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Original Research Article

Soil Quality Assessment of Bantay Clay Loam as Affected by Different Nipa Bioethanol Vinasse Concentrations

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Abstract: This study investigates the effects of nipa bioethanol vinasse, a distillery by-product of nipa sap on the chemical and microbiological properties of Bantay Clay Loam soil in the City of Batac, Ilocos Norte, Philippines. A pot experiment was conducted using Completely Randomized Design (CRD) with five treatments: 0%, 5%, 10%, 15%, and 20% vinasse concentrations. Each treatment was replicated three times to evaluate its influence on key soil parameters, including pH, organic matter, total nitrogen, available phosphorus, exchangeable potassium, and bacterial density. Preliminary analyses revealed that the vinasse contained low levels of nitrogen and phosphorus but moderate amounts of potassium, with a strongly acidic pH. Results showed no significant changes in soil pH, organic matter, nitrogen, or phosphorus levels following vinasse application. However, a significant increase in exchangeable potassium was observed at higher vinasse concentrations. Additionally, no inhibitory effects on bacterial colony growth were detected, suggesting that vinasse is microbiologically safe and may even support microbial activity in the soil. The study concludes that while nipa bioethanol vinasse may not significantly enhance all macronutrients, it serves as a promising organic source of potassium and poses no adverse effects on soil microbial populations. These findings contribute to the growing interest in sustainable waste-to-resource practices and support the integration of agro-industrial by-products into organic soil fertility management strategies. Further studies are recommended to assess its long-term effects, optimal application methods, and potential synergies with other organic or inorganic inputs.

Keywords: Bioethanol, Nipa Vinasse, Soil Ammendments, Concentration, Microbial Property.

INTRODUCTION

Various industrial and agricultural productions and processes produce other by-products aside from the goal product. One of such is the large-scale production of wastewater from the ethanol industry which is an unavoidable consequence of the modern world. These by-products were once considered waste and were frequently disposed of, causing a wide variety of environmental issues to arise due to its high nitrogen and phosphorus contents and high concentration of carbon and other nutrients. With the aim for more sustainable cities and communities and climate action as included in the '17 Sustainable Development Goals' of the United Nations, it has become widely accepted that by-products like this should be utilized and the ways and technology to reuse them should be studied and developed. In cases where organic matter concentration of the waste material is high, the agriculture sector provides a potential solution to these issues by employing the material as an alternative for chemical fertilizers to improve soil chemical, biological, and physical characteristics (Dotaniya, *et al.,* 2016)

The bioethanol production, which now became a great trend as a source of renewable energy, largely contributes to the production of this wastewater known as vinasse. The production of ethanol from sugar crops, starch crops, and/or cellulosic material, generates on average, 10–15 L of vinasse for each liter of ethanol produced, depending on the distillery equipment (Cortez *et al*., 1992). Vinasse was considered highly toxic to animals, plants, microbes, and microflora from freshwater. In addition, it disturbes marine animals that came to the coast to reproduce, and has a high pollution potential, approximately 100 times more than household

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sewage, due to the high organic matter content, causing a depletion of oxygen and high levels of biochemical demand for oxygen (BOD) (Kannan and Upreti, 2008). However, vinasse use as fertilizer became usual in sugarcane refineries since the beginning of the 1980s. When applied in nature to the soil, in controlled and small quantities to avoid the damaging effect, sugarcane vinasse helps fertilize the sugarcane crop, lowering the costs of chemical fertilizers (Laime *et al*., 2011).

The use of biofuels in the Philippines was initiated due to the oil crisis of the 1970s. However, the implementation did not immediately push through as the domestic cost of production of biofuels was higher than the cost of importing oil. Hence, to reduce the biofuels production cost and correspondingly its selling price, the Philippine Department of Agriculture (DA) is tasked to develop a sustainable and viable feedstock for the production of biofuels. For each feedstock assessed based on its availability and accessibility, a suitable and economically competitive conversion technology is applied, usually developed through the research programs of the Philippine Council for Industry, Energy and Emerging Technology Research and Development (PCIEERD) of the Department of Science and Technology (DOST) (Intech Europe, 2021).

The National Bioenergy, Research and Innovation Center (NBERIC), a directorate of Mariano Marcos State University (MMSU) is one of the largest producer of high-quality bioethanol from nipa sap in northern Philippines. Just like other production facilities, it also struggles with the disposal management of the wastewater produced during the distillation of nipahol. Thus, certain interventions and innovations should be made to determine possible utilization for the waste product.

Statement of the Problem

Vinasses has a high level of toxicity and has a great potential as pollutant however, when utilized effectively, can be a valuable input in the production of various crops. Majority of vinasses contains significant amounts of soil needed macro nutrients, which with appropriate concentrations, can contribute to the improvement of soil fertility. Therefore, its application can increase the productivity of any agricultural production by enhancing the chemical, physical, and biological soil attributes (Velásquez-Riaño *et al.*, 2019).

