Assessment of Fishpond Sludge in Bioremediation of Mechanic Workshop Polluted Soil

Stephen E.* Sau A. Jonathan F. Aminu S. and Abubakar S.
Department of Biological Sciences, Taraba State University, Jalingo, Nigeria.
* Corresponding author: psychsea07@gmail.com

Abstract: The use of fish pond sludge in bioremediation of mechanic workshop polluted soil was studied for a period of eight weeks. Soil samples were collected from mechanic village along Mayo Dasa in Jalingo, and transported to Biological Science laboratory Taraba State University, Jalingo. Fifteen kilogram (15 Kg) of the mechanic workshop soil was weighed into four different plastic perforated bowls labeled as A1, A2, B1 and B2. Fresh fish pond waste water was collected from a fish farm into three 20 litres plastic containers. The waste was poured through a muslin cloth and the residue collected and air dried at 28^0C for a week. The sludge was incorporated into A1 and A2 (FPS) while B1 and B2 were without fish pond sludge (PS). Two additional bowls labeled C1 and C2 (UPS) were filled with pristine soil obtained from soil opposite the mechanic workshop. Bacteriological analysis was carried by spread plate inoculation on Nutrient agar (NA) and the isolates that grew were subjected to microscopy, morphology and biochemical tests. pH, organic carbon, organic matter content, nitrate and phosphate of FPS, PS and UPS were determined bi-weekly to assess the effectiveness of the remediation process. The results showed higher bacterial population in FPS compared to PS and UPS all through the study. The bacterial count ranged from $1.1x10^4$ to $4.5x10^4$ cfu/g in UPS, 2.3 $x10^4$ cfu/g to 8.0 x 10^4 cfu/g in PS and $1.8x10^4$ cfu/g - $9.8x10^4$ cfu/g in FPS. There were significant differences in the bacterial counts at 0.05 probability limits. The organisms isolated and identified from the soil samples were genus of *Bacillus* spp, *Micrococcus* spp, *Pseudomonas* spp, *Proteus* spp, and *Staphylococcus* spp. pH ranged from 6.20 ± 0.17 - 6.41 ± 0.12. Higher concentrations of organic carbon, organic matter content, nitrate and phosphate were observed in PS compared to UPS and FPS. There were no significant differences in pH, nitrate, phosphate and moisture content at 95% probability limits while significant differences were observed in organic carbon and organic matter content. This study demonstrates the potential of fish pond sludge in bioremediation of mechanic workshop polluted soil.
Key word: Bioremediation, fish pond, sludge, mechanic workshop soil

INTRODUCTION

Soil pollution arising from the activities of automobile artisans is a salient environmental issue that has been reported as a threat to human health and eco-biodiversity (Margesin and Schinner, 2018). Mechanic workshops soil contamination often stem from the release of chemicals, trace metals, petroleum products and solvents (Mingorance and Delgado-Moreno, 2016). Bioremediation has gained prominent attention as reliable method in restoring the properties of contaminated soils. Bioremediation utilizes microbes and plants to degrade, immobilize or transform pollutants, thereby restoring the soil's natural state (Khan *et al.*, 2020). Cunningham and Lin (2017), reported that the use of materials, which consist of beneficial microorganisms would enhanced soil health and fertility. Sharma *et al.* (2017), weighed the option of using bio-fertilizers containing nitrogen-fixing bacteria for degradation of toluene-contaminated soil. The authors proved that bio-fertilizer could effectively accelerate the degradation of toluene in the soil. Gupta *et al.* (2019), reported that bio-fertilizers enhanced degradation of hydrocarbons in soil impacted with petroleum wastes. Singh *et al.* (2020), documented the fact that bio-fertilizer had the capability of enhancing the restoration of hydrocarbon polluted soil. Furthermore, bio-fertilizer was reported by Hanif *et al.* (2021), to improve soil fertility, nutrient cycling and microbial activity.

