



Effects of Vinasse and Press Mud Application on the Biological Properties of Soils and Productivity of Sugarcane

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Abstract The present study has been conducted to investigate the effects of vinasse and press mud (PM) (sugar industry by-products) applications on sugarcane soil microbial population, its enzyme activities and biomass carbon and nitrogen in order to work out its potential to be used as bio-friendly fertilizer. The treatments were compared with traditional chemical fertilizer (CF) applications. Vinasse and PM amended soils were found to have slightly lower pH and higher total N and C content, however, the difference was insignificant with CF amended soils. Both the treatments increased sugarcane yield compared to chemical fertilizer, but this increase was not significant in PM amended soils. The populations of fungi, bacteria and actinomycetes increased in vinasse and PM amended soils. This increase in former one was significant in PM amended soils only, and that of the latter in vinasse amended soil. The biomass C and N contents were also higher in both the treatments, however, only the former one had significant difference with CF. Amongst the soil enzymes, the

activities of cellulase, phosphatase and aminopeptidase were significantly higher in PM treatment while these were at par in vinasse and CF treatments. These results showed a potential possibility of substituting chemical fertilizers with vinasse and press mud which besides improving soil health and enhancing sugarcane productivity, can also solve the problem of their disposal in free environment.

Keywords Sugarcane soil · Vinasse · Press mud · Soil enzymes · Biomass C and N

Introduction

Since 1993, the Guangxi province is the top sugarcane and sugar producer of China and accounts for more than 65 % sugar production of nation (Li 2004). During the year 2009–2010, the sugarcane crop was cultivated on 103.14 million ha area and yielded 71.2 million tons of sugarcane. Besides producing sugar and alcohol, the sugarcane industries release a large amount of by-products, the disposal of which has become a great problem and threat to the environment. The sugar industry in Guangxi produces approximately more than 5 million tons of vinasse (a liquid by-product of alcohol industry) every year (Ou et al. 2002). During the past decades, the vinasse was discharged directly into the river which has caused serious water pollution and threat to the aquatic organisms. Since the year 2003, the researchers began to use the diluted concentrations of vinasse as liquid fertilizer in sugarcane fields. The results showed significant improvement in yield of sugarcane, *vis-à-vis*, soil's physico-chemical properties without any hardening symptoms (Li et al. 2007; Zhu et al. 2009). There have been some reports about the effects on soil properties including physical, chemical and microbial

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aspects (Su et al. 2008, 2009a, 2009b), however, more evidences are needed to illustrate the mechanisms of vinasse application on promotion of plant growth. Microflora and fauna are of immense importance to soils and any changes made to them would affect soils health as well as the yield of crops. In view of this, an experiment has been conducted to elucidate the effects of vinasse application in sugarcane fields on changes in bacterial and fungal composition of soil and activities of certain enzymes, and growth and yield of sugarcane.

Materials and Methods

Treatments

The experiment was conducted in the sugarcane fields of Qianjiang Farm (109°36'S, 23°36'E), Guangxi, China, the variety of sugarcane was used in "ROC 22", and consisted of three treatments. In the first treatment (T1), the suitably diluted (1:5; vinasse:water) vinasse @ (120 t ha⁻¹) was applied in furrows before planting sugarcane, in the month of February. In the second treatment (T2), press mud (obtained after cane juice filtration) was applied in the sugarcane fields @150,000 kg ha⁻¹. The third treatment (T3) included the application of N, P and K (in 8:5:7 ratios) fertilizer in the form of urea, P₂O₅ and K₂O of potash. Vinasse and press mud were supplied by the Qianjiang Sugar Mill Limited, Qianjiang, China. The soil samples from each treatment were collected at 6 months after treatment to analyze soil characteristics, number of bacteria and fungi, and the activities of some soil specific enzymes. Every sample was a mixture of soils taken from 5 to 10 locations from 0 to 30 cm depth. Each soil sample was sieved through stainless steel mesh (2 mm) and stored at °C in refrigerator for analysis.

