

EVALUATING POSSIBLE EFFECT OF MANAGEMENT PRACTICES ON VITAL AND RELEVANT ENZYME ACTIVITIES IN PETROLEUM POLLUTED SOIL

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ABSTRACT

This study aims to evaluate the possible impact of management practices on the activities of important and related enzymes in petroleum-contaminated soil through a four-factor phytoremediation method. The purpose of this experiment is to carry out a four-factor planting experiment according to the planting season in Nigeria in 2013, and choose a soil depth of 20 cm to represent a better community. The results indicate that the decrease in protease activity may be due to autolysis, and there is a time lag between sampling and analysis in different countries/regions, which may limit this study. It is observed that the higher β -glucosidase activity is related to organic matter content and respiratory activity.

KEYWORDS

Soil ecosystem; functional diversity; β -glucosidase; respiratory activity.

1. INTRODUCTION

Microorganisms and enzymes basically drive mineralization, degradation of organic matter and nutrient recycling, and collectively constitute soil biological processes. The possibility of understanding changes in soil organic matter using enzyme activities is well described by Gianfreda and Ruggiero and may be useful in the face of increased urbanization and industrialization with accompanying environmental hazards. Enzyme biochemical assays do not show actual extent of activity but represent potential activity and hence have been described by Bandick and Dick as indicators. This may have stemmed from their usual indirect measurements by determining their activity using biochemical assays in the laboratory. β -glucosidase, an enzyme involved in cellulose degradation, has been reported to be quick to respond to soil management and linked to its involvement in soil organic carbon cycle and thus gives an early sign of yield and changes associated with organic matter. Piotrowska and Koper underscored the significance of the product of enzymatic hydrolysis of cellulose, being the most bountiful organic compound in the biosphere, as a source of energy for soil microorganisms. Protein degradative pathway or proteolysis starts with the hydrolysis of peptide bonds linking amino acids in the protein, forming polypeptide chain[1-3]. This process, occurring in viruses, bacteria, archaea, plants and animals, is catalysed by the enzyme called protease often named peptidase or proteinase. They are involved in the invertebrate prophenoloxidaseactivating cascade. Intracellular enzymes and dehydrogenases however oxidize substrates by relocating hydride(s) (H^-) to an oxidising agent, normally a flavin coenzyme e.g. FAD or FMN or $NAD^+/NADP^+$. Enzyme activity is measured as a mounting effect of continuing microbial activity and agitation of the tenable population at sampling. Dick presented dehydrogenase as a typical illustration of an enzyme depicting activity of viable cells, which in theory cannot take place in settled soil complexes. Dehydrogenases

activity (DHA) is an indicator of the microbiological redox-systems and provides important determination in the soil samples, and gives useful information concerning biological properties of soil. Dehydrogenases are chiefly produced by anaerobic microorganisms but can utilize oxygen and other electron acceptors. Wolińska and Stępniewska reported that under anaerobic conditions, activities of soil DHA increase greatly. They reiterated the possible influences by soil pH, depth, temperature, organic matter content, available oxygen, oxidation-reduction potential, heavy metal pollution, season of the year, pesticide use, etc. Phosphatases describe a group of enzymes driving the hydrolysis of esters and anhydrides of orthophosphoric acid, thus performing a salient role of transforming organic P in litter into inorganic phosphate in soil, and this is readily available to soil and plants organisms. They have been reported to derive from plant roots and soil microorganisms with varying kinetic properties, and differ in their response to anthropogenic influences. They are also, predictors of soil function (nutrient cycling) with plant available phosphorus as significance. Absence of, reduced or poor enzyme activity may lead to an accumulation of dangerous chemicals in the environment that could hamper the activity of soil enzyme[4-6]. These activities can better describe the response of phosphorus transformation to climatic and other environmental influences. Respiration is the release of carbon via heterotrophic consumption from the soil in the form of CO_2 and is a major ecosystem process. Nwaichi et al. identified the dire need for provision of soil mechanism to solve the interwoven sustainable development and environmental crises in the Niger Delta, given high oil exploration and exploitation activities in the region. Limited accounts on the quality of crude oil polluted soils exist, to our knowledge as well as quality of wholly or partially recovered soils. This study therefore seeks to evaluate possible effect of management practices on vital and relevant enzyme activities in petroleum polluted soil with a four-factor phytoremediation recovery attempts[7-10].

