

halophilic archaea towards antibiotics affecting cell-wall synthesis is consistent with the lack of cell envelope peptidoglycan. Isolate number 67 showed presence of phosphatidyl glycerol methyl phosphate (PGP-Me) phospholipids, diglycosyl diphytanylglycerol (DGD-1) glycolipids, GL-2 unidentified and an unknown glycolipid<sup>18,19</sup>. PGP-Me and DGD-1 are archaeal polar lipids<sup>5</sup>. The presence of these lipids, lysis in distilled water, growth only above 10% NaCl concentration, resistance towards antibiotics affecting cell-wall synthesis, and presence of pigment of orange with reddish shade in isolate number 67 indicate the characteristic of true halophiles<sup>15,16</sup>.

Analysis of 16s rRNA of isolate number 67 was done. It was confirmed that it is haloarchaea and was identified as *Natronema thermotolerant*, a member of family Halobacteriaceae. This culture grew up to 50°C and tolerated 60°C temperature. Although the rest of the isolates showed presence of phosphatidic acid, they showed more halotolerant characters, rather than halophilic.

Thus if facility is not available for molecular studies, morphological, biochemical and physiological studies can also provide sufficient data for evaluating bacterial diversity from marine saltern ecosystem. Indian marine salterns are neglected for biodiversity studies. Thus this attempt provides considerable data regarding microbial diversity in such ecosystem. The study also provides information regarding applied value of halotolerant and halophilic isolates as they have industrial

importance. Out of 73 isolates, only six were studied; the remaining 67 isolates that tolerated up to 30% salt need further study.

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## Source of dissolved sulphate in the Alakananda–Bhagirathi rivers in the Himalayas

The natural sources of sulphate in river water are rainfall, groundwater and weathering of sulphide-rich minerals. In addition, human activities through addition of air pollutants, mining and processing of sulphide ores, petroleum refineries and chemical industries contribute enormously to dissolved sulphate content in river water. These multiple sources of sulphate can be distinguished by their specific isotopic signatures. For example, river sulphate derived from dissolution of evaporites has positive  $\delta^{34}\text{S}$  values (10 to 30‰),

whereas sulphate derived from oxidation of sulphides or from biogenic emissions may have strongly negative  $\delta^{34}\text{S}$  values<sup>1,2</sup> (–25 to –5‰) ( $\delta^{34}\text{S}$  denotes  $[\{({}^{34}\text{S}/{}^{32}\text{S})_{\text{sample}}/({}^{34}\text{S}/{}^{32}\text{S})_{\text{standard}}\}-1] \times 1000$ ). According to Ivanov *et al.*<sup>3</sup>, the annual sulphate flux from continents to oceans is  $6.8 \times 10^{12}$  mol, with contributions from rivers, anthropogenic emissions and groundwater of 48, 48 and 4% respectively. Berner and Berner<sup>4</sup> suggest that the significant sources of sulphate in river water in general, are rock weathering (33%) and pollution

(43%). Other sources could be natural biogenic-derived sulphate in rain (17%) and a minor fraction (5%) coming from volcanic activity.

In spite of the importance of rivers on global sulphur cycle, relatively little work has been reported on dissolved sulphur isotopes in the watersheds of both major and minor rivers of the world. In the present study, we have attempted to present data to understand the sources of dissolved sulphate in the Alakananda and Bhagirathi rivers in the Himalayas.