Soil Analysis

The solution of air dried soil with distilled water (1:2.5) was shaken for 1 h and measured with a glass pH electrode. Total C and N were analyzed with air dried soil samples using NC-analyzer (Sumigraph NC-80 AUTO, Sumitomo Chemical Co., Ltd., Japan). The amount of available phosphate (Truog-P) was determined using the vanado-molybdate method after extraction with 0.001 mol L⁻¹ H₂SO₄ at a ratio of 1:200 (w/v) (Truog 1930).

Microbial Numbers

Microbial number was determined using the method of agar plate dilution amended with cycloheximide (100 µg L⁻¹), as described by Martin (1950). Rose bengal-streptomycin agar medium and starch casein medium were used to determine

the number of fungi and actinomycetes in fresh soil samples as described by Miyashita (1997). The pH of medium was adjusted to 6.8 with HCl or NaOH. The microbial, fungi and actinomycetes were counted in 5 replicates.

Microbial Biomass

The contents of soil microbial biomass N (MBN) and soil microbial biomass C (MBC) were determined using the chloroform fumigation-extraction method as described by Brookes et al. (1985) and Vance et al. (1987).

Activity of Soil Enzymes

Cellulase Activity

β-Glucosidase (EC.3.2.1.21) and exocellulase (EC.3.2.1.91) assays were based on *p*-nitrophenol (*p*NP) release after cleavage of a synthetic substrate (*p*-nitrophenyl-β-D-glucoside and *p*-nitrophenyl-β-D-cellobioside, respectively). The color of released *p*-NP was measured at 400 nm in a spectrophotometer (UV-1700, Shimadzu, Japan). A standard curve was plotted using 0–80 µg mL⁻¹ concentrations of *p*-NP. The enzyme activities were expressed as *n* moles *p*NP released per g dry soil per minute (*n* mol *p*NP g⁻¹ min⁻¹).

Phosphatase Activity

Phosphatase (Phosphodiesterase and phosphomonoesterase) activity in soils was estimated by measuring the amount of *p*-NP released after incubating the samples with *p*-nitrophenyl-phosphate (Alef et al. 1995). In a reaction tube, 0.25 mL toluene, 4.0 mL modified universal buffer (5× MUB; pH 6.0; made by dissolving 12.1 g tris, 11.6 g maleic acid, 14.0 g citric acid and 6.3 g boric acid in 500 mL 1 M NaOH and making the volume 1 L) and 1.0 mL *p*-nitrophenyl-phosphate (15 mM) were added to 1.0 g soil sample and the mixture was incubated at 37 °C for 1 h. The reaction was terminated by adding 1.0 mL of 0.5 M CaCl₂ and 4.0 mL of 0.5 M NaOH to the mixture prior to filtration. The absorbance of released *p*NP was taken at 400 nm in a spectrophotometer (UV-1700, Shimadzu, Japan) and the phosphatase activity was expressed in mg *p*-NP g⁻¹ h⁻¹.

Protease Activity

Aminopeptidase activity was measured by the method as described by Pansombat et al. (1997) using 0.002 M *N*-benzyl-oxycarbonyl glycyl L-phenylalanine (ZGP). The absorbance was measured in spectrophotometer at 570 nm wavelength. All the analyses were conducted in 5 replicates.

Table 1 Physico-chemical characteristics of soil and millable cane yield of sugarcane

Soil sample	Depth (cm)	pH (H ₂ O)	TC (%)	TN (%)	C/N Ratio	Av. P ₂ O ₅ (mg kg ⁻¹)	Millable cane yield (t ha ⁻¹)
Vinasse (T1)	0–30	5.51bB	1.29aA	0.123aA	10.5	15.9aA	109.4aA
Press mud (T2)	0–30	5.08cC	1.17bB	0.116aA	10.1	9.8bB	104.5aA
Chemical fertilizer (T3)	0–30	5.70aA	1.09bB	0.096bB	11.4	14.9aA	104.2aA

Different small and capital letters are showing significant difference at $P < 0.05$ and $P < 0.01$

Statistical Analyses

Statistical analyses were carried out using multiple range test at a 0.95 level of probability) to determine significant differences ($p \leq 0.05$) between the treatments.