2. MATERIALS AND METHODS

2.1 Experimental design

Soil samples were taken from a freshly (2 weeks) and burnt crude oil spilled farmland in Oshie community of Akalaolu (a major host community to a big oil company in Nigeria) Ahoada West in the Niger Delta, after a field survey. Cause of spill was traced to sabotage. This experiment was designed to follow the 2013 planting season in Nigeria in a four-factor phytoremediation experiment and soil depth of 20 cm was chosen to have a better community representation. The design was a 90-d phytoremediation triplicate pot experiments using *V. subterranea*, *H. brassiliensis*, *C. citratus* and *F. littoralis*. Experimental control consisted of an agricultural soil in the same region with history of no pollution and poultry dung was used as soil amendment. Need-based watering was adopted and thinned plants were left on the soil on which it was grown. The amount of conditioner (animal dung) in this design was determined following Nwaichi et al. After 90 ds, collected rhizospheric soils were shipped in ice chest coolers to Environmental Microbiology laboratory at the Institute of Agrophysics Lublin Poland for analyses and were subjected to protocols outlined below:

2.2 β -Glucosidase activity

The activity of β -glucosidase was measured as given by Eivazi and Tabatabai. This method is based on the spectrophotometrical measure of released p-nitrophenol, PNP after 1 hour p-nitrophenyl- β -D-glucopyranoside (PNG)—laden soil incubation at 37°C with universal buffer as the substrate, though with modifications (pH 6.0). Measured enzyme activity was calculated as moles PNP released per kg dry soil per 1 hour ($\text{mM pNP kg}^{-1}\text{h}^{-1}$). Chemical properties, like organic carbon content (CORG), available P, total nitrogen content (NTOT) and pH in 1 mol KCl dm³, were determined according to standard methods accepted in soil science.

2.3 Protease (adapted from reported protocol by Alef and Nannipieri and Tabatabai)

Using a weighing balance (RADWIG PS1200/C/2 Max 1200 g Min 0.5 g e = 0.1 g, d = 0.01 g), 2 g of sieved (2 mm) moist soil were added to 15 ml Falcon (centrifuge) tubes (3 replicates and 1 control). Five millilitres of 1% sodium caseinate in TRIS HCl buffer pH 8.1 substrate (prepared over night and refrigerated), was added into test only to measure the amino acids released after soil incubation with sodium caseinate for 2 hr at 50°C using Folin-Ciocalteu reagent. Only TRIS HCl buffer pH 8.1 (5 mL) was introduced into control. This was shaken for 1 hr at 50°C and tubes placed in cold water to cool. An volume of 2 mL 17.5% TCA was introduced to test and control followed by centrifugation @ 3000 rpm for 2 mins. Into 2 mL supernatant in 5 mL test tube, 3 mL 1.4 M Na₂CO₃ was introduced to all tubes and was shaken thoroughly, then 1 mL FolinCiocalteu reagent (diluted 3 times) was added. After centrifugation for 2 mins at 200 rpm, absorbance readings (578 nm) of 96 Corning plates containing 200 μ L samples in Infinite M200 PRO TECAN Spectrophotometer were taken. Buffer was used as blank. Calibration curve was prepared, measured absorbance for the controls corrected, then calculation was done as follows: protease activity ($\text{mg tyrosine kg d}\cdot\text{m}^{-1}\text{h}^{-1}$) = $(C \times 15)/\text{dwt}$, where dm is dry weight of 1 g of soil, 15 is the final volume of solutions introduced to the soil during assay and C is the measured tyrosine concentration.

2.4 Dehydrogenases Activity (DHA)

DHA was measured following Casida et al. where specific dye, triphenyl tetrazolium chloride (TTC) that can specify the flow of electrons was employed as indicator of electron transport system (ETS) activity. Colorless and water soluble substrate (TTC) was reduced by dehydrogenases present in the soil environment, and this generates a red-colored insoluble product, triphenyl formazan-TPF that was quantified colorimetrically within the range of visible light (485 nm). TRIS-HCl pH 7.4 was used instead of CaCO₃ for buffering soil system. Developed colors (red) of high intensity correlated with higher activity in soil samples prepared for spectrophotometry while no-red coloration or light colors (red) pe-indicated lower DHA values.

