Detection of some minerals from natural wastes in modified soil

Fariha A. Alhijjaji

Department of biology science, Libyan academy, Misrata, Libya
E-mail: farihaalhijjaji18@gmail.com

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Abstract

Natural wastes such as keratin or seaweed wastes, affect soil's microbiological and mineral properties. Soils that are exposed to natural waste have higher populations of bacteria and other biological communities, as well as are richer in minerals than those which are not exposed to natural contaminants. In this research, experiments aimed to measuring nitrate, sulphate and ammonium in two types of soil (e.g. amended with keratin and seaweed) and comparing them with an agricultural soil. chemical production of minerals was investigated. The results showed that a Significant differences were observed over the entire period between modified soil and unmodified soil. These results suggest that seaweed and keratinous substrate can be favorable substrates for microorganisms and that these wastes undergoe microbial degradation in the soil leading to the release of organic substrates which are then returned to the ecosystem.

Introduction

Soil has a high bacterial population around the roots of plants (i.e. the rhizosphere), which provides the necessary nutrition for them. Also, the plant helps to provide a suitable micro-climate to increase bacterial population grows attached to or close to plant roots (Paul and Clark, 1989). Moreover, plant roots increase the nitrogen content in the soil by fixing atmospheric nitrogen. Soil microorganisms take part in the cycling of elements such as carbon cycle and cycles of sulphur, phosphorus and iron etc. (Fitter *et al.*, 2005).

Nitrogen cycle

The N-cycle involves the oxidation and reduction of a variety of different forms of nitrogen including:

Nitrogen (N): is an essential component of DNA, RNA, and proteins, the building blocks of life. All organisms require nitrogen to live and grow. Although the majority of the air we breathe is N2, most of the nitrogen in the atmosphere is unavailable for use by organisms. This is because of the strong triple bond between the N atoms in N2 molecules which makes it relatively inert. In fact, in order for plants and animals to be able to use nitrogen, N2 gas must first be converted to more a chemically available form, such as ammonium (NH4+), nitrate (NO3-), or organic nitrogen (e.g. urea - (NH2) 2CO). The inert nature of N2 means that biologically available nitrogen is often in short supply in natural ecosystems, limiting plant growth and biomass accumulation. Five main processes cycle nitrogen through the biosphere, the atmosphere, and the geosphere: nitrogen fixation, nitrogen uptake (organismal growth), nitrogen mineralisation (decay), nitrification, and denitrification. Microorganisms, particularly bacteria, play major roles in all of the principal nitrogen transformations (Harrison, 2003).

Ammonification N- mineralisation

Ammonification is the transformation of organic nitrogenous compounds to inorganic forms. This process is driven by a wide variety of microorganisms (bacteria, actinomycetes, fungi). In soils, some organic nitrogenous compounds become resistant to biodegradation because

they form complexes with either phenols and polyphenols, or both. Proteins are mineralised to NH4 according to the following sequence:

proteins ⟶ amino acids ⟶ deamination to NH4

Proteins are converted to peptides and amino acids by extracellular proteolytic enzymes (Bitton, 2005).

Nitrification

Nitrification is the ammonia (NH4+) oxidation to nitrite (NO2-) and the nitrite oxidization to nitrate (NO3-) by some microbial such as, chemoautotrophic bacteria (Henning *et al*., 1999). Firstly, *Nitrosomonas* bacteria oxidize the ammonium to nitrite. After that, the nitrite oxidate to nitrate by *Nitrobacter*bacteria (Tappe*et al*., 1999).

