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Determination of Available Nitrate, Phosphate and Sulfate in Soil Samples

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ABSTRACT: A study of soil samples was done to determine the concentration of the available phosphate, nitrate and sulfate. Samples were collected from different places at different depths. Before estimation of the anions, the samples were air-dried and sieved mechanically through a sieve with a 2 mm mesh. Sulfate and nitrate were extracted in water with ratio of (1:1), while phosphate was extracted with sodium bicarbonate solution at pH of 8.5. Phosphate was determined as available phosphorus by Spectrophotometric method in which the ammonium phosphomolybdate complex, which was formed first, was reduced by ascorbic acid in presence of antimony to give a distinct blue color which was measured at 880nm. The available nitrate was determined by phenoldisulphonic acid to give a yellow color which was measured spectrophotometrically at 410nm. Sulfate was analyzed by adding excess barium chloride to precipitate barium sulfate which be then determined by turbidimetry method and was measured at 420nm. Our results obtained were compared with previous studies for the same region by central lab for soil and water at 1982 and comparing the results with Libyan standard specification for soil for nutrients which was given by Phozyn international limited results.

Key word:* *Soil,* PO_4^{3-} *,* SO_4^{2-}*,* NO_3^- *ammonium phosphomolybdate, phenoldisulphonic acid, turbidimetry

INTRODUCTION

Soil may be defined as the weathered superficial layer of the earth's curst that typically is made up of decomposed and partly decomposed parent rock material with associated organic matter in various stages of decomposition. Soil is the natural medium in which the roots of most plants grow. From soil the plant absorbs water and solutes necessary for its continued well- being. If soil is fertile, it contains in a readily available form all the chemical elements essential for plant growth. Soil is a complex system which includes mineral (inorganic) matter, organic matter, water, air and organisms[1]. The chemical composition is determined by the nature of the starting materials from which the soil was formed and by the processes that it has undergone over time. Soils in the field are not a monolithic mass of unchanging composition. Rather, they are characterized by large spatial variability in both the horizontal and vertical dimensions. The chemical nature of soils is determined by the combination of mineral and organic matter that makes up the soil[2]. There are three important nutrients required by plants. Nitrogen and phosphorus are required in appreciable quantities, while needs for sulfur are less. Deficiencies of S are much less pronounced than those of micronutrients such as N and P, and S fertilization needs are also much lower. Sulfur and P are taken up by plant roots from soil solution as SO_4^{2-} and $H_2PO_4^-$, respectively. Sulfate deposition from the atmosphere and mineralization of organic sulfur usually provide adequate supplies of S to meet requirements[3].

Nutrients for healthy plant growth are divided into three categories: primary, secondary and micronutrients. (N), (P) and (K) are primary nutrients, which are needed in fairly large quantities compared to the other nutrients. (Ca), (Mg) and (S) are secondary nutrients which are required by the plant in lesser quantities but are no less essential for good plant growth than the primary nutrients. (Zn) and (Mn) are micronutrients which are required by plants in very small amounts. Most secondary and micronutrient deficiencies are easily corrected by keeping the soil at the optimum pH value. The capacity for holding anions increases with the decrease in pH. All anions are not adsorbed equally readily. The affinity for adsorption of some of anions commonly present in soil is of the order: $NO_3 < Cl < SO_4 < PO_4$. So the less anion is adsorbed on the soil, the easily lost by leaching. Available nitrogen is taken up by plant roots in the form of (NO_3^-) and (NH_4^+). The available forms of nitrogen are very water soluble and move rapidly through the soil profile with rainfall and irrigation[1,4].

The total content of phosphorus in soil is relatively low and it's in the soil solution is usually less than 0.1 µg\ ml and rarely greater than 1 µg\ ml. The concentration of P in saturated solutions of most phosphate fertilizers is many thousands of times greater than that of phosphorus in soil solutions, and much fertilizer phosphorus could be lost by leaching if this level of solubility persisted after addition of the fertilizers to soils. Most soils, however, have the capability of reacting rapidly with soluble phosphates and reducing their solubility[5].

