

BIOCHEMICAL ENZYMATIC STUDIES ON STATUS AND DISTRIBUTION OF SOIL ORGANIC-S FRACTIONS UNDER DIFFERENT DRAINAGE CONDITIONS

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ABSTRACT

This investigation was conducted under field conditions at three locations representing the semi-arid conditions of Kafr El-Sheikh Governorate. The selected soils were irrigated for a long time with different sources of wastewaters in non-rational rates under different drainage conditions. A novel conceptual diagram was established to integrate main fractions of labile and stable organic-S and the ways by which their values are determined and calculated.

The obtained results, could be summarized as follows:

- Grand mean value of arylsulfatase activity was 173.3 $\mu\text{g } \rho\text{-nitrophenol produced. g}^{-1}\text{ soil. hour}^{-1}$. Whereas, dehydrogenases activity was 9.4 $\mu\text{g TPF produced. g}^{-1}\text{ soil. hour}^{-1}$.
- Grand mean values of total-S and organic-S were 300 and 188.9 mg S. kg^{-1} soil. Organic-S was the main S-constituent in studied soils accounted for 62.23% of total-S, but its value was lower than reported from other regions.
- Grand mean value of ester sulfate-S was 56.4 mg S. kg^{-1} soil, accounted for 17.9% of total-S and 28.7% of organic-S. Sulfate esters constitute a major source of S for inorganic-S through biochemical enzymatic processes. This fraction was much more lower than reported from other regions.
- Grand mean value of organic-bonded S was 132.5 mg S kg^{-1} soil, represented 44.4% of total-S and 71.3% of organic-S, which was lower than reported from other regions. This fraction constitutes a greater proportion of total-S than ester sulfate-S.
- Grand mean value of amino acids-S and organic unidentified-S were 49.4 and 83.1 mg S. kg^{-1} soil. Amino acids-S value accounted for 16.7% of total-S, 27.7% organic-S and 39.3% of organic-bonded S. Amino acids-S content was greater than reported from other regions.

Generally, soil enzymatic-S activities and either total-S or all organic-S compounds had their superiority values in traditional drained soils in winter seasons. Whereas it reached their minimum values in badly drained soils in summer seasons. These findings in year 1999/2000 were markedly pronounced than those obtained in year 2000/2001. Monthly maximum activities of the studied enzymes were happened in winter months (Feb.), while their minimum activities were in summer month (July). These activities were negative correlated with max. air temp. and soil temp. (20 cm depth) which prevailing in studied area. Arylsulfatase and dehydrogenase activities had a significant positive correlations with total-S and all different organic-S fractions; furthermore with water table depth, soil pH values organic-C and -N contents. These activities were negative highly significant correlated with C/S ratio; ionic strength and bulk density.

Keywords: Arylsulfatase; dehydrogenase; total-S; organic-S; ester sulfate-S; amino-acids-S; sulfur fractionation; modified Johnson-Nishita apparatusu.

INTRODUCTION

It is well known that S is an essential plant nutrient element, which occurs in soils in a variety of chemical fractions, both organic and inorganic in different valence states (Blanchar, 1986). Additions of N-and K- as sulfate salts and P as single superphosphate [$\text{Ca} (\text{H}_2\text{PO}_4)_2 \text{CaSO}_4$] often met an unrecognized crop requirement for supplemental S (Paul and Clark, 1989).

A sufficient S supply is crucial to maintain a high yielding crops (El-Kammah & Ali, 1996 and Li *et al.*, 2001). In Egypt, although some soils suffering from S-shortage, neither direct S-fertilization nor balanced fertilization are used. Intensification of agriculture with high yielding crop varieties and multiple cropping, coupled with use of high analysis low or lacking S-fertilizers has accelerated the depletion of S reserves (Sammi Reddy *et al.*, 2002 and Hu *et al.*, 2002).

The amount of inorganic-S in soils is only minor compared to the organic S pool and with the escalating costs of S-containing fertilizers, S-mineralization potential of soil contributes to the S supplying of crops through maintaining adequate soil S reserves and meeting S requirements of crops, because plants take up S in form of inorganic sulfate-S. S-mineralization in soils is largely mediated by biological activity and the following enzymes are involved fulfill in the biochemical S-transformations of organic-S in soils: a) Arylsulfatase (EC 3.1.6.1, aryl-sulfate sulfohydrolase) hydrolyzes aromatic sulfate ester (R-C-O-SO_3^-) by fusion of O-S bond to phenol R-OH and inorganic sulfate-S (Elsgaard *et al.*, 2002 and Ekenler & Tabatabai, 2003)). b) Dehydrogenase (enzymes catalyzing dehydrogenation process) (Tabatabai, 1994). Relative much few reports have been devoted these enzymes in Egyptian soils. Organic-S in soils has been grouped into two main broad groups (i) organic-S that is bonded indirectly to C and is reduced to H_2S by hydroiodic acid. This organic fraction consists of S in the form of ester sulfate-S; compounds containing-C-O-S linkage; and sulfated thioglycosides N-O-S linkage, the second S atom in sulfocysteine-C-S-S- linkage and the C-N-S-linkage (sulfamates) and (ii) organic-S that is directly bonded to C and is reduced to inorganic sulfide by Raney-Ni in alkaline medium. This fraction consists of S in the form of amino acids-S. It includes a variety of S-groups such as $-\text{SO}_3\text{H}$ (sulfonate); $-\text{SO}_2$ (sulfones); $-\text{SH}$ (sulfhydryl, $-\text{C-S-S-C}$ (disulfide) and heterocyclic-S. Additionally, organic-S that is not reduced by either NI or Raney-Ni. This fraction is assumed to consists of S bonded directly to C, but not recoverable by the current methods (Freney *et al.*, 1970; Freney *et al.*, 1972; Tabatabai, 1982; Freney, 1986; Paul & Clark, 1989; Germida *et al.*, 1992; Hu *et al.*, 2002 and Mansfeldt & Blume 2002).

So, the objectives of the present biochemical study aimed to (i) assess the activity of enzymes involved in S-transformations in soils under different drainage conditions, (ii) determine all organic-S fractions and their distributions, (iii) study their seasonal and annual fluctuations and their correlations with biological activity and status of organic-S fractions.

MATERIALS AND METHODS

I. Locations description and soils sampling:

The experiment was conducted at three locations represented a temperate semi-arid conditions at Kafr El-Sheikh region, Kafr El-Sheikh Governorate (Coordinates: latitude 31°07'N, longitude 30°57' E and elevation 20 m above the sea level). Operationally, this studies elongated 21 months from Dec. 1999 to August 2001. Some seasonal micro-climatological features were obtained from Sedi-Salem Weather Observation Station (4) over this experimental period (Table 1.1). A main criterion for soil sampling before initiation this study, was the selection of sites that representing arable polluted alluvial salt-affected soils with different water table depths, under conventional tillage and common agricultural practices. Organic wastes have been supplied in non-rational rates and irrigated for a long-time with different sources of treated and untreated wastewaters under different drainage conditions. Composite surface soil samples (0-30 cm) were taken from different sites and analyzed for obtaining their main physical and chemical properties.

Location (1):

This site is located at Messier village (S₁) at Kafr El-Sheikh district and has a traditional drained alluvial slightly salt-affected soils with average water table depth 95 cm (Table 1.2). The selected soil has been received a heavy loaded sewage sludge applications and irrigated for a long-time with treated sewage effluents (sometimes for different undefined period of time with untreated sewage effluents). The preliminary obtained data of this location showed that, its particle size distribution was 58.7% clay, 24.7% silt, 10.8% fine and 5.8% coarse sand. Soil organic matter content, CaCO₃% and bulk density were 2.97%, 2.26% and 1.28 Mg m⁻³ respectively. Details physico-chemical characteristics of the selected soils are given in Table (1.2).

Location (2):

This site is located at Kafr Dokhmeas village (S₂) at El-Mahalla El-Kubra district, El-Gharbia Governorate. It has a poorly-drained salt-affected soils and average water table depth 75 cm (Table 1.2). The soil has been incorporated for undefined periods of time with chicken manures and irrigated for a long-time with contaminated agricultural drainage waters from El-Gharbia main drain (Cotchaner drain). Its particle size distribution was 51.4% clay, 30.9% silt, 14.3% fine and 3.4% coarse sand. Soil organic matter (%), CaCO₃ % and bulk density were 1.84%, 3.74% and 1.35 Mg m⁻³, respectively. Additional major properties of these selected soil are summarized in Table (1.2).