Nitrosomonas *Nitrobacter*

NH4+ ⟶ NO3- ⟶ NO2-

Denitrification

This is the reduction of nitrate to dinitrogen under anaerobic conditions; the organisms involved are anaerobic, heterotrophic bacteria. In contrast to the nitrification process, denitrification is an anaerobic process. It is a heterotrophic process, needs organic substrate. There are two types of denitrification: biological denitrification and chemodenitrification. Biological denitrification refers to biochemical reduction of $NO3^-$ to gaseous compounds. During denitrification, $NO3^-$ and $NO2^-$ are reduced to N oxides (NO, N_2O) and molecular N (N2) by micro-organisms. These gaseous products are not available for plant uptake (Hofman*et al*., 2004 ; Barnard *et al*., 2005).

NO3- ⟶ NO2- ⟶ NO ⟶ N2O⟶ N2

Biological Nitrogen Fixation

Rhizobium species living in symbiotic relationships in root nodules of legumes (e.g. soybean, clover, alfalfa, peas, beans) can convert atmospheric N2 gas to NH3, which is further converted to amino acids and proteins. In exchange, the legumes provide the *Rhizobium* species with the energy they need to grow and fix N2. Some non-leguminous trees and plants (e.g. alder, sugarcane) also are host N-fixing bacteria. Photosynthetic cyanobacteria are also N-fixing organisms and are especially important in rice paddies. The amount of N fixed varies greatly from crop to crop, ranging from a few kg to several hundred kg N ha-1 year-1. The process is depressed when other sources of N are abundant, and is also reduced in acid soils and in soils with low P availability (Hofman*et al*., 2004; Emerich*et al*., 2009).

Sulphur Cycle

Sulphur occurs in numerous forms in the environment, including gaseous sulphur dioxide (SO^2) and hydrogen sulphide (H2S). Both forms of sulphur enter the atmosphere as the result of volcanic eruptions or man-made activities including the burning of fossil fuels. Soil S occurs as organic sulphur compounds, sulphides (S-), elemental sulphur (S), and sulphate ($SO4^{2-}$), the latter being the major plant utilizable ion. Most soil sulphur occurs as organic-S and is unavailable to plants. Organic-S slowly breaks down via S-mineralization to the plant in the sulphateion. Microorganisms mineralize, assimilate, oxidize and reduce S in the environment. Sulphates are formed by mineralisation under aerobic conditions while hydrogen sulphide is formed anaerobically (i.e. desulphuration).A major S-decomposition product in the marine environment is dimethylsulphide. Sulphur is assimilated as sulphate by plants, algae and also most heterotrophic microbes. A few anaerobes are able to assimilate reduced sulphur as hydrogen sulphide. Sulphur oxidation is mediated by chemolithotrophs sush as *Beggiatoa, Thiothrix, Termothrix* (thermophile) and *Thiobacillus*(*T.thioparus* and *T.novellas*). *Thiobacillus*, an acid tolerant bacterium is able to oxidize elemental sulphur to sulphuric acid. The archaen genus Sulfolobus, which inhabits hot acidic environment, also oxidizes elemental sulphur. The oxidation of sulphur coincidentally results in the solubilisation and mobilization of phosphorous and mineral nutrients due to the production of mineral acids. Sulphur oxidation occurs at temperatures between 34-37ºC. S-reduction is achieved by anaerobic bacteria i.e. *Desulfovibrio desulphuricans, desulfovibriogiga, Desulphuro monasacetoxidans, Desulfobacte rcurvatum*. Sulphur reduction can be assimilatory or dissimilatory. In the former, the hydrogen sulphide produced is immediately incorporated into organic compound by the organisms. In dissimilatory sulphur reduction however, hydrogen sulphide is released in the environment where it can prove toxic. Members of the genus *Thiobacillus* can rapidly oxidize hydrogen sulphide and other reduced sulphur compounds to sulphate under aerobic conditions in both soils and sediments (Place, *et al*, 2007).

Oxidation

In the presence of available electron acceptors, reduced forms of S are oxidized by both chemical and microbial pathways:

$$\mathbf{HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-}}$$

A wide variety of organisms are capable of oxidizing S in an equally diverse range of environments. These organisms can be divided into three groups:

(1) photoautotrophs:

including species of green and purple sulphur bacteria.

