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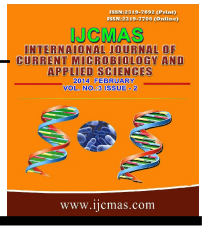

Original Research Article

Evaluation of physicochemical properties, microbial loads and enzymes activity studies of agrochemicals on the Imo-River basin farm in Imo State Nigeria

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A B S T R A C T

The impact of agrochemicals on soil physicochemical, soil microbial population and hydrolytic enzyme activities were evaluated at three different soil depths in the Imo–River Basin farm (IRBF) and compared with soil from a farmland located about 200 meters away from it, which served as the control. Soil physicochemical parameters, hydrolytic enzyme activities and microbial bioload were determined using standard methods. The results obtained showed significant reduction ($P < 0.05$) in electrical conductivity, moisture content and cation exchange capacity. Organic matter, pH, phosphate, sulphate, nitrates and potassium in the test samples increased significantly ($P<0.05$) compared with the control. There was a significant reduction in enzyme activities ($P < 0.05$) of the dehydrogenase, polyphenol oxidase, hydrogen peroxidase, acid phosphatase and alkaline phosphatase, in the test samples compared to control .There was also a significant increase ($P < 0.05$) in urease activity in the test soil especially at the topsoil compared to control. Soil microbial types and population were significantly reduced ($P < 0.05$) in the test soil compared to control. The result shows that agricultural activities affects soil properties adversely and calls for control of some agricultural practices.

K e y w o r d s

Enzymes; bioload; soil; agrochemicals; physico-chemical parameters; microorganism

Introduction

Mineral fertilizers, organic amendments, microbial inoculants, and pesticides are applied to the soil with the ultimate goal of maximizing productivity and economic returns, while side – effects on soil physicochemical properties and soil organisms are often neglected (Zwieten, 2006; Domsch *et al.,* 1984). The soil plays important role in human life, not only as the anchor of all agricultural activities but

also as sink to many wastes, some of which are hazardous (Prescot *et al.*, 2001). Soil needs to be at a minimum temperature and moisture level for active decomposition to occur. Air must be available for microorganisms to respire and decompose the dead organic matter. Over time, dead organic matter is reduced in size and volume, continually keeping the earth's surface clear of dead debris. (Griffiths *et al*., 2001).

Most important nutrient transformations in soil are biochemical reactions mediated by enzymes produced by the resident microbiota. . The use of soil enzyme to determine the extent of perturbation undergone by a soil has proved a successful approach to study the impact of various agricultural inputs on the physicochemical and enzymatic activities of the soil and hence soil fertility (Graham and Haynes, 2005; Griffiths *et al.*, 2001).

The use of agrochemicals especially fertilizers and pesticides have no doubt resulted in improvement in food production and control of diseases, but affects farmlands and domestic water supply sources. In addition to these effects, they also modify the physicochemical properties and microbial diversity of the affected soil (Wyszkowsk and Kucharsk, 2000; Sandrin and Maler, 2003).

Microbial culture techniques have been reported to be deficient in accessing microbial flora of soils because only culturable organisms are assessed while viable non culturable organisms are underestimated (Li *et al*., 2005; Wyskowska and Kucharski, 2000). The use of soil enzyme in the evaluation of soil quality becomes a more valuable tool since culturable and unculturable, even extra-cellular and intracellular enzyme activities are estimated in the process. The enzymes that can be investigated in polluted soils include dehydrogenase, urease, polyphenol oxidase, hydrogen peroxidase, acid and alkaline phosphatase (Oliveira and Pampulha, 2006; Li *et al.*, 2005; Wyszkowska and Kucharski, 2000; Nwaugo *et al.*, 2008).

In a like manner the physicochemical properties of soil that can be investigated in soil samples includes; pH, temperature, exchange acidity, CEC, conductivity, moisture content, organic carbon, organic matter, Sulphate Phosphates, Nitrates and a host of others (Oseni *et al*., 2007; Wyszkowska *et al*., 2001 This work reports the impact of agrochemicals and their agricultural practices on soil microbes and their enzyme activities.

