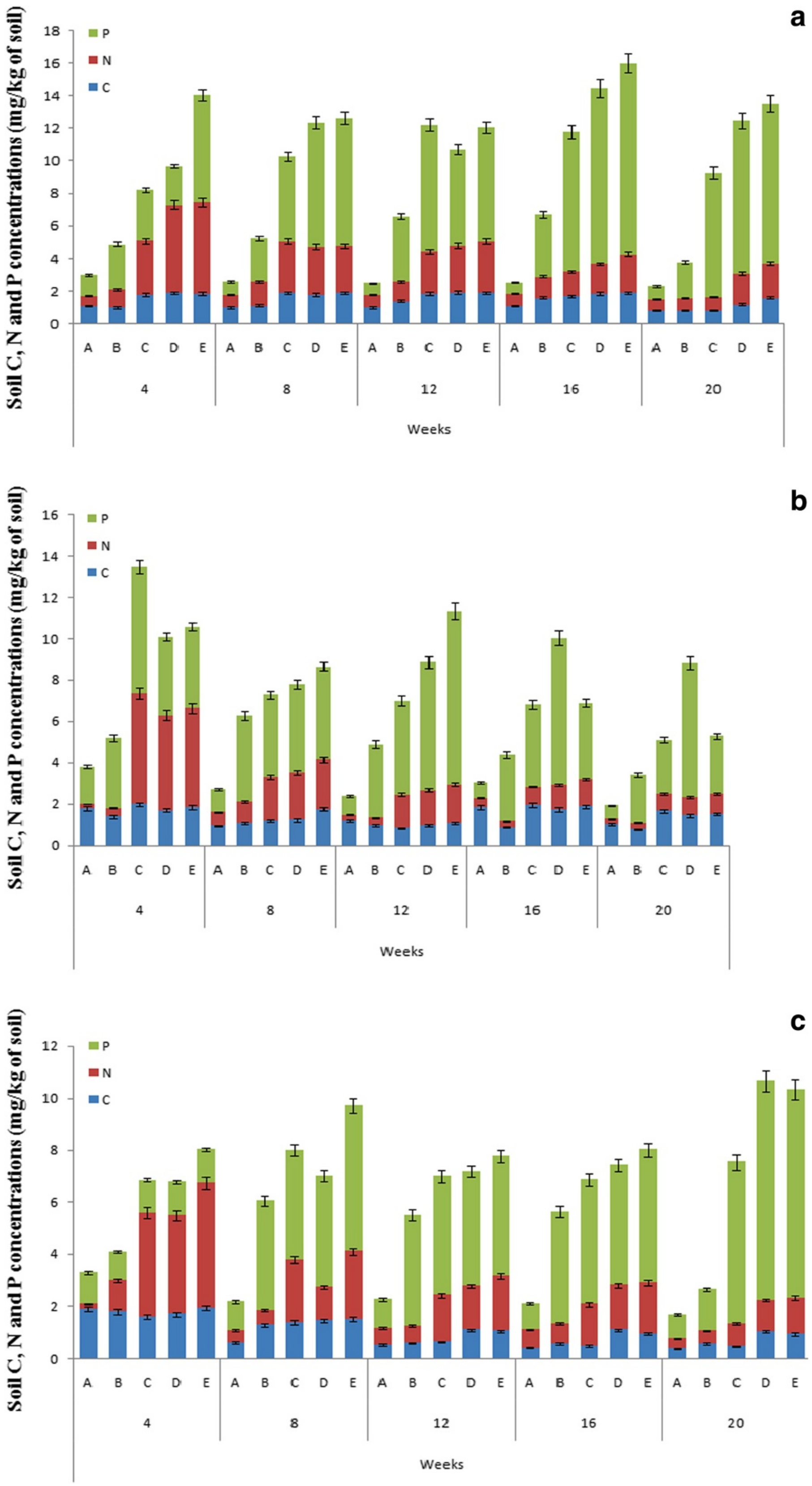

Figure 1 (See legend on next page.)