(2) chemolithotrophs: such as members of the *Thiobacilli.*

(3) heterotrophs: which include a wide variety of bacteria and fungi. While the first two occur generally in extreme environments such as hot sulphur springs, the latter named groups are largely responsible for oxidizing elemental-S in aerobic soils (Wainwright,1984). Hydrogen sulphide oxidation occurs in many unicellular and filamentous cyanobacteria (Jorgensen and Cohen, 1977). The filamentous *cyanobacterium oscillatoria limnetica* can perform anaerobic photosynthesis and use HS- as the electron acceptor.

Aim of the Study

This research aims to study important microbial processes related to soil fertility in two modified soils. The first is to study seaweed waste and the second one is keratin waste

Materials and methods

Materials

There are two types of soils were proposed for this study**:**
1-Agricultural soil amended with keratinous substrate.

2-Agricultural soil amended with seaweed substrate.

Methods

A- Determination of soil pH

Soil pH was determined in the laboratory using 1 : 1 soil sample - distilled water mix, after that shaken for 15 min on a reciprocal shaker (l00 revolutions min-1). The pH was then measured using a pH meter fitted with a glass electrode to measure of pH soil sample.

B- Preparation of standard curve for nitrate, sulphur and ammonium

It is necessary to create a standard curve for all minerals to obtain accurate results.

● Standard curve for nitrate

To obtain 1000µg $NO3^-$-N ml^{-1}, 1.37 g of sodium nitrate was dissolved in 1000 ml dH_2O. In order to the solution equivalent to 100µ g / NO_3^--N ml^{-1} (10 times dilution), 10 ml of sodium nitrate solution was mixed with 90 ml of dH_2O. Serial dilutions from previous solution were made with dH_2O to obtain 0, 10,25,50,75, and 100 µg NO_3^--Nml^{-1}. chromotropic acid methods was performed for all dilutions to evaluate nitrate ions (Sims and Jackson, 1971).

● Standard curve for ammonium

A calibration curve for ammonium ions was performed by dissolving 3.66 g of $(NH_4)_2SO_4$ ammonium sulphate in 1000 ml dH_2O which equals 1000µg NH_4^+-Nml^{-1}. 10 ml ammonium sulphate solution was mixed with 90 ml of dH_2O (10 times dilution) to obtain 100µ g / $NH4^+$-Nml^{-1}. Serial dilutions from the previous solution were made with dH2O to obtain 0, 10, 15, 25, and 50 µg NH_4^+-N ml^{-1}. Indophenol blue method was performed for all dilutions to evaluate ammonium ions (Wainwright and Pugh, 1973).

● Standard curve for sulphate-S

This curve was performed by dissolving 1.47 g of sodium sulphate (Na_2SO_4) in 100 ml dH2O which equal 1000µg SO_4^{2-}-Sml^{-1}. 10 ml of sodium sulphate solution was mixed with 90 ml of dH_2O (10 times dilution) to obtain 100µ g / SO_4^{-2}-S ml^{-1}. Serial dilutions from previous solution were made with dH_2O to obtain 0, 5, 25, 50, 75 and 90 µg SO_4^{2-}-Sml-1. Turbidimetric methods were performed for all dilutions to evaluate sulphate-S ions (Hesse, 1971)

C- Chemical analysis of degraded commercial algae (seaweed meal) in modified soil

This approach aimed to assess the amount of sulphur, nitrate and ammonium release from degraded seaweed using commercial algae. To demonstrate the potential of this approach and its suitability for the application Sulphate, nitrate and ammonium production from soil amended with seaweed is investigated.

