# THE EFFECT OF ADDITION SOME ORGANIC MATTERS IN **BIOLOGICAL OXIDATION OF AGRICULTURAL SULFUR AT DEFERENT LEVELS OF SOIL COMPACTION. II.-Population** densities of autotrophic and heterotrophic sulfur oxidizing Microorganisms <sup>+</sup>

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## Abstract:

This study was conducted to evaluate the interaction effect of different levels of agricultural sulfur, organic matter source and compaction on the biological oxidation of sulfur, through studying the variation in population densities of autotrophic and heterotrophic sulfur oxidizing microorganisms during different incubation periods. A factorial experiment was conducted according to a complete randomization design with three replicates., to know the effect of three agricultural sulfur levels (0, 1 and 2) gm S°. kg<sup>-1</sup> soil (S0, S1 and S2) respectively, with three organic matter treatments those without organic matter, addition of dried alfalfa and cow manure which added in one level 6 gm C. kg<sup>-1</sup> soil (C0, C1 and C2) respectively. Soil compacted to reach three levels of bulk density 1.25, 1.35 and 1.45 Mg. m<sup>-3</sup> (B1, B2 and B3) respectively. All the treatments was incubated at 28± 2°C for 15, 30 and 45 days (T1, T2 and T3) respectively with maintenance the soil moisture content for all treatments at 50% of the available water by weight method,: Through every incubation period the population density of autotrophic and heterotrophic sulfur oxidizing microorganisms were evaluated by M.P.N. method. The results obtained from this study could be summarized as follows:

- 1- population density of both autotrophic and hetrotrophic sulfur oxidizers differently affected by sulfur, organic source and compaction treatments. The higher average counts of the autotrophes were (16.58, 30.33 and 11.1 x 10<sup>2</sup> cells. g<sup>-1</sup> dry soil were recorded under treatments S1, C1 and B2 respectively, while those of the hetrotrophies (6.29, 7.62 and 5.55) x  $10^7$  cells. g<sup>-1</sup> dry soil were found in S0, C2 and B2 treatments. Respectively.
- 2- Generally, The interaction treatments showed the highest values at (S1C1B2) treatment after 30 days of incubation (T2). population densities of autotrophic and heterotrophic S-oxidizers recorded under this treatment were 7.98 x  $10^2$  and 7.98 x  $10^7$  cells. g<sup>-1</sup> dry soil, respectively.

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- ١-تاثرت الكثافة العددية للاحياء المجهرية المؤكسدة للكبريت بنوعيها الذاتية والمتباينة التغذية بمستويات كل من المتغيرات الثلاثة المدروسة ( الكبريت الزراعي ونوع المادة العضوية ورص التربة ) اذ بلغت اعلى معدلات الكثافة العددية للاحياء ذاتية التغذية ١٦,٥٨ و ١٦,٥٣ و ١١,١١ × ١٠ خلية . غم ' تربة جافة عند المعاملات (S1 ، C1 ، S1) على التوالي . في حين بلغت هذه المعدلات للاحياء (S0 ،
- ٢ اشارت نتائج تداخل المعاملات ان اعلى المعدلات بصورة عامة قد سجلت عند المعاملة (S1C1B2)
   خلال مرحلة القياس الثانية(T2) بلغت ٧,٩٨ × ١٠ أ و ٧,٩٩ × ١٠ <sup>٧</sup> خلية . غـم<sup>-1</sup> تربـة جافـة
   لاعداد البكتريا الذاتية والمتباينة التغذية على التوالي .

#### **Introduction:**

Most Iraqi Soils are ordinarily Calcareous.Carbonate percentage will reach to 400 gm. kg<sup>-1</sup> soil [1]. The presence of carbonate will lead to the formation of basic conditions. Which are negatively effect on some nutrients availability (ideal phosphorus , Iron , Manganese and Zinc). Whose have very important role in plant life. For this reason we are anticipating to reduction Soil reaction by any way will lead to increase the availability of these nutrients like information for us the addition of acidity affected matters as Sulfur was used from long period as device to basic Soil reduction.

When Sulfur added to soil it was exposing to biological oxidation process, which proceeded sulfur metamorphosis to oxides, this reaction found the formation of sulfuric acid.

