

## Microbial Oxidation of Elemental Sulphur as a Means of Increasing Sulphate Availability and Combating Alkalinity in Soils

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**Abstract:** Microbial oxidation of elemental sulphur was studied using three different soils and three different organic materials in order to assess sulphate production. The three soils were either amended with only elemental sulphur or with elemental sulphur and one of the organic materials at any of two different rates (5% and 20%). The experiment was laid out in a 3 x 3 x 8 Factorial in Completely Randomized Design and the treatments were replicated three times. They were brought to their field capacities and incubated at room temperature. At the end of each incubation period, a set of the treatment was sampled and analysed for the amount of sulphate formed. The change in pH in the various samples was also measured. The net sulphate formed increased significantly ( $P \leq 0.05$ ) with increases in incubation period in each of the three soils. The treatments amended with organic materials significantly produced more sulphate with time compared with the treatments without organic amendments. Higher rates (20%) of each organic material were significantly better than their lower rates (5%) with the plantain peels treatments producing the highest amounts of sulphates than the other organic materials in all the soils. Loam soil was significantly better than the other two soils in terms of sulphate formation with time while sandy soil produced the least amount of sulphate with time. The change in pH decreased significantly ( $P \leq 0.05$ ) with increases in incubation period for all the treatments. Loam soil samples showed the greatest amounts of pH reduction with time. Soils amended with organic materials also showed higher amounts of pH reduction than the soils without additional organic materials. Microbial oxidation of elemental sulphur using appropriate organic materials such as plantain peels is therefore recommended as a means of ameliorating sulphur deficiency and alkalinity in soils.

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### 1. Introduction

Sulphur is one of the essential elements needed for the proper growth and development of plants. It is immobile in plants and being a constituent of proteins, it is vital for the synthesis of the sulphur-containing amino acids - cysteine, cystine and methionine. The amino acids are indicators of the protein quality of plant produce. Leguminous crops and crops like cabbage and onion that have distinctive flavour and aroma require large amounts of sulphur since it is an essential element in aromatic oils (Vidyalakshmi *et al.*, 2009). In addition, elemental sulphur (sulphur in its simplest form) had long been found to control a disease of potato called potato scab caused by *Streptomyces scabies*. It does this by lowering the soil pH thus making the environment unfavourable for the growth of the organism (Hooker and Kent, 1950; Vlitos and Hooker, 1951).

Sulphur is usually found in small quantities in soils. In areas close to industrial centres, sufficient sulphur to nourish crops is brought down from the atmosphere by rain and snow (Millar *et al.*, 1962). Hence, deficiency of sulphur is rare in such areas. However, in countries where bush burning is common, for instance in the savannah belts of Nigeria

and Ghana sulphur deficiency has been recorded (Enwezor, 1976; Kang *et al.*, 1981; Acquaye and Beringer, 1989). Therefore to ameliorate this condition, inorganic fertilizer such as ammonium sulphate, single super phosphate, elemental sulphur and several other fertilizers and fertilizer ingredients containing sulphur are added to the soil. Soil dressing with organic manures that contain appreciable quantities of sulphur originating from the sulphur-containing amino acids of animal and vegetable proteins is also a common practice.

Studies have shown that the existence of nutrient elements in the soil in their right form and proportion is more important than the mere presence of these elements in the soil (Anyanwu *et al.*, 1969). Plants do not directly make use of sulphur. Thus, it must be oxidized to sulphate in order to provide plant-available sulphur. Oxidation of sulphur or sulphides is carried out by a number of gram negative bacteria of the genera *Thiobacillus*, *Thiomicrospora*, *Thiosphaera* and *Sulfolobus*. However, other heterotrophs like *Paracoccus*, *Xanthobacter*, *Alcaligenes* and *Pseudomonas* can also oxidize sulphur (Kuenen and Beudeker 1982; Vidyalakshmi *et al.*, 2009). All these bacteria produce sulphuric acid (hydrogen ions and

sulphate ions) as a metabolic product (Trudinger, 1971). The most significant of these bacteria is of the genus *Thiobacillus* of which *Thiobacillus thiooxidans* is considered the most significant species (Swaby and Fedel, 1973). Organic materials especially green manures when added to soils increase the sulphur oxidation process as they serve as the major source of energy for the heterotrophic micro-organisms present in soils (Tisdale and Nelson, 1966; Brady, 1974; Wainwright *et al.*, 1986).