1- Determination of nitrate in agricultural soil amended with seaweed

All samples of agricultural soil (50g) were placed in polythene bags and amended with 0.5g seaweed, (Sigma) and mixed thoroughly. A control was set-up lacking added seaweed. The modified soils were incubated in polythene bags closed with a small hole to allow for gas exchange. The bags were set up in triplicate and incubated for 28 days at 25°C. At zero time and at 7, 14, 21 and 28 days intervals samples were extracted. After incubation (1g) soils were then placed into screw capped glass bottle with (10 ml) deionised water used to extract nitrate; after shaking for 15min at 100 rpm on an orbital shaker, the samples were filtrated through Whatman No.1 filter paper.

Nitrate Determination

Nitrate was determined using the method of Sims and Jackson (1971). Chromotropic acid (CTA) reagent (7ml) was mixed with 3ml of filtrate the mixture was cooled in cool water and incubated at 40°C in the water bath for 45 minutes; the yellow colour CTA-NO_3 formed was measured at 410 nm in a spectrophotometer and the concentration of nitrate was determined by reference to a standard curve (0-100 NO_3-N ml-1) prepared from a standard solution of $NaNO_3$.

Reagents: Chromotropic acid

1- Stock solution

1.84 g of Chromotropic acid $C_{10}H_6O_8S_2Na_2$ was dissolved in 1 liter of sulphuric acid H_2SO_4 .The solution was stored at 4°C.

Working solution

Stock solution (100 ml) was diluted in 990 ml of concentrated H_2SO_4 and then added 10 ml of concentrated HCl. The solution was stored at 4°C for several weeks.

2- Determination of ammonium in agricultural soil amended with seaweed

Soil (50g) was placed in polythene bags and amended with (0.5g seaweed) and mixed thoroughly. A control was set-up lacking without added seaweed. The modified soil was incubated in polythene bags which were closed with a small hole to allow for gas exchange. The bags were set up in triplicate and incubated for 28 days at 25°C. At zero time and at 7, 14, 21 and 28 days, samples were extracted. Ammonium was extracted from the soil

with a solution of KCl (150 g KCl / 1000 water) in the ratio: (1g) soil: (10ml) KCl. The soil was shaken for 30min at 100 rpm on an orbital shaker and then filtered through filter paper Whatman No.1.

Determination of ammonium

Filtrate (2ml) was added to (1ml) of EDTA (6% w/v), (7ml) of distilled water, (5ml) of phenolate reagent and (3ml) of sodium hypochlorite solution (10%v/v). The reaction mixture was mixed thoroughly and incubated at 25°C for 20min in the dark. The volume was made up to 50 ml and mixed and the concentration of the indophenol-blue ammonium complex was measured at 630 nm using a spectrophotometer (Wainwright and Pugh, 1973). The concentration of ammonium intensity was then determined by reference to standard curve (0-50µg NH_4-N ml-1) prepared from a standard solution of ammonium sulphate $(NH_4)_2SO_4$.

Reagents

1)Ethylenediaminetetraacetic acid (EDTA):
60 g EDTA was dissolved in 900 ml of distilled water and then diluted to1L.
2) Phenol solution:
62.5 g Phenol was dissolved in ethanol (25 ml) and adding acetone (18.5ml) to make up to 100 ml. The phenol solution should be stored in the dark at 4°C.
3)Phenolate reagent: 20 ml of phenol solution was mixed with (20 ml) of hydroxide sodium (27% NaOH w/v) and diluted to 100 ml. The reagent was prepared fresh daily.

3- Determination of sulphatein agricultural soil amended with seaweed

The turbidimetric sulphate method was used to determine the oxidation of sulphur (Hesse, 1971). For this test, agricultural soil (50g) was placed in polythene bags and adjusted with 0.5g seaweed meal, then mixed well. Soil without modification was run as a control. The modified soil was incubated in polythene bags, and closed with small holes to allow for gas exchange. The bags were then set up in triplicate and incubated for 28 days at 25°C. At zero time, 7, 14, 21 and 28 days the samples were extracted. Soil samples (1g) were shaken with (10ml) of distilled water for approximately 15 min at 100 rpm using an orbital shaker. The samples were then filtrated through Whatman No.1 filter paper.