This process was occur by autotrophic microorganisms especially which following Thiobacillus species. also the heterotrophic microorganisms, in addition to some types of bacteria and actenomyces and fungi. [2, 3].

This oxidation process was effected by many factors like soil moisture, and soil aeration. The results of some researchers were showed [4, 5] that soil was expose to

compaction as a result of traffic heavy agricultural equipments on the soil during agricultural service process, which caused increasing the soil bulk density and penetration resistance which was decreasing soil porosity and its infiltration. That means the increasing in the Micro porous ratio comparison than Macro porous in the soil. Which lead to shortage in the soil air. This reason negatively affected on the growth and efficiency of sulfur oxidizer organisms in the soil. Generally we are know that most of these organisms were erobic in there nature. And most of the bacteria which follow Thiobasillus species sulfur oxidized was aerial compulsory [6].

There fore any variation in the soil oxygen quantity will later effect on these soil organisms efficiency. The studies of [7, 8] showed presence of the autotrophic and heterotrophic sulfur oxidizer microorganisms in more than 35 soil samples which are collected from Canadian cultivated soils. But about Iraqi soils [9] was pointed in his study to the absence of the role of autotrophic sulfur oxidize microorganisms in two different texture soil samples. But the existence of the heterotrophic thiosulfate  $(S_2O_3^{-})$  oxidize organisms. While the superiority showed to the heterotrophic organisms groups which was sulfur oxidize, and thiosulfate producer.

There fore the present study is designed to know the interaction effects for the addition of sulfur and organic matter under different levels of compaction on the population densities of autotrophic and heterotrophic sulfur oxidize microorganisms.

#### Materials and methods:

Soil samples was taken from upper horizon (0-30 cm) from alluvial soil which classified Typic Torriflovents from Al-Mamer region east of Al- faluji city at Al-Anbar governorate. Soil sample was aerial dried and crashed before sieving across screen which opens diameter 2mm. Some chemical and physical properties for soil before treatment were measured (table 1).

Agricultural sulfur (95 % S) which its specifications illustrated in the table (2) was added to the soil with three levels 0, 1 and 2 gm.  $kg^{-1}$  soil (S0, S1 and S2) respectively.

Two types of organic sources were used in this study the first dried alfalfa (C1) and the second cow manure (C2) (table 3) they are dried and crashed before sieving through 2 mm sieve, than they are added to the soil in one level (6 gm. kg<sup>-1</sup> soil) according to [10], addition to control treatment (with out addition O. M.).

Optimal moisture percentage for soil to compaction was limited with out and with addition of every organic matter sources to the soil. Also we are limiting the number of the hits which we are need to reach every studied compaction level. According to [11] which was mentioned in [12]. By using special apparatus industrialized similar to procter apparatus which was mentioned by [13]. After that agricultural sulfur and organic sources were added to the soil according to that's levels which are indicated above. Then treated soil was wetted to the optimal moisture percentage and compacted for every treatment.

After wards we limited the numbers of hits which we needs to reach the limitation bulk density for every treatment by expose every replicate to different number of hits to reach levels of bulk density 1.25, 1.35 and 1.45 Meq.  $m^{-3}$  (B1, B2 and B3) respectively. And for every above studied treatment. After wards we separated the middle ring by the thin wire than placed in plastic container (200 ml capacity). Also soil moisture content at the field capacity and wilting point permanent were limited for all treatments to know the available water percentage (table 4).

Experimental units were incubated at temperature (28 + 2 °C) [14]. The soil moisture content was fixed at 50% from available water for all treatments. After 15,

30 and 45 days of incubation (T1 , T2 and T3) respectively. Soil samples of every experimental unit was taken and mixed very well. Than we measured the population density of Sulfur oxidizes microorganisms were counted by using most probability number method(MPN).By preparation series of dilutions( $10^{-1}$ - $10^{-7}$ )using distill sterilized water for every one:

- **A.** Autotrophic thiosulfate oxidize microorganisms which was measured by method [15].
- B. Hetrotrophic sulfur oxidize microorganisms, measured according to [16].

Than the most probability numbers was accounted by using the tables which are demonstrated from [17] by taking the last three dilutions before the dilution which give negative test in the five replications for the two microorganisms groups.