2.5 Phosphomonoester's activity

Determination of p-nitrophenol released after soil incubation with p-nitrophenyl phosphate for 1 hr at 37°C is the underlying principle. Two sets of 1 g air-dried soils in Falcon tubes (3 replicates and 1 control) were prepared using weighing balance for alkaline and acid phosphatases. An aliquot of 0.25 mL Toluene was added to 1 g dry test and control soil and in a fume chamber 10 mins later, 4 mL buffer was introduced. To test, 1 mL PNP substrate was added and all samples were incubated at 37°C for 1 hr. Following this, 1 mL PNP was added to control. Again, an aliquot of 1 mL 0.5 M CaCl₂ and 4 mL 0.5 M NaOH were introduced to all samples and shaken in a Multi RS-60 BIOSAN Programmable rotator/mixer/shaker for 3 mins before Ependorf Centrifuge 5810 R centrifugation at 4000 rpm for 10 mins. At 485 nm using buffer as blank, microplates containing 200 μ L samples were read off in a spectrophotometer. Following preparation of calibration curve, control results were corrected and the PNP per ml of the filtrate were calculated by making reference to the calibration curve as in this formula:

$$\rho - \text{Nitrophenol (mmol PNP kgd}\cdot\text{m}^{-1}\text{h}^{-1}) = (C \times v) / (\text{dwt} \times \text{SW} \times t)$$

C = concentration of ρ -nitrophenol $\mu\text{g mL}^{-1}$ filtrate measured, dwt = dry weight of 1 g moist soil, v = total volume of soil suspension in ml, SW = soil weight, t = incubation time in hours.

For buffer preparation, 0.1 M HCl and 1 M NaOH mixture were corrected to pH 6.5 and 11 for acid and alkaline phosphatases respectively.

For substrate preparation, 1 mL acid buffer pH 6.5 was added to 0.05 g PNP (in a shielded beaker) per sample for acid phosphatase assay and 1 mL acid buffer pH 11 to 0.05 g PNP for alkaline phosphatases.

2.6 Respiratory activity

Substrate (Glucose) induced method adapted from reported protocol by Alef and Nannipieri was followed. To respiratory flasks in triplicate, 10 g < 2 mm screen soil was added to the outer jar and to the inner jar, 2.5 mL 0.2 M NaOH was added. One millilitre of glucose solution was evenly dropped onto the soil. Only 10 g soil-containing flasks for control, were autoclaved using Fedegari Autoclave AG and Classic Prestige Medical Autoclave for 20 mins at 121°C. Thereafter, 2.5 mL 0.2 M NaOH and 1 mL glucose solution were introduced. These were left to stand for 24 hr at room temperature. In a UV-lit gas chamber, 1 mL 1 M BaCl₂ was added to a 25 mL beaker containing transferred NaOH. Using 0.1 M HCl and Phenolphthalein indicator, titration was carried out while swirling and for calculation titre values were noted.

2.7 Statistical analysis

Means of triplicate data were analysed using Analysis of Variance, ANOVA. STATISTICA v 10 were employed to run Factor and Principal component analyses.

3. RESULTS AND DISCUSSION

Organic fertilization is known to beneficially increase organic C and N concentration in soil and affects organic matter in terms of quality and quantity. The pH values measured in 1 mol KCl dm³ ranged from 3.45 (in initial polluted soil) 6.8 (after 90 ds soil from manured Bambara cultivated regime). Total nitrogen content reached 1.7% in soil cultivated with Bambara and amended.

The class (Leguminosae) may have placed Bambara cultivated soils at an advantage in this regard. Observed influence of organic manure amendment in β -Glucosidase activity, BGA is as shown in Figure 1. Rubber-cultivated soils, when compared among plants, were significantly higher. Activity among plant-cultivation types and treatments responded positively to soil conditioners for Bambara, Lemon grass and Rubber-cultivated regimes but were not marked. This validates the environmentally friendliness of phytoremediation as a technique of soil clean-up. There was flooding in pots containing Fimbriatylis-cultivated designs and may have contributed to observed correlation with BGA activity. Protease activity (Figure 2) were highest in unpolluted soils for all plants.

Application of soil condition to phytoremediation design improved protease activity, though not marked ($p < 0.05$) and is indicative of community response to Carbon, Nitrogen and Sulphur limitations.

