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Research Article
Effect of Fresh-mixed Palm Oil Mill Effluent after Short-term Aging and Impact Assessment on Selected Soil Enzymes

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Abstract

Background and Objectives: The fresh palm oil mill effluent (POME) can be harmful to the soil if discharged untreated to the environment. The organic load and elemental content help to improving soil fertility and enzymes functionality. This study evaluated the impact of different regimes of POME on soil enzymes. **Materials and Methods:** The POME was mixed with chicken-droppings at varying quantities. The unmixed POME was determined after 4 weeks of aerobic aging. Soil samples were contaminated with fresh and aged POME and left for 24 h. The uncontaminated soil sample was used as a control. **Results:** The nitrogen, phosphorous and potassium in the mixed aged POME-soil samples were high compared to the control. Dehydrogenase and urease activities increased in all the soil samples over time. The mixed POME-soil sample had the highest activities (57.22±0.96, 61.40±1.20 and 68.76±0.83) for sample B5 and 1.79±0.01, 2.74±0.01, 2.84±0.06, respectively for samples B7 at week 4. The catalase activity indicates a reduction in the mixed samples of the control. **Conclusion:** The study concluded that mixing POME with poultry droppings increased soil fertility. Whereas, mixing fresh POME with poultry droppings allow for complete oxidation of the phenols in the fresh POME. Thus a combination of the fresh and aged POME may constitute a heavy pollutant to the soil, but the addition of chicken droppings greatly reduced POME impact on the soil enzymes.

Key words: POME-pollution, soil-improvement, soil-enzymes-activation, agro-waste, catalase activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The POME is an enduring left-over fluid (the liquid left after degumming) after recovery of oil[1,2]. New POME is a dense chocolate-like colloidal concoction of water, oil and fine suspended solids. It is hot (80-90°C) and holds high biological oxygen demand, with an[2,3] acidic pH of 4-5. Owing to the bulk of water used during oil processing, it has been projected that a milling plant with a capacity of 10 t of fresh fruit per hour could need water handling plant that is comparable to a population of million occupants[4].

The impact which POME may have on the soil enzymes and microflora may differ owing to their concentration and density that restrict the free circulation of oxygen[5]. However, minor application of this POME may instigate substantial upsurge in whole heterotrophic, phosphate solubilizing, nitrifying and lipolytic microbial biomass, whereas, a substantial application may produce a reduction in enzymatic performance[6]. The most affected could be the nitrifying bacteria followed by phosphate solubilizing bacteria, while the heterotrophic bacteria which usually release urease, lipase, dehydrogenase, phenol-oxidase, alkaline and acid phosphatase may struggle to increase their activities in the soil that is lightly impacted. The affected enzyme-alkaline phosphatase and dehydrogenase may persist in reciprocal reaction to boost bacterial flora and enzymatic activities[7]. Modest use of POME in the soil may increase nitrifying bacterial biomass and elevates dehydrogenase performance, acid phosphatase and oxidase activities in the contaminated soil.

Oil palm trees (*Elaeis guineensis*) are vastly cultivated as a source of oil in many parts of Nigeria. It has an extended lifespan of about 200 years. Crude palm oil is derived from the fleshy mesocarp of the fruits, whose oil contains fatty acid and esters of glycerol-triglycerides. The red pigment of the oil is because of fat-soluble carotenoids, vitamin E (tocopherols and α-tocopherols) content[8]. Oil palm mill industries have been recognized for economic growth and development of a nation. On the other hand, it has also contributed to environmental pollution, due to the generation of a high quantity of by-products during the extraction of oil. During the extraction of crude palm oil, about 50% of the water is waste as palm oil mill effluent (POME). In a previous study it was reported that the discharged of untreated POME can be detrimental to the soil ecosystem[9]. There was a report about the potential benefits of using pre-treated POME in crop production[1,10,11]. This study was aimed at evaluating the impact of fresh POME after short-term aging in combination with poultry droppings on soil fertility.