Traditionally, bio-fertilizers were sourced from organic waste materials, such as animal manure, plant residues and compost (Pichtel, 2015). According to Zhang *et al.* (2019), fish pond sludge with its high nutrient content and beneficial microbial diversity has been accorded a status of a bio-fertilizer. Fish pond sludge is rich in debris, phosphate-solubilizing bacteria, nitrogen-fixing bacteria and other microorganisms capable of stimulating plant growth as well as improving soil quality (Lanzén *et al.*, 2020).

Hence, this study sets out to assess the bioremediation potential of fish pond sludge in a mechanic workshop polluted soil bearing in mind its biodegradable and eco-friendly disposition.

MATERIALS AND METHODS

Study area: This study was carried out in the Department of Biological Sciences of Taraba State University, Jalingo, Taraba State. Jalingo lies within latitude 8.8933° N and longitude 11.3570° E with an estimated population of 518,000 (Emmanuel *et al*., 2024). Jalingo metropolis is drained by two rivers: Mayo-gwai and Lamurde which empties into River Benue. Jalingo has a tropical climate with well pronounced wet and dry season. The wet season starts around late April and peaks in September while the dry season usually starts in early November, terminating around late March (Emmanuel *et al*., 2024). The mean annual rainfall and temperature stands at about 1200mm and 29^0C. The region is characterized by fertile soils and rich agricultural practices- crops, livestock rearing and fish farming.

Sample collection: The mechanic workshop polluted soil samples were collected from mechanic village, Jalingo, Taraba State, Nigeria at surface layer of 0-10 cm into four bowls labeled as A1, A2, B1 and B2 with each containing 15g of the soil samples. The bowls were perforated underneath in order to prevent saturation of water. The Bowls A1 and A2 was incorporated with fish pond sludge (FPS) while B1, B2 contained mechanic workshop polluted soil only (PS). Lastly, 15 kg of pristine soil only was collected into bowls C1, C2 (UPS) to serve as control for the study.

Fish pond sludge: Fish pond waste water was collected from the bottom of a fish pond into sixty liters storage polyethylene cans (three cans of 20 litres each). The collected waste was passed through a muslin cloth. The residue was spread on a concrete platform to air dry for a period of one week.

Treatment of mechanic workshop polluted soil: The mechanic workshop polluted soil was gently crushed and sieved through a 2mm mesh sieve to remove non-soil particles and debris. Three kilograms (3 kg) of the sludge was incorporated into the mechanic workshop polluted soil samples contained in bowls labeled A1, A2 in order to achieve 20 % amendment level.

Sample analysis: Sampling was carried out bi-weekly for a period of 56 days (8 weeks) to assess the bacterial and physicochemical qualities of the mechanic workshop polluted soil undergoing bioremediation. The total viable bacteria count was determined aseptically using serial dilution on nutrient agar plates by pour plate method. The plates were incubated at 37°C for 24 hours. The bacterial colonies that grew on the nutrient agar plates were counted and sub-cultured on freshly prepared nutrient agar plates and stored as stock cultures on nutrient agar slants for biochemical and sugar fermentation tests.

Physicochemical properties such as pH, Phosphorus were determined as described by Onyeonwu (2000). Organic carbon, organic matter content, nitrate and moisture were determined as outlined by Ibitoye (2006). Briefly, Soil pH was determined at room temperature using glass electrode pH meter (Hannia, Italy).Ten grammes of soil sample was suspended in 25 milliliters of distilled water and mixed properly. The pH meter was standardized at pH 7.0 using phosphate buffer solution after which the pH of the samples was determined in duplicates by inserting the glass electrode in the soil suspension. The reading on the meter was recorded as the pH of the sample. (Onyeonwu, 2000).

Organic matter content was determined as described by Ibitoye (2006). Crucibles were dried at 160ºC for 20 minutes, cooled in a desiccator and weighed (W1). Five grammes (5g) of soil samples was weighed and added to the crucible (W2). The samples in crucibles were heated at 500ºC for seven hours. The crucible with ashes of samples was transferred into desiccator, cooled and reweighed (W3).The percentage organic matter content in each sample was calculated thus:

$$\% \text{ organic matter} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1= weight of the crucible without sample; W_2 = weight of the crucible with sample before drying; and W_3 = weight of the crucible with ash.