Location (3):

This area is situated at Kafr El-Sheikh city and has a badly-drained slightly salt-affected soils with average water table depth 60 cm (Table 1.2). This soil (S₃) has not been received sewage sludge or organic manures applications but irrigated for different undefined periods with contaminated waste-drainage water from Oil and Soap Company.

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Its particle size distribution soil was 59.8% clay, 28.5% silt, 7.2% fine and 4.5% coarse sand. Soil organic matter %, CaCO₃ and bulk density were 1.56%, 4.15% and 1.57 Mg m⁻³, respectively. Another main analytical characteristics of these selected soils used are listed in Table (1.2).

Table (1.2): Seasonal meteorological features at Kafr El-Sheikh region over the experimental period (21 months) during two successive winter and summer seasons 1999/2000 and 2000/2001

Successive seasons from December 1999 to August 2001 (21 months)		Air temperature °C				Relative humidity %				Soil temperature (°C)			
		Max.	Min.	Average	Dif.	Max.	Min.	Average	Dif.	Soil depth sections (cm)			Mean
										5	10	20	
Winter Dec., Jan., Feb.	1999/2000	16.14	5.41	10.77	10.73	88.29	40.81	64.55	47.48	12.76	12.98	13.32	12.98
	2000/2001	18.31	5.91	12.11	12.40	92.29	42.08	67.19	50.21	13.97	14.36	15.24	14.52
	Mean	17.23	5.66	11.44	11.57	90.29	41.45	65.87	48.85	13.36	13.67	14.28	13.75
Spring Mar., Apr., May	2000	22.00	8.96	15.48	13.04	87.70	30.97	59.34	56.73	-	-	-	-
	2001	25.73	12.66	19.20	13.07	91.56	33.44	62.50	58.12	-	-	-	-
	Mean	23.87	10.81	17.34	13.06	89.64	32.21	60.92	57.43				
Summer Jun., Jul., Aug.	2000	29.00	18.28	23.64	10.72	88.50	36.97	62.74	51.53	31.03	30.72	30.06	30.60
	2001	30.99	19.68	25.34	11.31	94.96	42.56	68.76	52.40	32.85	31.96	30.66	31.83
	Mean	30.00	18.98	24.49	11.02	91.73	39.77	65.75	51.97	31.95	31.34	30.05	31.28
Autumn Sept., Oct., Nov.	2000	25.42	13.83	19.63	11.59	89.93	39.72	64.83	50.21	-	-	-	-
	2001	26.98	15.21	21.10	11.77	92.17	39.81	65.99	52.36	-	-	-	-
	Mean	26.20	14.52	20.37	11.68	91.05	39.77	65.41	51.29				
Annual grand mean	2000	23.14	11.62	12.38	11.52	88.61	37.12	62.87	51.49	21.89	21.85	21.69	21.79
	2001	25.50	13.37	19.44	12.14	92.75	39.47	66.11	53.27	23.41	23.16	22.65	23.10
Overall average		24.32	12.49	15.91	11.83	90.68	38.30	64.49	52.39	22.65	22.51	22.17	22.52

Generally, during both consecutive winter seasons (Dec.-Feb.) 1999/2000 and 2000/2001 as well as successive summer seasons (June-August) 2000 and 2001, representative disturbed and undisturbed soil samples were monthly collected in duplicate from the soil surface (0-30). It was established a permanent sampling plot circular area with a diameter of 6 m (28.28 m²). Prior to analysis, the homogenized soils samples passed through a 2 mm-metal sieve, were subdivided into two main groups. The first one was immediately maintained in deep freezer and then thawed just prior to analysis for determination arylsulfatase activity. The second group was air-dried, gently crushed with cylindrical wooden roll and again sieved < 2 mm for chemical analysis. A subsamples of this group were finely ground in an agate mortar to pass a 100-mesh sieve, mixed thoroughly and stored in a plastic air tight containers at room temp. for total-N, organic-C, CaCO₃% and sulfur

fractions analysis. Undisturbed vertical cylindrical volumes of field moist soil samples were monthly obtained using cylinder core samplers for estimating soils physical properties.

II. General soils properties: were determined by the following routine work analysis:

Physical characteristics: Bulk density (Mg m^{-3}), total porosity (%), and particle size distribution were analytical determined using classical methods (Klute, 1986 and Carter, 1993).

Chemical and organic characteristics: soil reaction (pH\value); $\text{CaCO}_3\%$; organic-C g kg^{-1} soil; total-N mg kg^{-1} soil; total soluble cations and anions (meq/L) in saturated soil paste extracts were determined as described in details by Page *et al.* (1982), Carter (1993); and Rowell (1996). Ionic strength was calculated using an equation reported by Tan (1993) as follows:

Ionic strength = $\frac{1}{2} \sum_{i=1}^{i=n} M_i Z_i^2$, where M_i =conc. of ion (i) in mmoles L^{-1} and Z_i = charge of ion (i). The summation was taken over all the cations and anions.

III. Fractionation and chemical forms of sulfur in soils:

1. Total sulfur fraction (Total-S pool): Fraction (1)

Alkaline oxidation with sodium hypobromite (NaOBr) was selected and used as described by Tabatabai (1982) and Guthrie & Lowe (1984). This method involved reduction of sulfate to hydrogen sulfide by acidic reducing mixture containing hydriodic acid (HI), hypophosphorus acid (H_3PO_2) and formic acid (HCOOH) at ratio 4:2:1 (v/v), using a modified Johnson and Nishita apparatus. The H_2S thus liberated was immediately absorbed in a buffer containing zinc- and sodium acetate and subsequently treated with acidic ρ -amino dimethylaniline hydrochloride and ferric ammonium sulfate solutions for methylene blue color development. The extinction readings (E-values) of the methylene blue color were spectrophotometrically determined at wavelength of 670 nm. Total-S concentrations (C) in $\mu\text{g S. } 100 \text{ ml}^{-1}$ of the samples analyzed by reference to a calibration graph stock solution $1000 \mu\text{g S ml}^{-1}$).

Standard curve equation obtained as follows:

$$E = 0.010354 C \mu\text{g S}/100 \text{ ml at wavelength } 670 \text{ nm}$$

The obtained total-S fractions (1) were recorded as (mg-S. kg^{-1} soil).