Results and Discussion

Soil Properties and Cane Yield

Both the vinasse and press mud treatments significantly decreased the pH of soil, however, this decrease was more pronounced in press mud treatment. Generally, both of these by-products have significant amount of nutrients and organic matter, which is useful for soil health. Slightly acidic soils facilitate the availability of micronutrients to the plants and enhance the growth and development processes. Both the treatments also recorded higher C and N content in the soils compared to T3, however, no significant differences were observed between treatments T1 and T2. The soils treated with T1 and T2 recorded lower C/N ratios which indicate greater N availability and its solubilization in the treated soils. Vinasse treatment enhanced available P content of the soil, but it decreased in T2 compared to T3. The improvement in soil mineral and organic matter content and facilitated nutrient availability due to application of vinasse and press mud resulted in slightly higher cane yield in both treatments compared to T3, however, the increase in cane yield was not significant in the press mud treatment (Table 1). These results suggested a potential possibility of vinasse and press mud to be used as fertilizers which besides improving soil health and enhancing sugarcane productivity, can also solve the problem of their disposal in free environment.

Microbial Numbers

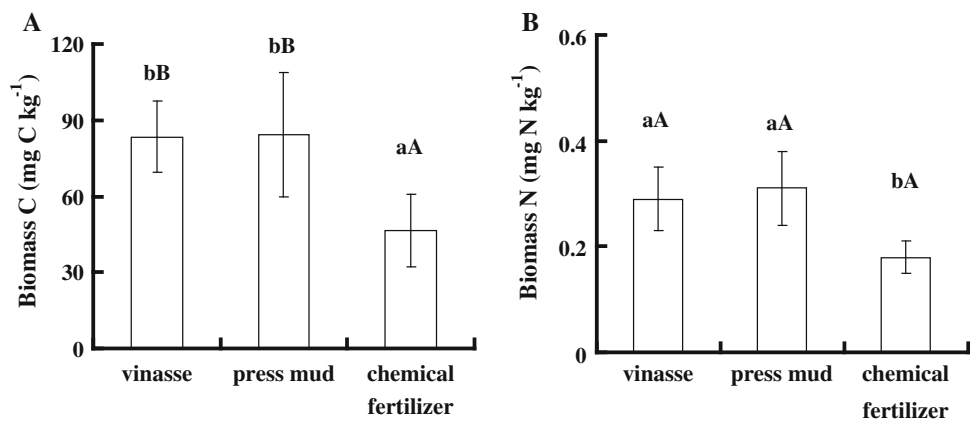
Fungi along with bacteria and actinomycetes play a vital role in the decomposition of organic matter in soil, thus releasing the nutrients locked up in the dead organic matter of plant, animal and microbial matter and bringing about the recycling of nutrients in nature. In soil, microbes oxidize organic carbon to CO₂ and liberate bound materials.

All the treatments showed similar trend of the bacterial and fungi population. The soils treated with press mud recorded maximum population of these two microorganisms which was significantly highest amongst all the treatments. Vinasse was not found to alter the bacterial or fungal population as it showed no significant difference with the soils treated with chemical fertilizer. On the other hand, actinomycete population differed significantly amongst all the treatments. Application of vinasse recorded the highest number of actinomycete followed by the press mud and chemical fertilizer treatments. The population of microorganism in soil was affected greatly by its organic matter content (Pansombat et al. 1996). The organic residues or substances of soil are first attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi. The presence of high number of actinomycetes in soils treated with vinasse might be due to its complex organic compounds.