Nitrate was determined using the micro Kjeldahl method as described by Association of official Analytical Chemist, AOAC (2005) and Ibitoye (2006). Two grams of air dried soil sample was put in a clean dry Kjeldahl flask containing 20 ml concentrated tetraoxosulphate (vi) (H_2SO_4) with one catalyst tablet. The flask was heated gently to avoid splashing until the liquid became clear with pale straw colour. The heating was continued further to ensure complete digestion and then allowed to cool. Fifty (50) ml of de-ionized water was added to the digest and mixed well. The volume was made up to 100 ml with distilled water in a standard flask and distilled with 10 ml of 40 % sodium hydroxide (NaOH) solution added to the digest. Distilled ammonia was liberated into 100ml conical flask containing 5 ml of 2% boric acid. The distillate was titrated with 0.01M HCl. Blank distillations (all the reagents without soil sample) was carried out and titrated. The percentage nitrogen was calculated from the formula:

$$\% \text{ Nitrogen} = \frac{V_1 - V_0}{10 \times \text{weight of sample}}$$

Where, V_1 = Titre value of sample; and V_o = Titre value of the blank.

The phosphorous content of the soil sample was determined as outlined by Onyeonwu (2000). Two grammes of finely ground soil was weighed into 25 ml capacity conical flask after which 30 ml of $HClO_4$ was added and digested in a fume cupboard at 130^0C until the solution appeared clear. The flasks were removed and cooled to room temperature; 50 ml of distilled water was added to the digest and filtered into 100 ml capacity flask. The absorbance of the coloured solution was determined at 882 nm wavelength after 30 minutes using a spectrophotometer (Genesys 20, USA).

The moisture content of the soil was determined using the dry weight method as described by Ibitoye (2006). Empty crucible was dried in an oven at 105^oC for a few minutes, locked in a desiccator and weighed (W_1). A 5 g of sample was placed in a crucible (W_2). The crucible with the sample was dried in an oven at 105^oC until a constant weight was achieved. The crucible was then transferred to a desiccator to cool and weighed with minimum exposure to the atmosphere (W_3). The loss in weight of the sample after drying was the moisture content. It was calculated using the formula:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1= weight of the crucible without sample; W_2 = weight of the crucible with sample before drying; and W_3 = weight of the crucible with sample after drying.

Statistical analysis: Descriptive statistics and analysis of variance (one way ANOVA) was performed using procedure of statistical package for social sciences (SPSS) version 16 (2007). Experimental precision achieved was reported at $p \leq 0.05$ level.

RESULTS

The total bacterial count in UPS, PS and FPS during the study is shown in figure 1. The bacterial count decreases gradually in UPS from 4.5×10^4 - to 1.1×10^4 cfu/g. The counts in PS ranged from 8.0×10^4 cfu/g to 2.3×10^4 cfu/g while FPS ranged from 1.8×10^4 cfu/g -9.8×10^4 cfu/g. There were significant differences in the bacterial counts at 0.05 probability limits. This decline in bacteria counts observed in UPS and PS may be due to natural fluctuations in the nutrient status of the soil as well as the presence of pollutants that might have impacted negatively on microbial growth. In contrast, the polluted soil treated with fish pond sludge (FPS) showed a different trend. Initially, the bacterial count was lower compared to UPS and PS. However, significant increase was recorded in FPS after the first week. This increase might be due to the utilization of fish pond sludge as bio-stimulant contributing to the proliferation of bacteria (Smith *et al.,* 2019). Fish pond sludge has been reported to be rich in organic matter and nutrients that can influence microbial growth in soil ecosystems (Gupta *et al.,* 2019).