Fractionation of Soil Organic Sulfur Components

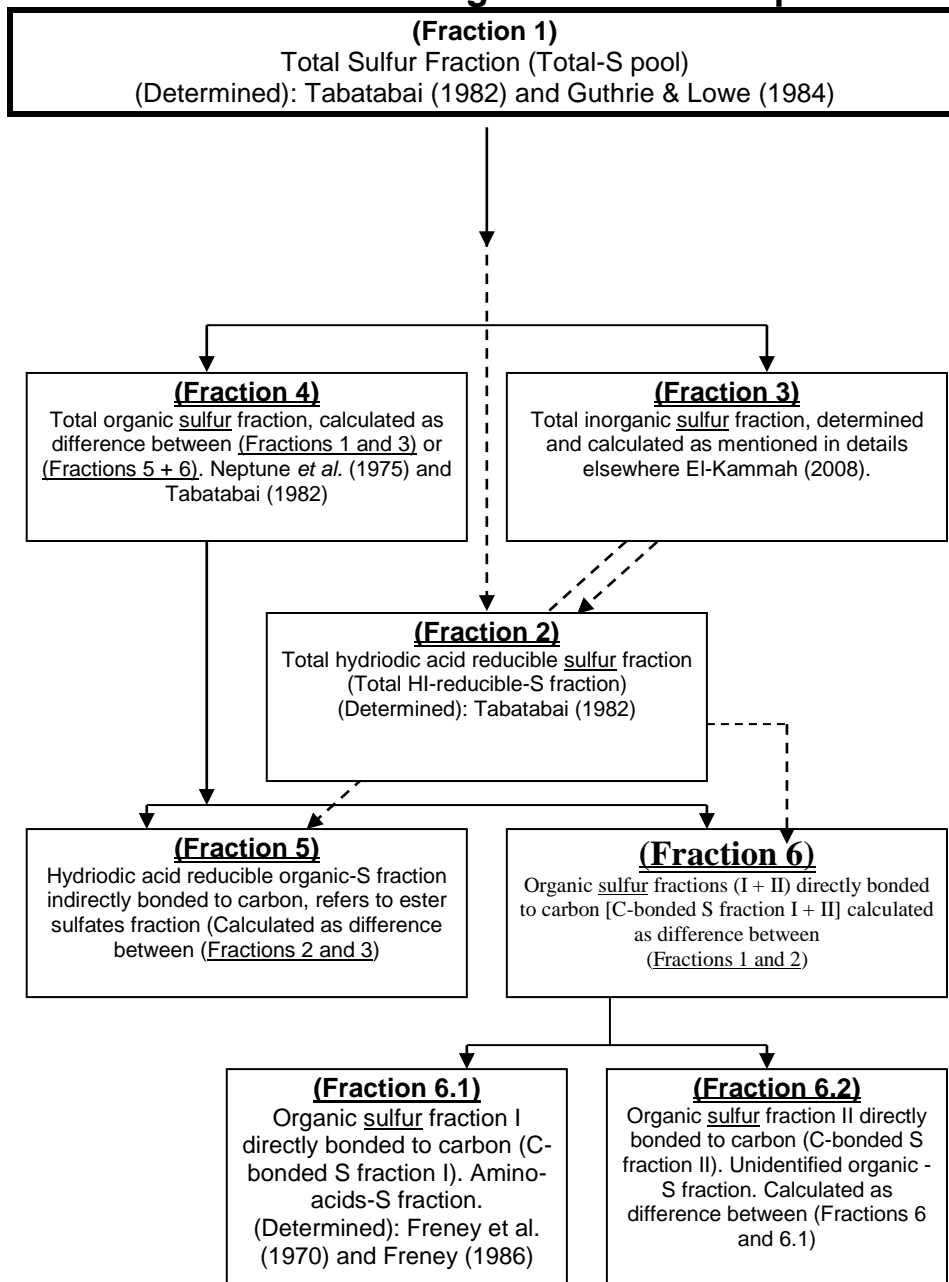


Fig. (1): A conceptual experimental diagram that integrates main forms of labile and stable organic sulfur fractions in selected soils and the ways by which their magnitudes are quantitatively determined and calculated

2. Total hydriodic acid reducible-S fraction: Fraction (2)

Directly digestion-distillation technique using a strong acid reducing mixture has enable to reducing all inorganic S compounds and insoluble plus co-precipitated/co-crystallized with CaCO_3 as well as organic S compounds in which S is not bonded directly to carbon, to hydrogen sulfide as shown in Fig. (1). This technique does not recover the S in organic compounds in which the S is directly bonded to carbon. Practically, finely ground soil samples < 100 mesh sieve were pretreated with 40% hydrofluoric acid (HF) at 100°C for 2 hours to dissolve silicates and release any sulfur protected from reaction with HI acid. After removing the acid, the pH 6.5 acidified soil samples were directly digested with strong acidic reducing mixture using the modification digestion-distillation technique as described by Tabatabai (1982). Calculations were made in a manner similar to that used for total-S fraction (1) after subtraction of insoluble plus co-precipitated co-crystallized with CaCO_3 the amount from obtained results (El-Kammah, 2008).

3. Total inorganic sulfur fraction (Fraction 3):

This fraction was calculated involving summation of its forms, i.e. the amount of inorganic-sulfate-S fraction (Fraction 3.1) plus the amount of the total inorganic non-sulfate-S fraction (Fraction 3.2) as shown in Fig. (1). Details informations about the determination and calculation of the inorganic-S fraction and its S-compounds are elucidated, and illustrated elsewhere (El-Kammah, 2008)

4. Total organic-sulfur fraction: (Fraction 4):

Direct determination of total organic-S fraction in soils are not possibly by present analytical methods. An estimate of total organic-S fraction as frequently calculated by difference involving subtraction the amount of total inorganic-S fraction: (Fraction 3) estimated independently by a separate analysis from determined total-S fraction: (Fraction 1) as reported by Neptune *et al.* (1975) and Tabatabai (1982) as shown in Fig. (1).

4.1. Organic ester sulfate-S fraction: Fraction (5):

Organic sulfur fraction indirectly bonded to carbon, which is reduced to hydrogen sulfide by hydriodic acid was indirectly accomplished by difference involving subtraction the value of total inorganic-S (Fraction 3) obtained by a separate analysis from the value obtained for total hydriodic acid reducible-S fraction: (Fraction 2) as shown in Fig. (1).

4.2. Total Organic-S fraction directly bonded to carbon: Fraction (6):

Organic bonded-S fractions (I + II) is difficult to analysis, it is usually calculated as being the difference between value of total-S fraction (1) and the value of total HI-reducible-S fraction: (Fraction 2) as shown in Fig. (1).

4.2.1. S-containing amino acids: Fraction (6.1):

Application of Raney-nickel catalyst to analysis of organic sulfur directly bonded to carbon (C-bonded S fraction I) in soils was originally estimated by the method developed by Freney *et al.* (1970) and Freney (1986). The used method involves desulfurization of the soil samples by Raney nickel catalyst in the presence of alkali for 30 minutes in Johnson and Nishita digestion-distillation apparatus. The H_2S thus liberated after acidification with excess HCl during boiling was received in buffer H_2S -

absorbing solution and estimated spectrophotometrically as methylene blue as described by Tabatabai (1982). However, this method recovers sulfides and other reduced inorganic S-compounds (Tabatabai, 1982), therefore a separate analysis was independently performed by boiling the soil sample in 6 N HCl and determined the H₂S evolved. Finally, the values obtained for C-bonded S fraction I were corrected for the amount of sulfides and other reduced inorganic-S compounds that may evolve as H₂S upon boiling in excess HCl.

4.2.2. Unidentified organic-S fraction: Fraction (6.2)

Organic-S fraction (II), that is directly bonded to C of soil organic matter, but not recoverable by current methods used for estimating of C-bonded S i.e. not reduced by either HI or Raney nickel catalyst. Practically, this fraction was calculated by difference involving subtraction the value of amino acids-S fraction from the calculated value of total organic-S directly bonded to C (fractions I + II).

4.3. Standing stocks:

Standing stocks of different sulfur fractions were calculated and expressed as kilo-gram per feddan, as
$$\frac{\text{S-fraction}}{\times 10^6} \times \frac{4200 \times 15 \times \text{Db}}{10^3}$$
 Where:

depth section 15 cm, Db = bulk density Mg m⁻³ and S-fraction in mg kg⁻¹ soil.

IV. Enzymes involved in S-transformations:

These enzymes activities were performed at their optimal pH and temperature in field-moist soil samples < 2 mm.

1. Arylsulfatase activity (EC 3.1.6.1.): was measured according to the method described by Tabatabai (1994) modified by Elsgaard *et al.* (2002) using of toluene as bacteriostatic and plasmolytic agent. This method based on colorimetic determination of *p*-nitrophenol released, by arylsulfatase activity when the soil sample was incubated at 37°C for one hour with toluene and buffered 0.05 M potassium *p*-nitrophenol sulfate solution as the substrate (chromogenic ester).

The yellow color intensity of the filtrate was spectrophotometrically measured at wavelength 420 nm. The concentration of *p*-nitrophenol released was calculated by reference to a calibration graph using the standard curve equation:

$$E = 0.005896 C \mu\text{g } p\text{-nitrophenol/ 5 ml at wavelength 420 nm.}$$

The arylsulfatase activity was recorded as $\mu\text{g } p\text{-nitrophenol released. g}^{-1} \text{ soil. hour}^{-1}$ at 37°C. pH 5.8.

2. Dehydrogenase activity:

Soil dehydrogenase activity was determined using the procedure reported by Tabatabai (1994). This method involves colorimetric determination of 2, 3, 5-triphenyl tetrazolium formazan TPF. produced by the reduction of 2, 3, 5-triphenyl tetrazolium chloride TTC by the dehydrogenase enzymes. TPF was extracted after incubation at 37°C and pH 8 for 24 hours by methanol. The extinction-readings of the reddish color of TPF in the filtrate was spectrophotometrically determined at a wavelength 485 nm. The produced amount of TPF was calculated by reference to a calibration graph

prepared from TPF standard solutions (μg TPF/100 ml). Results were recorded as μg 2, 3, 5-triphenyl tetrasolium formazan (TPF) produced (or H_2 consumed) per gram of soil per one hour at 37°C and pH8 using the standard curve formula:

$$E = 0.00033592 C_{\mu\text{gTPF}/100 \text{ ml}}$$

Statistical analysis:

All tabulated experimental and analytical data were statistically analyzed to clarify the obtained results as described by Gomez and Gomez (1976).

RESULTS AND DISCUSSION

1. Total sulfur fraction:

Data regarding total-S pool Fraction (1) in studied soils are given in Tables (1.3 and 2) and illustrated in Figs. (1 and 2). Analytical results revealed that total-S contents were 463.3, 237.5 and 200 $\text{mg S}\cdot\text{kg}^{-1}$ soil (avg. 300.25) for Messier (S_1); Kafr Dokhmeas (S_2) and Kafr El-Sheikh (S_3) soils regardless of their bulk densities respectively. Their average standing stock regarding their bulk densities was 257.6 $\text{kg S}\cdot\text{fed}^{-1}$.