Materials and Methods

Study Area

The study area Emeke-Obibi is in Owerri-North Local Government Area of Imo State. The area is located in the humid tropical forest of South–East Nigeria. The Imo River Basin Demonstration (IRBD), farms cover an area of about 4 kilometres square. Maize, melon, garden egg, vegetable and cassava are the major crops grown in the farms which are divided into sections and two seasons of planting are observed each year. For good yields mineral fertilizer (NPK) and pesticides (Mostly fungicides and insecticides) are constantly being used. These activities have been going on for about 20 years resulting in soil contamination, water pollution and reduction in yields despite the amount of NPK fertilizer applied.

Collection and preparation of Soil Samples for Analysis

The samples were collected eight weeks after the cultivation of the farms. The farms were divided into four quadrants for proper coverage. Soils were sampled at three depth levels from the IRBD farms and a farmland located about 200m away which served as the control. A1, A2 and A3 were labelled as test samples from 0 - 10, 10 - 20 and 20 – 30cm respectively while B1, B2 and B3 were labelled as control samples from 0 -10, 10 - 20 and 20 – 30cm respectively. Samples were randomly obtained from each quadrant, mixed to ensure homogeneity and to prepare one composite sample. Plant material and other debris were removed from the sample by hand picking. Microbial analysis was done on freshly collected samples from the soil solution. Samples for the determination of physicochemical parameters were air dried and crushed with a wooden roller, sieved with 2mm sieve and stored in a soil sac. Some samples for the soil enzyme analysis were equally air dried to a constant weight.

Soil Physicochemical Properties

Temperature and pH of the soil were determined directly at the site using multipurpose tester (Jenway HANNA 1910 model). Soil moisture was carried out using dry to constant weight method as in the publication of the American Public Health Association (ALPHA, 1998). Electrical conductivity was determined using the conductivity meter (1:2 soil/water ratios) (Whitney, 1998). Soil organic carbon and organic matter were estimated according to the method of Osuji and Adesihan (2005) as described by Akubugwo *et al*, (2007). Ammonium saturation method was used in the determination of cation exchange capacity (Dewis and Freitas, 1970). Soil sulphate, phosphate and nitrate were determined using the method described by Dewis and Freitas (1970). Buck scientific Atomic Absorption/Emission spectrophotometer – 205 was employed according to AOAC (2005) in the determination of potassium level.

Soil Enzyme Activities

The soil enzyme assays carried out in this study include dehydrogenase, hydrogen peroxidase, urease, polyphenol oxidase, acid and alkaline phosphatase. The soil dehydrogenase was determined with the method described by Cassida *et al*, (1964) as modified by Li *et al*, (2005). Hydrogen peroxidase activity was estimated using $KMnO_4$ titration method (Alef and Nannipieri 1995). The method described by Tabatabai, (1997) and Tabatabai and Bremner, (1969) was used to determine acid and alkaline phosphatase. Soil polyphenol oxidase was determined using colorimetric method as described by Li *et al*, (2005); modified from Tabatabai and Bremner (1969) and urease was estimated using colorimetric methods (Nannipieri *et al*., 1980; Kandeler and Gerber, 1988).

Estimation of the Soil Bacteria Bioload

Fungi and bacteria were isolated from the soil sample by the standard spread plate technique according to Collins and Lyne, (1980). Serial dilution of each of the soil samples collected was carried out by adding 1g of each of the soil sample into 9ml of sterile normal saline in a flask and shaken very well and finally diluted to 10^{-5}. Exactly 1ml aliquots of the dilutions were spread in triplicates on the surface of plates of sabouraud dextrose agar (SDA),

malt yeast extract peptone agar (MYPA) and nutrient agar (NA) using sterilized bent glass rod.

The cotton wool swabs of the soil solution were also used to inoculate separate plates of NA, SDA, and MYPA by streaking the plates with the swab. Plates of nutrient Agar were incubated for 24 – 48hrs while plates of SDA and MYPA were incubated up to 4 days at room temperature ($30\pm2^{o}C$). Fungal isolates were identified by reference to standard descriptions (Frey *et al*., 1981). Bacterial isolates were also identified with reference to morphological, colonial and biochemical tests (Collins and Lyne, 1980).