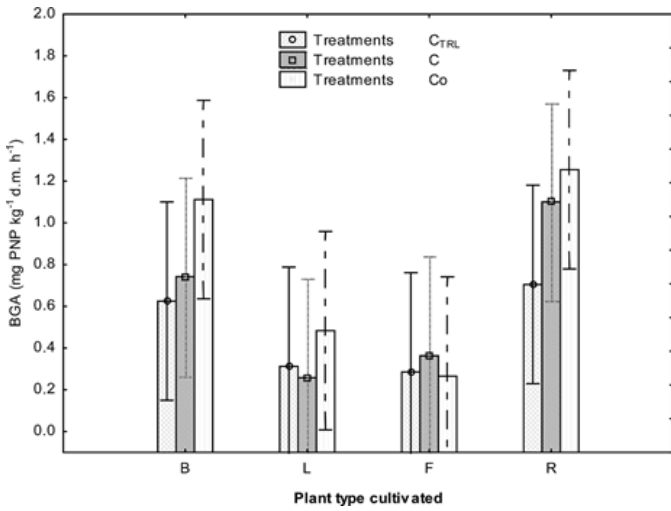


Figure 1. Effect of Organic manure application on β -glucosidase activity, BGA in different study soils. Vertical bars denote 0.95 confidence intervals. Values are means ($n = 3$) \pm SD. B, L, R and F denotes Bambara, Lemon grass, Rubber, and Fimbristyliscultivated soils; attached ctrl, c, and co denotes control (unpolluted), crude oil polluted and organically amended-crude oil polluted agricultural soils respectively. Increased activity with conditioner but for F-cultivated soil.

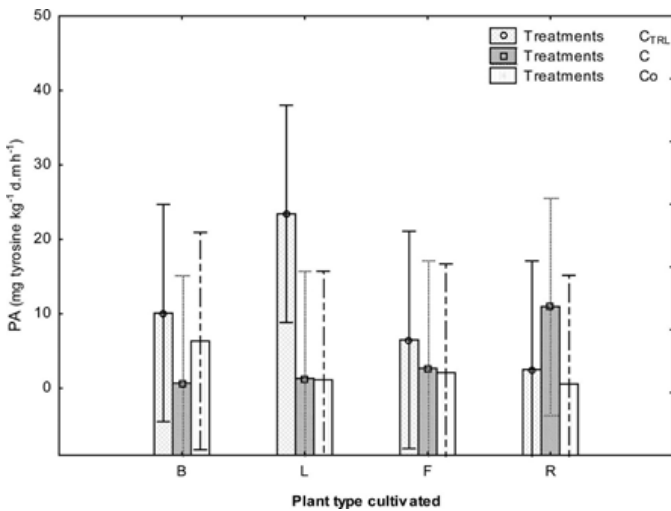


Figure 2. Protease activity, PA pattern in studied variants. PA denotes protease activity. No significant influences with conditioner was observed.

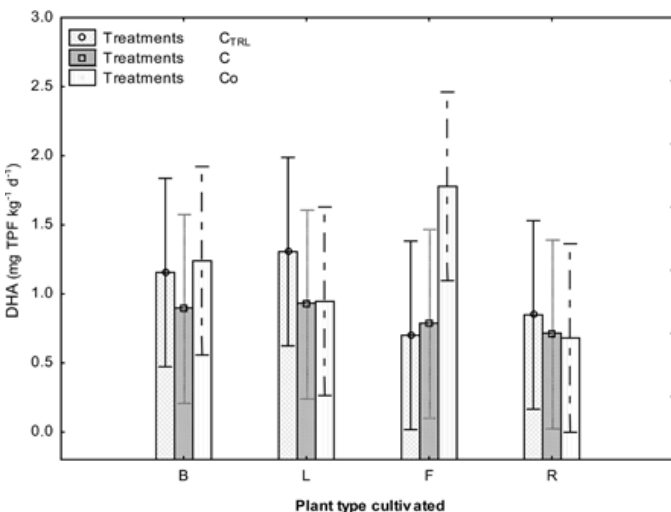


Figure 3. Dehydrogenases activity, DHA among plants and treatments. Vertical bars denote 0.95 confidence intervals. No significant influences with conditioner.

Observed rate of consumption for TTC-specific substrate for dehydrogenases, DHA, did not decrease for unamended contaminated designs leading to lower DHA activity (Figure 3).

