

*Full Length Research Paper*

# Effect of pesticides on microbial transformation of sulphur in soil

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**A pot study was conducted in the laboratory of Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India in the year 2007 to 2008 to investigate the effect of pesticides, on the microbial transformation of sulphur (S) in soil. Insecticide (Endosulfan), fungicide (Dithane M-45), herbicide (2,4-D) were added to the soil at their recommended doses, respectively and their effect on the proliferation and potentiality of thiosulphate oxidizing bacteria, aryl sulphatase, available and total sulphur were investigated in soil. The results of the present investigation revealed that insecticide, endosulfan effectuated a significant detrimental effect on some microbiological, biochemical and chemical properties in soil whereas fungicide and dithane M-45 caused a significant detrimental influence during the later stages in spite of stimulating influence at early stages incubation period. However, herbicide and 2,4-D, brought about a beneficial influence on the microbiological, biochemical and chemical properties in soil. Among the pesticides used in the study, the performance of the herbicide was favorable in all respect.**

**Key words:** Pesticide, sulphur, soil microbiological, biochemical, chemical properties.

## INTRODUCTION

Sulphur (S) is an essential element for all biological systems and has been recognized as a major nutrient for optimal plant growth (Morris et al., 1988). It is used by plant for synthesis of amino acid, biotin, thiamine, glutathione, co-enzyme-A, formation of chlorophyll, glucoside oils, disulphide and sulphydryl groups and activation of sulphurylase. Nowadays, S fertilization is an essential part of modern agriculture. It leads to increase in the crude protein content of forages, oil content of oilseeds, persistence of legumes' stand and winter hardiness and drought tolerance of crop plants. It also improve quality of cereals, uniformity and quality of vegetables, control of some soil borne diseases and

ultimately to higher yield. Total S may indicate the total pool of available S in soil but it has got little value in describing short time availability of S in soil. Therefore, for the element, to be available to plant, is readily metabolized in soil in a cyclic manner. There are four distinct processes: organic compound decomposition, that is; microbial assimilation or immobilization of simple compounds of sulphur; oxidation of inorganic compounds such as sulphide ( $S^{2-}$ ), thiosulphate ( $S_2O_3^{2-}$ ), sulphite ( $SO_3^{2-}$ ), polythionates and elemental S; reduction of sulphate and other anions of sulphide. Apart from these abiotic factors, one of the major process to convert S to available form for plant uptake is microbial oxidation, mainly by thiosulphate oxidizing bacteria, of the unavailable element and reduced S to plant available  $SO_4^{2-}$  (Jensen et al., 1995).

It is the fact that the intense external input oriented agriculture, which was part of Green Revolution Strategy of our country (India), has raised the pesticide

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**Table 1.** Physico-chemical, biochemical and microbiological properties of experimental soil.

S/N	Parameters	Values
<b>A</b>	<b>Physico-chemical properties</b>	
1.	Textural class	
a.	Coarse sand (%)	44.54
b.	Fine sand (%)	20.42
c.	Silt (%)	16.82
d.	Clay (%)	18.21
2.	pH	6.82
3.	Organic carbon (mg kg <sup>-1</sup> )	7080
4.	Total nitrogen (mg kg <sup>-1</sup> )	690
5.	Available nitrogen (mg kg <sup>-1</sup> )	88.10
6.	Available phosphorus (mg kg <sup>-1</sup> )	10.62
7.	Available potassium (mg kg <sup>-1</sup> )	66.28
8.	Available sulphur (mg kg <sup>-1</sup> )	6.76
9.	Total sulphur (mg kg <sup>-1</sup> )	104.22
<b>B</b>	<b>Biochemical properties</b>	
10.	Aryl sulphatase activity (n kat 100 g <sup>-1</sup> )	2.89
<b>C</b>	<b>Microbiological properties</b>	
11.	Thiosulphate oxidizing bacteria (CFU × 10 <sup>3</sup> g <sup>-1</sup> dry soil)	32.8
12.	Thiosulphate oxidizing potentiality (mg of thiosulphate oxidized / 19.3 mg reduced sulphur [75 mg Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ] / 0.15 g sucrose / g soil)	0.33

consumption as high as 43584 ton (technical) in 2000-2001 from 2330 ton (technical) in 1950 to 2001 As high as 43584 tonne (technical) in 2000-01 from 2330 tonne (technical) in 1950. Pesticides after its application and subsequent degradation mostly accumulated within topsoil (0-10 cm) (Harris and Sans, 1967), which is considered as the zone of maximum microbial activity. The pesticides, metabolized by several soil microorganisms, are involved in several transformation of nutrient elements like solubilization of phosphorus, mineralization of nitrogen as well as oxidation of sulphur (Shidhar et al., 2000), through constitutive enzymes in rhizosphere and dissipated from soil slowly as the time progresses.