The oxidation of elemental sulphur to sulphate results in acidification of the soil. The acid produced from the oxidation process helps in reducing the alkalinity of alkaline soils (Cox and Koenig, 2010). This favours the growth and development of certain acid tolerant crops such as rice (Chapman, 1980; Moore *et al.*, 1994; Slaton *et al.*, 1998; Slaton *et al.*, 2001). Other crops like cashew and tobacco that grow well on acid soils may also be favoured by this acidification process.

Earlier studies outlined above, indicate that the microbial oxidation of elemental sulphur in soils is of great importance since it not only enables the plants to make use of sulphur in the right form and proportion but it also corrects soil alkalinity. However, comparative studies of textural variations, plantain and yam peels as well as spear grass are rare in literature. Therefore, the objectives of the study were to assess sulphate production in sandy and loamy soils amended with three organic materials and also to determine the effect of this production on soil pH.

## 2. Materials and Methods

The soil types used in this study were loam, sandy clay loam and sandy soils. The loam and sandy clay loam soils were obtained from two different farm lands at the University of Nigeria, Nsukka (UNN). The sandy soil was obtained from another location but also at UNN. Soil survey had earlier been conducted in these areas in order to ascertain the various soil types that were present before the soil samples were collected. These soils have been classified as Ultisols (Igwe, 2001). They were collected at a depth of 0 – 20cm, air dried and passed through a 2mm sieve.

The organic materials used in this experiment were plantain (*Musa paradisiaca*) peels, yam (*Dioscorea rotundata*) peels and spear grass (*Imperata cylindrica*). Plantain peels and yam peels were obtained from New Point Restaurant, UNN. Spear grass was collected from a land belonging to Veterinary Faculty, UNN. These materials were selected because of their availability in the study area. They were air dried and cut into small pieces of about 5.0mm each.

Elemental sulphur was sourced from Department of Soil Science, UNN. In its pure form, elemental

sulphur is a yellow, inert, water-insoluble crystalline solid. It was crushed and passed through a 2mm sieve.

### 2.1 Incubation Technique

Each of 216 plastic beakers had 30g of the appropriate soil sample, 3g of elemental sulphur (excluding the control) and 5% or 20% of one of the organic materials (excluding the control) by weight. Three incubation periods (15, 30 and 60 days) were then applied. The treatments are defined as follows:

T<sub>1</sub>: S (S = sandy soil, loam soil or sandy clay loam)

T<sub>2</sub>: S + S<sup>o</sup> (S<sup>o</sup> = elemental sulphur)

T<sub>3</sub>: S + S<sup>o</sup> + 5% PP (PP = plantain peels)

T<sub>4</sub>: S + S<sup>o</sup> + 20% PP

T<sub>5</sub>: S + S<sup>o</sup> + 5% YP (YP = yam peels)

T<sub>6</sub>: S + S<sup>o</sup> + 20% YP

T<sub>7</sub>: S + S<sup>o</sup> + 5% SG (SG = spear grass)

T<sub>8</sub>: S + S<sup>o</sup> + 20% SG

Adequate water was added to bring the soils to their respective field capacities. The beakers were then covered with aluminium foil to minimize loss of water by evaporation and also to ensure aeration (since the aluminium foil was not air tight). The treatments were arranged in a 3 x 3 x 8 factorial in completely randomized design (CRD), replicated three times and incubated at room temperature. The water content was maintained with deionized water at weekly intervals. At the end of each incubation period, 72 treatments (24 from each soil) were analysed.