EXPERIMENTAL PART

The methods are taken from references no. [3], [6], [7] and [8]

I Preparation of soil samples

1. Soil samples were collected from Al Guarshah agricultural project, south of Benghazi.
2. Five samples were collected from different places by using auger at different depths (from 0 to 100cm). The auger is suitable for sampling hard soils. It consists of a sharpened spiral blade attached to a central metal rod which can be screwed into the soil. The auger was screwed to the desired depth and the sample was withdrawn.
3. Soil samples were transferred to plastic bags and were labeled. and toke the symbol S_aS_b where S_a is the soil sample number (a = 1, 2, 3, 4, 5 respectively) and S_b is the sub soil depth in cm (b = 0-20, 20-40, 40-60, 60-80, 80-100 cm respectively).
4. In the lab, the samples were air-dried, grass and any external objects were removed.
5. Sieving was done in mechanical engineering lab by mechanical sieving apparatus which consists of different sizes of meshes (10.0- > 0.075 mm) after rolling the samples to break down the large masses of the soil particles.
6. The sieved samples (> 2.0 mm) were preserved in labeled plastic bags .

II.Extraction of the samples

1. 50 g of each of the soil samples were weighed, transferred to 250 ml stopper conical flask and was shaken with exactly 50 ml of distilled water (1:1 ratio). The shaking was done by using mechanical shaker for 10 minutes.
2. After shaking, the equilibration was taken place by leaving the samples for 30 minutes.
3. The samples were filtered into Buchner funnels by using filter papers Whatman no. 42.
4. In the case of turbid filtrates, they were centrifuged by using 3000 cycle/min centrifuge for 5 min.

Note:

The extraction for phosphate determination was done by 50 ml of $NaHCO_3$ at pH 8.5 because it has been known for a long time that phosphate do not leach from soils but are retained in forms that may be removed only by extraction with various salts, acid, alkaline solutions[1] .

III. Materials and Reagents

All reagents were of analytical grade.

a. *Analysis of phosphate*

- Extraction solution for phosphate: 42.00 g of sodium bicarbonate was dissolved and was adjusted to pH of 8.5 with 50% NaOH and acetic acid.
- Mixed reagent
  1- Standard solution of ammonium molybdate was prepared by dissolving 12.7g of the salt in 250ml of distilled water.
  2. Antimony potassium tartarate was prepared by dissolving 0.291g in 100ml of dist. water.
  3. Both of the dissolved reagents (of 1 and 2) were added into 1000ml of 5N H_2SO_4 , mixed thoroughly, made to 2000 ml with dist. water and were stored in a Pyrex glass bottle in a dark compartment.
- Color developing reagent
  Ascorbic acid was prepared by dissolving 2.625 g in distilled water and diluting to 500ml.
- *Stock phosphate solution (1000 mg/L P):* 4.3937 g of the oven dried KH_2PO_4 salt was dissolved in dist. water, 1ml of concentrated H_2SO_4 was added and the solution was diluted to 1L in a volumetric flask.
- *Intermediate phosphate solution (250 mg/L P):* it was prepared by diluting 25 ml of the stock solution up to 100 ml using redistilled water.
- *Standard phosphate solution (2.5 mg/L P):* it was prepared by diluting 10.00 ml of the intermediate phosphate solution up to one liter with extracting solution.

b. *Analysis of Nitrate*:

- 0.01N KNO_3 (= 1011 *mg/L*) was prepared by dissolving 1.011g of the salt in distilled water and the volume was completed to the mark into1L volumetric flask.
- 0.02N Ag_2SO_4 was prepared by dissolving 3.12g of the salt in 1 liter distilled water.
- Phenoldisulphonic acid was prepared by dissolving 25g of phenol in 150 ml of conc. sulfuric acid, 35ml of fuming sulfuric acid (30% SO_3) was added and the solution was heated at 100 ^{o}C for 2 hours on water bath.
- Concentrated ammonium hydroxide.

c. *Analysis of Sulfate:*

- Conditional reagent was prepared by mixing (50) ml glycerol with a solution containing 30ml conc. HCl, 300ml distilled water, 100ml(95%) ethanol and (75g) NaCl.
- Standard sulfate (0.1N) was prepared by drying Na_2SO_4 at 110 ^{o}C in oven about 2 hours, and cooling in a desiccator, then 7.102g from it was dissolved in 1L of distilled water.
- Barium chloride crystal.