(See figure on previous page.)
Figure 1 Soil C, N, and P concentrations during cultivation of (a) tomato, (b) brinjal, and (c) chili. With different soil treatments, **A**: soil, **B**: soil + DAP, **C**: soil + BBRDM (composed of one part of blood and one part of rumen digesta, 1:1), **D**: soil + BBRDM (composed of two parts of blood and one part of rumen digesta, 2:1), **E**: soil + BBRDM (composed of three parts of blood and one part of rumen digesta 3:1). Available N was measured following Subbiah and Asija (1956), available P according to Bray and Kurtz (1945), and organic C as described by Walkely and Black (1934) before and during the cultivation in each tub every 2 weeks. For details of the methods, please see the complete articles, particulars of which are given in the list of references. Error ranges indicate one standard deviation from the mean (*n* = 6).

plants, N has to be in the inorganic form, like nitrate or ammonia (Savvas et al. 2010). Some organic residues are slowly mineralized due to low N content. When they are added to the soil, N and P contents can be temporarily reduced due to microorganism immobilization (López-Piňeiro et al. 2008). In those materials, N is slowly released, and the residual effect is expected to last longer (Cabrera et al. 2005). BBRDM, on the contrary, was mineralized extremely fast, as seen in Figure 2.

The availability of N was considerably higher in BBRDM (3:1) compared with the other two ratios. No significant differences in available P were observed among the three preparations of BBRDM (Figure 2). Available N and available P increased significantly on the sixth day of drying of BBRDM and subsequently leveled off. The high standard deviations may arise from variations during weighing as BBRDM is a coarse granular powder. A large quantity of N was released in a short period of time; high concentrations of mineral N (nitrate and ammonia) caused plant toxicity when applied at the time of planting to very young plants. Phytotoxicity caused by excess of N usually occurs due to accumulation of NH_4^+ (Savvas et al. 2010). As BBRDM was rich in N and P and mineralized fast, this material enhanced yield and productivity when applied to mature plants after 15 days of plantation. Blends of castor meal and husks containing higher than 4.5% N caused reduction in castor oil plant growth and even plant death (Lima et al. 2011). Castor meal was used as an organic fertilizer for wheat plants (Gupta et al. 2004). When the meal was added to the soil on the sowing day, increased mineral-N doses caused reduction in wheat plant growth because N content in the soil was increased to a toxic level.

Characterization of the microbial population of soil

The fertilizers were added at the start of the cultivation (after 2 weeks) and after 6 weeks. Therefore, microbial counts were obtained after the 2nd, 8th, and 14th weeks. Initial abundances of each type of microorganism in all the pots (at day 0) varied within 5%. In all the three crops cultivated, in general, higher numbers of bacteria, fungi, *Azotobacter*, and phosphate solubilizers and higher amount of cyanobacterial biomass were obtained from soils treated with BBRDM (3:1) or BBRDM (2:1) than those treated with DAP with one exception. During tomato cultivation, a higher number of phosphate solubilizers were obtained in soils treated with BBRDM (1:1) (Tables 4, 5, and 6). Error ranges indicate one standard deviation from the mean (*n* = 6).

Phosphorus is an essential component of the energy compounds (ATP and ADP) and phosphoproteins. Phosphate solubilizers liberate P in soil and make it available for plants. Phosphate-solubilizing bacteria release organic and inorganic acids which reduce soil pH leading to change of P and other nutrients to available forms ready for uptake