Therefore, it is necessary to investigate an intensive microbiological study for sulphur transformation in soil. That is, the population of thiosulphate oxidizing bacteria, thiosulphate oxidizing potentiality and aryl sulphatase activity upon pesticide.

## MATERIALS AND METHODS

### Soil analysis

A laboratory based pot culture experiment was conducted with a

typically gangetic alluvium (Inceptisols) soil at the Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India during 2005-2006.

Surface soil samples (0-15 cm depth) from monoculture (*Kharif* rice) cultivated field were collected, air dried, ground and passed through a 80 mesh sieve. The soils were analyzed for pH (1:2.5 soil-to-water) (Jackson, 1973), texture (International pipette method) (Piper, 1966), organic carbon by 1N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution method (Walkley and Black, 1934), CEC by 1N NH<sub>4</sub>OAc, pH 7.0 solution method (Hesse, 1971), total nitrogen by modified Kjeldahl method (Jackson, 1973), available P<sub>2</sub>O<sub>5</sub> by ammonium molybdate extractable method (Olsen et al., 1954), available K<sub>2</sub>O by 1N NH<sub>4</sub>OAc, pH 7.0 solution method (Hanway and Heidel, 1952) and available S turbidimetrically by 0.15% CaCl<sub>2</sub> extractable method as well as total sulphur by turbidimetrically using BaCl<sub>2</sub> and gelatin, after digesting the soil sample with di-acid mixture (perchloric acid and nitric acid 1: 2 v/v) (Tabatabai, 1974) (Table 1).

### Pot experiment

Small earthen pots were filled with 100 g of soil and adjusted to field capacity with deionized water. Each of the four pots were treated with no pesticides (control), with endosulfan 35% EC at 0.5 kg a.i. ha<sup>-1</sup>, dithane M-45 at 1.52 kg a.i. ha<sup>-1</sup> and 2,4-D 38% EC at 2.5 kg a.i. ha<sup>-1</sup> as their general recommendation doses for the field crops with thrice replication, using a completely randomized block design through SPSS statistical software. Irrigation was provided as required, using deionized water. The pots were then incubated at

**Table 2.** Effect of pesticides on total sulphur content ( $\text{mg kg}^{-1}$ ) in soil.

Treatments	Days after incubation						Mean
	5th	10th	15th	30th	60th	90th	
Control	107.8	106.2	104.5	102.4	101.8	102.6	104.2
Endosulfan	108.9	107.1	105.2	102.8	103.7	104.8	105.4
Dithane M- 45	106.3	103.8	101.5	100.9	102.0	103.1	102.9
2, 4- D	105.1	102.9	102.1	99.6	97.7	98.2	100.9
Mean	107.02	105.0	103.3	101.4	101.3	102.2	
<b>Source</b>	<b>Treatment</b>		<b>Days</b>		<b>Treatment × Days</b>		
SEm ( $\pm$ )	0.274		0.335		0.670		
CD (0.05)	0.782		0.958		1.916		

$37^\circ\text{C} \pm 1$  up to 90 days.

#### Sample collection and analysis

The samples were collected from the respective pots periodically (5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days after incubation) and analyzed for total sulphur (S) turbidimetrically using  $\text{BaCl}_2$  and gelatin, after digesting the soil sample with di-acid mixture (perchloric acid and nitric acid 1: 2 v/v) and available S by 0.15%  $\text{CaCl}_2$  (extractable) turbidimetrically as described by Tabatabai (1974). Thiosulphate oxidizing bacteria were measured by serial dilution technique and pour plate technique using thiosulphate oxidizing bacteria medium as described by Pramer and Schmidt (1965). Thiosulphate oxidizing potentiality of soil was calculated as the difference between the total S content of a sterilized and non-sterilized soil (Parker and Frisk, 1953). Aryl sulfatase activity was estimated on the basis of the determination of concentration of p-nitrophenol released after incubation of soil with p-nitrophenol sulphate for 1 h at  $37^\circ\text{C}$  temperature and measured the p-nitrophenol concentration at 400 nm by spectrophotometer (Tabatabai and Bremner, 1970).