IV. Instruments

UV/ Visible Spectrophotometer. pH meter . Glass electrode, Mechanical shaker, Centrifuge. Water bath

V. Measurements

►**Nitrate**

1. First, the concentration of the chloride in the sample was determined, the reading of chloride was divided by 10 and an amount of silver sulfate equivalent to the amount of chloride was added.
2. (2-5) ml of the sample was taken in centrifuge test tube and the volume was completed to 10 ml with distilled water. The tube was centrifuged for 10 min until solution was clear.
3. 5 ml of the clear solution was taken in glass evaporating dish, put on water bath, evaporated to dryness and it was cooled.
4. 1 ml of phenoldisulphonic acid was added after 10 min. then 10 ml of water was added and was transferred to 100 ml volumetric flask and was made alkaline by the addition of conc. NH_4OH, diluted to volume and was mixed .
5. The absorbance at 410 nm was measured by using Spectronic 21D UV/ Visible Spectrophotometer and glass cells.

Preparation of standard calibration curve: by taking volumes of 0.00 to 12 ml from the standard solution of KNO_3 into glass evaporating dishes and treating as above omitting the addition of silver sulfate

►**Phosphate**

10 ml aliquot was placed in a 50 ml measuring flask and added amount of dist. water. 10 ml of the color developing reagent was added, stirred, stand for 15 min, then measured at 880nm by using Spectronic 21D UV/ Visible Spectrophotometer and glass cells.

Preparation of standard calibration curve: by taking volumes of 0.00 to 16 ml from the standard solution of KH_2PO_4 into 50 ml volumetric flasks and treated as above.

►**Sulfate**

The analysis for sulfate was done by taking (5 or 2) ml of sample in a volumetric flask, and diluting to 100ml dist. Water, transferring into 250 ml conical flask. Add 5.0 ml conditional reagents, mixing in stirring apparatus, during stirring, add spoon full $BaCl_2$ crystals (about 0.2 – 0.3 g). Begin timing immediately, stirring for 1 min. at constant speed. Immediately after stirring period has ended (at 30 sec intervals 4 min) pour solution in 4 cm sample silica cell. Measure the absorbance at 420 nm by using Jena Model UV-VIS Spectrophotometer

Preparation of standard calibration curve: by taking volumes of 0.00 to 5.0 ml interval 0.5ml from the standard solution of sodium sulfate in volumetric flask, and dilute to 100 ml distilled water. Treating standard solution with specified amount of all reagents as above procedure. Set the spectrophotometer at zero by using dist. water as blank.

The calibration curves of Phosphate, Nitrate and Sulfate are shown in the following figures.

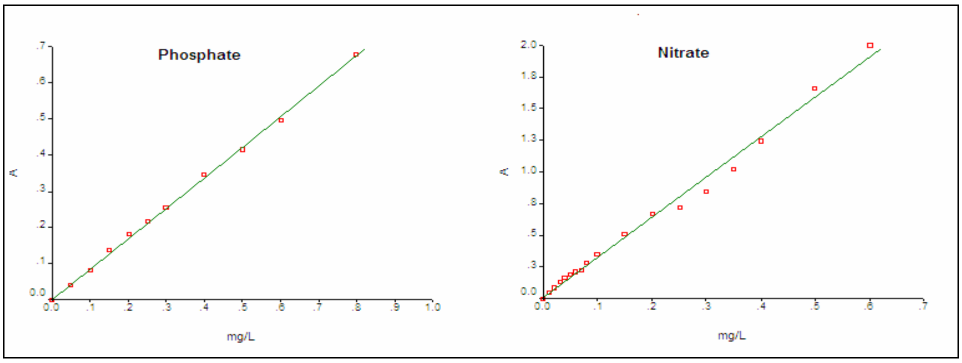

Fig1: Calibration curves of Phosphate (left) and Nitrate (right)