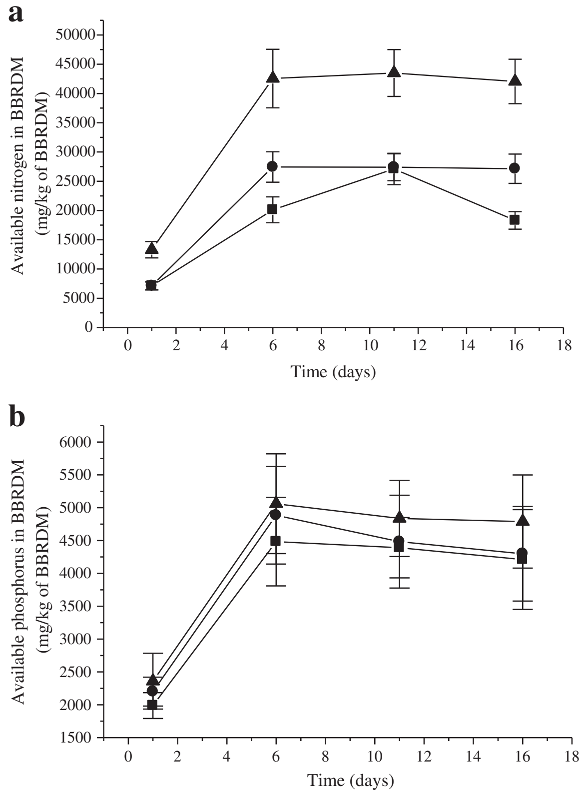

Figure 2 Available (a) nitrogen and (b) phosphorus during sun drying of BBRDM after preparation. Symbols: square-BBRDM (composed of one part of blood and one part of rumen digesta, 1:1), circle-BBRDM (composed of two parts of blood and one part of rumen digesta, 2:1), and triangle-BBRDM (composed of three parts of blood and one part of rumen digesta 3:1). Available N was measured following Subbiah and Asija (1956) and available P according to Bray and Kurtz (1945). For details of the methods, please see the complete articles, particulars of which are given in the list of references. Error ranges indicate one standard deviation from the mean (*n* = 6).

Table 4 Soil microbial abundance during tomato cultivation

Number of microorganisms	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Fungi ($\times 10^3$/ml) in CRB	6.44 ± 1.13^{b}	7.83 ± 1.5^{b}	9.39 ± 1.6^{b}	14.33 ± 2.64^{a}	14.44 ± 2.5^{a}
Bacteria ($\times 10^7$/ml) in NA	23.99 ± 1.69^{d}	29.33 ± 1.56^{c}	29.67 ± 1.62^{c}	35.61 ± 2.11^{b}	45.67 ± 2.93^{a}
Azotobacter ($\times 10^7$/ml) in AM	4.11 ± 0.66^{c}	5 ± 1.19^{c}	19.61 ± 0.71^{b}	26.5 ± 1.13^{a}	25.78 ± 2.28^{a}
Phosphate solubilizers ($\times 10^7$/ml) in PVK	5.83 ± 0.75^{e}	11.5 ± 0.91^{d}	19.17 ± 0.62^{a}	13.5 ± 0.62^{c}	17.5 ± 0.62^{b}
Chlorophyll *a* content (µg/g of soil)	0.49 ± 0.04^{c}	1.68 ± 0.07^{b}	2.13 ± 0.36^{ab}	2.2 ± 0.47^{a}	2.49 ± 0.14^{a}

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in a row or column indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture; CRB, Cooke Rose Bengal medium; NA, nutrient agar medium; AM, Ashbys mannitol medium; PVK, Pikovskaya medium.

by plants. Furthermore, phosphobacteria augment plant growth through the biosynthesis of growth-promoting substances like vitamin B_2 and auxins. The results of the present investigation are in confirmation with the findings in brinjal (Nanthakumar and Veeraraghavathatham 2000), in chili (Govindarajan and Thangaraju 2001), and in tomato (Kumaran et al. 1998; Reddy 1999). Nitrogen-fixing microbes are known to improve the growth and productivity of crops. *Azotobacter* species are free-living nitrogen-fixing bacteria and normally fix molecular nitrogen from the atmosphere without symbiotic relationship with plants, although some *Azotobacter* species are associated with plants (Kass et al. 1971). In addition, *Azotobacter* also synthesizes auxins, thereby stimulating plant growth (Ahmad et al. 2005; Rajaee et al. 2007). Significant increase in plant height, number of leaves, leaf area, leaf area index, and early flowering was observed upon application of BBRDM. This may be attributed to the increased uptake of nutrients in the plants leading to enhanced chlorophyll content and carbohydrate synthesis as well as increased activity of hormones produced by nitrogen-fixing and phosphate-solubilizing bacteria.

