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The soil microbiological properties assessment due sugarcane vinasse application

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Abstract

The sugarcane vinasse is a residue from the sugar-ethanol industry which is high of organic matter and nutrient and it can effect on soil properties, crop growth, and production. The research aims to know on testing the effect of sugarcane vinasse on soil microbiological condition also to know dose range of sugarcane vinasse application to soil microbiological condition. The field experiment was conducted with five sugarcane vinasse doses are $0m^3.ha^{-1}$, $20m^3.ha^{-1}$, $40m^3.ha^{-1}$, $60m^3.ha^{-1}$, $80m^3.ha^{-1}$ and established in a Randomized Complete Block Design with five replications. Sugarcane vinasse contains high organic matter and a number of nutrients such as N, P, K and a slightly acidic pH. Application of sugarcane vinasse to the soil is the same when other liquid organic fertilizer applications. The presence of organic matter and nutrients in sugarcane vinasse makes the application of sugarcane vinasse can affect some soil microbiological properties. The obtained data revealed that the application of sugarcane vinasse had significant differences to C/N ratio soil, microbial biomass carbon, and total microorganism.

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Introduction

Sugarcane vinasse is a residue from the sugar-ethanol industry, characterized as being an acidic suspension, high of the Chemical Oxygen Demand (COD) and the Biological Oxygen Demand (BOD) values, unpleasant odors and dark brown color (Gomez and Rodriguez, 2000; Jiang *et al.*, 2012; Christofoletti *et al.*, 2013). Sugarcane vinasse has macronutrient such as C, N, P, K, Ca, Mg, S and micronutrient such as Cu, Mn, Fe, Zn and B (Gopal and Kammen, 2009; Tajeda, 2010). Organic components of sugarcane vinasse such as ethanol, acetic acid, glycerol, lactic acid (Dowd, 1994; Decloux and Bories, 2002), melanoidin, humic acid, phenolic and carboxylic acids (Fitz Gibbon, 1998; Mohamed A. F. *et al.*, 2015).

The amount of sugarcane vinasse produced depends on ethanol distillation process. Sugarcane vinasse production variations range from 7-20 liters of sugarcane vinasse per 1 liter of ethanol produced (Braunbeck & Cortez, 2000; Espana Gamboa *et al.*, 2011, Luis A.B.C., *et al.*, 2014). Vinasse had BOD level at 39.5gL^{-1} and COD level at 84.9 - 95.0 g L^{-1} (Eckenfelder, 2008; Espana Gamboa *et al.*, 2011) and pH at 4.46 - 4.80 (Espana Gamboa *et al.*, 2011).

The chemical analysis result in Indonesia on sugarcane vinasse showed organic matter level at 10.62%, N total at 2.56%, P total at 0.482% and K total at 4.82% (Bistok H.S, 2013). Based on the sugarcane vinasse characters, it can be used as organic matter and nutrient source for organic fertilizer. Vinasse, like other organic fertilizers, has high organic matter, N, and K contents (Madejon *et al.*, 2001).

In some areas, research has been done on sugarcane vinasse application to changes in soil properties, growth, and crop yields. However, generally, research on sugarcane vinasse applications on soil and plants is generally more focused on observations on soil physical and chemical properties and their effects on agricultural crops (Cunha *et al.*, 1987; Gemtos *et al.*, 1999; Armengol *et al.*, 2003; Jiang *et al.*, 2012). Meanwhile, research on sugarcane vinasse on soil biological properties especially on soil microbiology is still limited. Sugarcane vinasse with high organic matter could affect soil microbiological condition when applied on soil (Meng *et al.*, 2009; Ueno *et al.*, 2014).

Soil microbiology is studying soil microorganisms about their function and effects on soil. The soil microbiology study range is the overall living component of soil microorganisms and their activity (Handayanto & Hairiah, 2007), which can improve soil health and fertility (Saraswati *et al.*, 2007).

Soil is a complex system in which soil health and fertility depends on the biomass, metabolites, and activity of microorganisms (Michael Lehman R, *et al.*, 2015; Vivek D and Prafulla S, 2011). Soil microbiological could affect soil chemical attributes due directly or indirectly involvement in nutrients cycle. Soil microbiological also could affect soil physical attributes, such as aggregate stability, bulk density and soil total pore (Handayanto & Hairiah, 2007).