Consequently, total-S content could be arranged in the following order as:

Traditional drained > poorly drained > badly drained soils

It can be noticed that, total-S contents were within the normal range which found in different soils of other regions. Similar trends were obtained by Neptune *et al.* (1975); Tabatabai (1982), Li *et al.* (2001) and Mansfeldt & Blume (2002). Total-S content had a positive high significant correlation with water table depth ($r = 0.93^{**}$). Total-S content in winter seasons was (335.5 $\text{mg S}\cdot\text{kg}^{-1}$ soil, and its standing stock 281.2 $\text{kg S}\cdot\text{fed}^{-1}$). These values were higher than those obtained in summer seasons: (265.0 $\text{mg S}\cdot\text{kg}^{-1}$ soil and its standing stock 234.9 $\text{kg S}\cdot\text{fed}^{-1}$). Statistical data revealed that, total-S content in year 1999/2000 was 325.2 $\text{mg S}\cdot\text{kg}^{-1}$ soil and its standing stock 274.1 $\text{kg S}\cdot\text{fed}^{-1}$. These findings were higher than those obtained in year 2000/2001: 275.3 $\text{mg S}\cdot\text{kg}^{-1}$ soil and its standing stock 240.8 $\text{kg S}\cdot\text{fed}^{-1}$. Total-S value had a negatively significant correlations with ionic strength ($r = -0.72^{**}$) max. air temp. ($r = -0.56^*$) and soil temp. ($r = -0.71^*$). However, total-S had a positive high significant correlations with organic-C ($r = 0.89^{**}$), total-N ($r = 0.85^{**}$) and clay content ($r=0.72^{**}$). On the other hand, total-S value was negatively correlated with C/N ratio ($r = -0.49^{**}$) and C/S ratio ($r = -0.57^{**}$) Kariarpanond *et al.* (1991) and Mansfeldt & Blume (2004), confirmed these results. They concluded that enrichment of soils in sulfur fractions was evidenced by narrow C/S ratios.

Table (1.3):Distribution percent of total organic-S fraction and its components.

Main soil-S fractions	Content mg S. kg ⁻¹ soil	Percentage distribution values		
		Total S pool	Total organic-S fraction	Total organics bonded
1. Total-S fraction (Fraction 1)	300.2	100		
Inorganic-S fraction (El-Kammah, 2008)	111.3	37.7		
2: Organic-S fraction (Fraction 4)	188.9	62.2	100	
2.1.: Ester sulfate-S fraction (Fraction 5)	56.4	17.7	28.7	
2.2.: Organic-bonded S fraction (Fraction 6)	132.5	44.3	71.2	100
2.2.1.: Amino acids-S fraction (Fraction 6.1)	49.4	16.6	27.7	39.3
2.2.2.: Unidentified organic S-fraction (Fraction 6.2)	83.1	27.6	43.5	60.7

Table (2):Total-S and total hydriodic acid reducible-S contents in studied soils.

Successive seasons from December 1999 to August 2001 (21 months)	Total sulfur fraction (Total-S pool) (FRACTION 1.1)						Total hydriodic acid reducible sulfur fraction (total HI-reducible-S fraction)						
	Messier (S ₁) Traditional drained		Kafir Dokhmeas (S ₂) Poorly drained		Kafir El-Sheikh (S ₃) Badly drained		Messier (S ₁) Traditional drained		Kafir Dokhmeas (S ₂) Poorly drained		Kafir El-Sheikh (S ₃) Badly drained		
	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	mg S kg ⁻¹ soil	Standing stock kg S fed ⁻¹	
Winter seasons Dec., Jan, Feb.	1999/2000	567	447.2	296	231.0	250	242.1	360.33	284.21	153.01	126.21	154.01	149.13
	2000/2001	474	384.3	244	207.0	182	173.0	269.05	218.15	119.67	95.49	123.48	117.39
	Mean	520.50	416.3	270	219.9	216	207.2	314.69	251.68	136.34	111.69	138.74	133.12
Summer seasons Jun., Jul., Aug.	2000	416	321.0	215	191.8	207	211.4	211.64	163.33	92.42	90.85	130.52	133.29
	2001	396	340.0	195	175.1	161	165.7	207.76	178.4	95.48	78.05	95.28	98.08
	Mean	406	330.9	205	183.5	184	188.6	209.70	170.85	93.95	84.47	112.90	115.76
	Grand mean	463.25	373.6	237.5	203.0	200	198.5	262.20	211.27	115.15	98.94	125.82	124.87

2. Total organic-S fraction:

Data concerning total organic-S fraction (Fraction 4) are given in Table (1.3 and 3) and illustrated in Figs. (1, 2, and 3). The organic-S contents were from 294.1, 167.7 and 104.8 mg S. kg⁻¹ soil (avg. 188.9) for S₁, S₂ and S₃, respectively. The average standing stock was 160.8 kg S. fed⁻¹. It could be said that, total organic-S content in traditional drained soils had the superiority value than those obtained under other drainage conditions. Total organic-S content had a positive highly significant correlation with water table depth (r = 0.96**).

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Percentage distribution of total organic-S content expressed as % of total-S accounted for 62.23%. Commonly total organic-S content and its percentage distribution in winter seasons were 209.7 mg S. kg⁻¹ soil, referred as 62.6% of total-S and its standing stock (174.6 kg S. fed.⁻¹).

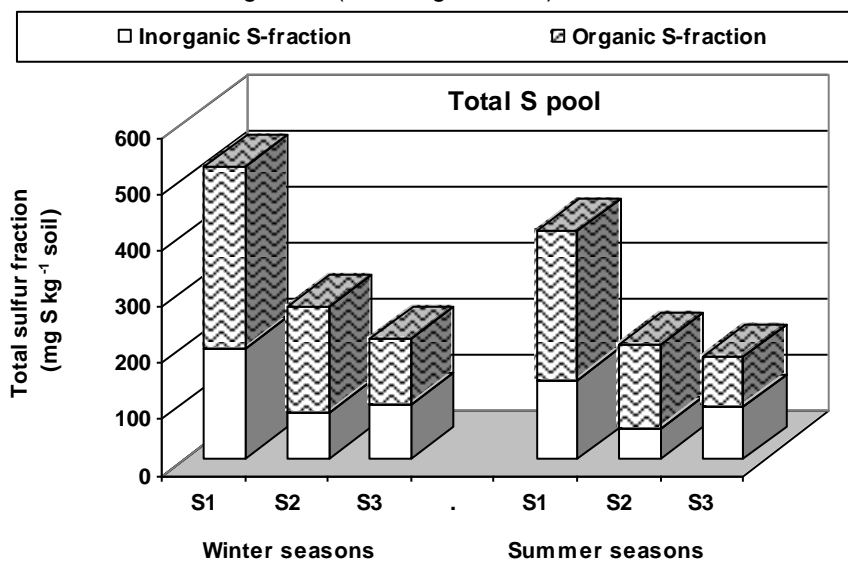


Fig. (2): A schematic diagram shows total-S pool and the distribution of its components in the studied soils.

These findings were greater than those obtained in summer seasons: 168.1 mg S. kg⁻¹ soil, referred as 61.8% and its standing stock 147.0 kg S. fed.⁻¹. Statistically, total organic-S content and its percentage distribution in year 1999/2000 were 205.9 mg S. kg⁻¹ soil, referred as (63.1% of total-S and its standing stock 172.2 kg S. fed.⁻¹. These values were much more in comparison with the values in year 2000/2001: 171.85 mg S. kg⁻¹ soil, referred as (61.4% of total-S content its standing stock 149.5 kg S. fed.⁻¹. Thus, it can be deduced that, total organic-S fraction was the main S-constituent in the investigated top soils, which is normally found in agricultural soils. This is consistent with many other studies and was shown for mineral top soils of various land use (Kraira Pamond *et al.*, 1991; Hu *et al.*, 2002 and Mansfeldt & Blume, 2002). Commonly, total organic-S content in Egyptian soils (Semi-arid region) is lower than those reported for the surface layers of soils from temperate and subtropic regions. It may be due to low level of humidified organic substances and the raising of acceleration rates of soil organic matter decomposition. Hu *et al.* (2000) confirmed these observations. Total organic-S content was negatively correlated with ionic strength (main soil parameter) ($r = -0.82^*$), max. air temp. ($r = -0.45$) and soil temp. ($r = -0.84^{**}$), and was positively correlated with organic-C ($r = 0.88^{**}$), total-N ($r = 0.90^{**}$).

