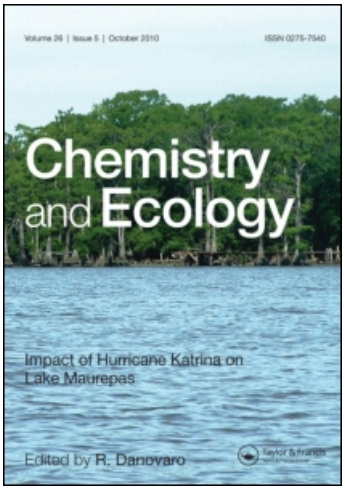

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Effect of Pollution On the Occurrence, Distribution and Sulphur Transformation Ability of the Thiobacilli in the River Ganga

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EFFECT OF POLLUTION ON THE OCCURRENCE, DISTRIBUTION AND SULPHUR TRANSFORMATION ABILITY OF THE THIOBACILLI IN THE RIVER GANGA

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The occurrence, distribution and nature of ambient thiobacilli along with their ability to oxidize different sulphur species under simulated natural and *in vitro* culture conditions were studied in the polluted and unpolluted sites of the River Ganga.

Thiobacillus thioparus, *T. thiooxidans* and *T. denitrificans* were isolated from the river water. The former two occurred in both polluted and unpolluted sites, while *T. denitrificans* occurred in polluted areas only. The paper pulp mill effluent discharge area contained the highest population of *T. thioparus*. The sewage drainage area showed relatively higher populations of *T. thiooxidans* and *T. denitrificans*.

The present study revealed that only biological oxidation of either thiosulphate or elemental sulphur occurred in the river water. All the thiobacilli screened oxidized thiosulphate, and three-fourths of them oxidized elemental sulphur. Some strains were found to be very good acidifiers. In spite of such acidification by the ambient thiobacilli, the pH of the river water remained alkaline. The specific rates of thiosulphate (0.18 – 0.51 μmol h^{-1} mg^{-1} cell) and sulphur (1.3 – 6.2 Normality day^{-1} mg^{-1} biomass) oxidations under simulated natural condition were found to be higher in polluted areas when compared with the unpolluted one (sulphur: 0.8 – 1.0 Normality day^{-1} mg^{-1}).

Further, addition of thiosulphate or elemental sulphur in the river water in simulated *in vitro* condition resulted in the increase of respective oxidation rates. The variations in the nature of pollutants discharged into the river water influenced the oxidation rate of thiosulphate or sulphur.

Keywords: *Thiobacillus thioparus*; *T. thiooxidans*; *T. denitrificans*; river pollution; thiosulphate and sulphur oxidation

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INTRODUCTION

Several species of *Thiobacillus* could utilize sulphate and thiosulphate while some do so when grown on trithionate and tetrathionate. Some thiobacilli are able to oxidize the elemental sulphur or sulphuric acid and thereby lower the ambient pH of their microniches.

The River Ganga, flowing through densely populated areas, is being increasingly polluted as a result of discharge of various pollutants into it. However, very little information is available on the acidification problem in freshwater ecosystem in India, which may be caused by the sulphur oxidizing thiobacilli in such an ecosystem.

In the present study attempts are made to isolate different *Thiobacillus* spp. from the River Ganga, to characterize them; to note their occurrence and distribution in the polluted and unpolluted zones of the river with a view to assess the impact of pollutions on the bacterial populations and to find their ability to oxidize sulphite, thiosulphate and sulphur *in vitro* and in a simulated ecological set up. The specific rates of such oxidation were calculated and their impact on the ecosystem is discussed.

METHODS

Four sites of the River Ganga (Fig. 1) flowing between Tribeni and Ichhapur (23°N, 88°E), West Bengal, India were selected for this investigation: S1 receiving no allochthonous input, S2 receiving rayon factory effluent, S3 receiving paper pulp mill effluent and S4 a sewage drainage area.

Water samples were collected aseptically with sterilized Ruttner Sampler. The pH and temperature of the river water were recorded *in situ*. The dissolved oxygen (DO) (APHA, 1981), amounts of sulphite and elemental sulphur (Troelsen and Jørgensen, 1982), thiosulphate (Sörbo, 1957), present in water samples were determined. Attempts were made to isolate the following species of *Thiobacillus* by using selective media depicted in the references; *Thiobacillus thioparus* (Beijerinck, 1904), *T. thiooxidans* (Waksman and Starkey, 1923), *T. denitrificans* (Lieske, 1912), *T. novellus* (Keyrh and Suzuki, 1977),