Determination of sulphate

Sulphate was determined as described by (Hesse, 1971). Filtrate (5ml) in 50ml volumetric flask was mixed with (1g) of barium chloride and (2ml) of gum acacia (0.25% w/v). The solution was made up to 25ml with distilled water. The resultant white suspension was measured at 470 nm by using spectrophotometer. The concentration of sulphate was then determined by reference to a standard curve (0-100 µg $SO4^{-2}S$ ml^{-1}) prepared from a standard solution of Na_2SO_4.

D- Chemical analysis of degraded commercial keratin (horn meal)in modified soil

Chemical analysis was aimed to detect sulphur from decaying keratinous substrate (horn meal).

Determination of the oxidation of sulphur in agricultural soil amended with horn meal

The keratinous substrate, hoof and horn meal were used in this experiment. This substrate is commercially produced by heating hoof and horn at high temperatures and pressure in order to use it as animal feed and fertilizers (Tapia and Simões, 2008). the same methods (determination of sulphur) described above were done for this test.

Statistical analysis of data

All observations were presented as mean. Sigma Plot© (Version12.0) was run to analyze data. T-test and ANOVA were performed to check whether there were significantly different.

Results and discussion

standard curve for nitrate, sulphur and ammonium

A calibration curve for sulphate, Nitrate and ammonium ions given in figure 1,2 and 3.

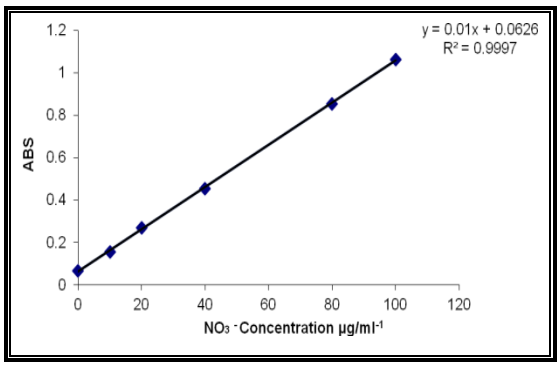

Figure 1: Standard curve for nitrate

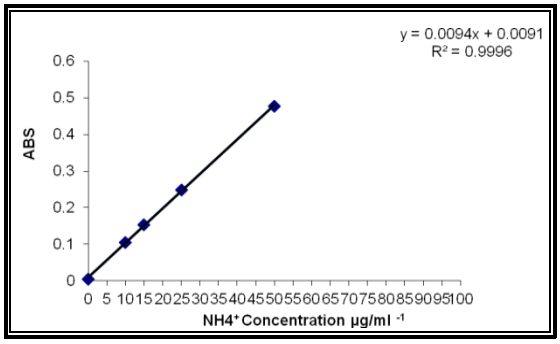

Figure 2: Standard curve for ammonium.

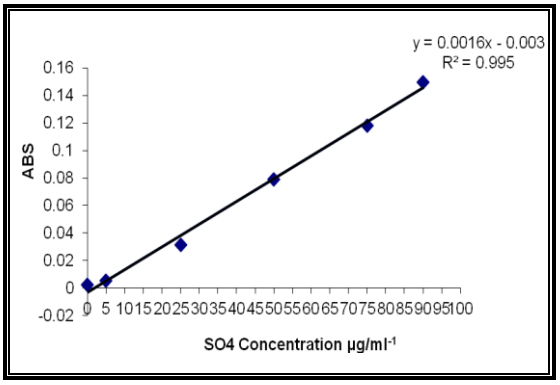

Figure 3: Standard curve for sulphur.

• Chemical Analysis of degraded commercial seaweed (seaweed meal)

The breakdown of commercial seaweed was also tested because of its use as a fertilizer and soil conditioner.