2.1. Organic ester sulfate-S fraction:

Contents of ester sulfate-S compounds (Fraction 5) are given in Table (4) and illustrated in Figs. (1 and 3). Ester sulfate-S contents were 93.1 to 45.4 and 30.7 mg S. kg⁻¹ soil (avg. 56.4) for S₁, S₂ and S₃ respectively. Their percentage distribution expressed as % of total-S and of organic-S fractions accounted for 17.9% and 28.7%. Ester sulfate-S contents were negatively correlated with ionic strength ($r = -0.93^{**}$); max. air temp. ($r = -0.66^{**}$) and soil temp. ($r = -0.61^{**}$). It had positive correlations with water table depth ($r = 0.65^{**}$), organic-C ($r = 0.97^{*}$), total-N ($r = 0.87^{**}$) and organic-S ($r = 0.84^{**}$). Results reported by (Neptune *et al.*, 1975; Hu *et al.*, 2002 and Mansfeldt & Blume, 2002) confirmed these results.

Generally, it could be said that, percentage of total-S as ester sulfate-S in Egyptian soils (17.8%) was much more lower than reported in other soils in temperate and subtropical regions such as Brazilian soils (40%), Australian soils (52%) Iowa soils 50% Canadian Soils (53%). Statistically, ester sulfate-S content and its percentage distribution in winter seasons was 70.7 mg S. kg⁻¹ soil, referred as (20.4% of total-S and 33.2% of organic-S fractions). This value was higher than those obtained in summer seasons: 42.01 mg S. kg⁻¹ soil referred as 15.3% and 24.2%, respectively. Ester sulfate-S content and its percentages in year 1999/2000 was 64.4 mg S. kg⁻¹ soil, referred as (18.8% of total-S and 29.8% of organic-S fractions). This value was more pronounced than those obtained in year 2000/2001: 48.31 mg S. kg⁻¹ soil, referred as 16.9% and 27.6%, respectively. As the result of their universal existence and labile nature, sulfate ester-S constitute a major source of S for the inorganic-S pool in soil, but this S pool is only available for plant uptake after hydrolysis to inorganic-S through enzymatic hydrolysis (Kariapanond *et al.*, 1991; Germida *et al.*, 1992 and Elsgaard & vitnther, 2004). Thus, arylsulfatase play an important role in the process whereby organic-S are mineralized in soils and made available to plant (Elsgaad & Vinther, 2004).

2.2. Total organic-S directly bonded to carbon fraction:

Data considering total organic-S-compounds in which S is bonded directly to carbon (Fraction 6) are given in Table (4) and illustrated in Figs. (1 and 4). Total C-bonded S fraction consists of two fractions. (i) Raney-nickel reducible organic-S "amino acids-S" and (ii) unidentified organic-S fraction. Total C-bonded S content in studied soils was 132.5 mg S. kg⁻¹ soil. Its percentage distribution expressed as % of total-S and of organic-S fractions accounted for 44.3% and 71.3%, respectively, which were lower than reported in Brazilian soils (avg. 51%), in Iowa soil (avg. 55%) (Neptune *et al.*, 1975).

2.2.1. Organic amino acids-S fraction:

Data considering Raney-nickel reducible organic-S content (Fraction 6.1) and its percentages are illustrated in Figs. (1, 3 and 4). Amino acids-S contents were from 74.1, 33.5 and 40.61 mg S. kg⁻¹ soil (avg. 49.42) for S₁, S₂ and S₃, respectively. Percentage distribution of this fraction, expressed as % of total-S, of organic-S and of organic directly bonded S fractions accounted for 16.6%, 27.7% and 39.3%, respectively. Analytical obtained results showed that amino acid-S content in Egyptian soils (avg. 16.67%) was

higher than those obtained in Brazilian and Iowa soils (Table 1.3). Amino acids-S content and its percentage distribution in winter seasons was: 57.6 mg S. kg⁻¹ soil, referred as (17.5%, 29.5% and 41.3%). These values were higher than those obtained in summer seasons: 41.19 mg S. kg⁻¹ soil, referred as (15.7%, 25.9% and 37.2%), respectively. Amino acids-S content in year 1999/2000: was 55.65 mg S. kg⁻¹ soil, referred as (17.4%, 28.3% and 40.2%). These findings were much more than those obtained in year 2000/2001: 43.19 mg S. kg⁻¹ soil, referred as (15.9%; 27.1% and 38.3%), respectively. Statistically, amino acids-S contents were negative corrected with ionic strength ($r = -0.90^{**}$); max. air temp. ($r = -0.58^{**}$) and soil temp. ($r = -0.65^{**}$). It had positively correlations with water table depth ($r = 0.72^{**}$), organic-C ($r = 0.95^{**}$), total-N ($r = 0.86^{**}$) and to total organic-S ($r = 0.89^{**}$).

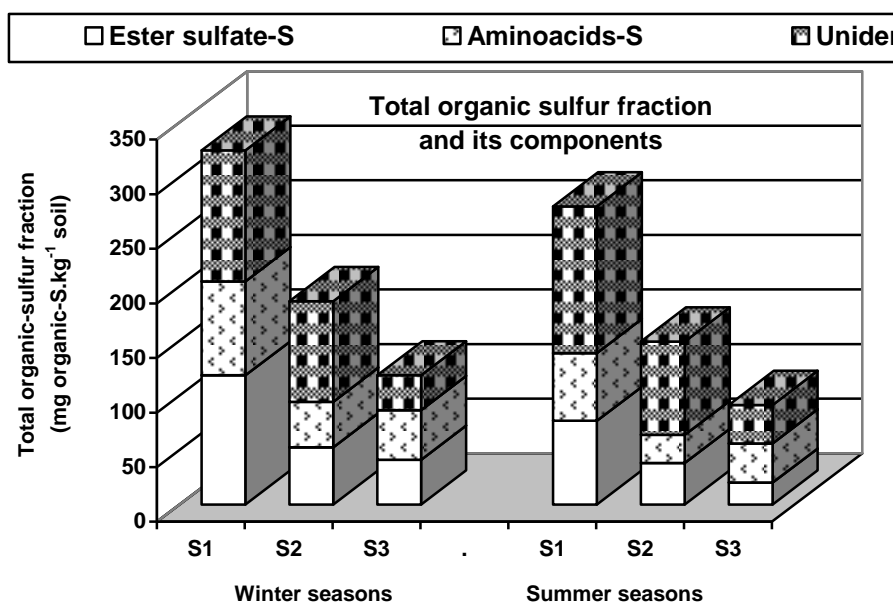


Fig. (3): A schematic diagram represents total organic fraction and the distribution of its components in the studied soils.

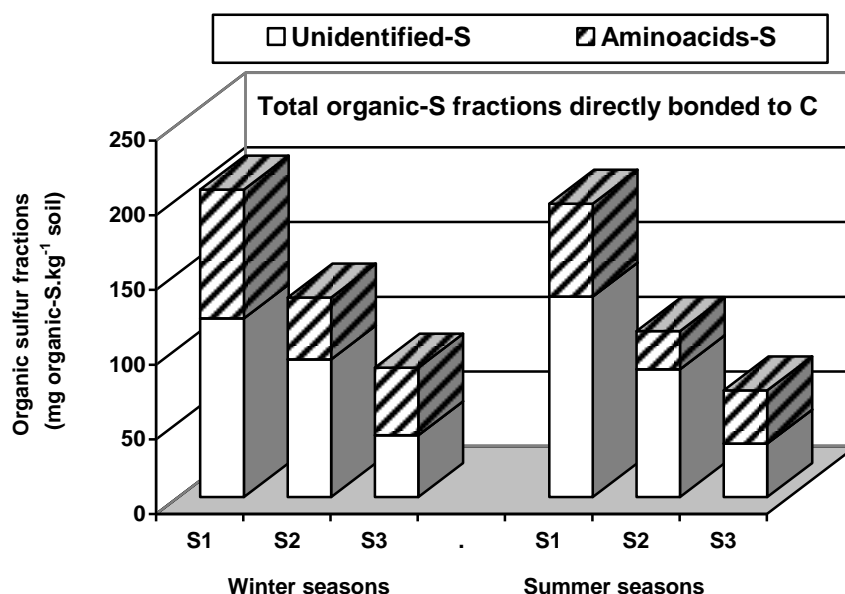


Fig. (4): A schematic diagram elucidates total C-bonded S fraction and the relative distribution of its components in studied soils.