The actinomycetes (yeasts) were also isolated from samples using standard dilution plate's procedures as described earlier. The soil samples were first dried at $45^{o}C$ for 2hrs in sterile Petri-dishes before the soil suspension for serial dilutions were prepared. Exactly 1ml aliquots of the dilutions in triplicates were spread over starch casein agar medium incubated at room temperature ($30\pm2^{o}C$) for up to 14 days. Distinction between actinomycetes and bacterial colonies was facilitated by the use of a hand lens. Microscopic and macroscopic features of the colonies were used for the identification of the isolates. Colonies observed were counted using colony counters. Only plates with 30 – 100cfu / ml were used.

Calculation: Bioload = Vol. used x dilution factor.

Statistical Analysis

Statistical analysis was done using analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple Range Test ($P < 0.05$).

Result and Discussion

The physicochemical properties observed in this work are as shown in Table I. The pH values of the Imo River Basin farm soils were significantly ($p<0.05$) higher compared to control. The electrical conductivity (dsm^{-1}) ranged from 0.31–0.74dsm^{-1} in the test soil samples and 0.42-0.68dsm^{-1} in the control with higher values found in the subsoil samples. The moisture content was higher at the topsoil in the control as against the topsoil from the polluted Imo River basin soils and ranged from 18.22% to 26.61% in control group and 13.78% to 18.51% test group. The CEC were 10.51Cmol Kg^{-1}, 10.27Cmol kg^{-1} and 5.69Cmol kg^{-1} in test group as against 16.62Cmol kg^{-1}, 15.25Cmol kg^{-1} and 5.05Cmol kg^{-1} in the control for the three different soil levels. The temperature (^{0}C) observed in the study for both the test samples and control were within 26.4–27.1^{0}C. A significantly ($p<0.05$) high values of nitrate, phosphate, sulphate and potassium were observed in topsoil samples of the Imo River Basin farm soils compared to control.

Soil enzymatic activities of Imo River Basin and control at different soil depth are as shown in table 2. Dehydrogenase, polyphenol oxidase, urease, acid phosphatase and alkaline phosphatase activities significantly ($P<0.05$) increased at the top soil (0-10) compared to the middle (10- 20) and sub soil (20-30) in both test soil samples and control. There was no significant ($P<0.05$) difference in hydrogen peroxidase activity within the different depths of the test soil sample but, a significant ($P<0.05$) increase was observed within the control groups. Generally, all the soil enzyme activities significantly ($P<0.05$) reduced in the test group compared to control except urease

Table.1 Physicochemical properties of test soil and control soil at different soil depths

Soil Depths (cm)	0 – 10 (A1)	0 – 10 (B1)	10 – 20 (A2)	10 – 20 (B2)	20 – 30 (A3)	20 -30 (B3)
pH	8.10 ±0.01c	6.82±0.02a	7.01 ±0.01b	6.62 ± 0.02a	6.75 ± 0.01a	6.73±0.02a
Temperature (^{0}C)	26.4 ±0.08a	26.8 ±0.02b	27.1 ±0.04b	27.1±0.04b	27.5 ±0.08c	26.6±0.06a
Electrical Conductivity (dsm-1)	0.31 ±0.00a	0.42 ± 0.02a	0.46 ± 0.01a	0.52±0.02a,b	0.74 ±0.01b	0.68 ± 0.01b
Moisture Content (%)	13.78±0.0a	26.61±0.02c	14.57±0.1ab	22.72±0.33c	18.50±0.04b	18.22±0.01b
Organic Carbon (%)	2.70 ±0.00d	1.16 ±0.01b	1.71 ± 0.01c	1.12 ± 0.01b	1.41 ± 0.01b	0.75±0.00a
Organic Matter (%)	4.65 ±0.03e	2.00±0.01b	2.95 ±0.01d	1.92± 0.01b	2.44 ± 0.02c	1.29±0.00a
CEC (mol Kg^{-1})	10.51±0.0b	16.62±0.01c	10.27±0.00b	15.25±0.01c	5.69±0.01a	5.05±0.00a
Nitrate (%)	0.97±0.01c	0.83 ± 0.00c	0.50 ± 0.02b	0.54 ± 0.00b	0.19 ± 0.00a	0.16±0.00a
Sulphate (%)	3.45±0.01d	1.49 ± 0.00a	2.63 ± 0.02c	1.62± 0.01a	1.60 ± 0.01a	2.15±0.03b
Phosphate (%)	0.59±0.01c	0.45± 0.00b	0.33 ± 0.01a	0.31 ± 0.01a	0.23 ± 0.01a	0.33± 0.01a
Potassium (PPM)	126.1±0.3c	76.1 ±0.40a	120.6±0.07b	79.8 ± 0.12a	81.7 ± 0.21a	80.7±0.05a

Values are means ± standard deviations, n=3. Values in the same row bearing the different superscript letters are significantly different at ($p<0.05$).