1-Sulphur production in agricultural soil amended with seaweed meal

Figure 4 shows the oxidation of sulphur in agricultural soil amended with seaweed over four weeks incubation period. The results show that microbial S-oxidation of sulphur increased from day 7 and continued increasing throughout 21 days incubation period while there was a slight rise in sulphate concentration in the control after 7 days. It can be clearly seen that the oxidation of sulphur reached a peak in week 3.

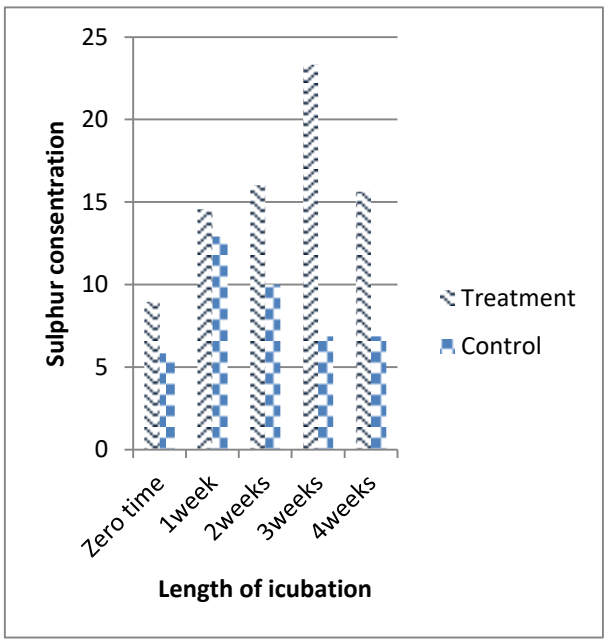

Figure 4: Sulphate production from oxidation of elemental sulphur in soil amended with seaweed.

2- Nitrate and ammonium production in agricultural soil amended with seaweed meal

The results given in Figures 5 and 6 show the amount of nitrate and ammonium in soil amended with seaweed. Figure 5 clearly shows that the highest nitrate production occurred at week 1 of the incubation period and then decreased after 2,3 and 4 weeks.

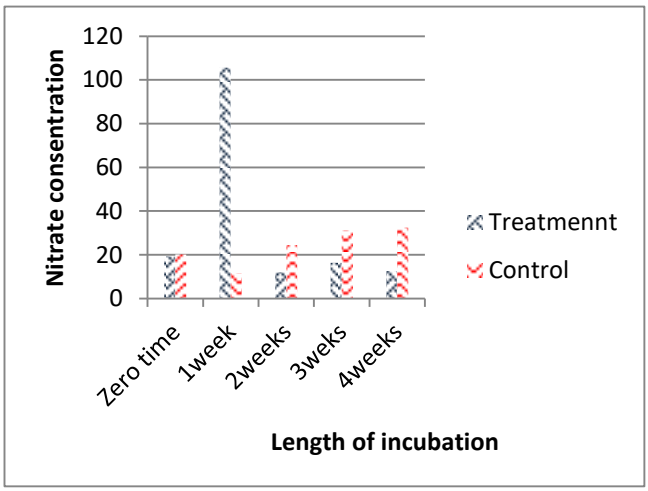

Figure 5: Nitrate production from the hydrolysis of seaweed.