Objectives

Generally, this study was conducted to evaluate the potential utilization of the nipa bioethanol vinasse (effluent) as soil amendment and its impact on the different soil characteristics of Bantay Clay Loam under the City of Batac, Ilocos Norte condition.

Specifically, it aimed to:

1. Identify the chemical composition of nipa bioethanol vinasse;
2. Determine the microbial density of vinasse;
3. Evaluate the effect of the different nipa bioethanol vinasse concentration in soil's chemical properties (organic matter (OM), pH, total nitrogen, available phosphorus, and exchangeable potassium);
4. Determine the relationship of vinasse concentrations on soil chemical properties of Bantay Clay Loam; and
5. Assess the effect of the different nipa bioethanol vinasse concentrations on the soil's microbiological property (bacterial plate count);

Conceptual Framework

The various concentrations of nipa bioethanol vinasse applied to Bantay Clay Loam serve as the independent of this study (see Figure 1). These treatments are thought to act as soil amendments that can affect a variety of soil quality indicators in addition to being nutrient sources. The study's dependent variables are the soil's chemical and microbiological characteristics, such as pH, electrical conductivity (EC), organic matter content, available macro- and micronutrients (like calcium, magnesium, sulfur, phosphorus, potassium, and nitrogen), microbial population density, and microbial enzymatic activity. When assessing the general fertility and health of the soil, these indicators are essential.

Byproduct of the production of bioethanol, nipa bioethanol vinasse is rich in organic matter, soluble salts, and nutrients that are vital to plants. Vinasse can improve the physicochemical and biological dynamics of soil by acting as a soil conditioner when added in different concentrations. It enhances microbial activity, improves nutrient cycling, and increases the amount of organic matter—all of which are critical for sustainable crop production. The chemical makeup of vinasse—which consists of organic carbon, nitrogen, potassium, and several trace elements—plays a major part in changing the microbial ecology and nutrient balance of the soil. Both direct nutrient supplementation and indirect effects, such as modifications in the structure of microbial communities, enzymatic activities, and rates of organic matter decomposition, are expected to cause these changes.

As a result, using nipa bioethanol vinasse is not only a way to enrich nutrients but also a possible tactic for sustainable agriculture and soil restoration. Vinasse is used as an organic input in agricultural soil management, especially in regions where Bantay Clay Loam predominates. The study aims to ascertain how each concentration level differently affects soil chemistry and biology.

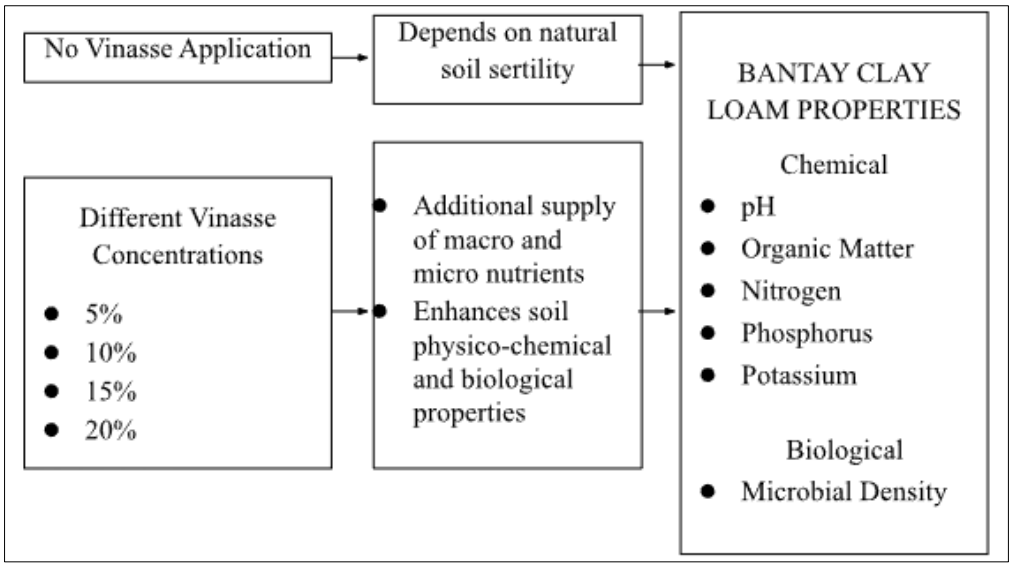

Figure 1: Conceptual Framework of the Study

MATERIALS AND METHODS

Research Design

The study involves a pot experiment laid-out in a Completely Randomized Design (CRD) with five treatments and 3 replications. The study have a total of fifteen experimental pots. Figure 2 shows the schematic lay-out of the experiment.