2.2.2. Unidentified organic-S fraction:

Analytical data concerning content of this fraction (Fraction, 6.2) and its percentage distribution are illustrated in Figs. (1, 3 and 4). Organic unidentified-S contents were 126.9 to 88.8 and to 33.5 mg S. kg⁻¹ soil (avg. 83.11) for S₁, S₂ and S₃, respectively. Its percentage distribution expressed as % of total-S, of total organic-S and of organic directly bonded S fractions accounted for 27.6%, 43.5% and 60.7%, respectively (Table 1.3). From the previous results, it could be concluded that content of organic-S directly bonded to C (Fraction 6) constitutes a greater proportion of total organic-S fraction than ester sulfate-S in well drained soils than those obtained in another drainage conditions. Similar trends were obtained by Krairapanond *et al.* (1991); and Mansfeldt & Blume (2000). It was demonstrated in literature using radio-labeled sulfate that, sulfate-S is incorporated into both C-bonded S and ester sulfate-S fractions resulting in C-bonded S as the major end-product. Referred to total organic-S the main proportion of C-bonded S was 54% and of ester sulfate-S 46% (Mansfeldt and Blume, 2002). A dominance of C-bonded S over ester sulfate-S indicating the importance of this fraction in soils and sediments. Additionally, carbon-bonded S was less important for the S nutrition of crops than ester-sulfate-S (Li *et al.*, 2001).

T4

Enzymes involved in organic-S transformations:

1. Arylsulfatase enzyme activity (Ec 3.1.6.1.):

Analytical results belong to arylsulfatase activity are illustrated in Figs. (5 and 6). Arylsulfatase activity values were 268, 162 and 90 μg p-nitrophenol produced. g^{-1} soil hour^{-1} at 37°C pH 5.8 (avg. 173.4) for S₁, S₂ and S₃, respectively. Arylsulfatase activity in winter seasons: was (231) and its monthly distribution: (192.5, 287.3 and 212.6 on Dec., Jan., Feb., respectively). These values were higher than those obtained in summer seasons: (115.7) referred as (152; 89 and 106 on June, July and August, respectively). Arylsulfatase activity in year 1999/2000: (196.7) was much more than those obtained in year 2000/2001: (150.05) over the experimental period.

Arylsulfatase activity was in the same range as reported from other soils under similar experimental conditions (Gupta *et al.*, 1993; Elsgaard *et al.*, 2002 and Elsgaard & Vinther, 2004). Statistically, the activity of arylsulfatase in studied soils was significant and positively correlated mainly with soil pH ($r = 0.86^{**}$). These findings confirmed by the observations of other studies reporting that enzyme activities of soils are often increased with increasing soil pH. It has been reported that the rate of synthesis, release and stability of this enzyme by soil microorganism are dependent on soil pH. (Acosta Martinez & Tabatabai, 2000). Arylsulfatase activity reached its maximum values in traditional drained soils in winter seasons, in year 1999/2000 on January. Meanwhile, it had the lowest values in badly drained soils, in summer seasons and in year 2000/2001 on July as illustrated in Figs. (5 and 6). These findings confirmed the observations of other authors reporting that, arylsulfatase activity increased significantly during the rainy seasons when the soils were continually moist, but when the soils dried at the end of the rainy seasons, a reduction in enzyme activity occurred (Ross *et al.*, 1984). Arylsulfatase activity was positively significant correlated with organic-C content ($r = 0.92^{**}$) in studied soils differing markedly in drainage conditions.

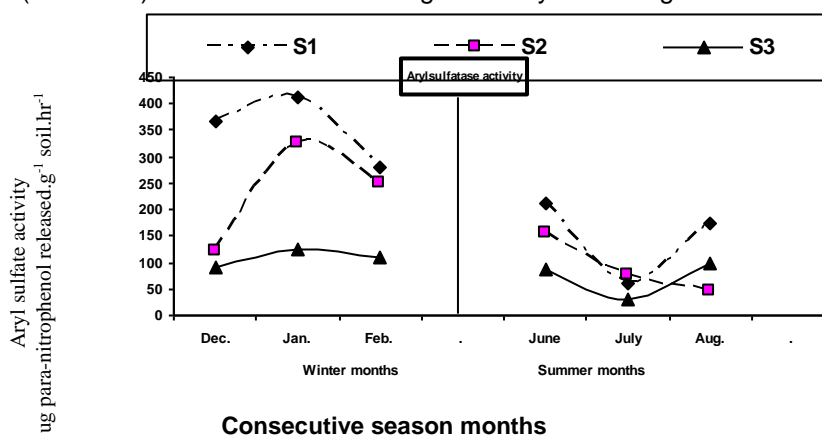


Fig. (5): Seasonal variations of soil arylsulfatase activity in studied soils.

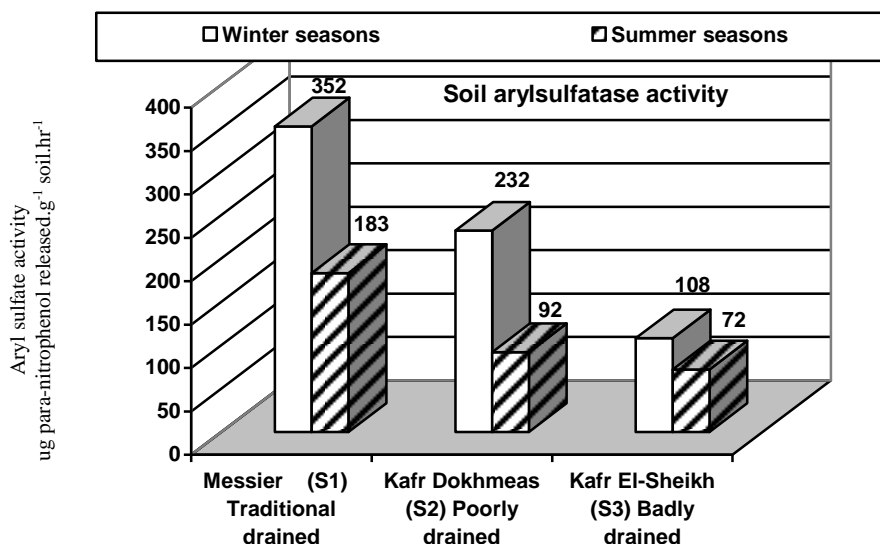


Fig. (6): A schematic diagram shows arylsulfatase activity in studied soils.

Furthermore, significant positive correlations were observed with total-S ($r = 0.53^*$); total organic-S ($r = 0.64^*$); ester sulfate-S ($r = 0.98^{**}$); bulk density ($r = -0.89^{**}$), amino acids-S ($r = 0.52^*$) and unidentified-S ($r = 0.48^*$). Similar trends were obtained by (Germida *et al.*, 1992; Mansfeldt & Blume 2002 and Elsgaard & Vinther, 2004). Moreover, arylsulfatase activity had a negatively significant correlations with ionic strength ($r = -0.83^{**}$), max. air temp. ($r = -0.69^*$) and soil temp. ($r = -0.76^*$). Also, they had a positively significant correlation with water table depth ($r = 0.92^{**}$).

2. Dehydrogenase enzymes activity:

Biological oxidation of organic-S compounds is generally a dehydrogenation process (Tabatabai, 1984) they transfer H from substrates to acceptors. Dehydrogenase activity values are illustrated in Figs. (7 and 8). and their activity values were 15.21, 9.44 and 3.56 $\mu\text{g TPF produced. g}^{-1} \text{ soil. hour}^{-1}$ at 37°C pH 8.5 (avg. 9.408) for S₁, to S₂ and S₃, respectively. Its value in winter seasons (12.33) was much more than those obtained in summer seasons: (6.47) in the course of the experimental period elongated 21 months. Analytical data declared also that monthly distribution values within winter seasons were 6.83, 12.08 and 18.07 on winter months Dec., Jan. and Feb. respectively. Meanwhile within summer seasons were 7.20, 3.77 and 8.45 on summer months June, July and August, respectively. Dehydrogenase activity in year 1999/2000. (12.143) was highly significant and higher than those obtained in year 2000/2001: (6.67). Dehydrogenase activity had the maximum values in traditional drained soils; in winter seasons; in year 1999/2000 and on February. However, it reached its minimum values in badly drained soil; in summer seasons; in year 2000/2001 and on July Figs. (7 and 8). Similar, trend were obtained by (Tabatabai, 1984; Frankenberger &

Johnson 1986, Erkenler & Tabatabai, 2003). Dehydrogenase activity values had positive significant correlations with all sulfur organic fractions: Total-S ($r = 0.46^*$); total organic-S ($r = 0.89^{**}$); ester sulfate-S ($r = 0.88^{**}$); amino acids-S ($r = 0.86^{**}$) and unidentified organic-S ($r = 0.43^*$). Also, they had a significant positive correlations with organic-C ($r = 0.89^{**}$), total-N ($r = 0.85^{**}$), and water table depth ($r=0.98^{**}$). However, they had a negative significant correlations with max. air temp. ($r = -0.58^*$) soil temp. ($r = -0.82^{**}$) and ionic strength ($r = -0.89^{**}$).

