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Assessment of physico-chemical properties, bacterial and fungal load of Agrochemical farm Soil in Jahun metropolis, Jigawa State, Nigeria

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Abstract
Soil is the solid material on the earth surface that results from interaction of weathering and biological activity on the parent material or underlying hard rock. This study was aimed at assessment of physico-chemical properties, bacterial and fungal load of Agrochemical farm soil in Jahun metropolis, Jigawa state. In this study, temperature, pH, moisture content, electrical conductivity, organic carbon, heavy metals and elements of the soil were assessed accordingly. Bacterial and fungal load were also determined using pour-plate methods. The temperature ranges between 14 to 30^{O}C. pH value ranges from 6.72 to 7.79, the moisture content ranges from 0.67% to 1.69%. The percentage of organic carbon range from 0.13% to 0.41%, whereas the electric conductivity ranges from 0.04 to 0.05 respectively. Sample from NPK and manure treated farm presented the highest bacterial count of 3.81×10^{7}cfu/g with the lowest in the NPK treated farm of 2.50×10^{6}cfu with no growth in the control river sand, whereas the control showed the highest fungal count of 3.20×10^{5}cfu/g with the least in NPK treated farm. In conclusion this study revealed that inorganic and organic management of the farm soil affect its physicochemical properties, size and activity of microbial population.

Keywords: Physico-chemical, Bacterial load, Fungal load, Agrochemical, Farm Soil

Introduction
Soil is the solid material on the earth surface that results from interaction of weathering and biological activity on the parent material or underlying hard rock. Soil develops as a result of the interplay of five factors; parent materials, climate, organisms, relief and time. Soil plays an important role in human life not only as anchor of all agricultural activities but also sink to many wastes some of which are hazardous. (Prescott *et al*., 2001).

Soil quality is the foundation of productive farming practices. Fertile soil provides essential nutrient to plants. Important physical characteristics of soil-like structures and aggregation allow water and air to infiltrate, roots to explore, and biota to thrive. Diverse and active biological communities help soil resist physical degradation and cycle nutrients at rates to meet plant needs. Soil health and soil quality are terms used interchangeably to describe soil that are not only fertile but also possess adequate physical and biological properties to "sustain productivity, maintain environmental quality and promote plant and animal health" (Doron, 1994) [6].

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).

Soil needs to be at a minimize temperature and moisture level for active decomposition to occur. Air must be available for microorganisms to respire and decompose the dead organic matter. Overtime, dead organic matter is reduced in size and volume, continually keeping the earth surface clear of dead debris (Griffiths *et al*., 2001) [12].

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 microbial diversity of the affected soil (Wyszkowska and Kucharsk, 2000; Sandrin and Maler 2003).

Heavy metals possess a great concern for contamination of soil and water because they are persistent and may affect vegetables, plant and human health. The heavy metal is generally a collective term, which applies to the group of metals and metalloids with atomic density of greater than 4gcm^{-3} or 5 times more-greater than water (Huton and Symon, 1986; Hawkes, 1997) [13]. The heavy metals are widely distributed throughout the environment.

Industrial discharge, fertilizer, mining wastes e.t.c might be some of the major source of heavy metals contamination in soil (Alloyway *et al*., 1988) [2]. When an element enters into the environment it follows some biochemical cycles being transported by air, water and gravity until they reach a geochemical sink. Soil is the ultimate sink for all elements where heavy metals may accumulate in soil with a short span of time (Kabata and Penias 1992) [15].

The use of chemical fertilizer is now widespread for supplementing nutrient. Some phosphoric fertilizers and pesticide are also adding various types of heavy metals like Cd, P, and Zn as impurities (Alloway *et al*., 1988) which after application may significantly increase their content in soil. It is reported that the major source of Pb intake for human being is food where the major absorption take place in gastrointestinal tract (WHO, 1972). It is obvious that heavy metal of contamination level can cause detrimental effect on crop production and health.