The application of sugarcane vinasse to soil was needed information for the range of sugarcane vinasse dose applications on soil microbiological. On the other hand, knowing the of sugarcane vinasse dose will be able to avoid the effects of sugarcane vinasse percolation into a deeper soil profile which will ultimately affect groundwater quality. Because if the application dose is more than optimum level, it could cause percolation and runoff, and worse even could cause nutrients poisoning on plant and anoxia to soil organism (Lahlah *et al.*, 2009). Therefore conducted research on the effects of sugarcane vinasse doses on soil microbiological. Based on the above mentioned, the purpose of this research is to know the sugarcane vinasse doses effect on the soil microbiology.

Material and methods

Location and description of experimental layout

The research was conducted on soybean cultivation in dryland system, soil classification of Latosol. Latosol is typically classified as Oxisols (USDA Soil Taxonomy). Research place in Kenteng Village, Susukan Sub-district, Semarang District, Central Java Province, Indonesia. Soil research characteristics can be seen in Table 1.

Table 1. Characteristics of soil research.

Soil property	Units	Values
Bulk density	$g.cm^{-3}$	1.17
Total pore	% volume	55.79
Permeability	$cm.hour^{-1}$	35.45
Soil texture	-	Silty-Clay (3.76% sand, 47.98 % silt, 53.26 % clay)
pH-H_2O	-	6.25
Cation exchange capacity (CEC)	$cmol.kg^{-1}$	8.10
Organic matter	%	8.34
N	%	0.10
P	ppm	1.83
K	ppm	1.07

The study laid out was used Randomized Complete Block Design (RCBD) with 5 doses of sugarcane vinasse treatment namely $0m^3$ ha^{-1}, $20m^3$ ha^{-1}, $40m^3$ ha^{-1}, $60m^3$ ha^{-1} and $80m^3$ ha^{-1}. Each treatment of sugarcane vinasse dose was repeated 5 times. Soybean varieties used are Grobogan varieties. Research activity begins with soil tillage and making a planting plot soybean with size 2 x $3m^2$ and then Rhizobium inoculation application. Application of sugarcane vinasse done 2 times ie first application of sugarcane vinasse was a half dose of the treatment given 1 week before the soybean seeds were planting and then the second application of sugarcane vinasse was a half dose from the remaining treatment given when 75% soybean was present in the flowering phase.

Data of observation and statistical analysis

The study observations were conducted on soil microbiological parameters are total organic carbon, total nitrogen, the ratio of C-N (C:N), microbial biomass carbon (C-mic), total microorganism and soil respiration were identified as indicators of soil quality in dry land management with sugarcane vinasse application. Measurement of total organic carbon by Walkley and Black titration method (Anderson and Ingram, 1993), total nitrogen based on a Kjeldahl oxidation method (Anderson and Ingram, 1993), microbial biomass carbon (C-mic) based on chloroform-fumigation-extraction method (Brookes *et al.*, 1985; Vance *et al.*, 1987; Anderson and Ingram, 1993), total microorganism by Pour Plate Count Method and soil respiration by Verstraete method (Anas Iswandi, 1989). The soil microbiological properties data were statistical analysis by Analysis of Variance test and then treatment means were compared using Honestly Significant Difference test of significance according to Steel & Torrie (1980) to know the significant differences between treatments sugarcane vinasse doses.

Result and discussion

Chemical characteristics of the sugarcane vinasse

Vinasse has a high content of organic matter, potassium, and calcium but moderate levels of nitrogen and phosphorus (Gomes and Rodríguez, 2000; Rajagopal Vadivel *et al.*, 2014). Based on sugarcane vinasse nutritional content (see Table 2), sugarcane vinasse has slightly acidic properties, high organic matter content, high nitrogen, high potassium, but phosphate has a lower content than nitrogen and potassium, so sugarcane vinasse applied to soil in soybean cultivation will be able to influence the soil microbiological properties, growth and yield of soybean crops.

Table 2. Characteristics of sugarcane vinasse.