The property	The value
	Soil partical size distribution
Sand	700 gm. Kg <sup>-1</sup>
Silt	120 gm. kg <sup>-1</sup>
Clay	180 gm. kg <sup>-1</sup>
Texture	Sandy loam
pH (1:1)	8.2
EC (1:1)	$2.0 \text{ ds. m}^{-1}$
CaCO <sub>3</sub>	201 gm. kg <sup>-1</sup>
Organic matter	9.5 gm. kg <sup>-1</sup>
Total Nitrogen	$0.53 \text{ gm. kg}^{-1}$
Available sulfate	185.3 mg. kg <sup>-1</sup>
Soil moisture content at tension 33kpa	27.00%
Soil moisture content at tension 1500 kpa	10.25%
Available water	16.75%
Autotrophic Microorganisms	$0.30 \times 10^2$ cell.gm <sup>-1</sup> dry soil
Heterotrophic Microorganisms	$3.90 \times 10^7$ cell.gm <sup>-1</sup> dry soil

 Table (1): Some properties for studied soil before treatment

Table (2): Standard specifications for used agricultural sulfur in the study

]	Mesh	Hydrocarbon %	Clay %	Total carbon %	CaSO <sub>4</sub> . 2H <sub>2</sub> O %	CaCO <sub>3</sub> %	Ca <sup>++</sup> ppm	EC (1:1) Ds. m <sup>-1</sup>	pH(1:1)	S° %
	325	0.06	1.5	0.12	0.0036	0.0	64	0.44	3.7	95

This measurements were gated from the general company for Meshrak sulfur

Table (3): Some chemical properties for organic matter sources used in the study

Property	Dried alfalfa (C1)	Cow manure (C2)
pH (5:1)	6.5	6.7
EC (5:1) (ds. m <sup>-1</sup> )	1.0	11.0
Total organic carbon (gm. kg <sup>-1</sup> )	418.0	454.0
Total nitrogen (gm. kg <sup>-1</sup> )	35.0	17.9
C/N ratio	12.0	25.4
Sulfur (gm. Kg <sup>-1</sup> )	13.2	1.8

The treatment	Moisture percentage at field capacity (33 kpa tension)	Moisture percentage at field capacity (1500 kpa tension)	Available water
C0	27.00	10.25	16.75
C1	27.30	10.29	16.71
C2	27.70	10.50	17.20

 Table (4): The soil moisture content for studied treatments at field capacity and wilting point permanent

These values measured at laboratory of the general company for soil and water resources.

## **Results and discussion:**

## 1- The population density of Autotrophic microorganisms.

Results of study showed the absence of the autotrophic oxide the sulfur element microorganisms in the soil, which treated with levels of sulfur and organic matter under studied compaction levels. The absence of this type of microorganisms was pointed to supporting poverty existence Iraiqin soils to these organisms because inclined there reaction to basic. This result was agreed with [8] who found the absence of the autotrophic sulfur oxidize microorganisms at basic reaction conditions. Also [9] obtained same results in the Iraqi soils, but he found the presence of the autotrophic thiosulfate oxidize microorganisms.

The results in table (5) showed significant increasing in the numbers of autotrophic thiosulfate oxidize microorganisms with increasing the level of sulfur addition to the soil. after first incubation time T1 (15 days) the addition of 1gm S. kg<sup>-1</sup> soil (S1) caused significant statistical increasing in the number of these organisms reached 15.67 x  $10^2$  cell. gm<sup>-1</sup> dry soil as mean comparison to control (S0)which showed 0.11 x  $10^2$  cell. gm<sup>-1</sup> dry soil. While S2 gave 14.98 x  $10^2$  cell. gm<sup>-1</sup> dry soil.

During the second and third periods after 30 and 45 days from incubation occurred significant increasing in the autotrophic microorganisms in both sulfur addition treatments (S1 and S2) comparison with S0 treatment.

Also increasing the incubation period was caused significant increase in the population density of these organism. The second level of sulfur addition (S2) treatment showed superiority during T3 period to get  $18.09 \times 10^2$  cell. gm<sup>-1</sup> dry soil.