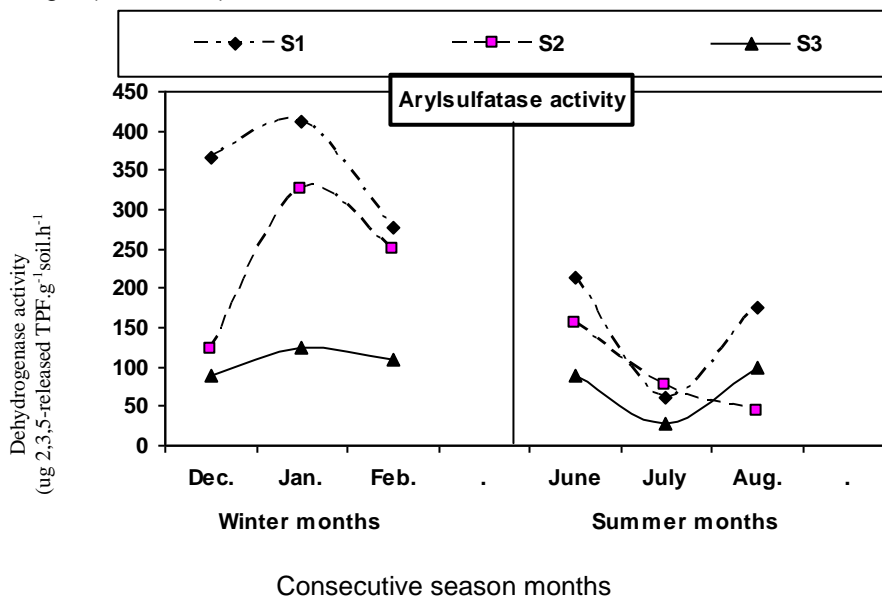


Fig. (7): Seasonal changes of soil dehydrogenase activity in studied soils..

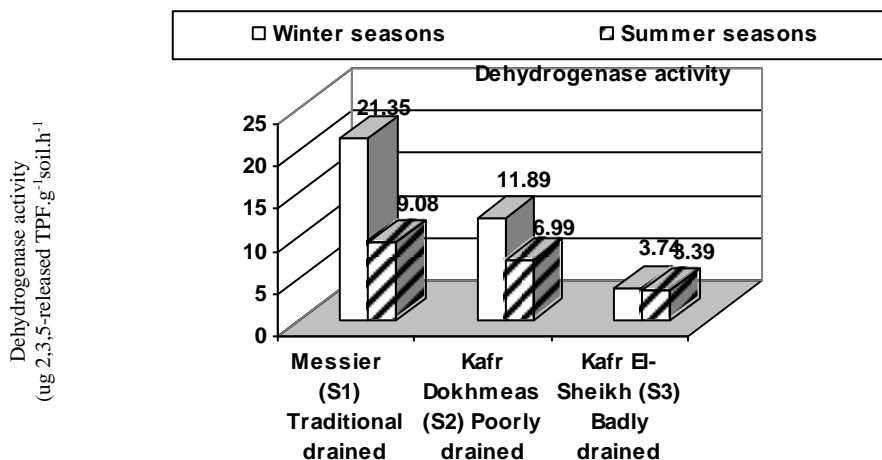


Fig. (8): A schematic diagram shows dehydrogenase enzyme activity in the studied soils.

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دراسات بيوكيميائية إنزيمية على حالة وصور الكبريت العضوية وتوزيعها في الأراضي الواقعة تحت ظروف صرف مختلفة

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أجريت هذه الدراسة تحت الظروف الحقلية في ثلاث مواقع بكفر الشيخ واقعة تحت ظروف صرف مختلفة ، ذات أعماق مستوى ماء أرضى متباينة ، تروى هذه المواقع بالمياه مختلفة مصادر التلوث لمدة طويلة. وكان الهدف من هذه الدراسة البيوكيميائية هو تقييم نشاط انزيمات تحولات الكبريت العضوى في هذه الأراضي وعلاقتها بحالة وصور الكبريت العضوية وتوزيعها ، وربط ذلك بالتغيرات البيئية والموسمية والسنوية. ولتقدير صور الكبريت العضوية المختلفة بالأراضي تم وضع ديجرام تصورى يتضمن الأساليب والطرق المختلفة لتقدير وحساب هذه الصور العضوية للكبريت في الأراضي تحت ظروف صرف مختلفة.

ويمكن تلخيص النتائج المتحصل عليها في الآتى:

- نشاط إنزيم الأريال سلفاتيز Arylsulfatase هو $173.35 \mu\text{g } \rho \text{ nitrophenol released. g}^{-1} \text{ soil.h}^{-1}$ ونشاط انزيم الـ Dehydrogenase $9.108 \mu\text{g TPF formed.g}^{-1} \text{ soil.h}^{-1}$ كمتوسط لتأثير جميع المتغيرات الدراسية التى تتضمن خواص الأراضي الكيميائية والفيزيائية والعضوية ، حالة الصرف في هذه الأراضي ، عمق مستوى الماء الأرضى ، الظروف البيئية (درجة حرارة الجو ودرجة حرارة التربة) السائدة بمنطقة الدراسة ، وربط التذبذبات الموسمية والسنوية بنشاط هذه الانزيمات وعلاقتها بصور وتوزيع مكونات الكبريت العضوية المختلفة في الأراضي.
- محتوى الأراضي من الكبريت الكلى والكبريت العضوى الكلى 300 ، 188.9 ميلجرام كبريت/كجم أرض ، ويمثل الكبريت العضوى 62.23% من الكبريت الكلى والباقى (37.77% معدنى). وعليه فإن الكبريت العضوى يمثل المكون الرئيسى للـ الكبريت في الأراضي تحت الدراسة إلا أن هذه النسبة أقل من تلك المقدره في المناطق المناخية الأخرى.
- محتوى الأراضي من الكبريت العضوى في صورة كبريتات استيرية Ester-sulfate-S وهى المركبات العضوية التى لا يرتبط فيها الكبريت مباشرة بـ كربون المادة العضوية هو 56.38% ميلجرام كبريت/كجم أرض تمثل 17.88% من الكبريت الكلى ، 28.74% من الكبريت العضوى الكلى تحت الدراسة وهذه النسب أقل من المقدره في المناطق المناخية الأخرى ، وتمثل هذه الصورة من الكبريت العضوى المصدر الرئيسى للـ كبريت المعدنى من خلال عمليات النشاط البيولوجى الإنزيمى أكبر من الصور العضوية الأخرى حيث يلعب إنزيم الأريال سلفاتيز دورا هاما في عملية أكسدة الكبريت العضوى في هذه الصورة.
- محتوى الأراضي من الكبريت العضوى الكلى الذى يرتبط فيه الكبريت مباشرة بـ كربون المادة العضوية Total organic-bonded S 132.5 ميلجرام كبريت/كجم أرض ، تمثل 44.35% من الكبريت الكلى ، 71.26% من الكبريت العضوى الكلى ، وهذه الصورة تمثل أكبر مكون من مكونات الكبريت العضوية بالأراضي عموما ، غير أن هذه النسب المتحصل عليها أقل من تلك المقدره في المناطق المناخية الأخرى.
- محتوى الأراضي من الكبريت العضوى في صورة أمينية كبريتية Amino acids-S هو 49.42 ميلجرام كبريت/كجم أرض ، تمثل 16.67% من الكبريت الكلى ، 27.72% من الكبريت العضوى الكلى ، 39.29% من الكبريت العضوى الكلى المرتبط مباشرة بـ كربون المادة العضوية ، وتعتبر القيم المتحصل عليها لهذه الصورة أكبر من تلك المقدره في المناطق المناخية الأخرى وهذا يعنى أن الكبريت العضوى في صورة أمينية يسود في أراضي المناطق شبه الجافة مقارنة بالمناطق المناخية الأخرى.