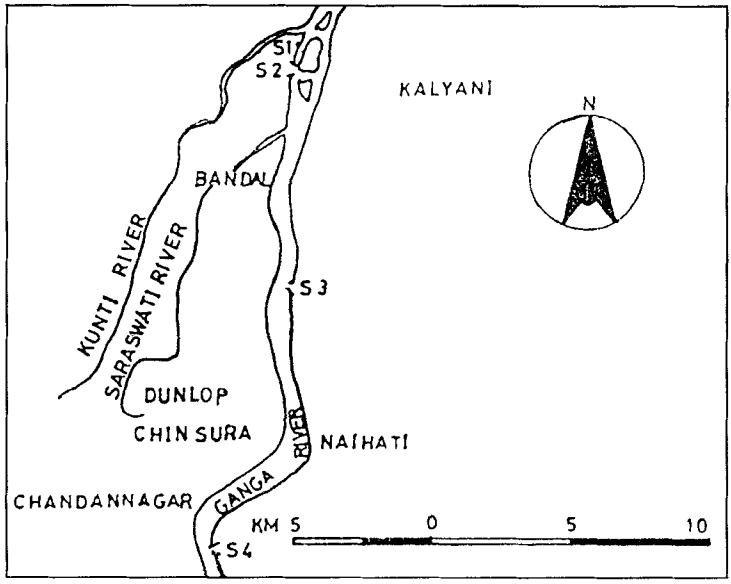

FIGURE 1 Map of the River Ganga with different sampling stations. S1 – unpolluted area, S2 – rayon factory effluent discharge area, S3 – paper pulp mill effluent discharge area, S4 – sewage drainage area.

T. ferro-oxidans and *T. neapolitanus* (Visnaic and Santer, 1957). For isolation of chemoheterotrophic sulphur oxidizing bacteria, 1% sucrose and 2% agar (Difco) were added to the medium of Parker and Prisk (1953). The water samples were inoculated into the selective medium for growth of different *Thiobacillus* spp. and incubated at $28° \pm 2°C$. One set was kept under aerobic condition and another in anaerobic condition. The bacterial growth was detected by observing the turbidity and noted by the drop in pH value due to the formation of acid in culture.

The population count of *Thiobacillus* spp. were made following MPN method in selective medium mentioned earlier. They were purified by employing dilution pour-plating technique in agar medium, followed by incubation at appropriate temperature. The isolated colonies were picked up and repeatedly subcultured to obtain pure culture. The purity of the culture was checked through microscopic examination.

Each bacterial isolate was characterized by studying the features shown in Table I. The oxidation of sulphur, thiosulphate and sulphide was studied using an inorganic medium containing these compounds separately as substrates, at a final concentration of $5\,g\,l^{-1}$. The utilization of organic nitrogenous compounds (asperagine, urea and peptone) was tested by supplementing them separately in selective medium in place of ammonium chloride, at a final concentration of $5\,g\,l^{-1}$. The optimal pH and temperature for growth were determined following conventional methods. The species of *Thiobacillus* were identified following Bergey's Manual of Systematic Bacteriology (1989).

The specific rates of oxidation of thiosulphate and elemental sulphur in a simulated condition *in vitro* were studied – (a) without changing the ambient bacterial population and thiosulphate/sulphur concentration, to obtain a tentative overall rate of oxidation of the two sulphur substrates under ambient condition of the riverine ecosystem; (b) after supplementing the water sample with either thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) or elemental sulphur (sulphur powder) as needed at the concentration of $5\,g\,l^{-1}$, to note whether the ambient concentrations of thiosulphate and elemental sulphur in the water sample are

TABLE I Relative physico-chemical characteristics of the Ganga River water and discharged effluents (annual mean ± SE)

Monitored sites	*pH*	*Temperature (°C)*	*DO*	*Sulphide*	*Thiosulphate*	*Sulphur*
S1	7.46 ± 0.05	24.8 ± 0.73	6.3 ± 0.48	3.3 ± 0.01	0	1.8 ± 0.33
S2	7.40 ± 0.12	26.8 ± 0.91	5.0 ± 0.49	0.9 ± 0.09	2.4 ± 0.59	3.5 ± 0.34
S3	7.26 ± 0.04	26.5 ± 0.78	5.3 ± 0.53	1.9 ± 0.16	0	2.8 ± 0.28
S4	7.58 ± 0.05	27.4 ± 0.39	5.1 ± 0.41	0.3 ± 0.01	0	8.8 ± 1.39
E2	3.03 ± 0.14	30.6 ± 0.89	0.8 ± 0.02	2.1 ± 0.2	9.9 ± 0.34	24.1 ± 0.91
E3	7.28 ± 0.02	31.5 ± 0.98	0.6 ± 0.05	4.4 ± 0.2	0	5.9 ± 0.38
E4	7.80 ± 0.06	27.1 ± 0.69	0.3 ± 0.01	0.8 ± 0.14	0	39.8 ± 1.11