However the addition of organic matter showed significantly negative effect on this characteristic after 15 days from incubation the average of this organism population reached (28.01, 1.57 and 1.17) x  $10^2$  cell. gm<sup>-1</sup> dry soil at C0, C1 and C2 treatments respectively. Also increasing the incubation period caused significant increasing in the population of this studied organism reached (30.83 and 32.14) x  $10^2$  cell. gm<sup>-1</sup> dry soil at T2 and T3 respectively for C0 treatment. Comparison with C1 and C2 whose gave population density (1.70 and 1.26) x  $10^2$  cell. gm<sup>-1</sup> dry soil at T2 and (1.77 and 1.30) x  $10^2$  cell. gm<sup>-1</sup> dry soil at T3 respectively.

The results in table (5) showed statically different between compaction treatments in order to there effects on autotrophic microorganisms. which its population densities reached (10.28, 10.30, 10.17) x  $10^2$  cell. gm<sup>-1</sup> dry soil for treatments B1, B2 and B3 respectively.

However the increasing in the incubation period will lead to increase the population of this organism to reach  $(11.27, 11.29 \text{ and } 11.24) \times 10^2 \text{ cell. gm}^{-1}$  dry soil

after 30 days of incubation and  $(11.74, 11.75 \text{ and } 11.73) \times 10^2 \text{ cell. gm}^{-1}$  dry soil after 45 days of incubation for B1, B2 and B3 treatments respectively.

The interaction between studied factors (sulfur and organic matter addition levels under studied compaction levels) also effected on the autotrophic sulfur oxidize microorganism. The S1C0B2 treatment recorded higher value to these organism reached 43.01 x  $10^2$  cell. gm<sup>-1</sup> dry soil at (T1) incubation period. But C1 and C2 at S0 level of sulfur addition at all studied compaction levels showed absence of this organism in the soil.

On the other side at T2 and T3 incubation periods generally all interaction treatments take the same direction which is recorded at the first period with simply increasing. Also superiority of interaction treatments for S2 level at 45 days incubation (table 5).

The lower population density of autotrophic sulfur oxidize microorganism that we noted during experiment stages may be clear pointer to the shortage importance and role of these organism in sulfur oxidize operation. Comparison to heterotrophic sulfur oxidize organism which limited with few types of bacteria that's majority followed Thiobacillus species whose activity appear in the acidity soils. There fore generally there activity was limited in Iraqin soils.

The addition of 1 and 2 gm. Kg<sup>-1</sup> soil sulfur showed clearly increasing in the population density of autotrophic microorganism in the soil this action may be connected with the formation of thiosulfate  $(S_2O_3^{=})$  as result of activity of many heterotrophic microorganisms, So that the use of this product by autotrophic microorganisms [18, 19, 20]. And because of the great types of these autotrophic microorganisms were aerial compulsory therefore the studied compaction levels B1 and B2 showed increasing in the population density of these organism comparison to level B3. From another direction the addition of dried alfalfa and cow manure to the soil cause significant negative effect on the population of studied microorganism and this action was connected with biological analysis processes of organic matter which have toxic effect [10, 21].

#### 2- The population density of hetrotrophic thiosulfate oxidize microorganism.

The results in table (6) showed higher population density as average 6.19 x  $10^7$  cell. gm<sup>-1</sup> dry soil was recorded at S0 (soil not treated with sulfur) comparison with S1 and S2 treatments which gave population density (5.60 and 4.45) x  $10^7$  cell. gm<sup>-1</sup> dry soil respectively at T1 incubation period. The same affect to sulfur addition was showed at T2 and T3 incubation times .while significant positive effect was demonstrated when the two types of organic matter were added to the soil at level 6 gm C. kg<sup>-1</sup> soil, reached (7.66 and 7.01) x  $10^7$  cell. gm<sup>-1</sup> dry soil for C1 and C2 treatments respectively at T1 period. The same direction continuous at T2 and T3 incubation times.

