

EFFECT OF DUNG AND NPK ON MICROORGANISM QUANTITY IN ALFISOL SOIL

Hamisu Almu

Department of Soil Science, Kano University of Science and Technology, Wudil
P.M.B 3244, Kano State

ABSTRACT

The DNPk experiment was laid down at samaru for more than 60 years to determine whether yield could be maintained under continuous cultivation, and the best combination of organic and inorganic fertilizers needed to achieve this. The experiment consisted of 4 treatments (Dung, nitrogen, phosphorus, and potassium) each at 3 levels (0, 1 and 2) applied annually in all possible combination. Each combination has one replicates only and there are 81 plots arranged in 9 blocks. The laboratory experiment involves an analytical study of the number of micro-organisms in the soil as influence by organic and inorganic fertilizers. Generally, in the experiment, soil amendments have been to affect microbial numbers to certain extent. Where phosphorus has been continuously applied at 2 and 1 levels, the total microbial counts were observed to be higher than where no amendments have been made. The application of potassium gave effects on total microbial populations.

KEYWORDS: *Dung, microbial counts and NPK*

INTRODUCTION

The soils forms a complex mosaic of micro-habitat within which it is difficult to determine the individual factors which control the population density of the micro-organisms. The quantities of micro-organisms in the soil are so numerous that their population cannot be determined with precision in any given soil sample. As such estimation was accomplished (Burgess and Raw, 1967). A total number of individual species of micro-organisms constitutes the majority of living organisms and by weight, makes up a significant of the earth biomass. The agricultural importance lies in their capacity to break down and transform the organic and inorganic materials of the soil making nutrients available to crops (Skinner, 1975). Soil contains five major groups of micro-organisms: the bacteria, actinomycetes, fungi, algae, and protozoa. However, most important of these are bacteria, fungi, and actinomycetes. Research on the number of micro-organisms in the soil has been conducted in Europe and many parts of the world (Burgess and Row, 1967). The numbers of micro-organisms was found to occur at a depth below the soil surface (2.5-5cm) (Corbet, 1990). He found out that the surface soil was not rich in life, doubtless on account of the sterilizing action of the sun.

Numbers of micro-organisms in profile of surface and buried horizons on mt. Kenya estimated by dilution plate counting were consistently lower than those from soils in different geographical regions (Boyer and Mahaney, 1985). The lower numbers are probably characteristic of the poorly weathered Inceptisols and Entisols usually found in the alpine zone. Bamforth, (1984) reported the number of micro-organisms for desert and semi-desert woodlands in Arizona, U.S.A. According to him, Bacteria numbered 1 to 5 x 10⁸ cells, fungi from 10 to 30cm of mycelium and protozoa from 2 to 10³ cells g⁻¹ dry weight in soils. Higashida and

Takao,(1985) observed the seasonal fluctuation patterns of microbial numbers in the surface soil of grassland soil and noted that bacteria numbers in the 0-2cm soil layer fluctuated with peaks occurring in May and August while fungal spore counts were maximal in August in the 0-2cm soil layer with a concomitant decrease in the hyphal length. He also found out that microbial numbers in the deeper layers were small and constant. Micro-organism plays a vital role in the many processes that occur in the soil. These environmental characteristics in turn affect the composition of the microbial community both qualitatively and quantitatively. It has long been recognized that a relation exist between the micro organisms of the soil and its fertility. The activities of soil micro organisms markedly influence the fertility of the soil and the growth of cultivated and uncultivated plants in the soils. The aims and objectives of this study is to determined the quantity of microorganism under continuous cultivation and the best combination organic and inorganic fertilizers needed to achieve this.

MATERIALS AND METHODS

The experiment was solely a laboratory research. It involved microbiological studies of soils and routine soils analysis. The work was conducted in the soil microbiology laboratory of Department of soil science, Faculty of Agriculture, Ahmadu Bello University, Zaria. (Dilution plate method is used in the study:

Experimental site

Site Description. The trial was conducted at the research farm of the Institute of Agricultural research (IAR) Samaru Zaria. Samaru is located at latitude 11° 11 'N and longitude 7°38'E in the northern guinea savanna of Nigeria at an altitude of 686 meter above sea level. The soils are developed from drift sand overlaying basement complex (Klinkenberg and Higgins, 1968) and classified as Alfisol (Awujoola, 1979, IAR, 1987).

Long term average annual rainfall is 1060 mm confined to between May and October. Global radiation is evenly distributed throughout the year, ranging from 440 cal cm⁻² day⁻¹ August to 550 cal cm⁻² day⁻¹ in April and May (Kowal, 1972).

The DNPk experiment was laid down at Samaru for many years to determine the quantity of microorganism under continuous cultivation, and the best combination of organic and inorganic fertilizers needed to achieve this.