### 2.2 Methods of Soil and Plant Analyses

The three soils used for this experiment were analysed before they were incubated. The parameters determined include: particle size composition (Bouyoucos, 1962), pH (Juo, 1979), organic carbon following Walkley and Black dichromate (wet) oxidation method (Nelson and Sommers, 1982), total nitrogen by the Kjeldahl method (Bremner, 1965), available phosphorus using Bray II method (Olsen and Sommers, 1982), calcium and magnesium by the complexometric titration method (Jackson, 1958), potassium by flame photometer, total sulphur using Morgan solution as the extracting solution and iron (Alloway, 1995). The relative sulphate was determined from the total sulphur in the soils. The organic carbon was converted to organic matter by multiplying with the factor 1.724.

At the end of each incubation period, the change in pH in the treatments was determined using the method described by Juo (1979). The net sulphate formed was also determined using the method described by Rehm and Caldwell (1968); the values obtained (in ml) were

converted to  $\text{tha}^{-1}$  based on the assumption that 1ha of soil weighs  $2.25 \times 10^3 \text{t}$ .

The organic materials used for this experiment were analysed for their chemical properties based on the procedure described by (Juo) 1979.

### 2.3 Statistical Analyses

GENSTAT Statistical Package was used for all the data analyses. The net sulphate formed with time in all the treatments together with the change in pH were analysed using a  $3 \times 3 \times 8$  Factorial in Completely Randomized Design (CRD). Fisher's Least Significance Difference (F-LSD) was used in detecting the difference between means (Obi, 2001).

### 3. Results and Discussion

Tables 1 and 2 show the results of the initial soil properties and chemical properties of the various organic materials respectively. Table 1 shows that loam soil was more acidic than the other two soils. It also had the highest amounts of organic matter, nitrogen, potassium, sulphur and iron. Sandy clay loam contained more phosphorus than the other soils, while sandy soil had the highest pH value and it also contained greater amounts of calcium and magnesium than loam and sandy clay loam soils. Plantain peels had greater percentages of carbon, nitrogen, phosphorus and sulphur than the other organic amendments (Table 2). Yam peels had the greatest amount of potassium, while, spear grass was the highest in calcium and magnesium.

**Table 1:** Some soil physicochemical properties

TC	Particle Size Composition					pH		OM	N	P	K	Ca	Mg	S	Fe
	CS	FS	TS	Silt	Clay	H <sub>2</sub> O	KCl								
	← % →							%		← mg/kg →					
LS	32.9	22.1	55.0	29.0	16.0	4.5	3.3	1.42	0.04	0.66	0.51	4.00	6.00	0.39	3.36
SCL	36.3	23.7	60.0	28.0	22.0	4.6	3.4	0.94	0.03	3.73	0.31	6.00	6.00	0.21	1.68
SS	52.1	37.1	89.0	2.8	8.0	5.9	5.4	0.20	0.01	0.93	0.25	8.00	8.00	0.11	0.56

TC = textural class, CS = coarse sand, FS = fine sand, TS = total sand, OM = organic matter, LS = Loam soil, SCL = sandy clay loam, SS = sandy soil.

**Table 2:** Chemical properties of organic materials

Organic Material	C	N	P	K	Ca	Mg	S
	← % dry weight →						
PP	25.0	1.10	0.21	0.60	0.60	0.70	0.21
YP	23.0	0.91	0.08	0.61	0.60	0.83	0.18
SG	21.0	0.71	0.06	0.52	0.80	0.86	0.16

PP = plantain peels, YP = yam peels, SG = spear grass.