Figure 2: Lay-out of the study

Variables of the Study

The independent variables of the study are different concentrations of nipa bioethanol vinasse and the dependent variables are the chemical and microbiological properties of the soil. The different concentrations of nipa bioethanol vinasse used are as follows:

T1- No Application (0ml)
T2- 5% Vinasse Concentration (15ml)
T3- 10% Vinasse Concentration (30ml)
T4- 15% Vinasse Concentration (45ml)
T5- 20% Vinasse Concentration (55ml)

Soil Sample Collection and Analysis

The chemical and microbiological properties of the soil specimen used was determined prior to the conduct of the study. Soil samples were collected from the homogenized soil sample used in the study. A kilogram of the composite soil were sent to the Department of Agriculture-Regional Field Office I, Regional Soils Laboratory at Sta. Barbara, Pangasinan, Philippines for routine chemical analysis. Results were recorded for future reference. Another kilogram was used for bacterial plate count at the Molecular and Microbiological Laboratory of the College of Arts and Sciences (CAS), MMSU, City of Batac, Ilocos Norte, Philippines. Results and other observations were recorded.

Vinasse Chemical and Microbiological Analysis

Adequate samples of the nipa bioethanol vinasse used in the experiment were also examined before the conduct of the study. A liter of the distillery effluent was sent to the Department of Agriculture-

Regional Field Office I, Regional Soils Laboratory at Sta. Barbara, Pangasinan, Philippines for routine chemical analysis.

Another 10 ml of the sample undergone bacterial plate count at the Molecular and Microbiological Laboratory of the College of Arts and Sciences (CAS), MMSU, City of Batac, Ilocos Norte, Philippines.

Soil Water Field Capacity Calculation

The soil's field capacity was first calculated to determine the amount of vinasse concentration to be applied for each experimental pot. Ten kilograms of composite soil sample were placed in 3 experimental pot (the pots were perforated below) and weighed. The pots were watered to saturation and covered with sack and placed in a dark area to avoid evaporation. After 48 hours, the pots were weighed again. The field capacity was calculated using the given formula.

Field Capacity = P_2 -P_1.

Whereas P_2=saturated soil weight after 2 days and P_1-oven dried soil weight.

Treatment Formulation

After determining the volume of solution needed (vinasse with water), the nipa bioethanol vinasse was sourced out from the National Bioenergy, Research and Innovation Center (NBERIC). The material was diluted to water with concentrations depending on the treatments. One time drenching was done as application method of the solution. This was calculated using the given formula:

$C_1V_1=C_2V_2$

Where C_1=concentration of the starting solution, V_1= volume of the starting solution, C_2=concentration of the final solution, and $V_{2=}$ volume of the final solution.

The treatments, vinasse concentrations, and the resulting final pH obtained through the potentiometric method was presented in Table 1.

Table 1: Treatments, vinasse concentrations, formulations, and the final pH obtained through the potentiometric method of the solution used in the study

TREATMENT	VINASSE CONCENTRATION	FORMULATION	pH
Treatment 1	0% Diluted Concentration	3L Water	7. 32
Treatment 2	5% Diluted Concentration	15 ml Vinasse + 2.85 L Water	7.29
Treatment 3	10% Diluted Concentration	30 ml Vinasse + 2.70 L Water	7.27
Treatment 4	15% Diluted Concentration	45 ml Vinasse + 2.55 L Water	7.24
Treatment 5	20% Diluted Concentration	55 ml Vinasse + 2.45 L Water	7.21

Water Management

The pH of irrigation water used in the study was also examined using the potentiometric method accordingly. The water pH reading was 7.32 which falls under the neutral pH rating. The experimental pots were irrigated with approximately 100 ml of water every three days and maintained under field capacity.

Data Gathering Procedures

Vinasse Analysis and Evaluation

The nipa bioethanol vinasse sample was sent to the Regional Soils Laboratory of Department of Agriculture-Region 1 for chemical quality assessment using the Potentiometric Method for its pH; the Kjeldhal Method for its total N; Vanadomolybdate method for its available P; and the Flame Atomic Emission Spectroscopy method for its exchangeable K. On the other hand, the microbial quality of the vinasse sample submitted to the Molecular and Microbiological Laboratory of CAS was examined using the bacterial plate count.

Soil Chemical Analysis and Evaluation

A kilogram of composite soil sample used prior and after the conduct of the study were also submitted to the Regional Soils Laboratory of Department of Agriculture-Region 1 for a series of analyses namely Potentiometric Method for soil pH; Walkey and Black Spectrophotometer method for organic matter; Olsen/Bray 1 Method available phosphorus; and Ammonium Acetate Extraction-Atomic Absorption/Emission Spectroscopy Method for exchangeable potassium. Post-conduct samples were collected from the 30 days after application (30 DAA) and 60 days after application (60 DAA).

Soil Microbiological Analysis

A gram of composite soil sample were collected from each experimental pot to determine the density of bacteria present. Sample were placed in a sterilized container and brought to the Molecular and Microbiological Laboratory of the College of Arts and Sciences (CAS), MMSU, City of Batac, Ilocos Norte, Philippines for inoculation and isolation of soil bacteria.

Sterilization of Materials

Prior to the conduct of the microbial analysis, all the tools and materials were first sterilized using the prescribed oven heated at 100 degrees Celsius for an hour. Furthermore, the water used was sterilized in a pressure cooker at 15 pounds per square inch (psi) for 15-20 minutes and cooled. A 9 ml solution was poured in each test tube and was used for serial dilution. All test tubes were plugged using cotton balls and were sterilized again using the same method.