It is generally known that increasing the concentration of soil N inhibits the effect of nitrogen-fixing bacteria. In the present study, the number of azotobacteria increased in spite of increasing soil N content. Would nitrogen fixation be affected? In the study by Mikanová et al. (2009) in the Czech Republic, soils were sampled from the plots with four variants of fertilization: without fertilization, mineral fertilization, farmyard manure, and farmyard manure with mineral fertilization. Counts of *Azotobacter* spp. and potential nitrogenase activity were very closely related to the nitrogen content in soil. According to the authors, nitrogen fertilization in organic form (farmyard manure) increased the counts of *Azotobacter* spp. and subsequently also the potential nitrogenase activity. Inorganic (mineral) fertilization had no effect on the measured characteristics. Their results were in accordance with those published by Czakó et al. (2007), where the authors reported significantly increased counts of *Azotobacter* spp. in dump substrate upon the addition of 400 metric tons of compost per hectare during a recultivation study conducted in the North Bohemian coal basin, Czech Republic. It was concluded that soil conditions, especially a sufficient supply of organic matter that increases the supply of nitrogen to soil, are suitable for the development of *Azotobacter* spp. (Mikanová et al. 2009). Significant correlation between the number of *Azotobacter* spp. and the contents of organic C and total N in Polish soils indicated that soil fertility was an important factor influencing colonization of soils by *Azotobacter* (Martyniuk and Martyniuk 2003). There was a significant correlation between soil pH, total nitrogen, and organic carbon with *Azotobacter* population as reported by Mazinani et al. (2012) in their study on Iranian soils. The number of bacteria in unit gram of soil increased with increasing concentrations of C and N.

Table 5 Soil microbial abundance during brinjal cultivation

Number of microorganisms	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Fungi ($\times 10^3$/ml) in CRB	4.17 ± 0.62^{d}	8 ± 0.79^{c}	14.11 ± 0.72^{b}	18.28 ± 1.67^{a}	17.39 ± 1.61^{a}
Bacteria ($\times 10^7$/ml) in NA	11.45 ± 1^{e}	22 ± 1.35^{d}	99.61 ± 6.84^{c}	177.95 ± 8.29^{a}	118.67 ± 7.26^{b}
Azotobacter ($\times 10^7$/ml) in AM	4.33 ± 0.87^{d}	10 ± 1.38^{c}	15.39 ± 1.61^{b}	22.83 ± 3.42^{a}	20.06 ± 2.17^{a}
Phosphate solubilizers ($\times 10^7$/ml) in PVK	6.89 ± 0.93^{d}	22.67 ± 2.73^{c}	21.33 ± 1.25^{c}	34.89 ± 2.66^{b}	46.56 ± 1.8^{a}
Chlorophyll *a* content (µg/g of soil)	0.5 ± 0.03^{d}	1.93 ± 0.03^{b}	2.38 ± 0.03^{a}	1.63 ± 0.03^{c}	2.35 ± 0.04^{a}

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in the same row indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture; CRB, Cooke Rose Bengal medium; NA, nutrient agar medium; AM, Ashbys mannitol medium; PVK, Pikovskaya medium.

Table 6 Soil microbial abundance during chili cultivation

Number of microorganisms	Soil treatments				
	Soil	Soil + DAP	Soil + BBRDM (1:1)	Soil + BBRDM (2:1)	Soil + BBRDM (3:1)
Fungi (×10^3/ml) in CRB	9.28 ± 1.1^c	12.17 ± 0.81^b	17.56 ± 1.89^a	19 ± 1.96^a	17.56 ± 1.96^a
Bacteria (×10^7/ml) in NA	20.22 ± 1.69^c	59.39 ± 1.83^b	60.45 ± 2.4^b	85 ± 2.9^a	86.05 ± 2.23^a
Azotobacter (×10^7/ml) in AM	2.5 ± 0.62^e	6.11 ± 0.72^d	13.11 ± 0.72^c	17.72 ± 1.47^b	20.39 ± 1.36^a
Phosphate solubilizers (×10^7/ml) in PVK	7.06 ± 0.88^e	15.22 ± 1.19^d	21.33 ± 1.3^c	34.67 ± 1.83^b	44.45 ± 1.86^a
Chlorophyll *a* content (μg/g of soil)	0.39 ± 0.02^e	1.06 ± 0.02^d	1.76 ± 0.03^b	1.56 ± 0.04^c	3.14 ± 0.03^a