The bacterial population observed in PS was comparatively lower than that observed in the FPS samples. This discrepancy suggests that the addition of fish pond sludge may have exerted a more pronounced stimulatory effect on bacterial growth in the polluted soil environment (Thompson *et al.,* 2016). Table 1 below shows the morphological and biochemical characteristics of bacterial isolates. The commonly isolated bacteria species were rods and cocci morphologically. The isolates were genus of *Staphylococcus* spp*, Proteus* spp*, Micrococcus* spp, *Bacillus* spp. and *Pseudomonas* spp.

Table 2 shows the findings of the physicochemical properties of the soil sample. The pH ranged from 6.41 ± 0.12 to 6.28 ± 0.17. The highest pH was observed in UPS while the lowest pH was observed in FPS. There was no significant difference in the pH observed in UPS, PS and FPS at 95% confidence interval. The low pH observed in FPS may be attributed to rich organic compounds in the fish pond sludge undergoing decomposition (Jones and Smith, 2017; Johnson *et al.* 2018). Jones and Smith (2017), are of the opinion that decomposition of organic matter impacts on pH level of soil undergoing biodegradation. Organic carbon ranged from 2.30 ± 0.40 to 3.17 ± 0.23. The highest carbon content was observed in PS followed by UPS while the least value was observed in the fish pond fortified soil, FPS. Similar trend was observed in the values of organic matter content i.e PS > UPS > FPS. Significant differences were observed in the organic carbon and organic matter contents of UPS, PS and FPS at 0.05% probability limits. The low values of organic carbon and organic matter observed in FPS compared to UPS and PS is consistent with report of Smith *et al.* (2019) and Ramos-Miras *et al.* (2019). These researchers in their separate findings, reported lower values in organic carbon and organic matter contents in soil undergoing bioremediation. The nitrate contents of UPS, PS and FPS ranged from 0.61 ± 0.25 mg/kg to 0.90 ± 0.06 mg/kg. The highest nitrate content was observed in PS followed by UPS while the least value was observed in FPS. There was no significant difference in the nitrate contents observed in UPS, PS and FPS at 0.05 probability level. The low nitrate content observed in FPS is an indication of active utilization of nitrates by microbes present in the soil. The findings in this study is in contrast to the reports of Johnson *et al.* (2018). These authors reported elevated nitrates in soil enriched with organic manure.

The pattern of phosphate concentration was UPS >PS >FPS. There was no significant difference in the phosphate concentration in UPS, PS and FPS at 5% probability level. The low level of phosphate observed in FPS may be an indication of phosphate utilization by soil microbes. This is consistent with earlier reports by Krasner *et al*. (2009). These authors were of the opinion that soil enriched with organic matter has reduced phosphate and nitrate levels due to their utilization during biodegradation process.

Moisture content ranged from 2.71 ± 0.75 % to 7.20 ± 4.42 % in all soil samples. The highest moisture content was observed in FPS followed by UPS and PS. There was no significant difference in the moisture content in UPS, PS and FPS. According to Rodriguez *et al.* (2017) water-holding capacity of soil increases in the presence of organic manure.