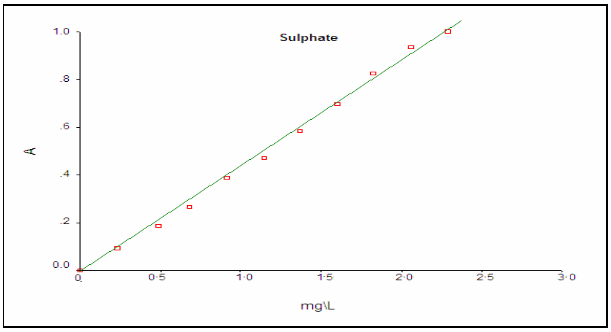

Fig2: Calibration curve of Sulfate

Results and Discussion

In this work, we determined the concentrations of available nitrate, sulfate and phosphate in five sample soils with their depths and the results which were obtained can be expressed in mg/L and meq/L of the water extract and also in mg/Kg and meq/100g of the soil sample[3] . The mean values and the standard deviations for each sample soil, individually, are summarized in the following five tables:

Symbol	$H_2PO_4^-$		SO_4^{2-}		NO_3^-	
	meq\L	mg\L	meq\L	mg\L	meq\L	mg\L
S_1S_0	0.023±0.000	0.70±0.00	0.60±0.013	29.00±0.635	0.20±0.00	12.40±0.00
S_1S_1	0.015±0.000	0.47±0.00	0.56±0.019	27.00±0.924	0.80±0.00	49.60±0.00
S_1S_2	0.018±0.000	0.55±0.00	0.79±0.012	38.00±0.583	0.21±0.00	1.32±0.14
S_1S_3	0.024±0.000	0.73±0.01	0.37±0.024	18.00±1.15	0.02±0.00	1.24±0.00
S_1S_4	0.033±0.000	1.02±0.00	0.87±0.005	42.00±0.234	0.20±0.00	12.40±0.00

Table1: Results of available P, SO_4^{2-} and NO_3^- for soil sample no.1

From table (1), the concentrations of available phosphate, sulfate and nitrate for the soil sample 1 and its depths are 0.47-1.02, 18.00-42.00 and 1.24-49.60 mg/L, respectively.

Symbol	$H_2PO_4^-$		SO_4^{2-}		NO_3^-	
	meq\L	mg\L	meq\L	mg\L	meq\L	mg\L
S_2S_0	0.109±0.0021	3.39±0.072	0.9 ±0.026	46.00±1.27	0.40±0.000	24.80±0.000
S_2S_1	0.006±0.0011	0.20±0.002	0.37±0.010	18.00±0.50	0.40±0.000	24.80±0.000
S_2S_2	0.024±0.0004	0.75±0.000	0.37±0.006	18.00±0.288	0.40±0.000	24.80±0.000
S_2S_3	0.054±0.0028	1.68±0.000	0.21±0.006	10.00±0.292	0,80±0.000	49.60±0.000

Table2: Results of available P, SO_4^{2-} and NO_3^- for soil sample no.2

From table (2), the concentrations of available phosphate, sulfate and nitrate for the soil sample 2 and its depths are 0.20-3.39, 10.00-46.00 and 24.80-49.60 mg/L, respectively.

Symbol	$H_2PO_4^-$		SO_4^{2-}		NO_3^-	
	meq\L	mg\L	meq\L	mg\L	meq\L	mg\L
S_3S_0	0.114±0.0017	3.53±0.020	0.18±0.022	9.00±1.042	0.20±0.000	12.40±0.000
S_3S_1	0.056±0.0000	1.73±0.000	0.37±0.006	18.00±0.288	0.20±0.000	12.40±0.000
S_3S_2	0.022±0.0008	0.69±0.028	1.23±0.031	59.00±1.50	1.20±0.810	74.40±24.80
S_3S_3	0.022±0.0004	0.68±0.006	0.77±0.018	37.00±0.866	1.33±0.468	82.00±0.000

From table (3), the concentrations of available phosphate, sulfate and nitrate for the soil sample 3 and its depths are 0.68-3.53, 9.00-59.00 and 12.40-82.00 mg/L, respectively.