Table 3 shows that the effects of the different treatments and their interactions were significant ( $P \leq 0.05$ ) on the net sulphate formed. The treatments that had organic materials ( $T_3 - T_8$ ) produced more sulphate than the treatments without organic materials ( $T_1$  and  $T_2$ ). This is not surprising because according to Brady (1974), organic matter/material is the principal energy source for soil micro-organisms; biochemical activities would be impossible without them. Hence, the added organic materials favoured the heterotrophic micro-organisms involved in sulphur oxidation, thus increasing the net sulphate formed in these treatments. The degree of their effects were in the increasing order of  $T_4 > T_3 > T_6 > T_8 > T_5 > T_7$ . This shows that the different organic materials (at their different rates) affected sulphur oxidation in distinctive ways. It is obvious from the study that the higher rates (20%) of the organic amendments produced more sulphate than

the lower rates (5%). The plantain peel treatments were significantly better than those of yam and spear grass. The reason for its increased efficiency may have been because it contained more sulphur than the other organic materials (Table 2). According to Tisdale and Nelson (1966), mineralization of sulphur from different organic materials depends on the sulphur content of the material; larger amounts of sulphates are released from materials with higher sulphur content. Therefore, more sulphur from the plantain peels may have been oxidized to sulphate.

Nitrogen rich materials decompose faster than materials that are not so rich in nitrogen (Allison, 1973; Barbarick, 2006). In fact, organic materials that are low in nitrogen are capable of inducing nitrogen deficiency in plants (Barbarick, 2006). Therefore, plantain peels which had the highest percentage of nitrogen decomposed faster than the other amendments whose

nitrogen values were lower. Thus, it hastened the formation of sulphate in treatments T<sub>3</sub> and T<sub>4</sub>.

Cellulose and lignin tend to slow down decomposition process in plant materials (Alexander, 1977). It is assumed in this study that plantain peels may have contained smaller amounts of cellulose and lignin than the other organic materials especially spear grass. Thus, it decomposed faster than others and hence, treatments amended with it produced the highest amounts of sulphate with time.

In the treatments without additional organic materials, T<sub>2</sub> was significantly better than T<sub>1</sub>. This was probably because of the additional sulphur source (elemental sulphur) in T<sub>2</sub> which was absent in T<sub>1</sub>.

The effects of soil types on sulphate production (Table 3) indicate that loam soil was significantly the best followed by sandy clay loam and then sandy soil. The reason for its increased efficiency may be linked to its lowest pH value (Table 1). According to Tisdale and Nelson (1966), oxidation of added sulphur occurs at a faster rate in soils that are acidic. Another reason may have been because of its organic matter content. It had the highest amount of organic matter compared with the other two soils. Organic matter is known to aid sulphur oxidation in soils (Solberg *et al.*, 1982; Wainwright *et al.*, 1986).

The increase in incubation period had a significant effect ( $P \leq 0.05$ ) on the net sulphate formed. The relative increase in the amount of sulphate formed in the 3 soils after 15, 30 and 60 days were more than 320 times, 390 times and 570 times the original values in the soil (Table 3). These high values indicate the high efficiency of microbial oxidation of elemental sulphur when those organic materials are used as soil amendments. Day 60 showed the highest amount of sulphate formation. This was probably because the increased incubation period allowed more of the added organic material to decompose. It is this decomposed material that the heterotrophic organisms involved in sulphur oxidation need for their development (Kuenen and Beudeker, 1982; Deshpande, 2010).

The results on Table 4 show that the effects of the different treatments and their interactions were significant ( $P \leq 0.05$ ) on the change in pH. The treatments with organic amendments had lower pH values than those without organic amendments. This was probably because the organic materials aided the oxidation process, thus, producing more sulphuric acid and hence significantly ( $P \leq 0.05$ ) reducing the soil pH. Higher rates of each organic material gave rise to lower pH values and the plantain peel amendments produced more sulphuric acid (had the lowest pH values) than the soils amended with the other organic materials. It is important to note that the decomposition of the organic materials produced hydrogen ions that may have also contributed to reduction of the soil pH.

Loam soil showed the greatest amount of pH reduction compared with the other soils. This could be attributed to its initial pH value; it had the lowest pH value prior to incubation (Table 1).