## RESULTS AND DISCUSSION

### Total sulphur

The results showed that application of endosulfan resulted in a significant elevation in the level of total S in soil over the control, indicating that the extent of mineralization of organic S compounds was slower due to the detrimental effect of insecticide on proliferation of microorganism, particularly, thiosulphate oxidizing bacteria as well as their potentiality (Table 2). On the contrary, the proliferation and potentiality of the thiosulphate oxidizing bacteria was higher in soil by the application of either dithane M- 45 (fungicide) or 2,4-D (herbicide) than that of control. The growth and activities of heterotrophic microorganism, in particular, thiosulphate oxidizing bacteria was enhanced that in turn mineralized the organic matter followed by volatilization of organic

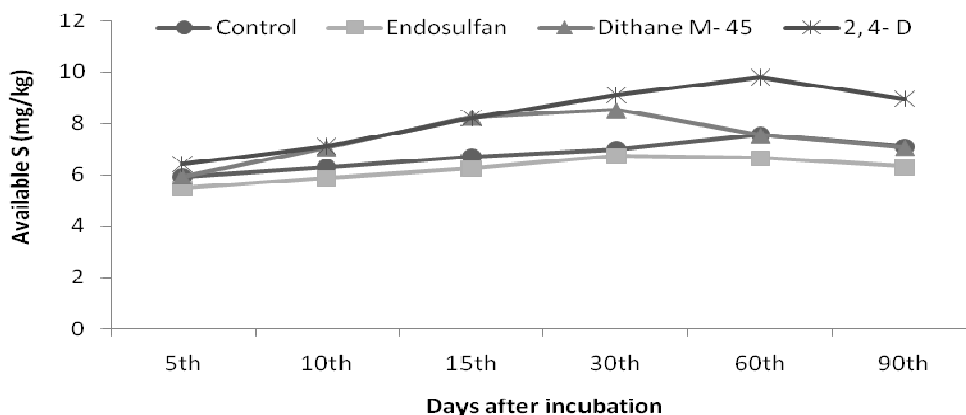
and inorganic S compound to derive energy and carbon for their cellular constituent resulting in lower build up of total S in soil. The significant negative (-ve) correlation between the level of total S and thiosulphate oxidizing bacteria ( $r = -0.98$ ) as well as their potentiality ( $r = -0.99$ ), respectively also reflects the above statements (Table 3). However, among the pesticides 2, 4-D gave the greatest loss followed by dithane M- 45 and endosulfan. The results substantiate the earlier reports of Balamani et al. (1995).

### Available sulphur

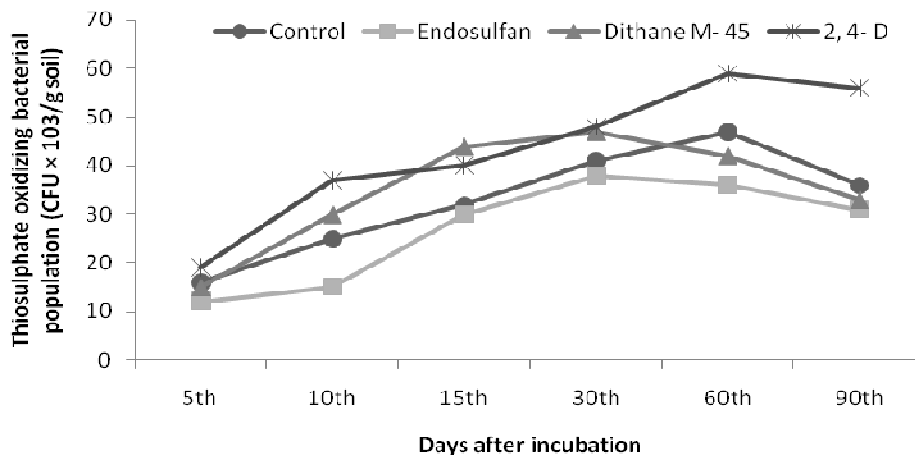
The influence of endosulfan resulted in a significant reduction of available S in soil whereas 2, 4 -D and dithane M- 45 resulted in significant rise in the level of available S over that of the control (Figure 1). The significant positive (+ ve) correlations were also obtained between available S and thiosulphate oxidizing bacteria ( $r = 0.98$ ) as well as their potentiality ( $r = 0.98$ ), respectively (Table 3). The results were in the line with that of Bezbaruah et al. (1990).

### Thiosulphate oxidizing bacteria

2,4-D and dithane M-45 gave a significant stimulation of thiosulphate oxidizing bacteria in soil as compared to that of control (Figure 2). The rise in the population of thiosulphate oxidizing bacteria was due to nutrient and energy sources from the pesticides in soil for their cell synthesis. Conversely endosulfan caused least proliferation of thiosulphate oxidizing bacteria in soil which was even significantly less than that of control due to the toxic effect of endosulfan and their activated compounds in soil. The reports were in line with earlier investigation by Balamani et al. (1995). Again in all the treatments, the population of thiosulphate oxidizing



**Figure 1.** Effect of pesticides on available sulphur content ( $\text{mg kg}^{-1}$ ) in soil at different days after incubation.