Symbol	$H_2PO_4^-$		SO_4^{2-}		NO_3^-	
	meq\L	mg\L	meq\L	mg\L	meq\L	mg\L
S_4S_0	0.043±0.000	1.34±0.05	3.85±0.052	185.0±2.52	11.00±2.84	682.00±86.80
S_4S_1	0.057±0.000	1.75±0.00	0.71±0.00	34.00±0.00	0.80±0.00	49.60±0.00
S_4S_2	0.026±0.000	0.81±0.00	0.63±0.043	30.40±2.08	0.73±0.23	45.46±0.00
S_4S_3	0.018±0.000	0.55±0.00	0.76±0.012	36.40±0.58	0.80±0.41	49.60±12.40
S_4S_4	0.019±0.000	0.58±0.00	0.56±0.005	27.00±0.23	1.67±0.23	103.30±7.16

Table4: Results of available P, SO_4^{2-} and NO_3^- for soil sample no.4

From table (4), the concentrations of available phosphate, sulfate and nitrate for the soil sample 4 and its depths are 0.55-1.75, 27.00-185.0 and 45.46-682.00 mg/L, respectively.

Symbol	$H_2PO_4^-$		SO_4^{2-}		NO_3^-	
	meq\L	Mg\L	meq\L	mg\L	meq\L	mg\L
S_5S_0	0.110±0.000	3.40±0.00	5.33±0.08	256.0±4.04	0.80±0.00	49.60±0.00
S_5S_1	0.033±0.001	1.03±0.03	0.69±0.06	33.00±2.79	17.87±7.97	1107.73±243.72
S_5S_2	0.031±0.001	0.96±0.03	0.73±0.009	35.20±0.45	32.67±2.34	2025.30±71.59
S_5S_3	0.019±0.000	0.58±0.00	0.58±0.007	28.00±0.36	17.20±5.97	1066.00±182.60

Table5: Results of available P, SO_4^{2-} and NO_3^- for soil sample no.5

From table (5), the concentrations of available phosphate, sulfate and nitrate for the soil sample 5 and its depths are 0.58-3.40, 28.00-256.0 and 49.60-2025.30 mg/L, respectively.

From the above values, we noted that the highest values for the three anions are present in sample 5 which was an agricultural soil .

The distributions of each ion in the soil according to the depths for the above parameters were studied and summarized in the following paragraph:

1. **Phosphate**:

Phosphorus occurs almost entirely as phosphate and both organic and inorganic forms are of major importance in plant-soil-water interaction, and in the general phosphorus biogeochemical cycling in natural systems. The majority of agricultural soils usually can not meet crop demands for P, and fertilization is required[3] . Plants generally absorb most of their P as $H_2PO_4^-$ and smaller amount as HPO_4^2 depending on the pH i.e. lower pH values will increase the absorption of $H_2PO_4^-$ ion, whereas, higher pH values will increase the absorption of HPO_4^{2-} ion[9] .

Figure 4 shows the relationship between the phosphate concentration and the depths of the soil. We note that:

i. Most of the phosphate is found in the top soil. This may be because the samples were collected on the month of April in which no raining for leaching the anions.

ii. The highest amounts are found in samples no. 3, 4 and 5 in which these samples are agricultural soils, and these amounts decrease with increasing the depths of the soil due to the absorption of them with the plant

iii. Increasing the amounts of P with increasing the depths in samples no. 1 and 2 (arid soils).

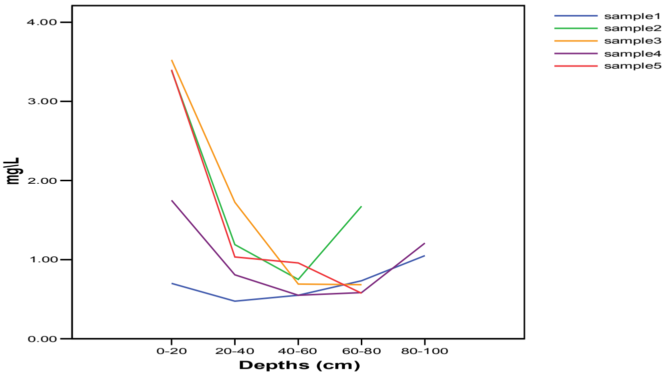

Fig3: The relationship between the depths of each soil sample and the phosphorus concentration

This result enhances the fact that P moves very slowly from the point of placement, for the phosphate ion is almost immobile in the soil and the water-soluble phosphorus sources move short distances[3] .

By comparing the concentration of phosphate at top soil obtained from our results and the results obtained from the soil and water laboratory for the same region in (1982) [7] and the optimum requirements of the phosphate to the plant obtained from Phosyn International Limited[10] , we obtained figure (4).