It is noteworthy that these regimes were more acidic than control (unpolluted) and organic manure-amended counterparts. Levyk et al. demonstrated in their work that acidic soil conditions in the pH range of 1.5-4.5 resulted in strong DHA inhibition. Weakening of enzymatic activity with increased soil acidity was linked by Frankenberger and Johanson with the subsequent destruction of hydrogen bonds and ion in corresponding enzyme active centre. The increases with soil conditioner were however, not marked. Heavy metals such as Cd, Pb and As (data not shown) were observed at initial characterization to exceed their set limits in agricultural soils. Their presence may have reduced DHA as they can interact with the enzyme-substrate complex, bringing about denaturation of the enzyme protein or interactions with the protein-active groups. We therefore think that DHA and microbial biomass may not be proportional given the peculiarity and complexity of contaminated soil with varied activity.

The highest acidic phosphatase activity, ACPA (Figure 4) was measured in the unamended Bambara cultivated soils and was influenced by depleted available P (Figure 9) measured. Nannipieri et al. showed negative correlations between this enzyme activity and available P in sandy soils. Our study soil is a clayey loam. A significant connection was also observed in activity with organic matter-increased organic matter content improved ACPA. There were no significant increases observed with soil conditioner application.

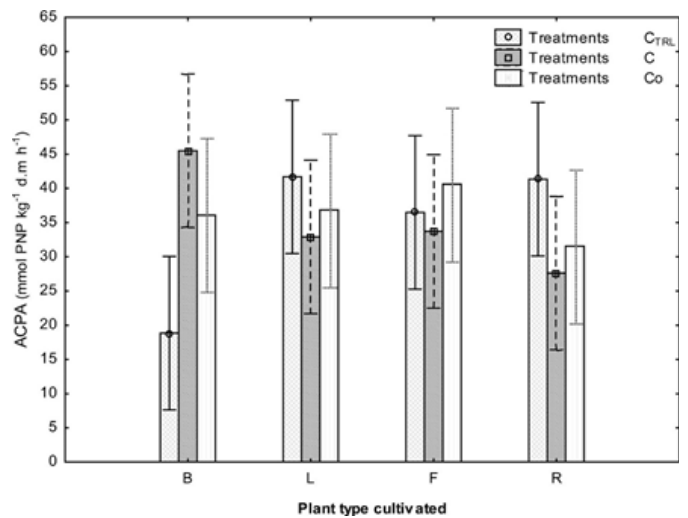


Figure 4. Acid phosphatase activity, ACPA among plants and between treatments as influenced by conditioner. Influence of conditioner was significant only in B-cultivated soils.

As shown in Figure 5, alkaline phosphatase activity, ALPA was low or undetectable in most soils investigated in the present study. Higher levels obtained for amended Bambara planted regime is attributable to its pH KCl of 6.8 (data not shown), which exceeded maximum optimum of 6.5 for acid phosphatases. These results do not agree with previous findings that soil pH correlates with optimum pH for phosphomonoester's activity. As reported by Eivazi and Tabatabai, there is no excretion of alkaline phosphomonoester's by plant roots, so we attribute activity to soil microorganisms. No clear influence of the clean-up technique was evident on soil ALPA. However, when organic manure was applied, significant differences were found.

Similarly, comparing respiratory activity, RESA among plant type cultivated (Figure 6), coincided with observed BGA pattern. The choice of Bambara and native specie, Rubber ere most beneficial. We found a direct relationship between cellulose breakdown, measurable with BGA, organic matter content and CO₂ release, measurable with RESA within all soils in the present study. These observed marked increases (Figure 7) with soil conditioning may implicate beneficial relationship with the abundance of microbial populations.

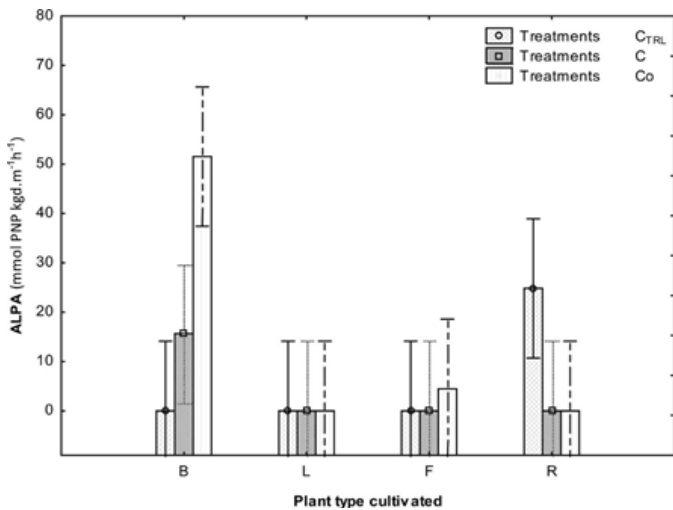


Figure 5. Alkaline phosphatase activity, ALPA among plants and between treatments as influenced by conditioner.