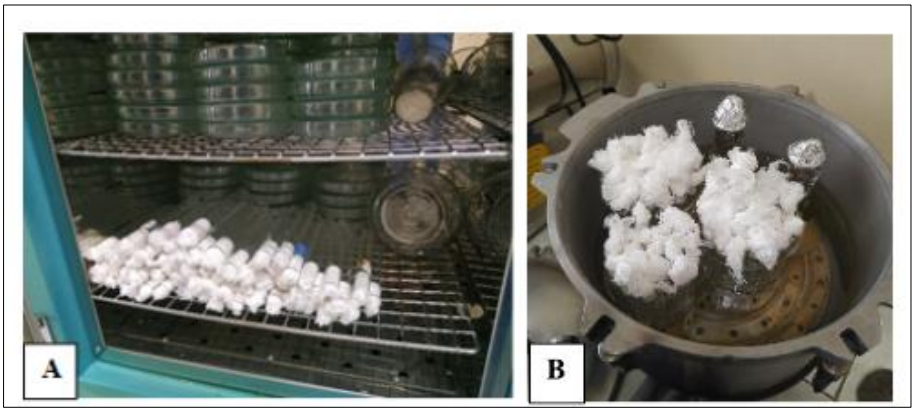

Figure 4: Materials sterilized using oven (A) and pressure cooker (B)

Preparation of the Culture Media

A 13g of nutrient agar was dissolved in a 1 liter distilled water. To allow complete dissolution, the solution was heated to moderate flame temperature. This was sterilized using pressure cooker at 15 psi for 15-20 minutes. Afterwards, the solution was poured on the petri dishes for isolation.

Figure 5: Pouring of culture media to be used in bacterial isolation

Serial Dilution of Samples

Seven test tubes were used for each sample. Each test tube was filled with a 9 ml previously prepared distilled water. A 1 ml of the sample was diluted in a test tube which served as the first dilution and placed in a rotary shaker for 15 minutes at 150 RPM to facilitate mixing. From the first dilution, 1 ml aliquot was obtained and diluted in another test tube. The procedure was repeated until the last test tube. The 5th test tube was placed in a vortex mixer to facilitate mixing. The aseptic technique was strictly employed during the dilution.

Figure 6: Transferring of soil sample (A) and serial dilution (B)

Plating of Samples

One ml was obtained in each test tube from dilution 5. A pipette was used to pour dilutions on to the petri dishes containing solidified culture media. The plates were incubated at 32°C in an inverted manner for 48 hours.

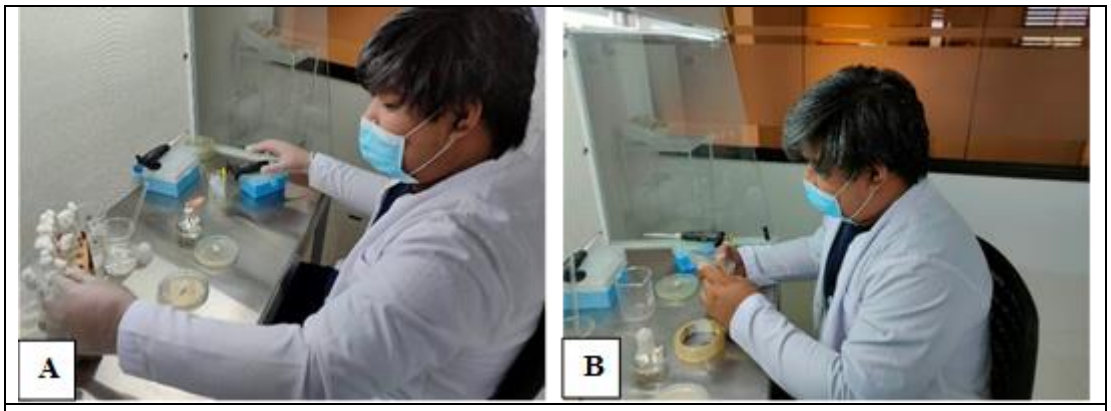

Figure 7: Bacterial isolation (A) and sealing of the petri dish (B)

Bacterial Colony Count

The density of soil bacteria was determined before and during the scheduled data gathering days following the microbial procedure for bacteria. The number of colonies formed were determined by colony counting.

Data Analysis

Data analysis was carried out using the one-way ANOVA in CRD using the Statistical Tool for Agricultural Research (STAR 2.0.1) software to detect significant effect of the different vinasse concentrations on the different soil properties. Treatment means was further compared using the Least Significant Difference (LSD) test, the relationship between the dependent and the independent variables was assessed using correlation analysis.

RESULTS AND DISCUSSIONS

Preliminary Analyses and Evaluations

Nipa Bioethanol Vinasse Chemical and Microbiological Quality

The summary of the collected data from the routine chemical analysis of the fertilizer material is shown in Table 2. Results show that the vinasse material has a very low pH and only contains a minimal amount of macronutrients. As of the writing, there's no published literature regarding the chemical component of the said fertilizer material.