S1, unpolluted area; S2, rayon factory effluent discharge area; S3, paper pulp mill effluent discharge area; S4, sewage drainage area; E2, rayon factory effluent; E3, paper pulp mill effluent; E4, sewage. DO dissolved oxygen; SE standard error; all parameters are expressed as $\mu g\,ml^{-1}$ except pH and temperature.

limiting for the oxidation or not; (c) in water samples devoid of ambient bacterial population obtained by passing the water sample through a sintered glass filter (G-5, Borosil, India), to note non-biological oxidation, if any; (d) in sterilized water samples as in (c) inoculated with river water of sites, to note the oxidation of thiosulphate/sulphur by the ambient microflora; (e) in sterilized water samples as in (c) inoculated separately with purified isolate of strains of thiobacilli, to note the heterogeneity amongst the isolated strains of thiobacilli relating to their ability to oxidize thiosulphate/elemental sulphur, following the experimental designs depicted below.

In case of (a) and (b), a large volume of water samples were collected from the four sites separately and were kept under normal temperature, day-night regime with the flow rate of water samples maintained at $1.1\,ml\,s^{-1}$ in a transparent container. In case of (c), 100 ml of water samples from 4 sites of the river were taken in sterilized glass containers separately, after sterilization through a Seitz filter. Each container was incubated at $28° \pm 2°C$. In case of (d), 100 ml of bacteria free river water samples, obtained as in (c), was inoculated with 1 ml of freshly collected river water of sites (those contained ambient bacterial flora as inoculum) and incubated at $28° \pm 2°C$. In case of (e), for the determination of thiosulphate oxidation rate, instead of river water samples containing ambient bacterial flora as inoculum, pure culture of *T. thioparus* isolated from site S2 was inoculated into the river water samples collected from the same site after sterilization in the manner mentioned earlier. However, for the determination of oxidation rate of sulphur, the sterilized water samples of S2 was inoculated with the pure culture of *T. thiooxidans* isolated from the same site.

The thiosulphate/elemental sulphur oxidation rate *in vitro* culture by the purified isolates of *T. thioparus*, *T. thiooxidans* and *T. denitrificans* in the $S_2O_3^{=}/S°$ – containing medium were determined by inoculating 0.1 ml of 24 h broth culture of each strain separately into the thiosulphate or sulphur containing medium as needed and incubated at the optimal temperature.

In all these cases, an initial concentration of thiosulphate or elemental sulphur, as the case may be, was measured at zero time and then at regular intervals of 10 h. Thiosulphate concentration was determined each time by the method of Sörbo (1957). The concentration

of elemental sulphur was determined by observing the increase in titrable acidity expressed as Normality per day per mg of bacterial biomass ($N\,d^{-1}\,mg^{-1}$) following Vogel (1978), as elemental sulphur was being oxidized to sulphuric acid. The rates of oxidation of $S_2O_3^{=}$ / S° was calculated from the regression plotted from time period against concentration. The regression coefficients were then developed.

The mean, standard error, correlation and ANOVA were determined following the procedures described in Michael (1984).

RESULTS

Water Quality

The annual mean values of physico-chemical parameters of the Ganga water and of the discharged pollutants are given in Table I. Both the river water and the effluents were alkaline. Only rayon factory effluent was acidic (pH 3.03). The dissolved oxygen was high in the unpolluted water ($6.3\,\mu g\,ml^{-1}$) (site S1), lower in the polluted ones ($5.0 - 5.3\,\mu g\,ml^{-1}$) (site S2) and very low in the effluents ($0.3 - 0.8\,\mu g\,ml^{-1}$) (sites S3, S4). The sulphide and elemental sulphur were low (sulphide 0.3 ± 0.01, S°: $1.8\,\mu g\,ml^{-1}$) in the unpolluted water, relatively high ($S^{=}$: $0.3 - 1.9\,\mu g\,ml^{-1}$; S°: $2.8 - 8.8\,\mu g\,ml^{-1}$) in the polluted areas and higher ($S^{=}$: $0.8 - 4.4\,\mu g\,ml^{-1}$, S°: $5.9 - 39.8\,\mu g\,ml^{-1}$) in the discharged effluents (Tab. I). The thiosulphate was detected only in the rayon factory effluent and in river water receiving it (Tab. I).