Table.2 Soil enzymatic activities of Imo River Basin and control at different soil depths

Enzymes	0-10 A1	0-10 B1	10-20 A2	10-20 B2	20-30 A3	20-30 B3
Dehydrogenase (mg/g/6h)	2.17 ± 0.01^{a}	4.44 ± 0.00^{d}	3.06 ± 0.02^{b}	3.53 ± 0.01^{c}	3.07 ± 0.01^{b}	3.16 ± 0.02^{b}
Polyphenol oxidase (mg/g/3h)	1.98 ± 0.01^{b}	2.72 0.01^{d}	1.48 ± 0.02^{a}	2.26 ± 0.01^{c}	$1.78\pm0.00^{a,b}$	1.95 ± 0.01^{b}
Hydrogen peroxidase(mL/g/h)	2.00 ± 0.01^{a}	2.62 ± 0.01^{c}	$2..18\pm0.03^{a}$	2.26 ± 0.01^{b}	2.09 ± 0.01^{a}	2.07 ± 0.01^{a}
Urease (mg /g / 5h)	5.37 ± 0.05^{d}	3.45 ± 0.01^{c}	2.96 ± 0.01^{b}	2.92 ± 0.01^{b}	2.49 ± 0.01^{a}	2.43 ± 0.02^{a}
Acid phosphatase (mg/g/h)	1.32 ± 0.01^{b}	1.63 ± 0.00^{c}	$1.04\pm0.01^{a,b}$	1.23 ± 0.02^{b}	0.91 ± 0.01^{a}	1.25 ± 0.02^{b}
Alkaline phosphatase (mg/g/h)	1.50 ± 0.01^{b}	1.85 ± 0.00^{c}	$1.32\pm0.02^{a,b}$	1.44 ± 0.01^{b}	1.20 ± 0.00^{a}	1.42 ± 0.00^{b}

Values are means ± standard deviations, n=3. Values in the same row bearing the different superscript letters are significantly different at ($p<0.05$).

Table.3 Bioload of test soil and control at different soil depths

Location	Number of colonies	Bacteria count g soil (x 10^5 CFU/g)
0-10 A1	2	1.23^{b}
0-10 B1	2	3.60^{e}
10-20 A2	2	1.20^{b}
10-20 B2	1	2.20^{d}
20-30 A3	1	0.59^{a}
20-30 B3	1	1.70^{c}

Values in the same row bearing the different superscript letters are significantly different at ($p<0.05$).

which significantly ($P<0.05$) increased in the test group compared to control.

The bioload of test soil and control at different soil depths are as shown in table 3. The top soil (0-10) were found to have the highest bioload with 1.23×10^5cfu/g in test samples and 3.6×10^5cfu/g in control while sub soil (20-30) have the lowest bioload with 0.59×10^5cfu/g in the test soil and 1.7×10^5cfu/g in the control. Gam positive bacteria were identified in all the depths while gram negative bacteria were only found in 0-10 B1 and 10-20 A2.

Table.4 shows the prevalence of microorganism species isolated from the various depths in both the Imo River Basin farm soils and control. All the organisms have their highest occurrence at the top soil followed by middle soil and sub soil in both the test soil and control. Fewer organisms were isolated in the test soil compared to control.

In this study it was observed that the Imo River Basin soil samples were more alkaline than the control. This may be due to the method of application of the fertilizers (broadcasting method) which leaves lots of it on the topsoil; hence it has the highest pH value of 8.10. Similar finding has also been reported by Han.*et al*., (2002). The similarity in the pH of the same soil depth in both test soil and control implies that the change in the topsoil sample from the Imo River Basin soil may have been caused by the agricultural practices or applied agrochemicals that are localized on the topsoil. The temperatures of the two sites (test and control) were within the temperature range of 26.4 – 27.1^0C which is good for most enzyme and microbial activities (Barross and Morita, 1978).