Microbial culture technique have been reported to be deficient in accessing microbial flora of soil because only culturable organisms are assessed while viable non culturable organisms are under estimated (Li *et al*., 2005; Wyskowska and Kucharski, 2000) [18].

In a like manner the physiochemical properties of soil that can be investigated in soil sample include; pH, temperature, exchange acidity conductivity, moisture content, organic carbon, organic matter, sulphate phosphates, Nitrates and heavy metals (Osem *et al*., 2007; Whyszkowska *et al*., 2001) Microbial characteristics of soils are being evaluated increasingly as sensitive indicators of soil health because of the clear relationships between microbial diversity, soil and plant quality, and ecosystem sustainability (Doran *et al*., 1994). While understanding Corresponding author, microbial properties such as biomass, activity and diversity are important to scientists in furthering knowledge of the factors contributing to soil health, results of such analyses may also be useful to extension personnel and farmers in diversifying practical measures of soil quality.

Materials and Methods

Determination of Physicochemical Parameters of Soil

Temperature

The temperature of the soil was determined by means of mercury-in-glass thermometer. The thermometer was first shaken and then brought into contact with the collected soil sample (before putting the sample into the flask) and the mercury level was allowed to settle for about two (2) minutes. The temperature of the soil sample was then read are recorded accordingly.

pH

The pH of the soil sample was determined by means of digital laboratory (Jenway, 3150) pH meter, 10g of the soil sample was weighed into 2 different beaker one containing 25ml of distilled water (pH 7.0) another containing 25ml 0.01M of calcium chloride ($CaCl_2$) and shaken using a mechanical shaker for 30 minutes. The suspension were filtered by the use of whatman No 1 filter paper. The filtrate were used for pH determination. The pH meter was switched on and allowed to warm up for about 15 minutes. It was then calibrated with Buffer of pH 4 and 7. After calibration, the electrode was rinsed and cleaned using cotton wool. The electrode was then dipped into a beaker containing the soil filtrate. The pH value of the soil with distilled H_2O and 0.01M calcium chloride was read from the digital screen of the pH meter.

Determination of Moisture Content of the Soil

The moisture content was estimated using the procedure described by Morris (1999) [19] as follows: firstly, a small metal container was weight and record as Wo. Three grams of the soil sample was weighed into the container, and then weighed again as W_I. The soil sample in the metal container was dried in an oven for 24 hours at 110^0C. The dried soil sample in the metal container was weighed until a constant value was obtained and the result was recorded as W_2. The weighed of the undried soil was $W_1 - W_O$, while the weight of the dried was $W_2 - W_O$. Assuming that, $W_1 - W_O = A$, and $W_2 - W_O = B$, then the percentage moisture content on weight basis of the soil represented by C was given by the relationship,

$$C = (\frac{A-B}{A}) \times 100.$$

Determination of Electrical Conductivity

The electrical conductivity of the soil was determined by a means of conductivity bridge, 10g of soil sample was weighed into 100ml polythene tube, 50ml of distilled water was added, the tube was stoppered and shaked on a mechanical shaker for 30 minutes, it was allowed to stand for 1 hour and returned to shaker for 2 hours. The mixture was centrifuged and the supernatant solution was carefully decanted and used to measure the electrical conductivity. The electrical conductivity bridge was switched on and allowed to warm up for about 15 minute, and calibrated using 0.01m KCl. The electrode of the machine was dipped into the tube containing the solution, the electric conductivity value was read from the digital screen of the conductivity meter. (Eno et al., 2009) [8].