Table 2: Chemical contents of nipa bioethanol vinasse used in the study

ROUTINARY PARAMETER	READING/CONTENT
pH	3.46
Total Nitrogen (%)	0.04
Available Phosphorus (ppm)	BDL
Exchangeable Potassium (cmol/kg)	0.31
*BDL-Below Detection Limit	

Microbial analysis of the vinasse used proves the presence and survival of microorganisms in the material. The results obtained from the analysis is presented in the Table 3. Unfortunately, there are also no published research or study evaluating the microbial load of the nipa bioethanol vinasse.

Table 3: Bacterial colony count of the soil sample used in the study

COUNT	LEVEL
14*	Low
*At dilution #5	

Preliminary Soil Chemical and Microbiological Analysis and Evaluation

The routine analysis done prior to the conduct of the study shows the initial characteristics of the soil specimen. Table 4 provides the summary of the findings. The chemical analysis results are similar to the definition of the Philippine Rice Research Institute (2015) of the different chemical characteristics and properties of the Bantay Clay Loam. Unfortunately, there is no existing literature about the microbial load of the aforementioned soil series under its oven-dry weight state.

Table 4: Soil chemical analysis results of the composite soil sample used in the study

ROUTINE PARAMETER	READING/CONTENT	REMARKS
Chemical Properties		
pH	7.03	Neutral
Organic Matter (%)	0.79	Very Low
Total Nitrogen (%)	0.04	Very Low
Available Phosphorus (ppm)	5.51	Low
Exchangeable Potassium (cmol/kg)	0.65	Medium
Microbiological Properties		
Bacterial Colony Count	24*	Low
*At dilution #5		

Evaluation and Interpretation of Experimental Findings

Soil pH

The soil negative logarithm of hydrogen ion concentration (pH) was measured through the Potentiometric Method. Table 5 summarizes the results of the analysis. Means from the different time frame reflects a quantitative increase in soil pH. However, there is no qualitative differences observed in terms of its effect between the 30 DAA and 60 DAA. Furthermore, analysis of variance reveals that there are no significant differences on the effect of the different concentrations of nipa bioethanol vinasse used in the experiment. All treatment has resulted to neutral pH regardless of the time of observation.

Ippolito and Bauder (2022) states that changing soil's pH is dynamic. They further point out that raising soil pH is relatively easy but lowering soil pH is difficult. Though the soil media was applied with a strongly acidic nipa bioethanol vinasse, it might not be enough to change the overall soil pH considering its frequency of application and the presence of other cultural and natural factors such as irrigation, parent material, and mineralization rate which can also alter the soil pH (USDA, 2023).

Table 5: Soil pH readings of the different treatments on the 30 days after application (30 DAA) and 60 days after application (60 DAA) and their corresponding pH ratings

TREATMENT	30 DAA	REMARKS	60 DAA	REMARKS
	ns		ns	
0% Concentration	7.05	Neutral	7.12	Neutral
5% Concentration	7.02	Neutral	7.13	Neutral
10% Concentration	7.01	Neutral	7.13	Neutral
15% Concentration	7.05	Neutral	7.14	Neutral
20% Concentration	7.03	Neutral	7.11	Neutral
Mean	**7.03**	**Neutral**	**7.12**	**Neutral**
CV(%)	**0.55**		**0.36**	
ns-Not significant				
CV-Coefficient of variation				

Soil Organic Matter

The soil organic matter was measured through the Walkey and Black Spectrophotometric Method. The result of the statistical analysis provides that there are no significant differences on the effect of the different concentrations of nipa bioethanol vinasse both on 30 DAA and 60 DAA. All the results still fall under the 'Low" nutrient rating for soil organic matter. Table 6 summarizes the data gathered after the analysis. The results agrees with Bot and Benites (2005) who indicates that it is difficult to increase soil organic matter content as it requires sustained effort which includes application of high-residue crops and other sources frequently. Chemical analysis of the vinasse used reflects a very low content of organic matter present in the material. Given such, the routine analysis will only have a small chance of detecting a true effect of the material (Bhandari, 2021) to the experimental soil.

Table 6: Soil organic matter (OM) readings on the 30 days after application (30 DAA) and 60 days after application (60 DAA) and their corresponding nutrient ratings

TREATMENT	30 DAA	REMARKS	60 DAA	REMARKS
	ns		**ns**	
0% Concentration	1.10	Low	1.29	Low
5% Concentration	1.11	Low	1.22	Low
10% Concentration	1.11	Low	1.26	Low
15% Concentration	1.23	Low	1.25	Low
20% Concentration	1.28	Low	1.19	Low

Mean	**1.16**	**Low**	**1.24**	**Low**
CV(%)	**7.49**		**11.17**	
ns-Not significant				
CV-Coefficient of variation				

Soil Total Nitrogen

There is a direct relationship between carbon and nitrogen in soils as the availability of organic matter in the soil dictates directly the total nitrogen present (Wibowo and Kasno, 2021). Most OM averages about 5% nitrogen so that the N:C ratio is 5:58 or 1:11,6, however, soils will vary from 1:8 to 1:12 in their N:C ratio, with most values falling between 1:10 to 1:12 (VELP Scientifica, 2019). Therefore, by multiplying the %OM by 0.05, an approximate value for the % nitrogen in soils was obtained.