The electrical conductivity observed from this study showed that it increased in the sub-surface soils both in the test soils and the control. It means that despite the higher pH of the test soil, the amount of the dissolved salts were still low. This can be traced to the method of application of fertilizer or pesticides that resulted in low dissolution of ions on surface soil. There was however no significant difference in the values found in the test soils compared with the control. High electrical conductivity may occur as a result of poor irrigation, water quality, excessive use of fertilizers and contamination from various chemicals or industrial waste (Arias *et al*., 2005; Singer and Munns, 1999). In general electrical conductivity value between 0 and $0.8dsm^{-1}$ are acceptable for general growth of plants (Arias *et al*., 2005).

Moisture content observed in this study was low compared with the control. While it increased with soil depth in the test soils the control follows the reverse. This finding was in agreement with that of Oseni *et al*. (2007) and may be attributed to the constant tillage of the demonstration farm with heavy machine (tractors) which loses the soil particles and encourages erosion and leaching of both water and nutrients from the soil (Filimonova, 1997; Graham and Haynes, 2005). Soil moisture content varies widely in soils and affects microbial populations, their activities and biochemical transformations (Suhani *et al*., 2001). Organic matter content of the test soils was higher than the control. Apart from the topsoil samples (0 – 10cm) others were within the same range in the two sites. Organic matter reflects soil carbon, nutrient availability and substrate for most soil biological activity (Bunning and Jimenez, 2003). Organic matter is an important store for plant nutrient especially nitrogen and phosphorus which

Table.4 Prevalence of microorganism species isolated from the various depth

Microorganisms	**0-10 A1**	**0-10 B1**	**10-20 A2**	**10-20 B2**	**20-30 A3**	**20-30 B3**
Bacillus sp.	4	10	3	8	3	6
Pseudomonas sp.	3	6	3	3	2	3
Azotobacter sp.	2	6	2	5	4	4
Salmonella sp.	0	2	0	1	**	**
Staphylococcus sp.	2	7	2	6	3	3
Lactobacillus sp.	0	5	**	4	2	2
Rhizopus sp.	2	6	4	4	4	3
Mucor sp.	1	4	2	4	0	2
Actinomyces sp.	0	2	0	2	0	0
Thermoactinomyces sp.	0	0	1	2	**	2

0 = Means not isolated, 1 – 10 = Number of times the organism was isolated, ** = not analysed

may be released slowly upon decay (Nielsen and Winding 2002). The higher values in the test samples means that although they were present in the soil, they are not easily released because of reduced level of microbes and their enzymes that carries out their degradation.

James and Riha (1986) reported that soil organic matter is a major source of negative charges in many soils, thus acting as an important cation exchanger. This explained the lower CEC levels observed in the samples from the control soils. Exchangeable cations are available to plant through exchange with H^+ liberated repeatedly may add more nitrogen (nitrates) to the soil than can be used and will eventually result in soil levels that were toxic to both plants, micro-organisms and possibly to animals that consume the feed crops. The sharp decrease at the subsurface samples (20 – 30cm) in the test and control samples were attributable to the fact that nitrates are soluble in water and moves with soil moisture. Some may be lost by leaching in sandy soils. This was the case with Imo River Basin farm by roots. This exchange reaction could be responsible for the retention of freshly introduced cations into soil solution. In this way the CEC gives the soil a buffering capacity, which may slow down the leaching of nutrient cations and positively charged pollutants.

Nitrates and phosphates were higher in the test samples compared with the control with higher values found at the topsoil samples. These high values may be attributed to excessive use of chemical / inorganic fertilizers. The result agrees with the findings of Chen (2008) that applying large amounts of NPK fertilizer per acre soil which is tilled constantly.

Phosphates are usually part of NPK fertilizers and stimulate the growth of plants and hasten maturity (Lowell *et al*., 2008). Although they are essential, its mismanagement poses a threat to water and soil quality. Its high level leads to algae growth which later decreases the available dissolved oxygen as they decay and presents conditions that are very detrimental to fishes in water or

population of soil micro-organisms (Person, 1990). This may have contributed to the decrease in bioload noted in this report.