**Figure 2.** Effect of pesticides on the population of thiosulphate oxidizing bacteria ( $\text{CFU} \times 10^3 \text{ g}^{-1}$  dry soil) in soil at different days after incubation.

bacteria progressively increased from the 5th to 60th day of incubation which might be due to proto-cooperative or commensalic effect and thereafter their decrease was due to competition or amensalic effect.

### Thiosulphate oxidizing potentiality

The endosulfan caused a significant depletion in the potentiality of total thiosulphate oxidizing bacteria as compared to the control (Table 4). The impact of endosulfan on thiosulphate oxidizing potentiality of soil was in accordance with the population of thiosulphate oxidizing bacteria in soil. The result, thus, confirms the report of Germida and Janzen (1993). On the other hand the influence of dithane M-45 and 2, 4-D caused a significant rise in thiosulphate oxidizing potentiality in soil

as compared to that of control. The increase in thiosulphate oxidizing potentiality was due to higher proliferation of thiosulphate oxidizing bacteria in soil as dithane M-45 as well as 2, 4-D or their degradation product might furnish energy and nutrients to the thiosulphate oxidizers resulting higher proliferation in soil. As a consequence, there was a significant positive correlation ( $r = 0.99$ ) in between the potentiality and proliferation of thiosulphate oxidizing bacteria in soil (Table 3). The results confirm the reports of Banerjee and Dey (2004).

### Aryl sulphatase activity

Endosulfan exerted a significant decreased in aryl sulphatase activity over that of control. On the other

**Table 3.** Correlation values studied among the parameters.

Relationship between	Correlation coefficient (r)
Available sulphur vs total sulphur	-0.99
Available sulphur vs aryl sulfatase activity	0.97
Available sulphur vs thiosulphate oxidizing potentiality	0.98
Available sulphur vs thiosulphate oxidizing bacteria	0.98
Total sulphur vs aryl sulfatase activity	-0.96
Total sulphur vs thiosulphate oxidizing potentiality	-0.97
Total sulphur vs thiosulphate oxidizing bacteria	-0.99
Aryl sulfatase activity vs thiosulphate oxidizing potentiality	0.99
Aryl sulfatase activity vs thiosulphate oxidizing bacteria	0.98
Thiosulphate oxidizing bacteria vs thiosulphate oxidizing potentiality	0.99

\*Table value for 'r' = 0.95 at 5% level for 2 degrees of freedom.

**Table 4.** Effect of pesticides on the population of thiosulphate oxidizing potentiality (mg of thiosulphate oxidized / 19.3 mg reduced sulphur [75 mg Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>] / 0.15 g sucrose / 1 g soil) in soil.

Treatments	Days after incubation						Mean
	5 <sup>th</sup>	10 <sup>th</sup>	15 <sup>th</sup>	30 <sup>th</sup>	60 <sup>th</sup>	90 <sup>th</sup>	
Control	0.22	0.26	0.29	0.37	0.43	0.38	0.33
Endosulfan	0.17	0.23	0.27	0.33	0.29	0.26	0.26
Dithane M- 45	0.22	0.29	0.42	0.45	0.40	0.35	0.36
2, 4- D	0.25	0.31	0.36	0.49	0.57	0.53	0.42
Mean	0.22	0.27	0.34	0.41	0.42	0.38	
<b>Source</b>	<b>Treatment</b>		<b>Days</b>		<b>Treatment × Days</b>		
SEm (±)	0.004		0.004		0.009		
CD (0.05)	0.010		0.013		0.025		

**Table 5.** Effect of pesticides on aryl sulphatase activity (n kat 100 g<sup>-1</sup>) in soil at different days after incubation.

Treatments	Days after incubation						Mean
	5 <sup>th</sup>	10 <sup>th</sup>	15 <sup>th</sup>	30 <sup>th</sup>	60 <sup>th</sup>	90 <sup>th</sup>	
Control	2.34	2.52	2.81	3.07	3.46	3.16	2.89
Endosulfan	1.86	2.14	2.42	2.91	2.79	2.64	2.46
Dithane M- 45	2.36	3.01	3.39	3.41	3.14	2.97	3.05
2, 4- D	2.51	3.12	3.21	3.68	3.94	3.86	3.39
Mean	2.27	2.69	2.96	3.27	3.33	3.16	
<b>Source</b>	<b>Treatment</b>		<b>Days</b>		<b>Treatment × Days</b>		
SEm (±)	0.003		0.004		0.008		
CD (0.05)	0.010		0.012		0.024		

hand, dithane M-45, and 2, 4-D exerted significant enhancing influence on aryl sulphate activity in soil. So, effect of pesticides on aryl sulphatase activity was in accordance with the proliferation of thiosulphate oxidizing bacteria in soil (Table 5). However, a significant positive

correlation ( $r = 0.99$ ) was found between the aryl sulphatase activity and thiosulphate oxidizing bacteria which reveals thiosulphate oxidizing bacteria elaborate the enzyme aryl sulphatase and so mineralize organic S compounds in soil (Table 3). The results confirm the

report of Przybulewska et al. (2004).