Soil property	Units	Values
Organic matter	%	10.62
N	%	2.56
P_2O_5	%	0.48
K_2O	%	4.82
pH – H_2O	-	6.37
Electrical Conductivity	$\mu S\ m^{-1}$	173.4

Sugarcane vinasse effect on soil chemical and physical properties

Soil biological properties are interconnected with soil chemical and physical properties (Delgado Antonio and Jose A. Gomez, 2016). Information on soil chemical and soil physical properties is required to determine the soil biological processes. Sugarcane vinasse effects on soil chemical properties shown in the following Table 3.

Soil chemical properties observed in this study were pH-H_2O, electrical conductivity (EC), total nitrogen (N), available phosphorus (P) and available potassium (K)

Soil pH and EC could affect soil microorganism activity because microorganism needs favorable soil pH to grow and multiply. Soil EC increases could decline of soil microorganism activity. Soil EC had the negative correlation with soil microbial biomass carbon (Susilawati *et al.*, 2013). On the other hand, microorganism could affect soil pH, because in anaerobic condition some soil microorganism produces organic acids while in aerobic condition some could oxidate ammonia and sulfur which then release H+ and decrease soil pH. (Handayanto & Hairiah, 2007).

Table 3. The Effects sugarcane vinasse on soil chemical properties.

Sugarcane vinasse doses (m^3 ha^{-1})	pH-H_2O	Electrical conductivity (µS m^{-1})	Nitrogen total (%)	Available phosphorus (ppm)	Avaliable potassium (ppm)
0	6.28	190	0.09	1.83	1.07
20	6.22	278	0.16	1.86	1.07
40	6.28	300	0.19	1.95	1.15
60	6.30	340	0.22	1.88	1.27
80	6.24	436	0.22	1.99	1.23
Standard Deviation ±	0.033	89.851		0.066	0.091

Sugarcane vinasse has a high organic matter content (Bistok, 2013) will effect on soil physical properties. Neves *et al.* (1983) reported that the addition of vinasse combined with organic matter can improve soil physical properties and soil nutrients mobilization, as a result of higher solubility provided by vinasse liquids. Canellas *et al.* (2003) reported an increase level soil organic matter, thus improving the soil physical properties conditions as a result of vinasse applications throughout the years. The effects of sugarcane vinasse on soil physical properties shown in the following Table 4.

Table 4. The Effects sugarcane vinasse on soil physical properties.

Sugarcane vinasse doses (m^3 ha^{-1})	Water holding capacity (%)	Bulk density ($g.cm^{3-1}$)	Total pore (%)
0	48.30	1.14	57.23
20	46.05	1.15	56.76
40	49.33	1.10	58.95
60	48.85	1.09	59.15
80	49.73	1.09	59.28
Standard Deviation ±	1.445	0.029	1.185

The soil physical properties affecting soil microbiological are water holding capacity, bulk density, and total pore. The total pore could affect total microorganism and microbial biomass carbon due to its relation to soil aeration. Soil pore size and proportion also could affect soil microorganism colonization (Handayanto & Hairiah, 2007).

Sugarcane vinasse effect on the ratio carbon-nitrogen (C: N) soil

Carbon and nitrogen are main nutrition for plant growth and the ratio carbon to nitrogen (C: N) soil are used as indexes of soil quality assessment and indicator soil organic matter decomposition process (Zhao F., *et al.*, 2015; Shunfeng Ge *at al.,* 2013; Liu M. *et al.*, 2011; Sui YY. *et al.*, 2005). The carbon to nitrogen ratio (C: N) at soil organic matter (SOM) is the main quality indicator and it has been extensively used estimated available N release after SOM decomposition (Gervasio Pinero *et al*, 2006). The carbon to nitrogen ratio (C: N) soil is a sensitive indicator of soil quality (Shunfeng Ge *et al*, 2013).

Sugarcane vinasse contains organic matter and nitrogen so that it can be a source of soil organic matter and soil nitrogen. Sugarcane vinasse will decompose by microorganisms so that the organic matter and nitrogen material contained in the form of organic compounds will be mineralized or immobilized by soil microorganisms. The microorganisms in soil use carbon for energy and nitrogen for protein synthesis in the body to grow and develop.

The decomposition process of the sugarcane vinasse in soil can be seen from the value of ratio carbon to nitrogen (C: N) soil.