About compaction effect on this characteristic, there is reduction in the population density occur with increasing the compaction level at T1. It reached  $5.60 \times 10^7$  cell. gm<sup>-1</sup> dry soil at level B1 decreased to  $5.43 \times 10^7$  cell. gm<sup>-1</sup> dry soil at level B3. The same direction was recorded when incubation time increased, to gave lower value  $5.43 \times 10^7$  cell. gm<sup>-1</sup> dry soil at T3 at B1 and B2, decreased to  $5.38 \times 10^7$  cell. gm<sup>-1</sup> dry soil at B3 treatment.

However about incubation time, we can see from table (6) significant superiority to T2 which gave higher population to these organisms reached  $5.59 \times 10^7$  cell. gm<sup>-1</sup> dry soil, decreased after that to gave lower value at T35.41 x 10<sup>-7</sup> gm dry soil. The

interaction between studied treatments showed significant effect about interaction of two treatments or more about studied characteristic. S0C1B1 and S0C1B2 treatments at T1 incubation time superiority in growth of heterotrophic thiosulfate oxidize microorganism, which reached 8.45 x  $10^7$  cell. gm<sup>-1</sup> dry soil as mean, decreased to  $0.71 \times 10^7$  cell. gm<sup>-1</sup> dry soil at S2C0B3 treatment at T3 incubation time.

The negative affect of sulfur addition on biomass of these organism was indicated from [22]. bat positive affect of organic matter addition may be committed directly to exist the carbon source and energy from these matters which used by hetrotrophic microorganism to growth (increasing in the population density) and there activity (oxidize the reduction formula of sulfur) which found in the organic matter or the soil. The same relationship between the hetrotrophic population density and activity with organic matter was showed by [16, 20 and 23].

The superiority of dry alfalfa on the cow manure addition to soil in the population density of these organism through three measuring periods. Perhaps to turn back to lower percentage of C: N ratio in the alfalfa which cause easily microbial analysis, in addition to content cow manure to some compounds materials which negatively affected on growth of soil microbial. This result obtained was agreed with [9].

Since all hetrotrophic sulfur oxidize organisms was aerial compulsory, especially the fungi and actenomyces there for the improving soil aerial condition by reducing compaction level reflected positively on growth and activity of these microorganism. So this was elucidate the increasing of the population density of studied organism at B1 and B2 compaction treatments comparison with B3

Incubation periods			Т	'1			Т	2			Г	3	
Sulfur	0.M.	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean
treatment	treatment												
<b>S0</b>	CO	0.32	0.32	0.32	0.32	0.30	0.31	0.28	0.30	0.27	0.27	0.26	0.27
	C1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.11	0.11	0.11	0.11	0.10	0.10	0.09	0.10	0.09	0.09	0.09	0.09
S1	CO	43.00	43.01	43.00	43.00	47.03	47.04	47.00	47.02	47.00	47.00	47.00	47.00
	C1	2.40	2.41	2.39	2.40	2.51	2.51	2.50	2.51	2.51	2.51	2.50	2.51
	C2	1.60	1.62	1.60	1.61	1.60	1.61	1.60	1.60	1.60	1.61	1.60	1.60
		15.67	15.68	15.66	15.67	17.05	17.05	17.03	17.04	17.04	17.04	17.03	17.04
S2	CO	41.01	41.12	40.00	40.71	45.16	45.33	45.00	45.16	49.18	49.22	49.10	49.17
	C1	2.31	2.33	2.30	2.31	2.61	2.60	2.60	2.60	2.80	2.80	2.78	2.79
	C2	1.92	1.92	1.90	1.91	2.21	2.17	2.19	2.19	2.31	2.31	2.30	2.31
		15.08	15.12	14.73	14.98	16.66	16.70	16.60	16.65	18.10	18.11	18.06	18.09
The me	an of T		10	.25			11	.26			11	.74	
Mear	n CO	28.11	28.15	27.77	28.01	30.83	30.89	30.76	30.83	32.15	32.16	32.12	32.14
Mear	n C1	1.57	1.58	1.56	1.57	1.71	1.70	1.70	1.70	1.77	1.77	1.76	1.77
Mear	n C2	1.17	1.18	1.17	1.17	1.27	1.26	1.26	1.26	1.30	1.31	1.30	1.30
The I	mean	10.28	10.30	10.17	10.25	11.27	11.29	11.24	11.27	11.74	11.75	11.73	11.74
Г	L.S.D.		On	e fold		Two	fold	Th	ree fold		Four fo	d	