The analysis of variance indicated that there is no significant differences between the different concentrations of nipa bioethanol vinasse. The nutrient index rating for the nutrient further implies that all of the results from the treatment fall under the "Very Low" rating. All of the data obtained are presented in table 7 with their corresponding nutrient rating.

Table 7: Soil total nitrogen readings of the different treatments on the 30 days after application (30 DAA) and 60 days after application (60 DAA) and their corresponding nutrient ratings

TREATMENT	30 DAA	REMARKS	60 DAA	REMARKS
	Ns		**ns**	
0% Concentration	0.055	Very Low	0.065	Very Low
5% Concentration	0.055	Very Low	0.061	Very Low
10% Concentration	0.055	Very Low	0.063	Very Low
15% Concentration	0.061	Very Low	0.063	Very Low
20% Concentration	0.064	Very Low	0.060	Very Low
Mean	**0.06**	**Very Low**	**0.06**	**Very Low**
CV(%)	**9.84**		**10.90**	
ns-Not significant				
CV-Coefficient of variation				

Soil Available Phosphorus

The soil phosphorus was measured using the Olsen/Bray 1 Method. Analysis of variance did not reveal any significant differences from the results obtained from the 30 DAA. However, the 20% concentration on the 60 DAA provides a significantly higher quantitative increase in soil phosphorus compared to other treatments. This agrees with the results of the study conducted by Montero-Arellano *et al.* (2022) which also shows a very minimal to undectable increase on the soil phosphorus after applying a low phosphorus containing sugarcane vinasse on sugarcane fields. The summary of the results and interpretation is presented in Table 8.

Table 8: Soil available phosphorus readings on the 30 days after application (30 DAA) and 60 days after application (60 DAA) and their corresponding nutrient ratings

TREATMENT	30 DAA	REMARKS	60 DAA		REMARKS
	ns		*		
0% Concentration	4.70	Very Low	5.24 b		Low
5% Concentration	4.42	Very Low	5.33 b		Low
10% Concentration	4.56	Very Low	5.39 b		Low
15% Concentration	4.77	Very Low	5.49 ab		Low
20% Concentration	4.82	Very Low	5.77 a		Low
Mean	**4.65**	**Very Low**			**Low**
CV(%)	**4.07**		**3.29**		
ns-Not significant					
*-Significant at 5% level of significance					
CV-Coefficient of variation					

Soil Exchangeable Potassium

The soil potassium was measured through the Ammonium Acetate Extraction-Atomic Absorption/Emission Spectroscopy Method. Table 9 summarizes all of the results gathered after the analysis. Analysis of variance shows a positive correlation between the concentration and the quantitative amount of potassium added to the soil. Furthermore, it implies that the higher the concentration the higher the amount of potassium being added to the soil.

Same results were obtained by Jiang *et al.,* (2012); Ronaldo *et al.,* (2017); and Yin *et al.,* (2018) after testing and analyzing soils and crop fields treated with potassium containing sugarcane vinasse.

Table 9: Soil exchangeable potassium readings of the different treatments on the 30 days after application (30 DAA) and 60 days after application (60 DAA) and their corresponding nutrient ratings

TREATMENT	30 DAA	REMARKS	60 DAA	REMARKS
	*		*	
0% Concentration	0.63 e	Medium	0.58 e	Medium
5% Concentration	0.67 d	Medium	0.63 d	Medium
10% Concentration	0.71 c	Medium	0.67 c	Medium
15% Concentration	0.73 b	Medium	0.71 b	Medium
20% Concentration	0.77 a	Medium	0.76 a	Medium
Mean	**0.70**	**Medium**	**0.67**	**Medium**
CV(%)	**2.35**		**2.69**	
*-Significant at 5% level of significance				
CV-Coefficient of variation *Means with the same letter are not significantly different at 5% level of significance				

Soil Bacterial Density

The soil bacterial density was measured using the Plate Count Method, which remains a widely accepted and reliable approach for quantifying bacterial populations in soil samples. Results from the study revealed that there was no inhibition of soil bacterial growth, regardless of the amount of nipa bioethanol vinasse applied or the observation period. This finding is significant as it demonstrates that the application of nipa bioethanol vinasse does not negatively impact the microbial ecosystem in the soil, even when used in varying concentrations over time. Such outcomes indicate the compatibility of nipa bioethanol vinasse with maintaining healthy soil microbiota, an essential factor for sustainable agricultural practices.

Table 10 provides a detailed summary of the bacterial density measurements taken at 30 days after application (DAA) and 60 DAA, emphasizing that microbial activity remains robust across different treatment levels. These observations align with existing literature, further supporting the compatibility of vinasse with soil microbial dynamics. For instance, a study conducted by Miyamoto *et al*., (2013) found that soil treated with pure vinasse exhibited a higher microbial load compared to soils treated with sterilized vinasse and deionized water. This suggests that vinasse, as an organic by-product, may even enhance microbial proliferation due to its nutrient-rich composition.