- **عموما** فإن نشاط إنزيمات تحولات الكبريت (الأريال سلفاتيز والديهيدروجينيز) في الأراضي وكذلك محتوى الأراضي من الكبريت الكلى ومن جميع صور الكبريت العضوية المقدره تصل أقصى قيمتها في الأراضي جيدة الصرف في المواسم الشتوية والعكس فإنها تصل إلى الحد الأدنى لها في الأراضي رديئة الصرف وفي المواسم الصيفية ويرتبط ذلك أساسا بوجود علاقة معنوية عالية موجبة مع عمق مستوى الماء الأرضى ومحتوى الطين بالأراضي وعلاقة معنوية عالية سالبة مع (C/S ratio) علاوة على أن جميع القيم المتحصل عليها عام 2000/1999م أكبر من تلك المتحصل عليها عام 2001/2000م.
- يصل نشاط الإنزيمات أقصى قيمة له في مواسم الشتاء وبصفة خاصة في شهر يناير ويصل إلى أقل قيمة له في مواسم الصيف في شهر يوليو ويرتبط ذلك بوضوح بالتغيرات الموسمية لدرجة حرارة الهواء الجوى ودرجة حرارة التربة وتذبذب عمق مستوى الماء الأرضى حيث أنه في فصل الشتاء يكون أكثر عمقا من فصل الصيف.
- يرتبط نشاط هذه الإنزيمات ارتباطا معنويا موجبا مع الكبريت الكلى total-S وكل صور الكبريت العضوى بالأراضي (الكبريت العضوى الكلى Total organic-S ، الكبريت العضوى في صورة استرات Ester sulfate-S ، الكبريت العضوى في صورة أميضية Amino acids-S ، وكذلك الكبريت العضوى الكلى المرتبط مباشرة بكاربون المادة العضوية Unidentified organic-S) ، ويرتبط أيضا مع رقم تفاعل التربة Soil pH ، الكربون العضوى Organic carbon ، النتروجين الكلى Total nitrogen ، ومن ناحية أخرى يرتبط النشاط ارتباطا معنويا سالبا مع خواص التربة (القوة الأيونية لعجينة الأرض المشبعة Ionic strength ، الكثافة الظاهرية Bulk density ، وكذلك محددات الظروف البيئية مثل درجة حرارة الهواء Max. air temp. ، درجة حرارة التربة لعمق 20 سم Soil temp.

Table (1.1): Seasonal microclimatological features at Kafr El-Sheikh region over the experimental period (21 months) during two successive winter and summer seasons 1999/2000 and 2000/2001

Successive seasons from Dec. 1999 to August 2001 (21 months)	pH (1: 2.5)	ECe (dSm ⁻¹)	Ionic strength (mmoles L ⁻¹)	SAR ratio	Bulk density Mg.m ⁻³	Total porosity %	Hydraulic conductivity (cm.hour ⁻¹)	Organic-C (g.kg ⁻¹ soil)	Total-N (mg.kg ⁻¹ soil)	C/N (ratio)	C/S (ratio)	CaCO ₃ (%)	
													Saturation extracts (soil pastes)
Messier (S₁):													
Winter seasons	1999/2000	7.94	4.17	60.3	4.75	1.25	52.7	1.582	20.0	1737	11.5	35.3	1.9
	2000/2001	7.55	3.86	56.7	4.90	1.28	51.4	1.560	17.6	1823	9.7	37.3	2.3
	Mean	(7.74)	(4.01)	(58.5)	(4.82)	1.26	(52.0)	(1.571)	(18.8)	(1780)	(10.5)	(36.3)	(2.1)
Summer seasons	2000	7.75	4.34	64.1	5.24	1.225	53.7	1.393	16.7	1374	12.2	40.3	2.3
	2001	7.69	4.68	67.3	4.81	1.36	48.5	1.365	14.5	1362	10.6	36.7	2.4
	Mean	(7.72)	(4.50)	(65.7)	(5.02)	1.29	(51.1)	(1.379)	(15.6)	(1368)	(11.4)	(38.5)	(2.3)
Grand mean	7.73	4.26	62.1	4.92	1.282	51.6	1.475	17.2	1574	11.0	37.4	2.2	
Kafr Dokhmeas (S₂):													
Winter seasons	1999/2000	7.86	4.82	66.2	6.35	1.23	53.2	1.243	12.9	975	13.2	43.7	3.3
	2000/2001	7.93	4.67	69.8	6.27	1.34	49.1	1.205	12.5	1051	11.9	51.3	3.7
	Mean	(7.89)	(4.74)	(68.0)	(6.31)	1.29	(51.2)	(1.224)	(12.7)	(1013)	(12.5)	(47.5)	(3.5)
Summer seasons	2000	7.68	5.12	71.4	6.74	1.41	46.5	1.000	8.8	579	15.2	41.0	4.0
	2001	7.75	4.58	72.9	6.57	1.42	46.2	0.892	8.4	531	15.9	43.5	3.8
	Mean	(7.71)	(4.85)	(72.2)	(6.65)	1.42	(46.3)	(0.946)	(8.6)	(555)	(15.6)	(42.2)	(3.9)
Grand mean	7.80	4.80	70.1	6.48	1.35	48.8	1.085	10.6	759	14.0	44.9	3.7	
Kafr El-Sheikh (S₃):													
Winter seasons	1999/2000	8.36	6.54	82.67	6.85	1.53	42.0	0.933	10.6	723	14.7	42.66	3.501
	2000/2001	8.31	6.11	85.28	6.34	1.50	43.0	0.856	9.7	610	15.9	53.33	4.175
	Mean	(8.33)	(6.32)	(83.98)	(6.60)	1.52	(42.5)	(0.834)	(10.1)	(664)	(15.3)	(48.00)	(3.837)
Summer seasons	2000	8.13	6.92	87.98	7.24	1.62	38.8	0.634	8.3	466	17.8	40.16	3.929
	2001	8.24	6.83	89.73	7.55	1.63	38.3	0.609	7.7	418	18.4	47.95	4.997
	Mean	(8.18)	(6.88)	(88.84)	(7.40)	1.62	(38.5)	(0.622)	(8.0)	(441)	(18.1)	(44.06)	(4.463)
Grand mean	8.26	6.60	86.41	7.00	1.57	40.5	0.758	9.1	543	16.7	46.03	4.150	

Table (3): Total organic-S and total inorganic-S contents in studied soils.

Successive seasons from December 1999 to August 2001 (21 months)	*Total inorganic sulfur fraction (FRACTION 3)						Total organic sulfur fraction Difference [Fraction (1)-Fraction (3)] (FRACTION 4)					
	Messier (S ₁) Traditional drained		Kafr Dokhmeas (S ₂) Poorly drained		Kafr El-Sheikh (S ₃) Badly drained		Messier (S ₁) Traditional drained		Kafr Dokhmeas (S ₂) Poorly drained		Kafr El-Sheikh (S ₃) Badly drained	
	Winter seasons (Dec., Jan., Feb.)						Winter seasons (Dec., Jan., Feb.)					
Relations	1999/2000	2000/2001	1999/2000	2000/2001	1999/2000	2000/2001	1999/2000	2000/2001	1999/2000	2000/2001	1999/2000	2000/2001
mg S fraction.kg ⁻¹ soil	235.66 (196.27)	168.88	85.75 (83.66)	81.57	109.53 (97.59)	85.65	331.34 (324.23)	317.12	210.25 (186.34)	162.43	140.47 (118.41)	96.35
% of total-S fraction	41.56% (37.33%)	33.10%	28.97% (31.07%)	33.43%	43.81% (45.44%)	47.06%	58.44% (62.67%)	66.91%	70.03% (68.29%)	66.56%	56.19% (54.56%)	52.94%
Standing stock kg S. fed ⁻¹ (15 cm depth)	185.87 (156.53)	127.19	66.94 (68.08)	69.22	106.05 (93.74)	81.42	261.35 (259.24)	257.13	164.12 (150.98)	137.84	136.02 (113.81)	91.59
Relations	Summer seasons (June, July, August)						Summer seasons (June, July, August)					
mg S fraction.kg ⁻¹ soil	2000 123.69 (141.95)	2001 160.21	2000 54.30 (55.86)	2001 57.42	2000 106.30 (92.72)	2001 79.13	2000 292.31 (264.05)	2001 235.79	2000 160.70 (149.14)	2001 137.58	2000 100.70 (91.28)	2001 81.87
% of total-S fraction	29.73% (35.09%)	40.46%	25.26% (27.35%)	29.45%	51.35% (50.25%)	49.15%	70.27% (64.91%)	59.54%	74.75% (72.65%)	70.55%	48.65% (49.75%)	50.85%
Standing stock kg S. fed ⁻¹ (15 cm depth)	95.46 (116.52)	137.57	48.44 (49.99)	51.55	108.56 (95.01)	81.46	225.59 (214.03)	202.47	143.36 (133.43)	123.51	102.84 (93.56)	84.28
Grand mean (mg sulfur. kg ⁻¹ soil)	179.68 (169.11)	158.5	70.03 (69.76)	69.50	107.92 (95.15)	82.39	311.83 (294.14)	276.46	185.48 (167.74)	150.01	120.59 (104.84)	89.11

* Cited from El-Kammah (2008)