Population Count of Thiobacilli

Of the sites examined in the Ganga River, thiobacilli were found in variable numbers (290 to 13370 MPN l^{-1}), mostly in the waters receiving pollutants (Tab. II). Three species of thiobacilli (*T. thioparus*, *T. thiooxidans* and *T. denitrificans*) were recorded in the polluted river water. No *T. denitrificans* was detected in S1. The counts of *T. thioparus* and *T. thiooxidans* in the polluted zones were higher than the counts of unpolluted area (Tab. II). The highest count of *T. thioparus* (1790 MPN l^{-1}) was noted in S3 and that of *T. thiooxidans* (13370 MPN l^{-1}) was noted in S4. The highest count of *T. denitrificans* (7250 MPN l^{-1})

TABLE II Population of *Thiobacillus* spp. screened from different monitored sites of the River Ganga

Monitored sites	*Bacterial counts (MPN* l^{-1})		
	T. thioparus	*T. thiooxidans*	*T. denitrificans*
S1	290	11600	nd
S2	400	12790	6160
S3	1790	11450	7080
S4	1330	13370	7250
E2	100	17160	–
E3	582500	937500	–
E4	5412500	7150	–

"nd" – not detected, chemoheterotrophic sulphur oxidizing bacteria were not detected. For details of other abbreviations see Table I.

was detected in S4. The untreated municipal sewage drainage area of the river contained the largest population of all the three species in comparison to other pollutant receiving sites. The discharged sewage harboured the highest number of *T. thioparus* population while the paper pulp mill effluent contained a maximum number of *T. thiooxidans* (937500 MPN l^{-1}). However, none of the following thiobacilli: *T. novellus*, *T. ferrooxidans*, *T. neapolitanus* and chemoheterotrophic sulphur oxidizing thiobacilli were detected in the river.

Isolation and Screening of Thiobacilli

Altogether 45 isolates of thiobacilli were screened from the River Ganga, out of which 38 were from the polluted zones (S2, S3 and S4) and 7 from the zone (S1) receiving no allochthonous input (Tab. III). Nineteen isolates growing on the respective selective medium for *T. thioparus*, 15 isolates growing on specified medium for *T. thiooxidans*, and 11 isolates growing on defined medium for *T. denitrificans* were separated and axenically cultured from the different monitored sites of the Ganga.

Characterization and Identification of Thiobacilli

The morphological, biochemical and physiological studies of the 45 isolates of thiobacilli revealed that they might be placed in three categories (Tabs. I–III) on the basis of similarities and dissimilarities in their characteristic features (Tab. III), followed by comparing these

TABLE III Morphological, biochemical and physiological characteristics of *Thiobacillus* spp. isolated from the River Ganga

Characteristics	*Strains*		
	T. thioparus[a]	*T. thiooxidans*[b]	*T. denitrificans*[c]
Cell shape	r	r	sr
Cell size (μm)	1.5–2.0/0.4–0.5	1.0–1.2/0.5	0.6–1.2/0.4–0.5
Cell arrangement	s	s/p	s
Aerobic/anaerobic	+/–	+/–	+/+
Nutritional type	oa	oa	fa
Temperature optimum (°C)	28–30	28–30	28, 30
Gelatin liquefaction	nl	nl	sl
Preferred N source	NH_4Cl	$(NH_4)_2SO_4$	NH_4NO_3
Oxidation of:			
$S^=/S_2O_3^=/S^\circ$	–/+/+	–/+/+	–/+/–
Growth characteristics:			
Thiosulphate broth			
growth nature	pl	ut	ut
acid production	+	+	+
pH	3.6–3.8	0.5–1.0	5.0–5.2
Thiosulphate agar			
growth nature	sc	sc	sw
colour	wy	t	m
Sulphur broth			
acid production	+	+	+
pH	3.6–3.8	0.3–0.6	5.0–5.2

[a] isolates TICH 1, 2; TIPP 1, 2, 4, 6, 7; TR 3, 4, 6–8; TPN 1–7;
[b] isolates TTICH 3, 4, 8, 10, 12, 13; TTIPP 3, 5, 8; TTR 1, 2, 5, 9, 12, 13;
[c] isolates TDICH 5–7, 9, 11; TDIPP 9–12; TDR 10, 11.
r – rod; sr – small rod; s – single; p – paired; oa – obligate autotroph; fa – facultative autotroph; nl – non-liquefier; sl – slow liquefier; pl – pellicle; ut – uniform turbidity; sc – scanty; sw – slow; wy – whitish yellow; t – transparent; m – moist; + positive; – negative.
All isolates were Gram negative, capsulated, spore-former, motile, utilized urea, peptone, asperagine. None grew in thiosulphate agar stab.

features with Bergey's Manual of Systematic Bacteriology (Staley, 1989). The comparison revealed that bacterial strains: TICH 1, 2; TIPP 1, 2, 4, 6, 7; TR 3, 4, 6–8; TPN 1–7, belonging to category-I was *T. thioparus*; strains: TTICH 3, 4, 8, 10, 12, 13; TTIPP 3, 5, 8; TTR 1, 2, 5, 9, 12, 13 of category II was *T. thiooxidans*; strains: TDI CH 5, 7, 9–11; TDIPP 9–12; TDR 10, 11 of category-III was *T. denitrificans*.