**Table (5):** the effects of sulfur, organic matter and compaction treatments on the population density of autotrophic thiosulfate oxidize microorganisms  $(x \ 10^2 \text{ gm}^{-1} \text{ dry soil})$ 

Table (6): the effects of sulfur, organic matter and compaction treatments on the population density of hetrotrophic thiosulfate oxidize microorganism  $(x \ 10^7 \text{ gm}^{-1} \text{ dry soil})$ 

0.008

0.010

0.004

0.005

0.001

0.002

0.05

0.01

0.036

0.048

Incubation periods			]	Г1			]	Г <b>2</b>				Т3	
Sulfur					Co	mpacti	on trea	tments					
treatments	treatments	B1	B2	B3	mean	B1	B2	B3	mean	B1	B2	B3	Mean
	C0	3.98	3.98	3.92	3.96	3.95	3.97	3.90	3.94	3.82	3.83	3.71	3.79
<b>S0</b>	C1	8.44	8.44	8.33	8.40	8.45	8.46	8.30	8.40	8.20	8.20	8.16	8.19
	C2	6.66	6.66	6.57	6.63	6.68	6.69	6.66	6.68	6.60	6.60	6.57	6.59
The	mean	6.36	6.36	6.27	6.33	6.36	6.37	6.29	6.34	6.21	6.21	6.15	6.19
	CO	1.01	1.01	0.90	0.97	1.09	1.09	0.93	1.04	1.05	1.06	0.95	1.02
<b>S1</b>	C1	7.91	7.91	7.89	7.90	7.94	7.99	7.92	7.95	8.00	8.00	7.98	7.99
	C2	7.82	7.84	7.80	7.82	7.80	7.82	7.80	7.81	7.80	7.80	7.79	7.80
The	mean	5.58	5.59	5.53	5.57	5.61	5.63	5.55	5.60	5.62	5.62	5.57	5.60
	C0	0.95	0.95	0.88	0.93	0.92	0.93	0.89	0.91	0.81	0.82	0.71	0.78
<b>S2</b>	C1	6.85	6.85	6.31	6.67	6.81	6.80	6.78	6.80	6.26	6.26	6.28	6.27
	C2	6.77	6.78	6.23	6.59	6.76	6.78	6.72	6.75	6.30	6.29	6.31	6.30
The	mean	4.86	4.86	4.47	4.73	4.83	4.84	4.80	4.82	4.46	4.46	4.43	4.45
The mean of T Mean C0 Mean C1 Mean C2 The mean			5.	.54			5.	.59				5.41	
		1.98	1.98	1.90	1.95	1.99	2.00	1.91	1.96	1.89	1.90	1.79	1.86
		7.73	7.73	7.51	7.66	7.73	7.75	7.67	7.72	7.49	7.49	7.47	7.48
		7.08	7.09	6.87	7.01	7.08	7.10	7.06	7.08	6.90	6.90	6.89	6.90
		5.60	5.60	5.43	5.54	5.60	5.61	5.54	5.59	5.43	5.43	5.38	5.41

L.S.D.	One fold	Two fold	Three fold	Four fold
0.05	0.026	0.006	0.003	0.001
0.01	0.034	0.008	0.004	0.001

## **Conclusions and recommendation:**

- 1- Results of the study showed the absence of autotrophic sulfur oxidize bacteria but included the soil autotrophic thiosulfate oxidize bacteria.
- 2- The soil contanined high numbers of the hetrotrophic sulfur oxidize microorganism.
- 3- The addition of agricultural sulfur with 1 gm S° kg<sup>-1</sup> soil level gave significant positive effect on the autotrophic thiosulfur oxidize organism. but its addition significantly negatively effected on the hetrotrophic sulfur oxidize microorganism.
- 4- Soil compaction levels affected on the numbers of both microorganisms, the 1.35 Meg. m<sup>-3</sup> bulk density level showed higher values comparison to the 1.25 and 1.45 Meg. m<sup>-3</sup> levels.
- 5- Incubation times differ in there effects on the studied properties.
- 6- We are think the necessary to repetition this study under field conditions to know the interaction effect of sulfur addition and compaction under natural condition.

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