MATERIALS AND METHODS

Experimental design: Aging of POME was carried out in an open bucket. The fresh POME was added into 6 different buckets and labelled sample one to sample 6 (S1-S6) as follows:

S1 : 10 L of POME (unstirred, control1)
S2 : 12 L of POME (stirred)
S3 : 14 L of POME (stirred)
S4 : 14 L of POME+200 g of poultry droppings (stirred)
S5 : 14 L of POME+400 g of poultry droppings (stirred)
S6 : 14 L of POME+600 g of poultry droppings (stirred)

The POME was allowed to age for 4 weeks and stirred for 20 min at every 12 h. Sample one (S1) which served as a control for the aging of POME was not stirred.

A known weight (6 kg) of the soil was added into 7 perforations buckets and were labelled sample B1-B7. They were contaminated with 1 L of POME (labelled as S1, S2, S4, S5 and S6) thus:

B1 : Soil (control 2)
B2 : Soil+1 L of fresh POME
B3 : Soil+1 L of S1 (Aged POME and unstirred)
B4 : Soil+1 L of S2 (Aged POME and stirred)
B5 : Soil+1 L of S4 (Aged POME+200 g poultry droppings)
B6 : Soil+1 L of S5 (Aged POME+400 g poultry droppings)
B7 : Soil+1 L of S6 (Aged POME+600 g poultry droppings)

Collection, preparation and application of effluents: Fresh POME was collected from Iheaku, Udenu Local Government Area of Enugu state. The chicken droppings were collected from Odim area, University of Nigeria, Nsukka. The soil samples were collected at the depth-15 cm at the Department of Soil Science, University of Nigeria, Nsukka. The soil was sieved and air-dried for three days before analysis.

Location, duration and total time of experiment: The location of this investigation was at Iheaku in Udenu Local Government Area of Enugu State, Nigeria. The study was carried out in April, 2019 and lasted for 4 weeks with 4 h/ day.

Chemicals and equipment: The chemicals used for this study were of analytical grade.

Determination of soil total nitrogen: A 5 g of the soil sample was placed in a round bottom flask containing 10 mL of distilled water and left to stand for an hour. Thereafter,

20 g of catalyst digestion mixture and 10 mL of concentrated H_2SO_4 were added into the mixture and refluxed for 90 min. The agitated mixture was introduced into a 250 mL volumetric flask and made up to the 100 mL mark using distilled water. From the prepared volume, 20 mL was collected into a distillation flask, 20 mL of NaOH and pieces of zinc were added (anti-bump) into the mixture and gently swirled. A 50 mL of the sample from 250 mL volumetric flask was placed in a conical flask and added with 10 mL of boric acid (4%) and 2 drops of mixed indicator. The conical flask was placed below the condenser to let the tip of the condenser dip into the solution. After condensation, 150 mL of the condensate was collected and the flask removed to evade sucking back. The mixed indicator in the condensate turned blue owing to the dissolution of ammonia. The condensate was titrated against HCl (0.1 N) until a color change to light brown (original color of indicator) was observed[12]. Total nitrogen was then calculated using the equation as follows:

$$N\ (\%) = \frac{V_S - V_B \times N_{acid} \times 0.01401}{W} \times 100$$

Where:

V_S = Volume of titrant (HCl) for sample
V_B = Volume of titrant (HCl) for blank
N_{acid} = Normality of acid (0.1N)
W = Weight of the soil sample (g)

Determination of phosphates in soil: Phosphate was determined by following the method described[12]. About 6 g of soil was leached in a tight-covered bottle with 30 mL Bray and Kurtz mixture (0.03 N $NH_{4+}F$+0.1 N HCl), it was shaken for 1 min and filtered using Whatman No. 42 filter paper. A 2 mL of aliquot was taken from the filtrate into a 15 mL test tube and added with 6 mL of distilled water, 1 mL 2.5% NH_4^+ Molybdate-which was prepared by dissolving 2.5 g NH_4^+ Molybdate in 100 mL 15 N H_2SO_4 and 1 mL 2% fresh ascorbic acid. The mixture was shaken to give 10 mL solution. The test tube was immersed in a boiling water bath until a blue colour develops. The blue-mixture was permitted to cool and absorbance was taken at 882 nm with a spectrophotometer.

Determination of potassium concentration in the soil sample: Potassium content in soils was determined with flame photometer according to Jackson[13]. A 5 g of the soil sample was leached at pH 7 with ammonium acetate buffer to obtain 100 mL. An aliquot of the solution was taken into the flame photometer for quantification of potassium.