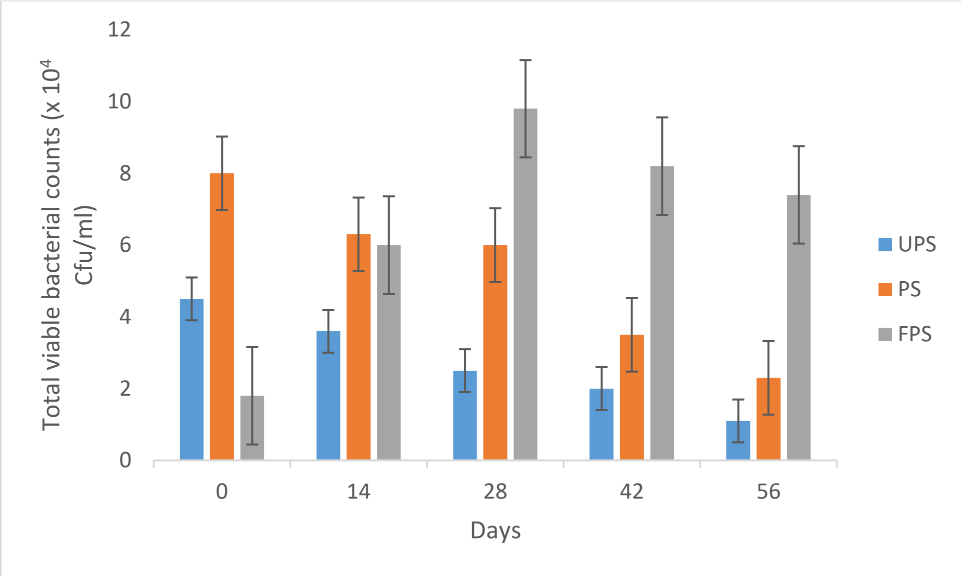

Figure 1: Total viable count of bacteria from mechanic workshop soil incorporated with fish pond sludge

Table 1: Morphological and biochemical characteristics of bacteria isolates from mechanic workshop shop undergoing bioremediation

Codes	Colonial characteristics	Gram reaction	Shape	Spore	Indole	Catalase	Mannitol	Glucose	Sucrose	Lactose	Coagulase	Probable isolate
A	Smooth yellow	+	Cocci	-	-	+	+	+	+	+	+	*Staphylococcus* spp
B	Milky white	-	Rods	-	-	+	-	+	+	-	-	*Proteus* spp
C	Small white opaque	+	Cocci	-	-	+	-	-	+	-	-	*Micrococcus* spp
D	Cream, rough edges, opaque	+	Rods	+	-	+	+	+	+	-	-	*Bacillus* spp
E	Creamy white, flat surface	-	Rods	-	-	+	-	+	+	+	-	*Pseudomonas* spp

Table 2: Physicochemical qualities of Mechanic Workshop Polluted soil undergoing bioremediation (M+SE) and control plot

Parameters	UPS	PS	FPS
pH	6.41 ± 0.12^a	6.28 ± 0.17^a	$6.20 \pm .0.17^a$
Organic carbon	$2.30 \pm 0.40^{a,b}$	3.17 ± 0.23^a	1.30 ± 0.66^b
Organic matter content	3.67 ± 0.50^b	5.42 ± 0.38^a	0.92 ± 0.13^c
Nitrate	0.75 ± 0.20^a	0.90 ± 0.06^a	0.61 ± 0.25^a
Phosphate	21.61 ± 2.30^a	16.62 ± 1.70^a	14.04 ± 4.70^a
Moisture content	3.03 ± 1.34^a	2.71 ± 0.75^a	7.20 ± 4.42^a

Key: a,b,c: means denoted by different superscripts along the same row are significantly ($p<0.05$) different. Values are means of four replicates. UPS: Pristine soil, PS: Mechanic workshop polluted soil, FPS: Mechanic workshop polluted soil amended with fish pond sludge.

CONCLUSION

This study showed that the use of fish pond sludge in bioremediation of mechanic workshop polluted soil increased the bacterial population more in FPS compared to UPS and PS. This increased bacterial population is required for an efficient bioremediation of hydrocarbon polluted soil. The pH, nitrate, organic carbon, organic matter were low in the soil fortified by fish pond sludges compared to UPS and PS suggesting fish pond sludge can be used to enhance bioremediation of mechanic workshop polluted soil.

REFERENCES

Association of official Analytical Chemist. (AOAC, 2005). *Methods of Analysis*, Washington D.C.

Cunningham, S. D. and Lin, Q. (2017). Phytoremediation assay for organic contaminants in soil and sediment. *Methods in Enzymology*, 595, 215-233.

Emmanuel, G, Zubairu, A.I., Danji, M.B.., Hassan, C.K. and Bakari, B.W. (2024). Adoption of organic farming practices among vegetable farmers in Jalingo Local government Area of Taraba State, *Nigeria. Journal of Arid Agriculture*, 25 (1): 130-137.