The ratio carbon to nitrogen (C: N) soil is considered a sign of process capacity in carbon and nitrogen organic decomposition or mineralization by the soil microorganism. The ratio carbon to nitrogen (C: N) soil, if it has a high value, shows the organic matter decomposition process is running slowly due to the limited ability of soil microorganism mineralization, while a low value the ratio carbon to nitrogen (C: N) soil shows rapid process of decomposition the soil organic matter by the soil microorganism. Soil microbial activity can be determining soil organic matter decomposition and the ratio carbon to nitrogen (C: N) soil (Jorg S., *et al.*, 2014; Richard L H., *et al.*, 2012).

Measurements on soil carbon organic and nitrogen content are done at 7 days before soybean crops harvest so that the sugarcane vinasse applied to the soil has advanced decomposition. Data in Table 5, showed sugarcane vinasse had significant differences on nitrogen and the ratio carbon to nitrogen (C: N) soil but had not significant on soil organic carbon because sugarcane vinasse has happened advanced decomposition in soil.

Table 5. The Effects of sugarcane vinasse on soil organic carbon, nitrogen and C/N ratio.

Sugarcane Vinasse doses (m^3 ha^{-1})	Soil carbon nrganic (%)		Nitrogen total (%)		C:N ratio	
0	3.40	a	0.09	a	35.87	c
20	3.06	a	0.16	b	20.56	b
40	2.69	a	0.19	c	14.96	ab
60	3.31	a	0.22	d	15.16	ab
80	2.81	a	0.22	d	12.65	a

Similar letters are not significantly different at the 0.05 level of probability according to Honestly Significant Difference (HSD) test.

The data show for various doses of sugarcane vinasse application gives significant difference on the ratio carbon to nitrogen (C: N) soils. The high value in ratio carbon to nitrogen (C: N) soil makes in the soil there is competition between plants and soil microorganisms in use nitrogen, so that in such conditions often occur nitrogen immobilization. Soil organic matter decomposition, ammonification, and nitrification would be changed the ratio of carbon to nitrogen (C: N) soil.

Table 3 shows that the higher dose of sugarcane vinasse applied will affect the lower ratio carbon to nitrogen (C: N) soil. The application of sugarcane vinasse could directly be increasing soil carbon and nitrogen, due carbon and nitrogen content on vinasse itself. But the increase of carbon level happened for only in brief time because carbon will be decomposed by the microorganism.

Influence of sugarcane vinasse on soil microbial biomass carbon (c-mic), total microorganism and soil respiration

Application sugarcane vinasse to optimize soybean production may effect in soil microbiological, thereby be affecting soil fertility. Soil microbiological parameters, notably microbial biomass carbon (C-mic), total microorganism and soil respiration were a sensitive indicator for the status and changes in the soil fertility levels.

Soil microbial biomass carbon (C-mic) is relation with soil organic matter decomposition and soil nutrient on nutrient cycling, turnover and transformation (Anita R. and Shang S.Y., 2015; DeLorenzo *et al.*, 2001; Jia Cheng Yang and Heribert Insam. 1991) so that soil microbial biomass carbon (C-mic) will play a role in ecosystem processes and pollutant degradation (Ananyeva, *et al*, 2008). Therefore, soil microbial biomass carbon can be considered as an important parameter for quantitative assessment of soil fertility.

Organic and inorganic fertilizer can significantly affect soil total microorganisms, microbial activities, populations, and communities are governed by environmental variables, such as soil type and texture, temperature, moisture, or pH, and management practices such as cropping, fertilization, and type of ecosystem also affect soil microbial activities (Raiesi and Asadi, 2006; Blagodasskii *et al*, 2008; Chu *et al*, 2007). The organic compound has a positive correlation with the microorganism population (Bhattarai *et al*, 2015), so that sugarcane vinasse contains organic matter and nutrients will be able to affect the total soil microorganisms.

The results of research by Leita *et al.*, (1999) and Cerny *et al.*, (2008) show that organic matter addition will significantly increase in total organic carbon and soil microbial biomass nitrogen and the carbon content in the soil in response to the increasing amounts of organic carbon added.