Determination of urease activity: The assay for urease activity was determined by measuring 5 g of air-dried soil sample and mixed with 5 mL of toluene, 20 mL of distilled water and 10 mL of 10% urea solution and incubation at 37°C for 24 h. Thereafter, the soil suspension was centrifuged at 4000 rpm for 5 min and 1 mL aliquot was treated with 4 mL of sodium phenol solution (containing 100 mL of 6.6 M phenol solution and 100 mL of 6.8 M NaOH) and 3 mL of 0.9% sodium hypochlorite solution. The ammonium released into the solution was quantified colorimetrically at 578 nm on a spectrophotometer. Urease activity was expressed as μg NH_4^+-N g^{-1} soil h^{-1}.

Determination of phosphatase activity: Phosphatase activity assay was determined with 5 g of air-dried soil which was mixed with 5 drops of toluene, 10 mL of disodium phenyl phosphate solution and 10 mL of distilled water. The suspension was incubated for 24 h, at 37°C and centrifuged at 4000 rpm for 5 min. The supernatant was colored with 0.25 ammonia-ammonium chloride buffer, at pH 9.6, 0.5 mL of 2% 4-amino antipyrine and 0.5 mL of 8% potassium ferrocyanide. The phenol content was determined colorimetrically at 510 nm in a spectrophotometer. Phosphatase activity was expressed as μg phenol g^{-1} soil h^{-1}.

Determination of catalase activity: Catalase activities were assayed with 2 g of the air-dried soil sample, mixed with 40 mL of distilled water and 5 mL of 0.3% H_2O_2. The soil slurry was shaken for 20 min at 150 rpm. The remaining peroxide was stabilized by adding 5 mL of 1.5 M sulfuric acid and the solution was centrifuged at 4000 rpm for 5 min. The peroxide in the supernatant was titrated with 0.05 M $KMnO_4$. Catalase activity was expressed as mL $KMnO_4$ g^{-1} soil h^{-1}.

Determination of dehydrogenase activity: Dehydrogenase activity was examined by using 3 g of air-dried soil, mixed with 3% triphenyltetrazolium chloride solution-substrate and 1.25-1.75 mL of distilled water. The soil slurry was mixed and incubated at 37°C for 24 h. Thereafter, triphenylformazan was extracted with methanol and quantified with a colorimetric device at 485 nm. Dehydrogenase activity was expressed as μg TPF g^{-1} soil h^{-1} and all enzyme activities were calculated as the mean of 2 replicates.

Statistical analysis: The data were analyzed using statistical package for social sciences version 16.0. Results were expressed as mean±standard. T-test and one-way analysis of variance were used to compare between groups and the acceptance level of significance was $p<0.05$.

RESULTS

The total nitrogen content of the soil samples was 7.74±0.07 mg mL^{-1}, while the POME soil had an increasing value in the total nitrogen content with sample B7 having the highest amount of nitrogen 10.71±0.05 mg mL^{-1} across week 1-4 shown in Fig. 1.

Figure 2 revealed that the control group had 2.05±0.04 mg mL^{-1} of potassium content and sample B2 recorded 2.49±0.01 mg mL^{-1}. The POME soil samples indicated an increasing value of potassium content, with sample 7 having the uppermost value of 3.27±0.14 mg mL^{-1}.

Figure 3 represents the phosphorous content of sample B1 was 0.046±0.10 mg mL^{-1} while sample B2 documented 0.07±0.10 mg mL^{-1}. Sample B7 noted the highest quantity of phosphorous 0.088±0.10 mg mL^{-1}. This was due to the amount of contaminants and volume of chicken droppings that was used to mix.