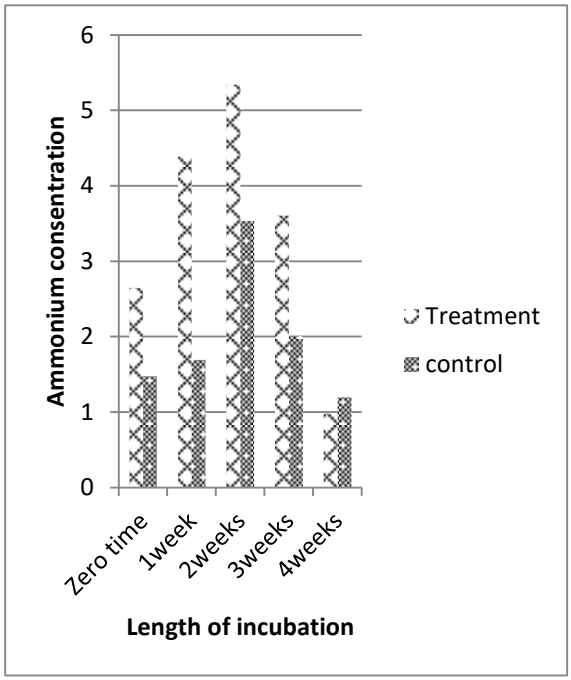

Figure 6: Ammonium production from the hydrolysis of seaweed.

Rapid ammonium production occurred in weeks 1and 2 , and then this level decreased sharply and remained stable (Fig.6)

• Chemical Analysis of degraded commercial keratin(hoof and horn)

The release of large amounts of sulphur in the soil is associated with keratin degradation (Rajak *et al*., 1992).

Determination of the oxidation of sulphur in agricultural soil amended with a keratinous substrate

Figure 7 shows the amount of sulphur produced in the agricultural soil amended with hoof and horn from 1 to 4 weeks of incubation. The results show that microbial S-oxidation of sulphur increased over the 3 weeks incubation period (treatment). It can be clearly seen that there was a significant increase between treatment and control throughout the length of incubation. Moreover, oxidation of sulphur reached a peak of week 3. A decline in the amount of sulphate in the last phase of the experiment might be result of an adaptation of microorganisms such as fungi to the use sulphate as a sulphur source. Sulphitolysis may refer to the extremely high content of sulphur in the hoof and horn (Rajak *et al*., 1992).

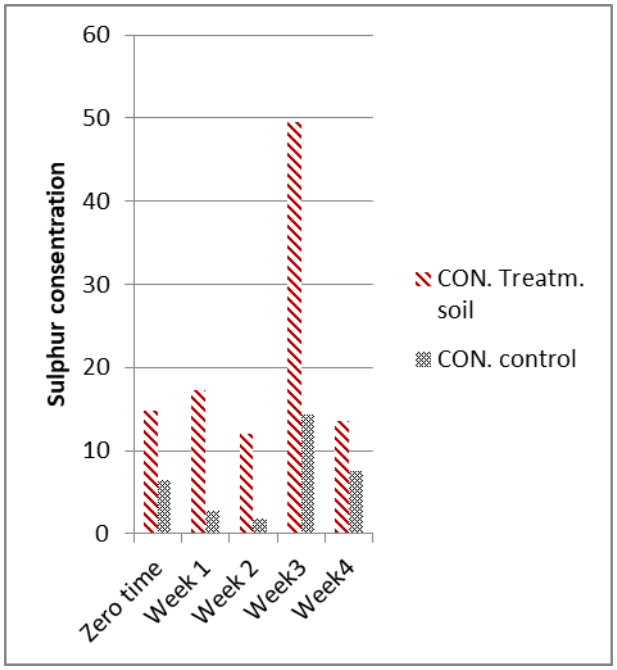

Figure 7: Sulphate production from oxidation of elemental sulphur in agricultural soil adjusted with keratinous substrate.

Conclusion

This research looked at some minerals: nitrate, ammonium and sulphur concentrations produced in modified soil. The general trend of nitrate was an increase in soil amended with seaweed in week 1, but the nitrate concentration in the agriculture soil sample was higher than the seaweed soil sample. In addition, it was noticeable that in all period of incubation, soil treated with seaweed was much higher in sulphur and ammonia than control. Also, elemental sulphur was oxidized to sulphate in soil amended with hoof and horn, the highest sulphate oxidation occurred in the presence of added hoof and horn in week 3. The overall conclusion is that the amended soil shows more biogeochemical activity than the agricultural soil.

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