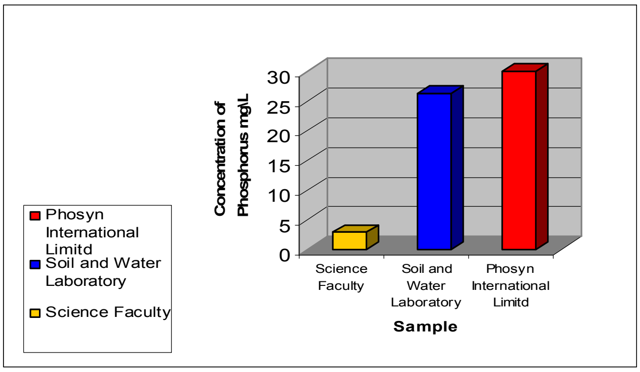

Fig 4: The Phosphorus concentration obtained from our results and Soil and Water Lab and comparing the results with the nutrient level in soil obtained from Phosyn

From the above figure, we note that the concentration of the phosphorus much decreased and by comparing with Phosyn value, the soil needs more phosphorus which can be obtained by fertilization. However, the effectiveness of phosphate fertilizers may be limited by adsorption to certain minerals and by transfer to the organic form. Phosphate from fertilizers can affect micronutrient availability to plants and reduce their migration within landscape[3] .

2. ***Nitrate:***

The determination of inorganic N, mainly NH_4^+ and NO_3^- in soil is often useful, because, despite their usually low levels, these inorganic forms are readily available for plant uptake[9] . In warm, well-aerated, slightly acid to slightly alkaline soils the nitrate is the nitrogen form absorbed by plant is the predominant form [9] .

Figure 5 shows the relationship between the nitrate concentration and the depths of the soil.

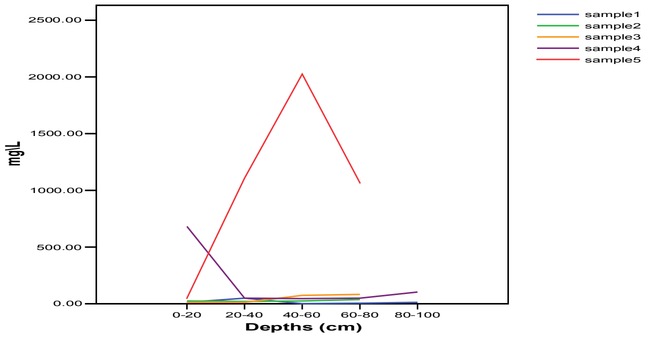

Fig5: The relationship between the depths of each soil sample and the nitrate concentration

We note that:

i. The highest concentration of nitrate for the soil samples studied is found in sample 5 at 40-60 cm.

ii. The movement of nitrate is readily and randomly and this is noted by comparing samples 4 and 5.

iii. Only samples 4 and 5 have quantities of nitrate (agricultural soils) and the other samples may be the nitrate is consumed or converted to insoluble form.

This result enhances the fact that the nitrogen salts move up and down in the soil solution, depending on direction of water movement. Of the two general types of nitrogen salts, nitrate moves more readily, for it does not attach itself to soil particles. On the other hand, ammoniacal nitrogen is adsorbed by the soil colloids. As it is converted to the nitrate, it becomes mobile[1] .

By comparing the concentration of nitrate, as in case of phosphate, we obtained figure (6).

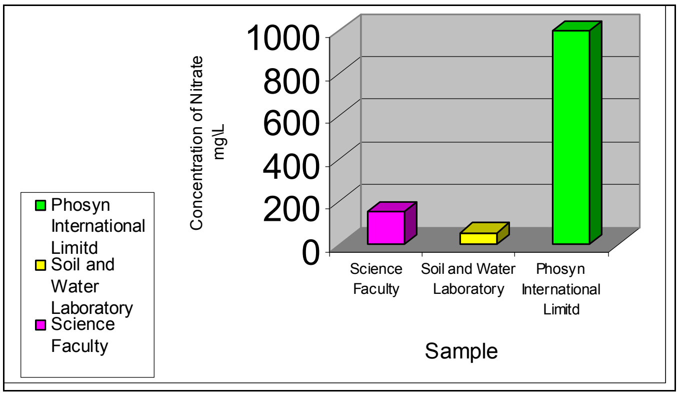

Fig 6: The Nitrate concentration obtained from our results and Soil and Water Lab and comparing the results with the nutrient level in soil obtained from Phosyn

From the above figure, we note that the concentration of the nitrate is also low as compared with Phosyn value although it is more than that obtained by Soil and Water Laboratory. So the soil needs more nitrate which can be obtained by fertilization.