Oxidation Rates of Thiosulphate and Sulphur

The oxidation rates of thiosulphate and sulphur in simulated natural conditions are shown in Table IV. The thiobacilli oxidised thio-

TABLE IV Rate of degradation of thiosulphate and sulphur in simulated natural condition

Monitored sites	*Degradation rate*							
	Thiosulphate ($\mu mol\, h^{-1} mg^{-1}$)				*Sulphur* ($\mu mol\, h^{-1} mg^{-1}$) $\times 10^{-4}$			
	Effluent	0 *m*	2 *m*	4 *m*	*Effluent*	0 *m*	2 *m*	4 *m*
S1	–	–	–	–	–	1.0	0.8	0.8
S2	0.86	0.51	0.48	0.18	8.0	1.3	1.5	1.3
S3	–	–	–	–	11.3	6.2	4.2	4.5
S4	–	–	–	–	6.0	5.5	5.2	4.9

For S1, S2, S3, S4 see Table I.

sulphate only in S2 at a specific rate of 5.1 $\mu mol\, h^{-1} mg^{-1}$. The specific rate of oxidation of elemental sulphur in polluted areas was higher than that observed in the unpolluted area.

The specific rate of thiosulphate (ambient plus supplemented) oxidation in the river surface water at S1 was 27.5 $\mu mol\, h^{-1} mg^{-1}$. Specific rates of oxidation of thiosulphate of 62.5 $\mu mol\, h^{-1} mg^{-1}$, 116.9 $\mu mol\, h^{-1} mg^{-1}$ and 84.6 $\mu mol\, h^{-1} mg^{-1}$ were noted in the river surface water near the effluent discharge area of the rayon factory, the paper pulp mill and sewage (Tab. V). The specific oxidation rates of thiosulphate in the river water sample of 2 m and 4 m depth of S1 was found to increase gradually (29.2 $\mu mol\, h^{-1} mg^{-1}$ at 2 m depth and 40.3 $\mu mol\, h^{-1} mg^{-1}$ at 4 m depth). The rate of thiosulphate oxidation remained constant in the water sample of 2 m and 4 m depths (64.5 $\mu mol\, h^{-1} mg^{-1}$) from the rayon factory effluent discharge area (S2) and also near the paper pulp mill effluent discharge area (S3). However, the river water sample at 2 m depth near the sewage outfall

TABLE V Oxidation of thiosulphate and sulphur (ambient plus supplemented) in the River Ganga

Monitored sites	*Oxidation rate*							
	Thiosulphate ($\mu mol\, h^{-1} mg^{-1}$)				*Sulphur* ($N\, d^{-1}\ mg^{-1}$) $\times 10^{-4}$			
	Effluent	0 *m*	2 *m*	4 *m*	*Effluent*	0 *m*	2 *m*	4 *m*
S1	–	27.5	29.2	40.3	–	1.4	1.8	3.0
S2	48.7	62.5	64.5	64.5	11.0	2.0	2.6	33.0
S3	119.7	116.9	116.9	116.9	17.3	16.5	32.5	42.0
S4	142.7	84.6	84.6	92.3	18.2	15.5	26.0	42.0

For S1, S2, S3, S4 see Table I.

area showed the rate of thiosulphate oxidation was similar to surface water sample (84.6 μ mol h^{-1} mg^{-1}) but a slightly higher rate (92.3 μ mol h^{-1} mg^{-1}) was noted in the water sample at 4 m depth.

The rayon factory and paper pulp mill effluent showed an oxidation rate of 48.7 and 119.7 μ mol h^{-1} mg^{-1} while the sewage showed a rate of 142.7 μ mol h^{-1} mg^{-1}. When water samples from all the monitored sites were supplemented with sulphur powder, a higher oxidation rate was observed than the ambient sulphur oxidation rate. In this case, the rates of increase in titrable acidity were found as 1.43×10^{-4}, 2.03×10^{-4}, 16.5×10^{-4} and 15.5×10^{-4} N d^{-1} in the surface waters of S1, S2, S3 and S4 (Tab. V). No oxidation of thiosulphate or sulphur was observed in the bacteria-free river water.

The oxidation of ambient thiosulphate and sulphur in a bacteria-free river water inoculated by water samples of corresponding sites, and water level containing natural microflora, showed that the river surface water near the rayon factory effluent discharge point showed thiosulphate oxidation rate of 0.33 μ mol h^{-1} mg^{-1}. The rate of thiosulphate oxidation at 2 m and 4 m depths of the river in this area was recorded as 0.31 and 0.32 μ mol h^{-1} mg^{-1} (Tab. VI). The rates of increase in titrable acidity were observed as 0.8×10^{-4}, 1.2×10^{-4}, 4.2×10^{-4} and 4.8×10^{-4} Normality day^{-1} in the bacteria-free river water samples containing ambient elemental sulphur, when composite water samples from the river surface of S1, S2, S3 and S4 were inoculated in that bacteria-free water, with the increase of depth rates were found to increase (Tab. VI). Oxidation of ambient thiosulphate and elemental