METHODOLOGY

In this experiment four treatments (Dung, Nitrogen, Phosphorus, and potassium) each at three levels (0, 1, 2) were applied annually in all possible combinations. Each combination has one replicate only and there are 3 x 3 x 3 x 3 or 81 plots arranged in 9 blocks (a 3⁴ factorial design with one replicate). Each plot is about 0.25ha. The experimental field has been cropped since 1970 with a rotational cropping system involving cereals, fibres and legumes.

The rate fertilizers and manures treatments during the experimental period (1970-2005) are presented in Table 1. In all cases level rates exactly half of level of level 2, while level 0 is nil.

Until 1989, the source of nitrogen fertilizer was ammonium nitrate (CAN) due to increased in the acidity of the soil. Before 1991, fertilizer rates were lower but rates of dung have remained unchanged. As from 1997 the phosphate fertilizer was half that of 1991 to correspond to the officials AERLS ext. Bull. 11, 1993. Individual's plots have received the same treatment combinations annually from 1970 to the present. All crops residues and weeds etc are removed from the field each year after the harvest of experiment and burnt in nearby trenches.

Also the spraying of the trace elements Zn, Mo, B, and Cu was carried out in 1987 and in the same cropping season, lime (CaCO₃) was applied.

In 1981 and 1982 four sub-treatments were super-imposed these are:

- (1) Sub-soiling to depth of 40cm + liming 2.5tons/ha.
- (2) Sub-soiling only to a depth of 40cm.
- (3) Liming only at the same rate as (1)
- (4) No sub-soiling and liming

Cotton was planted in 2005, cropping season before soil sampling was carried out.

Table 1 : Rate of fertilizer /manuring treatments used during the experimental period 1970-2005 (kg/ha)

Fertilizer element and manure	Treatment level			Year (s)
Dung (FYM)	0	2500	5000	1970-2005
Nitrogen (N)	0	24	48	1970-2005
	0	67.5	135	1992-2005
	0	33.5	67	2002
Phosphorus (p)	0	9	18	1970-2005
	0	27	54	1992-1998
Potassium (k)	0	13.5	27	1999-2005
	0	14.5	29	1970-2001
	0	29	58	2002-2005

Soil sample

Top soils (0-15cm) were sampled from nine different plots under study having DNPk application ratio as shown in table below:-

Table 2: DNPk Application Ratio

Plot	Dung	Nitrogen	Phosphorus	Potassium
1	0	2	0	0
2	0	0	0	0
3	0	1	0	0
4	0	0	2	0
5	1	0	0	0
6	0	0	0	1
7	2	0	0	0
8	0	0	0	2
9	0	0	1	0

During sampling, large pieces of plant materials and other impurities were hand picked. The collected soil samples were then put in polyethene bags and stored for subsequent analysis.

Sterilization of Bottles, Water, Petri-dishes, and Pipettes

This step was carried out in order to eliminate all micro-organisms that might cause contamination.

The water sterilization was done by putting the water in a large glass bottle and then taken into an autoclave and the pressure of the autoclave

adjusted to 15 pounds per square inches for minutes to allow for pressure development. The sterilization of the Petri-dishes, bottles, and pipettes was done by enclosing the Petri-dishes in a sterilized can taken to an oven together with the bottles. The temperature of the oven was adjusted to 160°C and left for one and half hours.

Preparation of Media

The medium for total microbial counts was prepared by mixing appropriately the following materials.

Materials	Amount
Dextrose (glucose)	1.0g
Potassium nitrate	0.5g
Di-potassium hydrogen phosphate	0.2g
Magnesium sulphate	0.2g
Calcium chloride	0.1g
Sodium chloride	0.1g
Ferric citrate	0.1g
Yeast Extract agar	15.0

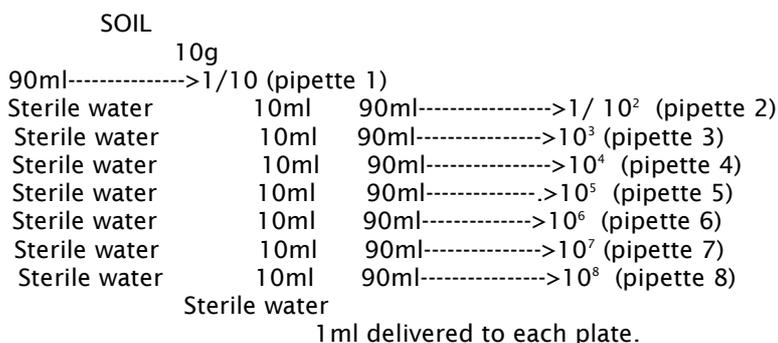
One litre of water was added. But about 500ml of the water was added first then the flask together with the media was placed on a hot plate until all the bubbles started to appear after which the remaining 500ml of water was added to make up to volume (one litre). The whole flask was then returned to the hot plate. The suspension was constantly stirred to ensure through mixing of the materials and the mixture was left on the hot plate to boil.

After the mixture has started boiling it was removed from the hot plate, allowed to cool a little and then put into the autoclave for sterilization at 15 pounds per square inches for 15 minutes.