Determination of organic Carbon

The organic carbon content of soil was estimated using Wakley – Black method (1934). One gram (1g) of the soil sample was weighed in a 500ml conical flask. 10mlof 1N potassium dichromate solution was added. This was followed by the addition of 20ml conc. sulphuric acid and then swirl by gentle rotation for 1 minute. The mixture was allowed to stand for 30 minutes after which it was diluted to 100ml with deionized water. And 2-3 drops of indicator was added. The whole mixture was titrated with 0.5N ferrous ammonium sulphate solution until the color changes from dull green to maroon colour. A blank titration was carried out in the same manner (except that it excluded the soil sampled. The analysis was runned triplicate and the main value was taken.

$$\% \text{ carbon air – dry soil}) = \frac{Blank\ titre - actual\ 0.3 \times m \times f)}{g\ of\ air\ dry\ soil}$$

Where f = correction factor = 1.33
M = concentration of $FeSO_4$

Determination of Heavy Metal and Other element

Nitrogen in the Soil

Nitrogen in the soil was determined by regular machro-kjeldahl method as follows: 1g of soil sample was weighed into a dry 100ml macro-kjeldahl digestion tube and 2g of $K_2SO_4H_gO$ and 1g of Cu_2SO_4 was added, then 10ml of concentrated H_2SO_4 was added by using automatic pipette

The digestion tube was placed on the digestion block and heated at low heat, the heat was increased until the digest has cleared the mixture was boiled for 3 hours, during which the heat was regulated so that the H_2SO_4 condenser about middle of the way up the neck of the tube. The tube was allowed to cooled and 100ml of water was added slowly to the tube. The digest was carefully transferred into another clean plastic container. All sand particles was retained in the original digestion tube because sand can cause severe bumping during, Kjeldahl distillation. 10ml of $H_3BO_3^-$ 2 – 3 drops of mixed solution was put into 100ml Erlenmeyer flask which is then placed under the condenser of the distillation apparatus 10ml of 10N NaOH solution was poured through distillation flask by opening the funnel stopcock and distillation was commenced immediately the distillation was continued until 50ml distillate was collected, then the NH_4 – N was determined in the distillate by titrating with 0.025N H_2SO_4, and the colour change was observe from green to pink. (end point).

Run a blank similarly but without sample

The percentage of N content in the soil was calculate as follows (Eno, 2009) [8].

$$\%N = \frac{0.014 \times VD \times N \times 100 \times TV}{wt\ of\ soil \times AD}$$

Where VD = vol of digest
N = Normality of acid
TV titre value
AD = aliquot of digest

Phosporus in the Soil

Phosphorus in the soil was determined by Bray No 1 extract method (Bray, 1945). 1g of soil was weighed into a plastic tube and 7ml of P – extract was add (P – extract include Ammoniun fluoride 1N and concentrated hydrochloric acid 0.5N). The suspension was mixed and centrifuge at 2000rpm for 15 minutes. 2ml of the aliquot was pipetted into 25ml V.F, 10ml metric flask of distilled water was added and 4.0ml of reagent B (ascorbic acid, ammonium molybdate, potassium antimonyl tartrate, conc. sulphuric acid) was added into the solutions to reach mark with distilled water and stand for 30 minutes.

Set of reference standard was prepared from the ppm phosphorus (KH_2PO_4) solution. The instrument was set to zero (& Abs) and then set full scale (zero Abs) using the blank solution (P – extraction solution only). The absorbance of standard and sample was measured and recorded at 860nm. A calibration curve graph prepared from the standard data and plot phosphorus concentration against absorbance. The graph was used to determined the phosphorus concentration in the sample solution and available phosphorus was calculated as follows:

$$\frac{P}{P(ppm)} = ppm\ from\ graph \times \frac{VE}{WS}$$

Ppm from graph = $\frac{Absorbance}{Standard}$
VE = volume of flask
WS = weight of sample

Potassium in the Soil

Exchangeable potassium was determined by ammonium acetate method (Reeuwijk, 2002). For the extraction 10g soil sample was weighed into a plastic bottle include two blanks. 100ml of 1m NH_4OAC was added and screened the cap. The bottle was placed into a mechanical shaker and extracted for 2 hours. The sample was centrifuged for 10 minutes at 6, 000rpm. The potassium was extracted. The standard and instrument blank was prepared in one molar NH_4OAC and calculate the K as follows:

$$\text{Potassium PPM gram} = \frac{Absorbance}{standard}$$

$$K = \frac{ppm\ gram \times V.E \times 100}{1000 \times W \times eqult\ of\ element}$$