Figure 4 showed an observable increase in the soil dehydrogenase activity of the POME soil. The non-POME contaminated soil had the lowermost activity 29.04±0.95 TPF mg^{-1} min^{-1} and was significantly higher ($p<0.05$), paralleled to samples 3-7 which offered

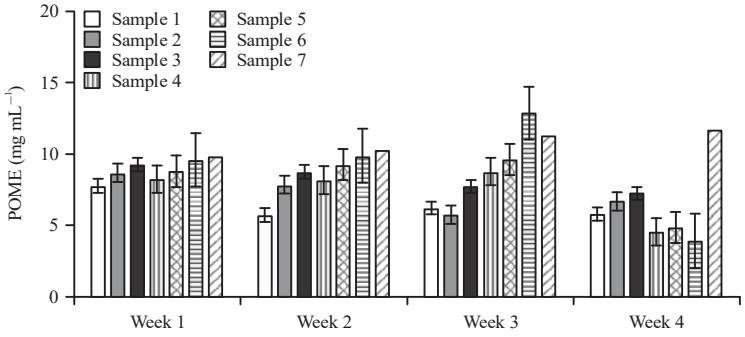

Fig. 1: Total nitrogen in soil contaminated with POME (mg mL^{-1})

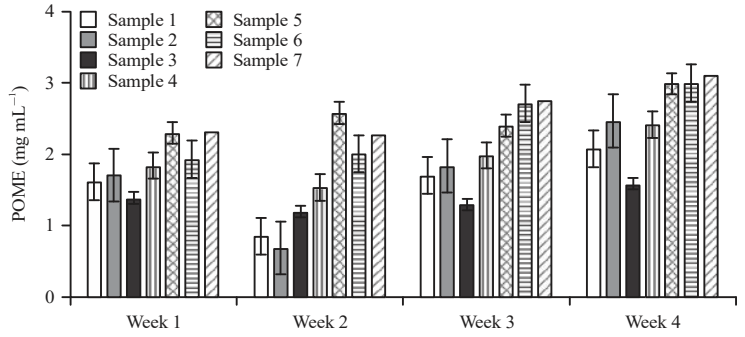

Fig. 2: Potassium in soil contaminated with POME (mg mL^{-1})

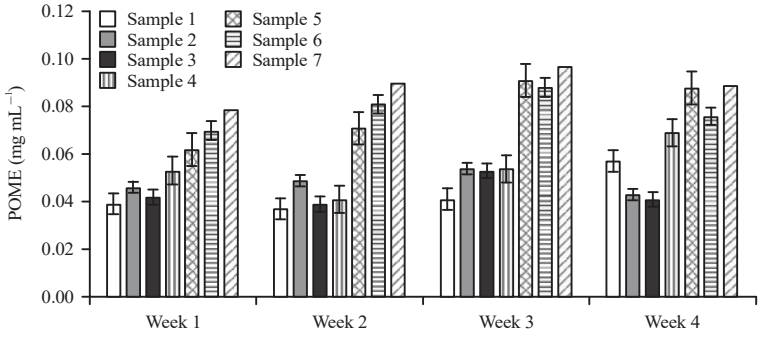

Fig. 3: Phosphorus in soil contaminated with POME (mg mL^{-1})

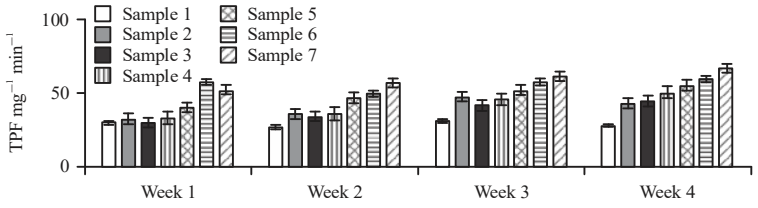

Fig. 4: Dehydrogenase activity (TPF mg^{-1} min^{-1})

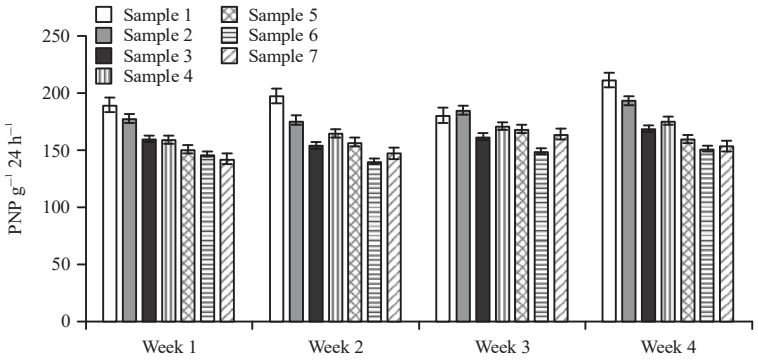

Fig. 5: Acid phosphatase activity (PNP g^{-1} 24 h^{-1})