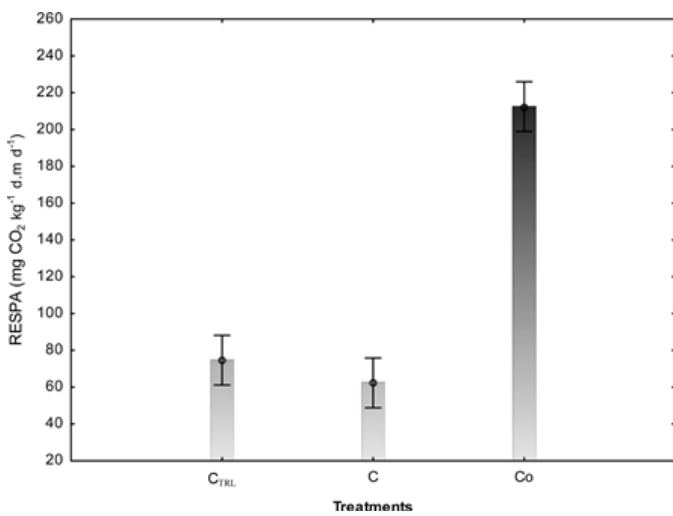


Figure 6. Respiratory activity, RESPA as influenced by soil conditioner among plant types cultivated.

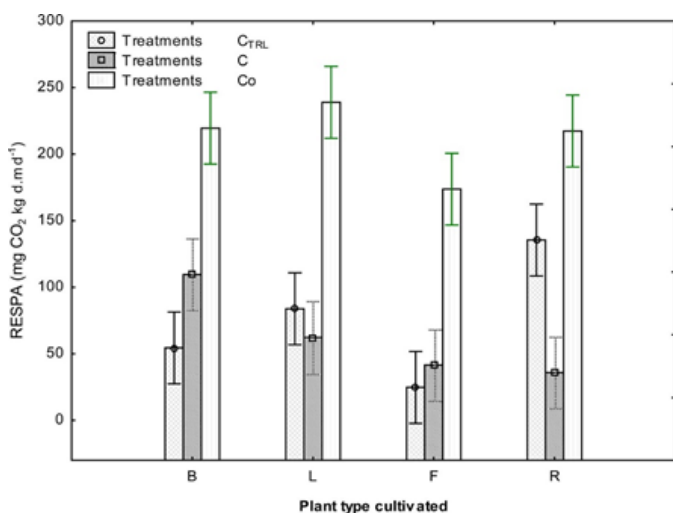


Figure 7. Respiratory activity, RESPA as influenced by soil conditioner among plants and treatments.

Soil respiration, also known as carbon mineralization, was statistically marked among treatments and cultivated plant types (Figure 6) and gives an indication of the innate ability to support soil life including

soil microorganisms, animals and crops by study soil, especially given application of conditioners. There was no activity prior to phytoremediation experiment. Also, this life-support capacity showed no positive effect owiththe application of organic manure for Fimbristylis-cultivated soils. Frequent flooding of pots cultivated with Fimbristylis may have induced incomplete mineralization of soil organic matter and resultant decreased aerobic soil microbial activity.

Factor analysis (Figure 8) was used to identify “invisible” factors, that portray the hidden “organizing principle” of anything being measured with a number of observable counts like dehydrogenases, proteases, phosphomonoesters, β-glucosidases and respiratory activities. Factor scores show how each “hidden” factor is analogous with the “observable” variables considered in this analysis. Factor loadings of 0.77, 0.72 and 0.73 show that alkaline phosphatases, respiratory and β-glucosidases activities can be used to give detailed account of hidden Factor 1; in other words, Factor 1 has features very similar to aforementioned activities. Other observable counts were not helpful in describing Factor 1. Similarly, Factor loadings of 0.80 and 0.74 show that Factor 2 has features very similar to dehydrogenases and acid phosphatases activities.