Gupta, V., Gupta, A., Sharma, S. and Singh, K. (2017). Soil microbial diversity: An overview. In Soil microbial diversity in sustainable agriculture (pp. 1-22). Springer, Singapore.

Hanif, A., Hussain, A., Javed, M. T., Tayyab, M., Akhtar, M. S., Fahad, S. and Khan, M. A. (2021). Microbial fertilizerfor sustainable agricultural productivity: An Innovative Perspective. *Trends in Plant Science*, 26(1), 63-68.

Ibitoye, A.A. (2006). *Laboratory Manual on Basic Soil Analysis* (2nd ed). Foladave Nigeria Limited, Akure, pp 30-37.

Johnson, T., Anderson, L. and Green, D. (2018). Organic manure-induced changes in soil porosity. *European Journal of Soil Science*, 71(4), 521-533.

Jones, A. and Smith, B. (2017). Soil structure improvement with organic manure. *Geoderma*, 290, 45-56.

Khan, S., Afzal, M., Iqbal, S. and Khan, Q. M. (2020). Plant–Microbial Interactions For sustainable soil remediation and reclamation. *Environmental Science and Pollution Research,* 27(24), 29354-29368.

Krasner, S.W., Westerhoff, P., Chen, B., Rittmann, B.E., Nam, S.N. and Amy, G. (2009). Impact of waste management treatment process on organic carbon, organic nitrogen and DBP precursors in effluent organic matter. *Environmental Science and Technology*, 43 (8):2911- 2918.

Lanzén, A., Øvreås, L. and Urich, T. (2020). Bioremediation of pollutants in aquatic ecosystems using microbiome engineering strategies. *Frontiers in Microbiology*, 10, 3045.

Margesin, R. and Schinner, F. (2018). Bioremediation of contaminated soils and sediments. *In Manual of Environmental Microbiology* (Pp. 1426-1439). ASM Press.

Mingorance, M. D.and Delgado-Moreno, L. (2016). Mechanic Workshops: A potential source of soil contamination by phthalate esters. *Science of the Total Environment*, 342-349.

Onyeonwu, R.O. (2000). Manual for waste/wastewater, Soil/Sediment, Plant and Fish analysis. Mac Gill Environmental Research Laboratory Manual. 81pp.

Pichtel, J. (2015). Soil and Water Contamination: From Molecular To Catchment Scale. CRC Press.

Ramos-Miras, J., Johnson, A., Smith, B., Yadav, R., Sharma, N. and Rodriguez, A. (2019). Effect of organic amendments on soil physicochemical properties: A review. *Geoderma*, 249, 193-207.

Rodriguez, A., Johnson, A., Smith, B., Yadav, R., Sharma, N. and Ramos-Miras, J. (2017). Impact of organic amendments on soil moisture content: A meta-analysis. *Journal of Hydrology, 545, 96-104.*

Sharma, N., Johnson, A., Smith, B., Yadav, R. Ramos-Miras, J.and Rodriguez, A. (2017). Influence of organic amendments on soil physicochemical properties: A Review. *Agriculture, Ecosystems & Environment*, 232: 185-199.

Singh, M., Aakansha Singh, R. and Mehrotra, S. (2020). Bioremediation in contaminated soils using phosphate-solubilizing bacteria. *Environmental Technology and Innovation*, 20, 101037.

Smith, J., Lee, Y. and Koopman, C. (2019). Assessment of petroleum hydrocarbon contamination levels using polycyclic aromatic hydrocarbons (Pahs). *Journal of Environmental Management,* 238, 146-154.

Thompson, J., Brown, K. and Davis, P. (2016). Title of the fifth study. *Environmental Microbiology Reports,* 8(6), 321-335.

Zhang, W., Walsh, C. and Abdel-Raheem, M. (2019). Utilisation of aquaculture solid waste as biofertilizer. *Aquaculture*, 505, 471-478.