Similarly, Eduardo *et al*., (2017) observed consistent results when evaluating the microbiological properties of soils irrigated with sugarcane vinasse. Their research indicated that vinasse not only supports but potentially enriches microbial populations, promoting a balanced soil ecosystem. These findings further reinforce the idea that vinasse, whether derived from sugarcane or nipa bioethanol production, does not inhibit soil bacterial growth but instead can serve as a beneficial organic amendment.

The results of this study highlight the potential of nipa bioethanol vinasse as an environmentally friendly input in agricultural systems. Its application supports soil microbial health, which is crucial for nutrient cycling, organic matter decomposition, and overall soil fertility. This aligns with the broader goal of sustainable agriculture by integrating organic by-products like vinasse into nutrient management practices without compromising the ecological balance of soil microbial communities.

The soil bacterial density was measured through the Plate Count Method. Results shows that there are no inhibition of soil bacterial growth regardless of the amount of nipa bioethanol vinasse applied and the time of observation. This also proves that the application of nipa bioethanol vinasse won't prevent the growth of bacteria in soil when applied in different concentrations. Table 10 summarizes the data from 30 DAA and 60 DAA and their corresponding measurements of bacterial growth. In a study conducted by Miyamoto *et al.* (2013), a higher microbial load was even observed on soils applied with pure vinasse rather than those applied with sterilized vinasse and deionized water. Eduardo *et al.,* (2017) also came with the same observation after assessing the different microbiological properties of soil irrigated with sugarcane vinasse.

Table 10: Bacterial density in soil as affected by the different concentrations of vinasse observed on the 30 days after application (30 DAA) and 60 days after application (60 DAA)

TREATMENT	30 DAA	REMARKS	60 DAA	REMARKS
0% Concentration	>500	TNTC	>500	TNTC
5% Concentration	>500	TNTC	>500	TNTC
10% Concentration	>500	TNTC	>500	TNTC
15% Concentration	>500	TNTC	>500	TNTC
20% Concentration	>500	TNTC	>500	TNTC
*TNTC-To Nocturnal To Count (Very High)				

Nipa Bioethanol Vinasse Chemical and Microbiological Quality

The collected data from the routine chemical analysis of the fertilizer material is shown in Table 2. Results show that the vinasse material has a very low pH and only contains a minimal amount of macronutrients. As of the writing, there's no published literature regarding the chemical component of the said fertilizer material.

Microbial analysis of the vinasse used proves the presence and survival of microorganisms in the material. The results obtained from the analysis is presented in the Table 3. Unfortunately, there are also no published research or study evaluating the microbial load of the nipa bioethanol vinasse.

Soil Chemical and Microbiological Analysis and Evaluation

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Evaluation and Interpretation of Experimental Findings

Soil pH

The soil negative logarithm of hydrogen ion concentration (pH) was measured through the Potentiometric Method. Table 5 summarizes the results of the analysis. Means from the different time frame reflects a quantitative increase in soil pH. However, there is no qualitative differences observed in terms of its effect between the 30 DAA and 60 DAA. Furthermore, analysis of variance reveals that there are no significant differences on the effect of the different concentrations of nipa bioethanol vinasse used in the experiment. All treatment has resulted to neutral pH regardless of the time of observation.

Ippolito and Bauder (2022) states that changing soil's pH is dynamic. They further point out that raising soil pH is relatively easy but lowering soil pH is difficult. Though the soil media was applied with a strongly acidic nipa bioethanol vinasse, it might not be enough to change the overall soil pH considering its frequency of application and the presence of other cultural and natural factors such as irrigation, parent material, and mineralization rate which can also alter the soil pH (USDA, 2023).

Soil Organic Matter

The soil organic matter was measured through the Walkey and Black Spectrophotometric Method. The result of the statistical analysis provides that there are no significant differences on the effect of the different concentrations of nipa bioethanol vinasse both on 30 DAA and 60 DAA. All the results still fall under the 'Low" nutrient rating for soil organic matter. Table 6 summarizes the data gathered after the analysis. The results agrees with Bot and Benites (2005) who indicates that it is difficult to increase soil organic matter content as it requires sustained effort which includes application of high-residue crops and other sources frequently. Chemical analysis of the vinasse used reflects a very low content of organic matter present in the material. Given such, the routine analysis will only have a small chance of detecting a true effect of the material (Bhandari, 2021) to the experimental soil.

Soil Total Nitrogen

There is a direct relationship between carbon and nitrogen in soils as the availability of organic matter in the soil dictates directly the total nitrogen present (Wibowo and Kasno, 2021). Most OM averages about 5% nitrogen so that the N:C ratio is 5:58 or 1:11,6, however, soils will vary from 1:8 to 1:12 in their N:C ratio, with most values falling between 1:10 to 1:12 (VELP Scientifica, 2019). Therefore, by multiplying the %OM by 0.05, an approximate value for the % nitrogen in soils was obtained.