Microbial Biomass

Soil microorganisms are the main participant in soil formulation and nutrient recycling (Spedding et al. 2004). Size and activity of microbial biomass are greatly stimulated by the addition of manure. Soil microbial biomass is the living component of soil organic matter (Zhang and Zhang 2003; Gil-Stotres et al. 2005). And it generally comprises 1–5 % of total organic matter content (Nsabimana et al. 2004). Microbial biomass measurements have been used to give an early indication of the changes in the organic matter content of a soil as a result of variation in soil management (Garcia-Gil et al. 2000; Hargreaves et al. 2003; Insam et al. 1989; De Varies et al. 2007; Zhang et al. 2004). Amongst all the treatments, application of press mud resulted in the highest microbial C and N, followed by the soils treated with vinasse and chemical fertilizer (Fig. 1). However, no significant difference was observed between the vinasse and press mud treatments. This might be due to the microbial activities during long time storage duration of vinasse and press mud. Higher total C and N contents in the soils with T1 and T2 treatments (Table 1) acted as an energy source for the autochthonous microorganisms (Perucci 1992) and significantly increased the microbial numbers (fungi, bacteria and

Fig. 1 Effect of the vinasse and press mud application on the microbial biomass C (a) and biomass N (b) contents in the soil. Bars represent the standard deviation from mean value. Different small and capital letters are showing significant difference at $P \leq 0.05$ and $P \leq 0.01$



actinomycetes) (Fig. 2). The results of the present study are in accordance with those of Leita et al. (1999) who reported that the soil microbial biomass, which represents approximately 1–4 % of total soil organic C, is a more sensitive indicator of changing soil conditions than direct analysis of the organic C content.

Soil Enzyme Activities

Cellulases Activity

β -Glucosidase and exocellulase (C_1 enzyme) activities play an important role in carbon metabolism in soils. Especially, the β -glucosidase, an enzyme involved in the carbon cycle, has been widely used in quality evaluation of soils subjected to different management procedures (Gil-Stotres et al. 2005). In the present study, the highest activity of β -glucosidase was found in press mud treated soils ($0.320\text{--}0.339 \mu\text{g } p\text{-NP g}^{-1} \text{ h}^{-1}$), followed by the chemical fertilizer treated

soils ($0.312\text{--}0.334 \mu\text{g } p\text{-NP g}^{-1} \text{ h}^{-1}$) (Fig. 3a). The soils treated with vinasse recorded the lowest activity. However, no significant difference was observed in exocellulase activity amongst different treatments. The difference between T1 and T2 was significant at 0.05 as well as 0.01 P level, however, T2 and T3 differed significantly at 0.05 P . Furthermore, contrary to the findings of Chang et al. (2008), significant negative correlations of β -glucosidase activity with biomass C ($r^2 = -0.32$) and biomass N ($r^2 = -0.20$) were observed (Table 2) which suggested higher activity of this enzyme was related with the decomposition of organic material (Dinesh et al. 2004), and difference in enzyme activity was due to different types of fertilizers (Chang et al. 2008). Besides this, positive correlations were also found between the enzyme activity and the number of fungi ($r^2 = 0.73$) and bacteria ($r^2 = 0.68$), suggesting their role in promotion on this enzyme. However, the β -glucosidase activity was found negatively correlated with the actinomycetes population.