Where V.E = volume of extraction
DF = Dilution factor
W = Wt of soil used

$$Eq = \frac{mol.}{volume}\frac{39}{1}$$

Sulphate in the Soil

Sulphate was determined in soil using the turbidimeteric method as follows; 5g of soil sample (air-dried, passed 2mm sieve) was weight into rubber bottle and 250ml of extraction solution was added (KH_2PO_4 – 500ppm) and shaked in a mechanical shaker for 30 minutes. The suspension was filtered with Whatman No.1 filter paper. 10ml filtrate was pipetted into 25ml volumetric flask, and distilled water was added to bring the volume to approximately 20ml, 1ml of gelatin – $BaCl_2$ (colour developer) and 4ml of distilled water were added to made up the volume. The mixture was mixed thoroughly and stand for 30 minutes. The Absorbance was read at 420nm within 30 – 60min with spectronic – 70 electric colorimeter the content of the flask was shaken before pouring into the photo test tube. A set of standard sulphate solution containing 0, 2, 4, 6, 8 and 10ppm SO_4 – S per 25ml.

Microbial Analysis of Soil Samples

Enumeration of Bacteria

Using a measuring cylinder 9ml of sterilized distilled water was added to 1g of soil and mixed thoroughly. After 15 minutes of waiting to allow sedimentation of the soil particles. The sample was serially diluted to obtained a range of 10^{-1} – 10^{-6} dilutions. Using a sterile pipette, 1ml aliquot of each dilution was aseptically transferred into each of the correspondingly labeled Petri-dishes, containing (15ml) of prepared nutrient agar. This was carefully and thoroughly mixed by swirling then allowed to solidify. The prepared dishes were inverted then incubated at 35^0C for 24hrs. The Petri-dishes containing 30 – 300 colonies were counted and recorded. Results were recorded and expressed as cfu/g of soil (Benson, 1994)

Enumeration of Fungi

Soil fungi were enumerated by using pour plate method. Using a measuring cylinder 9ml of sterilized distilled water was added to 1g of soil and mixed thoroughly, and allowed to settled down, the sample was serially diluted to obtained a range of 10^{-1} – 10^{-6} dilutions (AHO and Bartha, 1992). Using a sterile pipette one ml (1ml) aliquots of each sample dilutions of 10^{-3} – 10^{-6} were separately and aseptically transferred into each of the correspondingly labeled Petri-dishes, containing 15ml each of prepared potato dextrose agar (PDA), which has been acidified with sulphuric acid to a pH

of 4.8 to suppress the growth of bacteria. This was carefully and thoroughly mixed by swirling then allowed to solidify. The prepared dishes were inserted then incubated at room temperature for three to four days. Total fungal counts were made on any plate showing discrete colonies. Result were recorded and expressed as (cfu/g) of soil (Fawole, 1988; APHA, 1998).

Results and Discussion

The findings of this work showed that agricultural use of soil affect its chemical properties. The changes in these properties were associated with the organic and inorganic fertilizer management of the farm. Soil from organic (manure treated farm) showed an increase in organic carbon content (Table 1) compared to inorganic (NPK fertilizer) treated farm and control, this might be due to the addition of organic amendment as they are the sources of nitrogen and carbon to soils. This agree with the finding from several researchers, Kumar et al. (2000) [17] found that, the organic materials applies alone or in combination with organic fertilizer gave greater residual soil fertility in terms of increase in organic carbon content from 0.36% to as high as 0.61% also (Keng et al., 2005) [16] reported that application of organic manure significantly increased soil carbon content whereas chemical fertilizer had no effect. It also showed that inorganically treated farm showed high content of total nitrogen, available phosphorus and exchangeable potassium compared to control and organically treated farm (Table 1). This result is in accordance to the study done by Perham et al. (2002) where manure treated soil was compared to inorganic fertilizer treated soil in which inorganic fertilizer treated soil showed higher nitrogen content.