All plants were fertilized with 5.0 g BBRDM/kg of soil at the second and sixth weeks. Different superscript letters in the same row indicate significant differences between treatments ($P < 0.05$). DAP, diammonium phosphate; BBRDM, bovine blood and rumen digesta mixture; CRB, Cooke Rose Bengal medium; NA, nutrient agar medium; AM, Ashbys mannitol medium; PVK, Pikovskaya medium.

Although *Azotobacter*, in general, is a nitrogen fixer, the addition of nitrogen (as NO_3^- as well as NH_4^+) in the medium decreased the lag phase and generation time and thus increased the number of the bacteria (Vermani et al. 1997). Results of another investigation showed that the addition of NH_4Cl and $NaNO_3$ into the medium increased the growth of 1 wild and 13 mutant types of *Azotobacter vinelandii* (Iwahashi and Someyo 1992). Laane et al. (1980) presented evidence on the direct depressing effect of ammonium chloride on nitrogen fixation by *A. vinelandii* and attributed it to the inhibition of the electron transport system to nitrogenase. Abd-el-Malek et al. (1975), on the contrary, observed no relationship between NH_4^+-N concentration and densities of *Azotobacter* in the Nile valley soils of Egypt, indicating that the presence of NH_4^+-N had no effect on the multiplication of the organism. The absence or presence of NH_4^+-N did not affect the rate of sugar consumption by *Azotobacter* and corroborated the observation that NH_4^+-N did not affect their multiplication rate. The authors further showed that the addition of composted organic matter increased the efficiency of N_2fixation and prevented the competitive inhibition of ammonium salts. This could be attributed to the following factors: (a) soil organic matter possesses the property of cation exchange and its presence in a medium results in it adsorbing the NH_4^+-N, rendering them less available to *Azotobacter* than N_2, and (b) the presence of certain growth factors or trace elements which may increase the efficiency of fixation in addition to enabling the organism to overcome the unfavorable effect of ammonium salts on fixation.

In the light of these literature reports, the question of finding increased azotobacteria with increased N concentration as well as the putative relationship between soil nitrogen concentration and nitrogen fixation as apparent through the present study should be addressed as a separate detailed investigation. Further experiments on the determination of non-symbiotic nitrogen fixation by measurement of ethylene gas and nitrogenase activity are necessary to arrive at a definite conclusion.

Soil fungi perform vital functions within the soil in relation to nutrient cycling. It is now widely recognized that decomposition, mineralization, and plant-fungal mutualisms regulate the movement of soil-borne elements into the plant roots. Soil fungi are also responsible for disease containment and water dynamics; all of which help plants become healthy and robust (Christensen 1989). Soil cyanobacteria release secondary metabolites, which can be mineralized using the microflora and are thus beneficial to agricultural crops. These substances may be growth promoters and/or inhibitors for soil microflora. Cyanobacteria play an important role in soil chemical transformations and thus influence the bioavailability of major nutrients like P (Singh et al. 2011).

Organic fertilizers increase the numbers of beneficial microbes in the soil that contribute to soil fertility. For tomato cultivation, Tu et al. (2006) showed that microbial biomass and microbial activity were generally higher in organically managed soils, cotton gin trash being most effective, than conventional soil treatment with synthetic fertilizers. Similarly, cotton gin trash enhanced the populations of beneficial soil microbes during tomato cultivation as reported by Bulluck and Ristaino (2002). Microbial populations found at the rhizosphere, rhizoplane, and bulk soil due to the application of compost-based fertilizer, singly or in combination with urea, increased two- to five-fold relative to treatment with synthetic NPK fertilizer during cultivation of tomatoes (Taiwo et al. 2007).