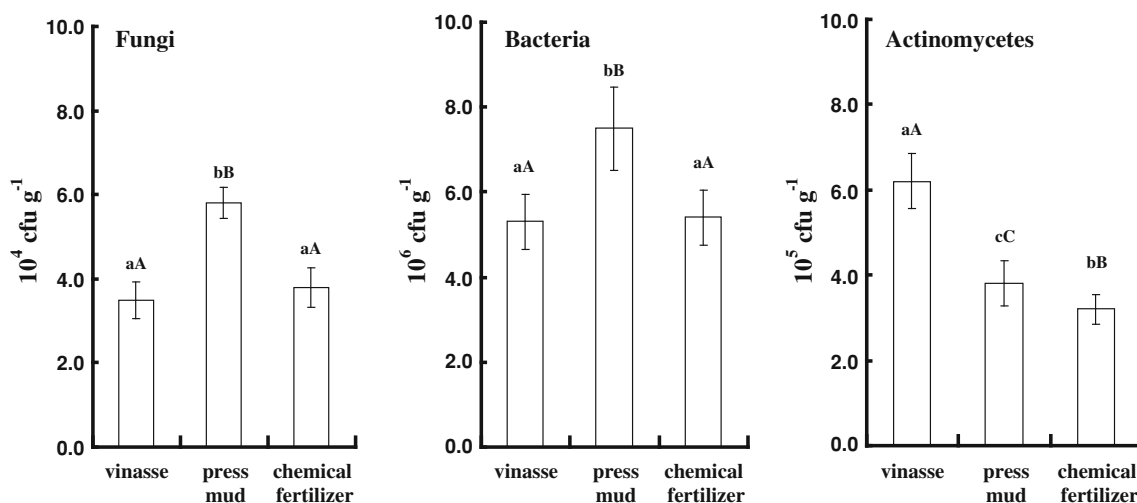


Fig. 2 The numbers of fungi, bacteria and actinomycetes in soil treated with vinasse, press mud and chemical fertilizer. Bars represent the standard deviation from mean value. Different small and capital letters are showing significant difference at $P < 0.05$ and $P < 0.01$

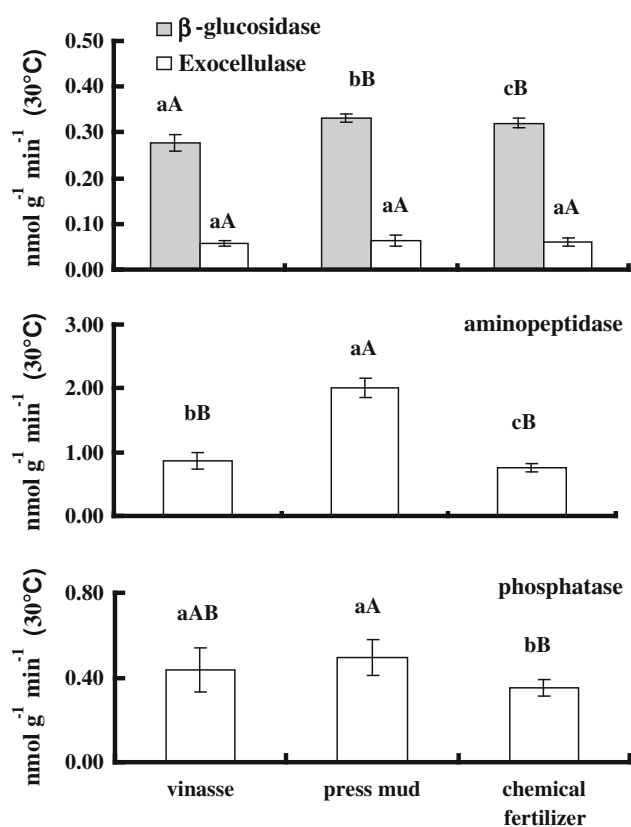


Fig. 3 Activities of cellulase, aminopeptidase and phosphatase in soils treated with vinasse, press mud and chemical fertilizers. Bars represent the standard deviation from mean value. Different small and capital letters are showing significant difference at $P \leq 0.05$ and $P \leq 0.01$

Protease Activity

Within organic farming in particular, increasing amounts of waste products from the food industry are being used in addition to manure to cover crop nitrogen (N) needs. These different organic fertilizers release N at different and often unknown rates. To achieve high N use efficiency with maximized yield return and minimized N loss to the environment, the availability of N from organic fertilizers must be synchronized with crop uptake. By documenting the course of mineralization under field conditions, it could be possible to estimate when as yet non-mineralized nitrogen becomes plant-available in relation to the time of fertilization. It needs to determine when to fertilize to get the maximum N utilization efficiency. The course of mineralization for a number of organic fertilizers has been studied

under controlled laboratory conditions (Griffin and Honeycutt 2000; Raupp 2005; Cordovil et al. 2007). In the present study, it was found that the different fertilizers application differently affected the soil aminopeptidase activity (Fig. 3b). Similar to phosphatase activity, the activity of aminopeptidase was also found the highest in press mud treated soils, followed by the vinasse treated soils. Loll and Bollag (1983) reported that soil amended with organic compounds, such as straw, increase the protease activity. Our results of total N contents (Table 1) in the soils are in accordance with the enzyme activity. The present study also revealed a significant correlation of biomass N content with the aminopeptidase activity. A strong positive correlation was also observed between the enzyme activity with bacterial and fungal populations (Table 2).