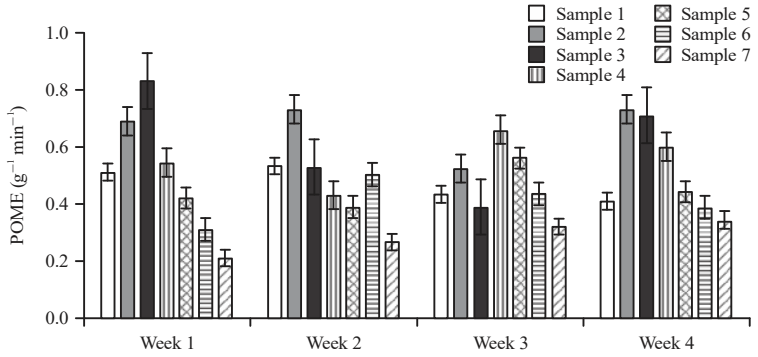

Fig. 6: Catalase activity in POME contaminated soil (g^{-1} min^{-1})

46.19±0.90, 52.22±0.76, 57.22±0.96, 61.40±1.20 and 68.76±0.83 TPF mg^{-1} min^{-1}, respectively. The non-POME soil had a minimum activity.

The acid phosphatase activity was detected and revealed observable increase in the non-POME soil (213.04±0.95 PNP g^{-1} h^{-1}). POME polluted soil presented a decrease in the phosphatase activity, with sample B7 taking the lowermost value (154.76±0.83 PNP g^{-1} h^{-1}) (Fig. 5).

Figure 6 indicated that sample B2 gave the highest catalase activity (0.73±0.01 g^{-1} min^{-1}), while sample B7 noted the lowest activity 0.342±0.01 g^{-1} min^{-1}. Sample B1 logged 0.41±0.01 g^{-1} min^{-1} while a reduction in the catalase activity was detected in the supplemented samples with an increase in the concentration of the poultry droppings.

The non-POME contaminated sample gave the lowest urease activity (1.432±0.01) at week 4, while an increase in the other samples was noted, with sample 7 having the highest value (3.85±0.02 ppm h^{-1}) at week 3, but reduced to 2.84±0.06 ppm h^{-1} at week 4 (Fig. 7).

DISCUSSION

Fresh POME was mixed with poultry droppings and allowed to age-within 12 h by stirring the broth. This was carried out for 4 weeks and thereafter, it was contaminated with the soil. The total nitrogen concentration in the soil samples was determined and presented in as Fig. 1. The control gave total nitrogen 7.74±0.07 mg mL^{-1}, while the

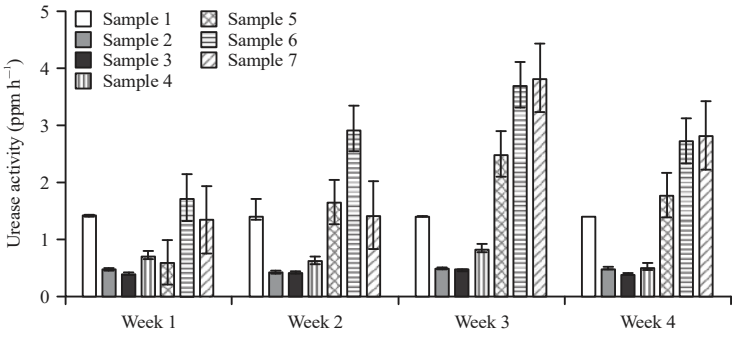

Fig. 7: Urease activity (ppm h^{-1})