Higher sulphate concentration found in this work at the topsoil surface of the test farm soil may have resulted from the excessive inorganic fertilizer or pesticides applied and their oxidation or atmospheric decomposition of sulphur compounds contained in them or even those contained in the natural soil that are eventually exposed due to constant tillage. Most sulphur inputs are eventually transformed into sulphuric acid and may acidify soil and water (Mitchell *et al*., 1989).

Higher values of potassium were found at topsoil samples from the test samples when compared with the control. Potassium concentrations in soils should ordinarily increase with soils depth, as in the control (Table 1). This change in order could be attributed to the NPK fertilizers constantly applied on the farms and constant tillage with heavy machinery. Most of this potassium is not available to plants as they do not dissolve but are trapped between layers of clay minerals (Rehm and Schmitt, 2002). Potassium uptakes by plants are affected by several factors which include soil moisture, soil aeration and oxygen level, soil temperature and the tillage system.

This study showed a reduction in the activities of dehydrogenase, polyphenol oxidase, hydrogen peroxidase, acid and alkaline phosphatases in the test soil samples compared to control while, urease activity was observed to be significantly ($P<0.05$) higher in the test soil than the control. Soil dehydrogenase, polyphenol oxidase and hydrogen peroxidase are the most sensitive to pollution, with the highest sensitivity observed in dehydrogenase while, the least affected of the soil microbial enzymes were acid and alkaline phosphatases. Dehydrogenase activity serves as an indicator of the microbiological redox system and may be considered a good measure of microbial oxidative activities in soil (Tabatabai, 1997). Also, dehydrogenase occurs only within living cells, unlike other enzymes which can occur in an extra-cellular state. The results obtained from this work is consistent with the observation made by previous researchers who worked on enzymes in polluted or contaminated soil (Oliveira and Pampulha, 2006; Nwaugo *et al*., 2008; Akubugwo *et al*., 2007).

Hydrogen peroxidase is another key oxidoreductase associated with aerobic microbial activities. It decomposes hydrogen peroxide into molecular oxygen and water, thus alleviating its toxicity to organisms (Daniel *et al*., 1992; Li *et al*. 2005). Polyphenol oxidase is associated with carbon cycle and its presence in soil environment is important to the formation of humic substances. Phosphatases and urease are involved in the biochemical cycles of phosphorus and nitrogen respectively (Matoche *et al*., 1994; Koper *et al*., 2005). There was an increase in the activity of urease at the topsoil of the test soil compared with the control. This could be attributed to the nature or types of fertilizers used which may contain lots of urea or urea containing components that encourages the growth of bacteria with such enzymes. Based on this one can assume that pollution or contamination of soil exhibits an inhibitory effect on soil enzymes which invariably affects soil organisms. In both study locations, the numbers of bacteria colonies were between one or two colonies of both gram-positive and gram negative organisms. However, the control soil at 0 – 10cm

depth had the greatest number of both fungi and bacteria count in both cases. This was supported by several researchers (Nwaugo *et al*., 2008; Oseni *et al*., 2007). The various depths in the two locations showed similar organisms. The observed wider distribution of some organisms in the Imo River Basin Farm soil samples may be due to the method of cultivation adopted.

In all cases, the number of bacteria count were significantly ($P<0.05$) lower in test soil compared to control in the three soil levels. The drastic reduction both in the number and species of the various micro-organisms isolated from the soil samples at various soil depths from the test soil, compared with the control may be attributed to the constant application of agrochemicals and tillage system. This finding was in line with that of Filimonova, (1997) who reported that agricultural practices such as monoculture and pesticides application contribute to a general reduction of species diversity and total population of organism.

In conclusion, agricultural practices such as monoculture and pesticide application contribute to general reduction of soil fertility, species diversity and total organism population. Long tillage history changes the soil environment affecting the number and kinds of soil organism, resulting in erosion that washes off the topsoil, lead to loss of plant residue or organic matter which is food for organisms. The affected soil needs remediation if it will continue to be used for agricultural purposes.

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