Phosphatase Activity

In soil, phosphorus exists mostly in organic and inorganic forms, both of which are important sources of P for plant and microbial uptake. The organic form exists mostly in humus and other organic materials. P availability is also controlled by environmental conditions such as soil organic matter moisture content and aeration which influence microbial activity and eventually microbial transformations of phosphorus. Soil phosphatase, the enzyme that transforms organic P to inorganic P is mostly of plant and microbial origin and consists of alkaline and acid types. The alkaline phosphatase is mostly of microbial origin. Loss of moisture likely to influence the activity of the microbial populations and in turn influences the phosphorus mineralization process. Phosphatases are important in soils because these extracellular enzymes catalyze the hydrolysis of organic phosphate esters to orthophosphate, thus they form an important link between biologically unavailable and mineral P (Kanazawa et al. 2005; Amador et al. 1997). Phosphatase activity is sensitive to soil pH, organic amendments and fertilizer additions etc. In the present study, the activity of phosphodiesterase enzyme was found the highest in soils treated with press mud followed by vinasse treatments. It varied from 0.360 to 0.574, 0.421–0.603 and 0.310–0.397 $\mu\text{g } p\text{-NP g}^{-1} \text{h}^{-1}$ in soils treated with vinasse, press mud and chemical fertilizer treatments, respectively (Fig. 3c). No significant variation was observed between the T1 and T2, however, both the treatments showed significant

Table 2 Linear correlation coefficients between selected biological properties and the enzymatic activities of the soils

	Biomass C	Biomass N	Fungi	Bacteria	Actinomycetes
β -Glucosidase	−0.316	−0.197	0.735*	0.678*	−0.930**
Exocellulase	−0.101	0.021	0.865**	0.822**	−0.830**
Phosphodiesterase	0.925**	0.964**	0.727*	0.780**	0.298
Aminopeptidase	0.579	0.675*	0.981**	0.994**	−0.257

* $P < 0.05$; ** $P < 0.01$

differences ($P \leq 0.05$) with the T3. The enhancement in enzyme activity in the T1 and T2 might be related with the decrease in soil pH (Table 1) (Dick et al. 2000).

Conclusions

The results of the present study clearly showed that the application of vinasse and press mud in sugarcane fields significantly improved the soil fertility status by enhancing soil C and N contents. Lower C/N ratios in the amended soils indicated higher N mineralization by microbial activities. Vinasse treatment enhanced available P content in the soil, however, it decreased in the press mud amended soils. High millable cane yield under vinasse amended conditions might be attributed to the higher micronutrient availability under slightly acidic conditions caused by vinasse application. In addition, enhancement in fungal and bacterial populations by application of press mud and actinomycetes population in vinasse treated soils suggesting their roles in decomposition of organic materials to release nutrients for plants growth and development. Furthermore, the higher biomass C and N contents in the soils treated with vinasse and press mud showed changes in soil organic matter content caused by microbial enzymatic activities. The present study revealed higher cellulase, phosphatase and protease activities in the vinasse and press mud treated soils which facilitated the process of carbon, phosphorus and nitrogen mineralization in the soils and improved the fertility status. These results suggested a potential possibility of vinasse and press mud to be used as fertilizers which besides improving soil health and enhancing sugarcane productivity, can also solve the problem of their disposal in free environment. Decomposition of soil organic matter, as well as the organic matter presented in vinasse and press mud, is a slow process. These organic fertilizers besides adding nutrients to the soil, sustained the microbial growth and a medium for enzymatic activities which might be more useful for enhancing the productivity of the ratoon crop, and will reduce the application of chemical fertilizers.

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