Procedure for Dilution and Total microbial Counts

Dilution Procedure

10g of each of the soil sample was weighed and suspended in 90ml. of sterile water to obtain a dilution of 1:10. This suspension was shaken vigorously for about five minutes. The coarse particles were allowed to settle and 10ml was withdrawn aseptically with a sterile pipette and then discharged in to the second dilution bottle containing 90ml of sterile water. This second suspension comprised dilution 10². The dilution and plate inoculation steps were completed according to the following diagram.



Total Microbial Counts Procedure

For each soil sample, eight different dilutions were set as above i.e. 10¹ - 10⁸. Each dilution was assigned to five Petri-dishes. One millilitre of each soil dilution was delivered by a separate sterile pipette into the five Petri-dishes and 15ml of the prepared media was added to each Petri-dishes.

A set of control was provided in this procedure, 1ml of sterile water and 15ml of the

media were poured into five Petri-dishes. This is to enable us check the sterility of the media. After all the plate inoculation steps had been accomplished, the plates were incubated in the inverted position for 8 days and the temperature of 28°C. After incubation, plates with well distributed colonies were selected and counted and the number of micro-organisms per gram of soil was calculated using the expression below:-

$$\text{Number of microbes/g of soil} = \frac{\text{Average count} \times \text{dilution factor}}{\text{Dry weight of a gram of soil}}$$

For total microbial count, the dilution factor used was 10⁸

RESULTS

Table 4: Multiple range tests for total microbial counts in soil

Soil	Counts	Means
9	4	1.03 x 10 ⁸ a
6	4	5.5 x 10 ⁷ b
4	4	4.83 x 10 ⁷ bc
7	4	4.20 x 10 ⁷ bc
1	4	4.13 x 10 ⁷ bc
2	4	3.65 x 10 ⁷ c
5	4	1.85 x 10 ⁷ d
3	4	1.75 x 10 ⁷ d
8	4	1.50 x 10 ⁷ d

- (1) Methods: 95 percent LSD Intervals
- (2) Soil means followed by similar letters are not significantly different at 5 percent probability level by Duncan's multiple range test (D.M.R.T.).

RESULTS AND DISCUSSIONS

Effect of Dung N.P.K on the Total Microbial Counts in Soil

The mean total microbial counts recorded are presented in table 4. The results indicated that there was a highly significant difference amongst the treatments. This was in agreement with findings of Marshal, 1997 who observed application of fertilizers and manures changed the population dynamics of soil micro-organisms so that some species were favoured whereas others were affected adversely.

The highest number of microbial counts was recorded for soil 9, which was significantly different from numbers in any other soil. On the other hand microbial numbers recorded for soil 6, 4, and 1 were not significantly different from each other. Similar differences amongst soil 4, 7, 1, and 2 were not significant statistically but there were different from those of 5, 3 and 8 which were statistically the same. Although micro-organisms behave differently, manures and fertilizers increase microbial population to certain extent. However, the magnitude of response varies with different organisms and the type of amendments (Tsao, 1999). Thalenhorst (1982) observed that single formulations particularly of N and Ca increased numbers of micro-organisms. However, the highest rate of increase was from complete and mixed fertilizers, especially when Ca accompanied these fertilizers (Muller, 1985). The application of some amendments like Sulphur and gypsum were shown to favour greater number of micro-organisms (Kevan, 1982). As shown in table 4, soil 9 has been receiving one level of P (table 2) yearly and recorded the highest microbial numbers. Hence, it might be said that continuous application of moderate

levels of single super phosphate fertilizers has a positive effect on the numbers of micro-organisms in the soil. However, this contradicts the results Hryniuk, (1982) who observed that continual use monocalcium phosphate, super phosphate with sodium nitrate and $(\text{NH}_4^+)_2\text{SO}_4$ depressed soil micro-organism. The soils were generally acidic (table 3) which has depressing effect on the growth of microbes especially bacteria (Myskow and Zieba, 2001). They showed that that fertilization with N.P.K, particularly N in the forms of $(\text{NH}_4^+)_2\text{SO}_4$, resulted in the acidification of the soil, strong development of bacteria and actinomycetes

SUMMARY AND CONCLUSIONS

Generally, in this study manures and fertilizers affects microbial numbers to certain extent. Among the fertilizers, Phosphorus and Nitrogen proved more important than Potassium. The application of phosphorus relatively increased the numbers of micro-organisms in the soil. The numbers of fungi and actinomycetes in the soil upon fertilization gave no definite trend. Soil micro-organisms are seen as producers of soil fertility through the decomposition of organic matter, producing humus, nitrates, phosphorus and compound of Ca, K and other elements great importance. However, the application of fertilizers and manures to the soil strongly affects microbial decomposition. Some conclusion can be drawn from the study:-

Although certain formulations may adversely affect a specific group of micro-organisms, manures and fertilizers generally increase numbers and species of micro-organisms. Continuous application of Dung to the soil is not advocated since it produced no marked effect on the microbial organisms.

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