TABLE VI Oxidation of ambient thiosulphate and sulphur in bacteria-free river water inoculated by water samples of corresponding sites and water level containing natural microflora

Monitored sites	*Oxidation rate*					
	Thiosulphate (μ mol h^{-1} mg^{-1})			*Sulphur* (N d^{-1} mg^{-1}) $\times 10^{-4}$		
	0 *m*	2 *m*	4 *m*	0 *m*	2 *m*	4 *m*
S1	–	–	–	0.8	0.8	1.0
S2	0.33	0.31	0.32	1.2	1.4	1.5
S3	–	–	–	4.2	4.5	4.8
S4	–	–	–	4.8	5.2	5.5

For S1, S2, S3, S4 see Table I.

sulphur in bacteria-free water inoculated by pure culture of *T. thioparus* and *T. thiooxidans* of corresponding sites and water level are summarized below.

The surface water of the river near S2 showed a thiosulphate oxidation rate of 0.38 μ mol h^{-1} mg^{-1} while samples at 2 m and 4 m water depths exhibited an oxidation rate of 0.18 μ mol h^{-1} mg^{-1} and 0.28 μ mol h^{-1} mg^{-1} (Tab. VII). The rates of increase in titrable acidity were noted as 0.8×10^{-4}, 1×10^{-4}, 4×10^{-4} and 4.2×10^{-4} Normality day^{-1} in the surface water of S1, S2, S3 and S4. The results of regression analyses of degradation of thiosulphate ions by the purified isolates of the three *Thiobacillus* species under *in vitro* cultures were shown in Table VIII. The rates of thiosulphate oxidation were also worked out from the slope of respective regression line. All the 45 isolates oxidized thiosulphate. The rates of thiosulphate oxidation by *T. thioparus* ranged from 86.8 μ mol h^{-1} mg^{-1} to 282.6 μ mol h^{-1} mg^{-1}. The strain TR 4 isolated from rayon factory effluent discharge area (S2), showed lowest thiosulphate oxidation rate (86.8 μ mol h^{-1} mg^{-1}) while TICH 1, an isolate from sewage discharge area (S4) showed highest thiosulphate oxidation rate (282.6 μ mol h^{-1} mg^{-1}). The rates of oxidation of thiosulphate ion by isolates of *T. thiooxidans* varied from 189.6 μ mol h^{-1} mg^{-1} to 247 μ mol h^{-1} mg^{-1} with the maximum and minimum rates noted in TTR 2 and TTICH 10. Isolates of *T. denitrificans* oxidized thiosulphate at rates varying between 159.9 μ mol h^{-1} mg^{-1} and 226.8 μ mol h^{-1} mg^{-1}. The highest rate was exhibited by TDICH 5 and the lowest by TDR 11 (Tab. VII). The increase in titrable acidity by *T. thioparus* isolates was found to vary between

TABLE VII Oxidation of ambient thiosulphate and elemental sulphur in bacteria-free water inoculated by pure culture of *T. thioparus* and *T. thiooxidans*

Monitored sites	*Oxidation rate*					
	Thiosulphate ($\mu mol\, h^{-1} mg^{-1}$)			*Sulphur* ($N\, d^{-1} mg^{-1}$) $\times 10^{-4}$		
	0 *m*	2 *m*	4 *m*	0 *m*	2 *m*	4 *m*
S1	–	–	–	0.8	0.7	1.0
S2	0.38	0.18	0.28	1.0	1.2	1.4
S3	–	–	–	4.0	4.2	4.2
S4	–	–	–	4.2	4.5	4.8

For S1, S2, S3 and S4 see Table I.

2.75×10^{-4} and 6.01×10^{-4} N d^{-1} (Tab. IX). The former rate was shown by the isolates TPN 1, 4; TTI CH 2, while the latter was shown by the isolates TR 3, 8. On the other hand, during oxidation of elemental sulphur the increase in titrable acidity ranged from 2.57×10^{-4} (TTICH 3, 8, 10) to 5.09×10^{-4} (TR 1, 2, 5; TIPP 8) N d^{-1}.

TABLE VIII Rate of *in vitro* thiosulphate oxidation by purified isolates of *Thiobacillus* spp.