## Conclusion

The Green Revolution of India primarily based on high value inputs like pesticides and fertilizers. The application of these inputs especially pesticides, may create a great problem on beneficial soil micro-organisms and microbial transformation of several primary and secondary nutrients. In our pot culture study, it was observed that the pesticides like endosulfan and dithane M-45 served a detrimental effect on transformation of S, whereas 2,4-D created a favorable beneficial effect on S transformation in soil environment. Although India produces 233 million tonnes (2009-10) of food grains per year at present, but our food grain production was stagnant from the last decades (Agriculture Survey by Hindu, 2010). The food grain requirement of India by 2025 would be 320 million tons (high demand scenario) and 308 million tons (low demand scenario) (Kumar et al., 2005). So it is the right time to be aware of the all agricultural inputs to use them properly and appropriately to maintain and sustain soil quality and health resulting in sustainable agriculture in India.

## REFERENCES

- Balamani B, Neelima S, Tarun B, Benbaruah B, Saikia N, Bora T (1995). Effect of pesticides on most probable number of microbes from tea plantation and uncultivated land enumerated in enrichment media. *Indian J. Agric. Sc.*, 65: 578-583.
- Banerjee MR, Dey BK (2004). Effects of different pesticides on microbial populations, nitrogen mineralization and thiosulphate oxidation in the rhizosphere of jute (*Corchorus capsularis* L. cv.). *Biol. Fert. Soils*, 14(3): 213-218.
- Bezbaruah B, Saikia N (1990). Pesticide influence on sulfur oxidation in soil and bacterial isolates. *Indian J. Agric. Sci.*, 60: 406-410.
- Germida JJ, Janzen HH (1993). Factors affecting the oxidation of elemental sulfur in soils. *Fert. Res.*, 35(12): 101-114.
- Hanway JJ, Heidel H (1952). Soil analysis methods as used in Iowa State College, Soil Testing Laboratory, Iowa State College Bull., 57: 1-131.
- Harris CR, Sans WW (1967). Absorption of organochlorine insecticide residues from agricultural soils by root crops. *J. Agric. Food Chem.*, 15: 861-863.
- Hesse PR (1971). A text Book of Soil Chemical Analysis. John Murry Publ., London, pp. 384-387.
- Jackson ML (1973). Soil chemical analysis. Prentice Hall of India pvt. Ltd. New Delhi.
- Jensen AJH, Sleyster R, Jochemsem A, Bontsema J, Lettinga G (1995). Biological sulfur oxidation in the fed batch reactor. *Biotechnol. Bioeng.*, 47: 327-332.
- Kumar R, Singh RD, Sharma KD (2005). Water resources of India. *Curr. Sci.*, 89(5): 794-811.
- Morris RJ, Shin JS (1988). Sulfur the fourth major plant nutrient. *International Symposium on Sulfur for Korean Agriculture*, pp. 6-9.
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circ.*, Washington, USA, p. 939.
- Parker CD, Frisk J (1953). The oxidation of inorganic compounds of sulphur by various sulphur bacteria. *J. Gen. Microbiol.*, 8: 344-364.
- Piper CS (1966). Mechanical analysis: Soil and Plant analysis (Reprint for Asia). Hans publications, Bombay, pp. 47-49.
- Pramer D, Schmidt ED (1965). Experimental soil microbiology. Burges Publishing Co., Minneapolis, pp. 107-109.
- Przybulewska K, Nowak A, Hoppen B (2004). Influence of temperature on pesticide action using the example of enzymatic activity of selected soil bacteria. *Folia University Agricultural Stetinensis. Agricultural*, 93: 333-340.
- Tabatabai MA (1974). Determination of sulfate in water samples. *J. Sulfur Inst.*, 10: 11-13.
- Tabatabai MA, Bremner JM (1970). An alkaline oxidation method for the determination of total sulphur in soils. *Soil Sci. Soc. Am. Proc.*, 34: 62-65.
- Walkley A, Black CA (1934). An examination of Digestion methods for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.*, 37: 29-38.