The analysis of variance indicated that there is no significant differences between the different concentrations of nipa bioethanol vinasse. The nutrient index rating for the nutrient further implies that all of the results from the treatment fall under the "Very Low" rating. All of the data obtained are presented in table 7 with their corresponding nutrient rating.

Soil Available Phosphorus

The soil phosphorus was measured using the Olsen/Bray 1 Method. Analysis of variance did not reveal any significant differences from the results

obtained from the 30 DAA. However, the 20% concentration on the 60 DAA provides a significantly higher quantitative increase in soil phosphorus compared to other treatments. This agrees with the results of the study conducted by Montero-Arellano *et al*., (2022) which also shows a very minimal to undectable increase on the soil phosphorus after applying a low phosphorus containing sugarcane vinasse on sugarcane fields. The summary of the results and interpretation is presented in Table 8 Soil.

Exchangeable Potassium

The soil potassium was measured through the Ammonium Acetate Extraction-Atomic Absorption/Emission Spectroscopy Method. Table 9 summarizes all of the results gathered after the analysis. Analysis of variance shows a positive correlation between the concentration and the quantitative amount of potassium added to the soil. Furthermore, it implies that the higher the concentration the higher the amount of potassium being added to the soil. Same results were obtained by Jiang *et al*., (2012); Ronaldo *et al*., (2017); and Yin *et al*., (2018) after testing and analyzing soils and crop fields treated with potassium containing sugarcane vinasse.

Soil Bacterial Density

The soil bacterial density was measured using the Plate Count Method, which remains a widely accepted and reliable approach for quantifying bacterial populations in soil samples. Results from the study revealed that there was no inhibition of soil bacterial growth, regardless of the amount of nipa bioethanol vinasse applied or the observation period. This finding is significant as it demonstrates that the application of nipa bioethanol vinasse does not negatively impact the microbial ecosystem in the soil, even when used in varying concentrations over time. Such outcomes indicate the compatibility of nipa bioethanol vinasse with maintaining healthy soil microbiota, an essential factor for sustainable agricultural practices. Table 10 provides a detailed summary of the bacterial density measurements taken at 30 days after application (DAA) and 60 DAA, emphasizing that microbial activity remains robust across different treatment levels. These observations align with existing literature, further supporting the compatibility of vinasse with soil microbial dynamics. For instance, a study conducted by Miyamoto *et al*., (2013) found that soil treated with pure vinasse exhibited a higher microbial load compared to soils treated with sterilized vinasse and deionized water. This suggests that vinasse, as an organic by-product, may even enhance microbial proliferation due to its nutrient-rich composition.

Similarly, Eduardo *et al*., (2017) observed consistent results when evaluating the microbiological properties of soils irrigated with sugarcane vinasse. Their research indicated that vinasse not only supports but potentially enriches microbial populations, promoting a balanced soil ecosystem. These findings further reinforce the idea that vinasse, whether derived from sugarcane or nipa bioethanol production, does not inhibit soil bacterial growth but instead can serve as a beneficial organic amendment.

The results of this study highlight the potential of nipa bioethanol vinasse as an environmentally friendly input in agricultural systems. Its application supports soil microbial health, which is crucial for nutrient cycling, organic matter decomposition, and overall soil fertility. This aligns with the broader goal of sustainable agriculture by integrating organic by-products like vinasse into nutrient management practices without compromising the ecological balance of soil microbial communities.

The soil bacterial density was measured through the Plate Count Method. Results shows that there are no inhibition of soil bacterial growth regardless of the amount of nipa bioethanol vinasse applied and the time of observation. This also proves that the application of nipa bioethanol vinasse won't prevent the growth of bacteria in soil when applied in different concentrations. Table 10 summarizes the data from 30 DAA and 60 DAA and their corresponding measurements of bacterial growth. In a study conducted by Miyamoto *et al*., (2013), a higher microbial load was even observed on soils applied with pure vinasse rather than those applied with sterilized vinasse and deionized water. Eduardo *et al*., (2017) also came with the same observation after assessing the different microbiological properties of soil irrigated with sugarcane vinasse.

CONCLUSION

This study assessed the potential of nipa bioethanol vinasse as a soil amendment by evaluating its effects on the chemical and microbiological properties of Bantay Clay Loam. The results revealed that while vinasse application had no significant impact on soil pH, organic matter, total nitrogen, or phosphorus levels, it significantly enhanced the soil's potassium content. Moreover, vinasse application did not inhibit soil microbial growth, suggesting that it is microbiologically safe for use as a soil input.

These findings support the potential of nipa bioethanol vinasse as an organic supplement for improving soil potassium levels in crop production systems. However, its limited impact on other macronutrients highlights the need for its integration with other soil amendments to achieve balanced nutrient availability. The study reinforces the idea that sustainable waste management strategies can transform agro-industrial by-products into valuable agricultural resources, promoting circular economy practices in farming communities.

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