POME polluted soil showed 10.71±0.05 mg mL^{-1}. Figure 2 represents the concentration of potassium in the polluted and control soil samples. The control had potassium 2.05±0.04 mg mL^{-1}, whereas, sample B2 had 2.49±0.01. The POME contaminated soil samples indicated an increase in the value of potassium content in sample 7-3.27±0.14 mg mL^{-1}. The phosphorous in sample B1 was 0.046±0.10 mg mL^{-1}, whereas, sample B2 had 0.079±0.10 mg mL^{-1}. Sample B7 gave the highest quantity of phosphorous 0.088±0.10 mg mL^{-1} (Fig. 3). The combined increase in nitrogen, phosphorus and potassium (NPK) was witnessed in the soil with an upsurge in the mixed sample. The increased NPK was owed to the high organic content of POME. These outcomes were aliketo[14], where, higher concentration in nitrogen (13.53±0.01) and potassium (11.02) content of POME contaminated soil was compared to the nation POME contaminated soil 2.10±0.01 and 5.50 nitrogen and potassium, respectively. The NPK contributes to soil nutrient. Augmenting the soil with elements that proliferates the nutrient quotient of the soil may enhance the soil nutrient quality. Thanks, to Onwusi and Nwuche[15], who recorded an increase in the nitrifying bacteria load, with an increase in POME concentration in the soil samples.

Increase in the soil dehydrogenase activity of the POME contaminated soil is presented in Fig. 4. The non-POME polluted soil exhibited the bottom activity (29.04±0.95 TPF mg^{-1} min^{-1}) and was significantly lower ($p<0.05$), linked to samples 3-7 which gave 46.19±0.90, 52.22±0.76, 57.22±0.96, 61.40±1.20 and 68.76±0.83 TPF mg^{-1} min^{-1}, respectively. The non-POME contaminated soil had the slightest activity. Increase in the dehydrogenase activity was due to high oxygen demand of the samples with an increase in the concentration of bacteria. The increase in dehydrogenase activity was attributed to organic content or microbial activities. Leirós *et al.*[16], who reported an increase in the activities of dehydrogenase to be proportional to increased concentrations of petroleum-contaminated wastewater, which served as the carbon source for microbial remediation[16]. This was contrary to the report of Ubani *et al.*[17] that recorded a decrease in the enzyme activity and was ascribed to the effect of heavy metals on some soil microorganisms[17]. The acid phosphatase activities were higher in the non-POME soil (213.04±0.95 PNP g^{-1} h^{-1}). The POME contaminated soil showed a decrease in the phosphatase activity, with sample B7 having the lowest value (154.76±0.83 PNP g^{-1} h^{-1}) as shown in Fig. 5. Sample B2 gave the highest catalase activity (0.73±0.01 g^{-1} min^{-1}), while sample B7 recorded the lowest activity of 0.342±0.01 g^{-1} min^{-1}. Sample B1 noted 0.41±0.01 g^{-1} min^{-1} while a decline in the catalase activity was perceived in the mixed samples with an increase in the concentration of the poultry droppings. In Fig. 6, the non-POME sample gave the lowest urease activity (1.432±0.01) at week 4, while an increase in catalase activity was perceived in other samples including sample 7 which have the highest value (3.85±0.02 ppm h^{-1}) at week 3 but reduced to 2.84±0.06 ppm h^{-1} at week 4. The decreased catalase activity witnessed in the POME contaminated soil was due to increased organic oxygen concentration and increased dehydrogenase activity. Ezirim *et al.*[18] reported a significant decrease in the hydrogen peroxidase activity of POME polluted site compared to the non-polluted site. Urease activity increased as a result of the increase in the organic content of the samples (Fig. 7). Yang *et al.*[19] recorded an increase in urease activity as a result of increased organic content and heavy metal activities. However, the outcomes of this investigation show that light use of POME in the soil might be good for agricultural purposes since soil fertility is enriched by augmenting microbial activities.

CONCLUSION

The disposed of untreated POME to the environment confers unfavourable effects to agricultural land, they do so by suffocates the soil organisms and increase the level of heavy metals and pH. Pre-treating fresh POME and mixing with poultry droppings may increase the soil microbial load and improve aeration.

SIGNIFICANCE STATEMENT

This investigation discovered that the co-mixing of agro-waste- fresh palm oil mill effluent and chicken droppings increases the activities of the soil enzymes, improved the soil fertility capability and reduces the negative impact of POME on the soil ecosystem. Therefore, adopting this technique will help the farmers and Agriculturist to remediate POME contaminated soil. This study will help researchers to undertake other needful areas that will add value to solving environmental pollution.

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