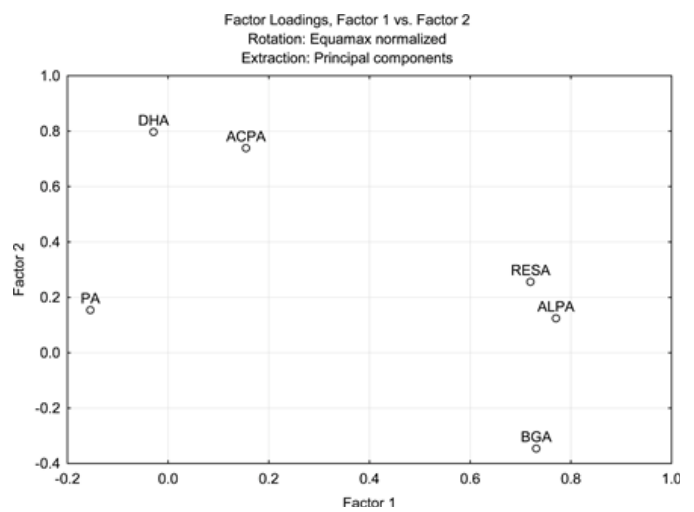


Figure 8. Multivariate factor analysis of expressed activities.

For organic matter (Figure 9), significant increases was observed with conditioner but for F-cultivated soil but were not marked (Figure 10) with observed levels of available Phosphorus except for F-cultivated soils.

Generally, results indicate the importance of incorporating leguminous plants in phytoremediation site and the influence of plant-soil-microbial interactions where there is a nutrient boost.

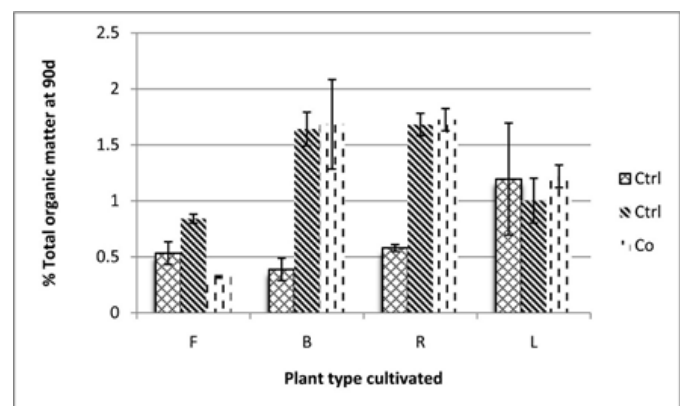


Figure 9. Total organic matter, TOM at 90 d of phytoremediation. Significant increases was observed with conditioner but for F-cultivated soil.

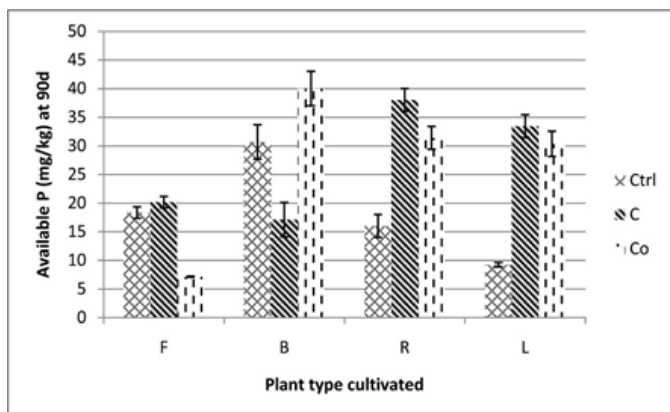


Figure 10. Available Phosphorus at 90 d of phytoremediation. Non-marked increases with conditioner except for F-cultivated soils.

4. CONCLUSION

Reduced activity of proteases may be due to autolysis with time lag between sampling and analysis in different countries and may constitute limitation to this study. Higher β -glucosidase activity was observed to be connected with organic matter content and respiratory activity. The legumes may be useful in phytoremediation design given observed trend with Bambara plants. Application of organic manure significantly improved CO₂ release by soil biota in hydrocarbon-stressed soil and may be explored for phytoremediation technique. Measurement of inherent changes in biological soil properties, therefore could complement microbial indicators for a more sensitive and rapid approach in understudying soil quality, especially the environmentally impacted types.

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