Appearance, taste, and food quality

Normal color of the fruits was attained, as may be seen in Additional file 2: Figure S1a,b,c. The taste of the vegetables in terms of sourness, sweetness, and hotness as reported by five individuals was identical to normal tomato, brinjal, and chili. The composition of vegetables obtained from cultivation with BBRDM (3:1) (per 100 g): tomato-carbohydrate 3.89 ± 0.45 g, fat 0.19 ± 0.04, protein 1 ± 0.2 g per; brinjal-carbohydrate 5.5 ± 0.4 g, fat 0.18 ± 0.03 g, protein 0.82 ± 0.02 g; chili-carbohydrate 8 ± 0.3 g, fat 0.39 ± 0.02 g, protein 2.1 ± 0.1 g. The composition of vegetables obtained from cultivation with DAP (per 100 g): tomato-carbohydrate 3.9 ± 0.4 g, fat 0.2 ± 0.01 g, protein 0.9 ± 0.05 g; brinjal-carbohydrate 5.7 ± 0.6 g, fat 0.19 ± 0.01 g, protein 1.01 ± 0.02 g, and chili-carbohydrate 8.8 ± 0.4 g, fat 0.4 ± 0.01 g protein 1.9 ± 0.02 g. Thus, the vegetables grown with BBRDM and DAP were of comparable quality.

Conclusions

Highly polluting slaughterhouse could be gainfully utilized, which would promote the preservation of a healthy environment around rural slaughterhouses. Field investigations are underway, and preliminary results have shown similar trends as observed in this pot study.

Additional files

Additional file 1: Composition of microbiological media used in this study. Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria. It is useful because it remains solid even at relatively high temperatures. Also, bacteria grown in nutrient agar grow on the surface and are clearly visible as small colonies. Pikovskaya's agar medium is used for culturing phosphate-solubilizing microorganisms. Both inorganic and organic phosphates exist in soil. Many naturally occurring soil fungi and bacteria are phosphate solubilizers, and they play an important role in maintaining phosphorus balance of crop plants. Ashby's agar media are used for the isolation of *Azotobacter*, a non-symbiotic nitrogen-fixing bacteria which uses mannitol as a carbon source and atmospheric nitrogen as nitrogen source. Various essential ions required for promoting the growth of *Azotobacter* are also available in this medium. BG-11 broth is the universal medium for the culture and maintenance of cyanobacteria. Used for studies of the photosystems, as well as to identify the primary targets of the photoprocess. Cooke Rose Bengal agar is a selective medium. A variety of inhibitory agents have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora.

Additional file 2: Photographs of fruits cultivated with BBRDM. Tomato, brinjal, and chili plant belong to the nightshade family. Fruits grown with BBRDM were more in numbers than those with DAP. The appearances of the fruits were also better. Tomato fruit is rich in lycopene, one of the most powerful natural antioxidants, which may have beneficial health effects. Brinjal is an important food in India. Owing to its versatile nature and wide use in both everyday and festive Indian food, it is often described as the 'king of vegetables.' Chilies contain large amounts of vitamin C. Their very high vitamin C content can also substantially increase the uptake of non-heme iron from other ingredients in a meal, such as beans and grains.

Abbreviations

ADP: adenosine diphosphate; AM: Ashbys mannitol; ANOVA: analysis of variance; ATP: adenosine triphosphate; BBRDM: bovine-blood-rumen-digesta-mixture; BG-11: medium for blue green algae; BOD_5: 5-day biochemical oxygen demand; C: carbon; COD: chemical oxygen demand; CRB: Cooke Rose Bengal; DAP: diammonium phosphate; K: potassium; N: nitrogen; NA: nutrient agar; P: phosphorus; PVK: Pikovskaya; TKN: total Kjeldahl nitrogen; TP: total phosphorus; TS: total solids; TSS: total suspended solids.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MR, SK, and PKS did the experimental work and statistical analyses. AD and JM were involved in the conceptualization of the study, interpretation of results, drafting of the manuscript, and overall supervision. All authors read and approved the final manuscript.

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