Soil respiration is a measure of carbon dioxide (CO2) released from soil microorganism metabolic activity, respiration of plant roots and soil fauna (Anas, 1989). The amount of soil respiration is an indicator of soil fertility primarily about a number of nutrients contained in the organic matter (Popelarova, *et al* 2007). The ability of soil microorganisms to perform their metabolic activities were determined the presence of materials added to the soil such as fertilizers, pesticides or other materials. One indicator of soil microorganisms metabolism activity is soil respiration. Application of sugarcane vinasse will affect the content of soil nutrients and soil organic matter that will affect the activity of soil microorganisms metabolism.

Data showed that sugarcane vinasse doses had significant differences to microbial biomass carbon, total microorganism but had not significant to soil respiration. The effects of sugarcane vinasse on microbial biomass, total microorganism and soil respiration shown in the following Table 6.

Table 6. The effects of sugarcane vinasse on soil microbial biomass carbon (C-mic), total microorganism and soil respiration.

Sugarcane vinasse doses (m^3 ha^{-1})	Soil microbial biomass carbon (C-mic) (μg g^{-1} soil)		Total microorganism (10^7)		Soil respiration (mg CO_2.kg^{-1}.$soil^{-1}$.day^{-1})	
0	845.02	b	6.62	b	42.47	a
20	762.18	ab	4.62	b	41.07	a
40	731.67	ab	4.58	b	38.54	a
60	680.56	a	2.04	a	37.66	a
80	660.86	a	1.97	a	36.46	a

Similar letters are not significantly different at the 0.05 level of probability according to Honestly Significant Difference (HSD) test.

Table 6 could be seen that the higher doses of sugarcane vinasse resulted in lower microbial biomass carbon level. According to Susilawati *et al.* (2013), the decreasing of soil microbial biomass carbon occurred for high Soil Electrical Conductivity (EC) level (Tabel 3.).

EC level had negative influence to microbial biomass carbon (Sarig and Steinberger, 1994). In this study, the higher doses of sugarcane vinasse made the higher level on soil EC level. Susilawati *et al.* (2013) stated that there is a positive correlation between total organic carbon with microbial biomass carbon, likewise this research. Even though the application of sugarcane vinasse decreasing the microbial biomass carbon level, it doesn't reach the critical threshold. Martens (1995) pointed out that microbial biomass carbon level at agricultural land was between 200-1000μg g^{-1} soil. Microbial biomass carbon at every dose of sugarcane vinasse was between those ranges.

Soil electrical conductivity (Table 3.) significantly had a negative exponential relationship to microbial biomass carbon (Susilawati *et al.*, 2013; Sarig and Steinberger, 1994), thus indirectly could negatively affect soil total microorganism. Soil total microorganism could also be affected by soil carbon organic (Table 5.), for carbon is the energy source to the microorganism. Therefore, the higher soil total organic carbon causing higher soil total microorganism, and vice versa. Data showed that sugarcane vinasse had a significant difference to soil total microorganism. The treatment without sugarcane vinasse resulted in the highest soil total microorganism, while vinasse application on 80m^3 ha^{-1} resulted in the lowest soil total microorganism. Even so, the whole doses of sugarcane vinasse could be maintained keep soil total microorganism within 10^7 level. However, application of doses sugarcane vinasse of 60m^3 ha^{-1} could significantly be decreasing the soil total microorganism.

Soil respiration is indicating the activity level of soil microorganism respiration rate, where it is conditioning of describing metabolic activity rather than amount or type of microorganism in soil. Data showed no significant differences between treatments of sugarcane vinasse on soil microorganism respiration. This could be occurred because of soil total microorganism was at a similar level at 10^7 (Table 6). Soil respiration had a positive relation to microbial biomass carbon and soil total microorganism (Table 6).

Conclusion

Sugarcane vinasse contains high organic matter and a number of nutrients such as N, P, K and a slightly acidic pH. Application of sugarcane vinasse to the soil is the same when other liquid organic fertilizer applications. The presence of organic matter and nutrients in sugarcane vinasse makes the application of sugarcane vinasse can affect some soil microbiological properties. Sugarcane vinasse application has significant differences to total nitrogen, C/N ratio, microbial biomass, and total microorganism.

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