The pH also decreased significantly ( $P \leq 0.05$ ) with increases in incubation period. This was probably because the increase in incubation period allowed more of the sulphuric acid produced during sulphur oxidation to accumulate and thus, this lowered the soil pH accordingly.

**Table 3:** Mean effects of treatments, soil types and incubation period on the net sulphate formed

Treatment	Mean Net Sulphate (tha <sup>-1</sup> )
T <sub>1</sub> (S)	8.06
T <sub>2</sub> (S + S <sup>o</sup> )	9.73
T <sub>3</sub> (S + S <sup>o</sup> + 5%PP)	15.85
T <sub>4</sub> (S + S <sup>o</sup> + 20%PP)	16.98
T <sub>5</sub> (S + S <sup>o</sup> + 5%YP)	13.45
T <sub>6</sub> (S + S <sup>o</sup> + 20%YP)	14.46
T <sub>7</sub> (S + S <sup>o</sup> + 5%SG)	12.08
T <sub>8</sub> (S + S <sup>o</sup> + 20%SG)	13.56
F-LSD <sub>(0.05)</sub>	0.01
Soil Type	
Sandy Soil	10.72
Loam Soil	14.39
Sandy Clay Loam	13.95
F- LSD <sub>(0.05)</sub>	0.01
Incubation Period	
Day 1(before incubation)	0.03
Day 15	9.86
Day 30	11.98
Day 60	17.22
F-LSD <sub>(0.05)</sub>	0.01
F- LSD <sub>(0.05)</sub>	0.01
T x S	0.02
T x P	0.02
S x P	0.01
T x S x P	0.04

T = treatment, S = soil type, S<sup>o</sup> = elemental sulphur, PP = plantain peels, YP = yam peels, SG = spear grass, P = incubation period.

**Table 4:** Mean effects of treatments, soil types and incubation period on the change in pH

Treatment	Mean Net Sulphate (tha <sup>-1</sup> )
T <sub>1</sub> (S)	4.5
T <sub>2</sub> (S + S <sup>o</sup> )	4.3
T <sub>3</sub> (S + S <sup>o</sup> + 5%PP)	3.5
T <sub>4</sub> (S + S <sup>o</sup> + 20%PP)	3.2
T <sub>5</sub> (S + S <sup>o</sup> + 5%YP)	3.9
T <sub>6</sub> (S + S <sup>o</sup> + 20%YP)	3.5
T <sub>7</sub> (S + S <sup>o</sup> + 5%SG)	3.9
T <sub>8</sub> (S + S <sup>o</sup> + 20%SG)	3.7
F-LSD <sub>(0.05)</sub>	0.1
<b>Soil Type</b>	
Sandy Soil	4.3
Loam Soil	3.5
Sandy Clay Loam	3.6
F- LSD <sub>(0.05)</sub>	0.1
<b>Incubation Period</b>	
Day 15	4.2
Day 30	4.0
Day 60	3.2
F-LSD <sub>(0.05)</sub>	0.1
F- LSD <sub>(0.05)</sub>	0.1
T x S	0.3
T x P	0.3
S x P	0.2
T x S x P	0.4

T = treatment, S = soil type, S<sup>o</sup> = elemental sulphur, PP = plantain peels, YP = yam peels, SG = spear grass, P = incubation period.

#### 4. Conclusion

From the study, increase in organic matter application and incubation period enhanced sulphur oxidation which resulted in the formation of more sulphate. Thus, they enhanced the efficiency of sulphate availability in the three soils used for this study. Based on the above findings, when a large amount of sulphate is required by a plant, the incubation period of organic materials need to be long. This study also shows that loamy soils give better conditions for sulphur oxidation than sandy soils. Plantain peel was found to aid sulphur oxidation better than yam peel and spear grass. It was also found that elemental sulphur could be added to soils in order to remediate soil alkalinity, but, this should be done with caution to avoid excessive acidification.

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