Agbenin and Goladi (1997) [1] also reported from their studies that the lower value of total nitrogen in organic treated soil could be as a result of crop intake, immobilization by microorganism and nitrogen loss through volatilization. The higher value of available P and exchangeable K in inorganically treated farm could be due to addition of NPK fertilizer compared to manure, and control soil which increase the content of P and K in inorganic farm.

The study also showed high amount of cadmium and lead in inorganic farm followed by control with the least in manure treated farm, while for nickel the high content is recorded in control soil followed by NPK fertilizer treated farm with the least in manure treated farm, this is true because huge amount of fertilizer are frequently applied to soil in concentrated farming system to deliver suitable N, K and P for crop growth. The complexes used to offer these elements comprise rare quantity of heavy metals (for example cadmium and lead) as contamination, after continual fertilizer application may meaningfully proliferate their quantity in the soil (Jones and Jarvis, 1981) [14].

The soils has pH ranges between 6.28 to 6.98 which indicates suitability of the soil for planting, because a California certified organic farm est. 1980 stated that a pH range outside (6.3 – 6.8) the nutrient in that farm become unavailable and soil biology is suppressed.

The Sulphate content ranges from 10 – 36mg/kg, with the highest concentration of 33.06mg/kg recorded in manure treated farm and the control soil having the least of 10.10mg/kg, this might be due to the addition of manure because a California certified organic farm est. 1980 state that many manures and some composts are significant source of sulphur.

Table 1: Mean Physicochemical Parameters of the Soil Sample

Parameter	NPKF	MF	C(RS)
Temperature	30	27	18
pH	6.28	6.72	6.98
Moisture contents	0.67	1.01	1.69
EC (ds/m)	0.04	0.04	0.05
Organic carbon %	0.15	0.41	0.13
Nitrogen %	0.36	0.14	0.07
Available phosphorus (mg/Kg)	9.70	6.14	7.02
Potassium (mol/kg)	0.39	0.21	0.24
Sulphate (SO4) (mg/kg	32.76	33.06	10.10
Cadmium (mg/kg)	1.82	0.45	0.91
Lead (mg/kg)	0.68	0.43	0.45
Nickel (mg/kg)	0.77	0.43	1.15
Manganese (mg/kg)	2.06	1.03	0.50

Key
NPKF = NPK fertilizer treated farm
MF = Manure treated farm, C(RS) = Control (river sand)

The bacterial and fungal count from this study (Table 2) showed high fungal and bacterial count in the manure treated farm compared to NPK fertilizer treated farm, without a single bacterial count in control soil, which may be due to the addition of organic amendment that might have a large impact on the size and activity of microbial population. Botton et al. (1985) [5] and Ramsey et al. (1986) reported increase in microbial count in response to fertilizer. In general, the bacteria population was higher than fungal population in manure treated farm which strongly agreed with the work of Paharm et al. (2003) reported that cattle manure application promoted the growth of bacteria but not fungi, when compared with the control soil in which the highest fungal count was recorded (Fraser et al., 1994) reported that addition of animal manures provided a significantly greater input of organic carbon which increased bacterial population. Moreover, other researchers have shown that incorporation of organic amendment increased soil microbial activity (Elliott and Lynch, 1994) [7], microbial density (Bruggen – van Semenov, 2004) [4] and density of bacteria (Girvan et al., 2004) [11]. Another possible reason is that manure promoted biological and microbial activities, which accelerated that break down of organic substances in the added manure. That enhances biological activities in the manure. There is need for enlighten the farmers as this will enable them to know the appropriate amount of fertilizers to be added on the soil if necessary needed. The continue application of inorganic fertilizer should be discouraged as it increase metals in the soil.

Table 2: Mean Bacterial and Fungal counts of soil from NPK and Manure used Farm

Site	Bacterial count (cfu/g)	Fungal count (cfu/g)
NPK treated farm	2.50×10^6	3.2×10^4
Manure treated farm	3.81×10^7	2.4×10^5
Control (RS)	Nil	3.20×10^5

Key: NPK= Nitrogen, Phosphorus and Potassium, C(RS)= Control (River Sand)

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