Bacteria	*Occurrence*	*Strain no.*	*Rate* (μ *mol* h^{-1} *mg*$^{-1}$)	*r-value*
1	2	3	4	5
T. thioparus	S2	TR 4	86.8	−0.61
	S3	TIPP 7	206.3	−0.98
	S1	TPN 2, 3; TIPP 4, 6; TR 3	210.8	−0.95
	S2	TR 7	213.6	−0.94
	S2	TR 6, 8; TPN 4, 6; TIPP 1, 2	219.9	−0.98
	S1	TPN 1, 7	228.7	−0.95
	S1	TPN 5	239.1	−0.98
	S4	TICH 2	251.0	−0.99
	S4	TICH 1	282.6	−0.99
T. thiooxidans	S4	TTICH 10	189.6	−0.98
	S3	TTIPP 3; TTR 1	208.2	−0.97
	S3	TTIPP 8; TTICH 12	215.6	−0.98
	S3	TTIPP 5	221.2	−0.98
	S2	TTR 9, 12	223.1	−0.95
	S2	TTR 5	227.4	−0.98
	S2	TTR 1	230.5	−0.99
	S4	TTI CH 4, 8	234.2	−0.98
	S4	TTI CH 3, 13	238.0	−0.87
	S2	TTR 2	247.3	−0.99
T. denitrificans	S2	TDR 11	159.9	−0.99
	S2	TDR 10	174.7	−0.98
	S3	TDIPP 10	179.7	−0.98
	S3	TDIPP 9, 11; TDICH 11	185.9	−0.98
	S4	TDI CH 9	190.7	−0.99
	S3	TDI PP 12	195.2	−0.98
	S4	TDI CH 6	211.4	−0.99
	S4	TDI CH 7	215.6	−0.94
	S4	TDI CH 5	226.8	−0.97

All *r*-values are significant at 5% except the value −0.61.
For details of other abbreviations ... see Table I.

TABLE IX Rate of elemental sulphur oxidation in the media by isolates of *Thiobacillus thioparus* and *Thiobacillus thiooxidans*

T. thioparus strains	*S°-oxidation rate (increase in titrable acidity)* $N\,d^{-1}\,mg^{-1} \times 10^{-4}$	*T. thiooxidans strains*	*S°-oxidation rate (increase in titrable acidity)* $N\,d^{-1}\,mg^{-1} \times 10^{-4}$
TPN 1, 4; TI CH 2	2.75	TTI CH 3, 8, 10	2.57
TPN 5, 6; TI CH 1	3.18	TTI CH 12, 13	3.27
TPN 2, 3	3.62	TTI CH 4	2.90
TR 6	5.14	TTR 13; TTIPP 3	3.56
TPN 7	5.36	TTR 9, 12	4.10
TR 4, 7	5.65	TTIPP 5	4.63
TR 3, 8	6.01	TTR 1, 2, 5; TTIPP 8	5.09

TPN isolates from unpolluted area; TR, TTR isolates from rayon factory effluent discharge area; TTIPP isolates from paper pulp mill effluent discharge area; TTI CH isolates from sewage drainage area.

Analysis of Variance Test

The ANOVA showed that the specific rates of elemental sulphur oxidation between S1 and S3, S1 and S4, S2 and S3 and S2 and S4 were found to differ significantly at 5% level. Nevertheless, the specific rates of sulphur oxidation at different layers of water column of an individual monitoring site had no significant differences between them.

ANOVA showed that the specific rates of thiosulphate and sulphur oxidation (ambient plus supplemented) were found to vary significantly between site to site. Such rates in case of sulphur oxidation were also found to differ from site to site as well as from depth to depth, when bacteria-free water samples were inoculated with the river water from sites.

DISCUSSION

The thiobacilli occurred in both the pollutant receiving and non-receiving sites of the Ganga River. The percentage of occurrence of thiobacilli was, however, relatively more in the polluted zones of the river than that of the unpolluted one (Tab. II). Such occurrence of thiobacilli were probably due to the presence of different sulphur species, such as sulphide ($0.3-3.3\,\mu g\,ml^{-1}$), thiosulphate ($0-2.4\,\mu g\,ml^{-1}$) and

elemental sulphur (1.8–8.8 $\mu g\,ml^{-1}$) in the river water (Tab. I), from which these bacteria derived energy for their growth, and also due to the favourable pH (7.2–7.5) and temperature (24.8–27.4°C) for growth (Tab. I). The screening of more thiobacilli from the polluted zones of the river might be a consequence of the availability of a higher quantum of sulphur substrates (Tab. I) and also as a result of allochthonous input of thiobacilli (Tab. II) into the river. The study further revealed that the monitored sites receiving no allochthonous input contained only *T. thioparus*, and *T. thiooxidans* and although in lesser numbers, while the other three polluted sites yielded *T. thioparus*, *T. thiooxidans* and *T. denitrificans* in higher numbers (Tab. II). These bacteria were found to be active participants in the sulphur transformation processes operating in the river ecosystem. In the monitored sites, *T. novellus*, *T. ferrooxidans* and *T. neapolitanus* were not detected. Furthermore, no chemoheterotrophic sulphur oxidizing thiobacilli were found to occur in the river water under study.