Crop demands for N can quite often exceed the natural supply and fertilization is then required to sustain high yields. However, excess N from fertilizer can have toxic effects[3] .

3. Sulfate

Under aerobic conditions, most of the inorganic S is present as sulfate[3] .

Figure 7 shows the relationship between the sulfate concentration and the depths of the soil,

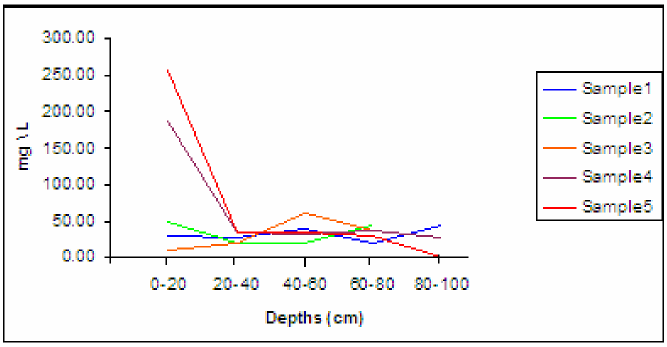

Fig7: The relationship between the depths of each soil sample and the sulfate conc.

From above figure and with comparing with P (fig, 3), we note that:

i. The behavior of sulfate is almost like that for phosphate in this soil.

ii. The highest amounts are found in samples no. 4 and 5 in which these samples are agricultural soils and the sources of sulfate in these two samples may be from the irrigation water (mostly untreated wastewater) , and these amounts decrease with increasing the depths of the soil due to the absorption of them with the plant

iii. Sample no.1 (arid soil) shows the increase of S amounts with increasing the depth and this due to leaching of S by raining may be from three months ago.

These results enhance that the relation between the amount of percolating water and the downward movement of sulfate was determined and it was found that the greater the water added, the greater the net downward movement of sulfate depending on the type of soil [9] .

By comparing the concentration of sulfate obtained, as in case of phosphate and nitrate, we obtained figure (8).

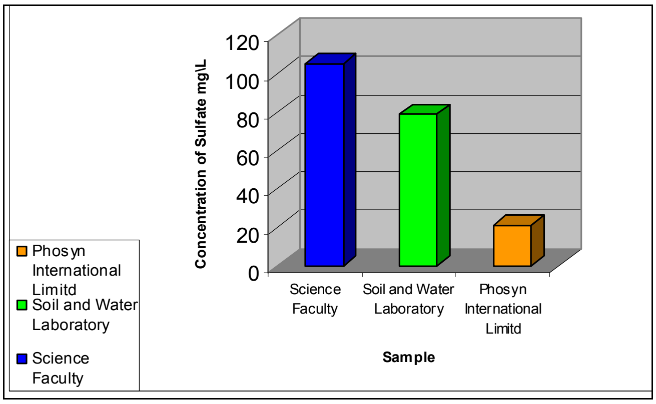

Fig 8: The Sulfate concentration obtained from results and Soil and Water Lab and comparing the results with the nutrient level in soil obtained from Phosyn

From the above figure, we note that the concentration of the sulfate is more than that obtained from Soil and Water Lab and by comparing with Phosyn value, the soil contains much more sulfate which may be obtained, as we said before, from the irrigation water (mostly untreated wastewater) and these amounts are unfavorable for the most crops.

CONCLUSION:

Our work is the analysis of available phosphate, nitrate and sulfate in five sample soils with their depths (from 0.0 to 100 cm) and comparing our results with the results obtained by Central Lab for Soil and Water for the same region at 1982.

Referring to the Phosyn Company guideline, we can conclude that the phosphate and nitrate obtained from our soil results are much less than the optimum values of that well-known company and the soil needs more fertilizers. In the case of sulfate, the results are much higher than that are needed by the crops and we should test the irrigation water.

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