All the screened thiobacilli were good oxidizers of thiosulphate (Tab. VII). Though thiosulphate was detected only in the rayon factory effluent discharge area of the river, its presence in other sites also could not be ruled out, as such sulphur species were known to occur in the transitional zones above anoxic waters and the same was also produced as a common intermediate during biological and chemical oxidation of sulphide (Cline and Richards, 1969; Tuttle and Jannasch, 1973; Jørgensen, 1982). The latter was present in the river water receiving either sewage or other industrial effluents. Further, the sulphide ions present in polluted zones, though they could not be oxidized directly by any of the isolated thiobacilli, would act as a source of oxidizable substrates to such thiobacilli through microbial oxidation involving *Beggiatoa*, *Rhodopseudomonas* (Sinha, 1992), *Thioploca*, *Thiovulum* (Maki, 1987), members of Chromatiaceae and Chlorobiaceae (Hansen *et al.*, 1975). Besides, three-fourths of the isolated thiobacilli were capable of oxidizing elemental sulphur present in the river water and produced a moderate to high acidity in their microniches (Tab. IX). However, the effect of such acidification on a macroscale was not evident in the Ganga River, where the annual mean pH values ranged between 7.2 to 7.5 only along the river stretch under study (Tab. I). This might be due to the presence of several neutralizing factors, viz. by a dilution factor, buffering ability

of the river water (Sinha and Banerjee, 1995), microbial transformation (Sinha, 1992). Nevertheless, *T. thioparus*, *T. thiooxidans* and *T. denitrificans*, isolated from the River Ganga, were very potent acidifiers as indicated by their ability to lower the pH to a greater extent *in vitro*. The isolates of *T. thiooxidans* lowered the pH of sulphur broth to a range between 0.3 and 0.6, similarly the isolates of *T. thioparus* lowered the pH of thiosulphate broth to a range between 3.6 and 3.8 and those of *T. denitrificans* brought down the pH of both sulphur and thiosulphate broths to a range between 5.0 and 5.2 (Tab. III). This situation noted that any untoward rise in the population density of these bacteria would pose an ecological problem by increasing acidification of the river. Several strains of *T. thiooxidans*, (TTR 1, 2, 5, 9, 12, 13; TIPP 3, 5, 8; TPN 7) and *T. denitrificans* (TDI CH 5–7) oxidized the sulphur species in *in vitro* culture and lowered the pH to such values which were markedly lower than the pH values depicted against these species in Bergey's Manual of Systematic Bacteriology (Staley, 1989). Further, the strains of thiobacilli showed marked heterogeneity with respect of the rate of oxidation of thiosulphate ions in *in vitro* condition. The *T. thioparus* strain TICH 1 showed three- and one- fourth fold higher rate of oxidation than that of the strain TR 4 which recorded the slowest rate (Tab. VIII). Amongst the three species of *Thiobacillus*, the average rate of thiosulphate oxidation was highest ($223.54\,\mu mol\,h^{-1}\,mg^{-1}$) in the *T. thioparus* strains, followed in decrease order by those of *T. thiooxidans* ($222.48\,\mu mol\,h^{-1}\,mg^{-1}$) and *T. denitrificans* ($186.30\,\mu mol\,h^{-1}\,mg^{-1}$).

Nevertheless, the rates of oxidation of both thiosulphate (Tab. VIII) and elemental sulphur (Tab. IX) were considerably high in most of the isolated strains of thiobacilli. A reflection of such high rate of oxidation was not evident in the river water for the reasons mentioned earlier.

The thiobacilli were very much involved in the oxidation of thiosulphate and elemental sulphur as evidenced by the pure culture of *T. thioparus* and *T. thiooxidans* in the ambient bacteria-free water sample of S2 in a simulated natural condition (Tab. VII).

Neither oxidation of thiosulphate nor of S° was detected in the ambient bacteria-free water samples when kept in simulated natural condition; indicating that no chemical transformation of $S_2O_3^{=}$ and S° could occur in the river water.

The highest rates of oxidation was noted when the isolated strains were cultured in a selective medium. The underlying reasons may be the presence of optimal concentration of nutrients, pH, temperatures, besides lack of ecological competition.

The statistical analysis indicated that significant site to site variation with respect to sulphur oxidation rate was noted, indicating that different pollutants influenced the microniche of the river water, which in turn, probably brought about such variations in the oxidation rates.

All the screened thiobacilli oxidized thiosulphate and three fourths of them oxidized elemental sulphur; the ultimate product of such oxidation was sulphuric acid. As a consequence of further pollution, the number of such acidifiers would be likely to increase, causing acidification problems in the river water. Some strains of *T. thioparus*, *T. thiooxidans* and *T. denitrificans* were found to be good acidifiers. The acidity was beyond the ranges normally seen (Staley, 1989) for such microbes.

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