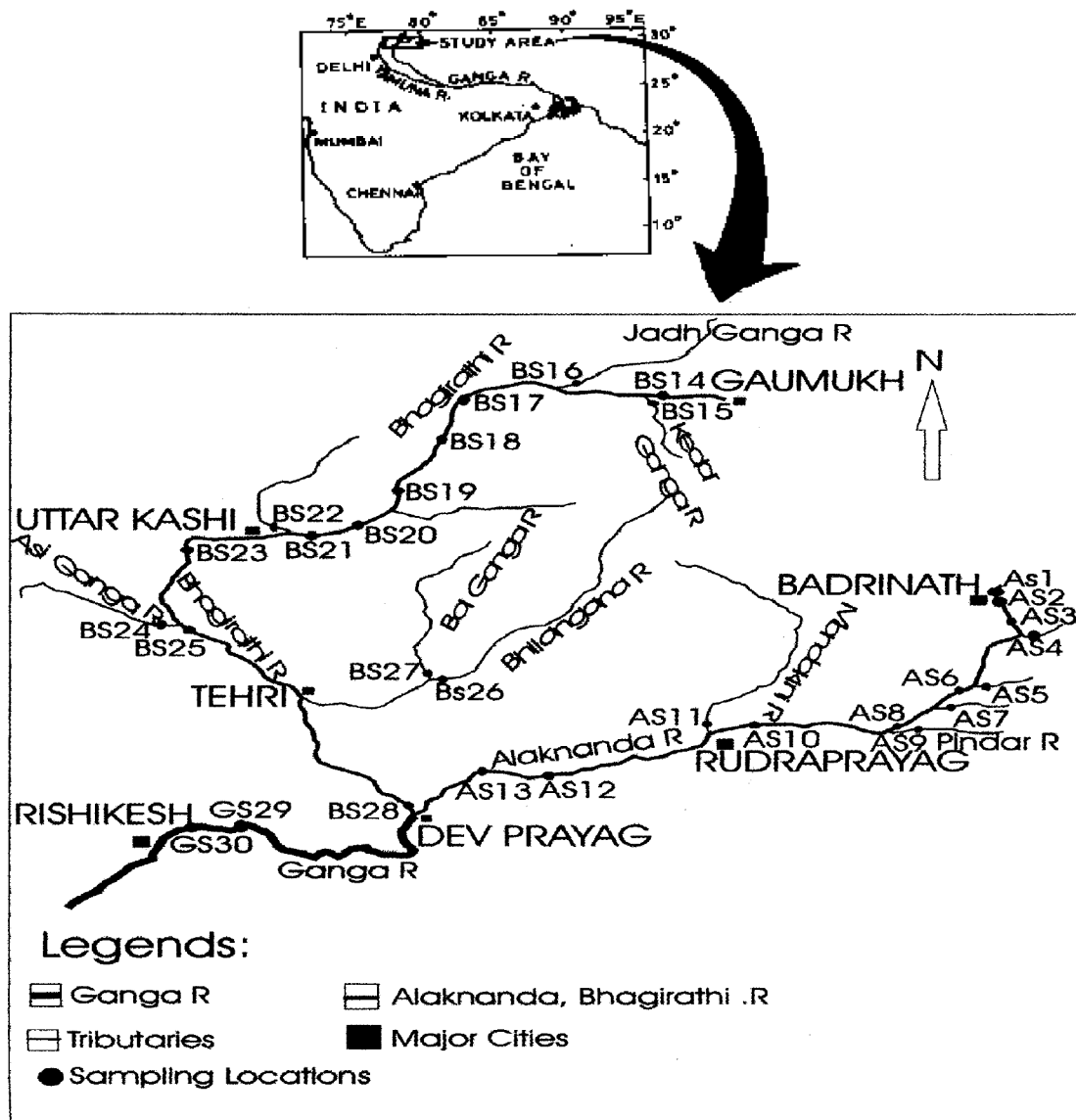


Figure 1. Location of Alakananda–Bhagirathi rivers and sampling sites for the present study (not to scale).

The study area covers the Ganga river in the Himalayan mountains up to Rishikesh. The source of the Ganga, the Gangotri glacier in the Kumaun Himalayas, is at an elevation of about 7000 m. The main stream of the Ganga is formed at Devprayag after the confluence of Bhagirathi and Alakananda, both originating in the Higher Himalayas. Near the source of the Alakananda and Bhagirathi rivers, Precambrian Central Crystalline rocks (medium to high grade metamorphic rocks) are exposed. The Alakananda flows through lithologies similar to the Bhagirathi river, which are the Higher Himalayan Crystallines in the upper reaches and the Kumaun Lesser Himalaya in the lower reaches. After passing through the Central Crystallines, the river traverses through limestones, marbles and quartzitic sequences of Tejam and Berinag formations. Before its confluence with the Bhagirathi, the stream passes through limestone and dolomite-bearing Uttarkashi Formation and the outcrops of phyllites and micaceous greywacke of the Chandpur Formation. Various stratigraphic formations exposed downstream of Devprayag are rocks of Nagthar Formation (phyllites, quartzite and sandstone), Chandpur Formation (phyllites, schist and quartzite), Deoban (limestone and shale), Mandhali Formation (limestone, slate and phyllite), Krol, Tal and Blaini formations (dolomitic limestone, silicic limestone, calcareous and pyritic shale and sporadic deposits of

gypsum and bands of phosphorites). The Bhagirathi and its tributaries predominantly drain the Kumaun Lesser Himalaya, characterized by three main lithologies, the autochthonous and allochthonous sediments and the crystalline nappes. The sediments mainly consist of greywacke, shales, slates, carbonates and quartzites. The main components of the crystalline nappes are granites, gneisses, schist, micaceous quartzites and calc-silicates. Presence of shales, phyllites and carbonates has also been reported in the crystallines<sup>5</sup>.

Water samples from 30 locations as shown in Figure 1, were collected from Alakananda, and Bhagirathi rivers in the Himalayan highlands and their major tributaries during May 2004. For  $\delta^{34}\text{S}$

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analysis, water samples were filtered through 0.45 µm cellulose nitrate membrane filter papers. To the filtered water samples, 10% BaCl<sub>2</sub> solution was added to precipitate dissolved sulphate. After addition of BaCl<sub>2</sub> solution, the samples were heated at 80°C overnight. Next morning, concentrated HCl was added to the sample solution until a pH of 2–3 was obtained to precipitate BaCO<sub>3</sub> that might have precipitated along with BaSO<sub>4</sub>. Finally, the solution was filtered through 0.45 µm cellulose nitrate membrane filter. BaSO<sub>4</sub> retained on the filter paper was rinsed thoroughly with distilled water in order to remove any chloride left from the addition of BaCl<sub>2</sub> solution. Any trace of chloride was tested with AgNO<sub>3</sub> solution. Finally the BaSO<sub>4</sub> was dried till constant weight was obtained.

<sup>34</sup>S analyses of BaSO<sub>4</sub> were performed after conversion of SO<sub>2</sub> in the presence<sup>6</sup> of V<sub>2</sub>O<sub>5</sub> and SiO<sub>2</sub> and subsequent determination of sulphur isotope composition with FINNIGAN MAT Delta S Mass Spectrometer at the Geological Institute of the Ruhr University, Bochum, Germany. The overall analytical precision was 0.2‰ (1σ). Dissolved sulphate was determined by Ion Chromatograph (Metrohm make) at the Geochemical Laboratory, IIT-Roorkee. The analytical results of total dissolved sulphate in the river waters and isotopic compositions of sulphur are presented in Table 1.

Dissolved sulphate concentrations in the samples vary appreciably (Figure 2) from a very low value of 47 µmol l<sup>-1</sup> in the Mandakini river at Rudraprayag to a high value of 535 µmol l<sup>-1</sup> in Jadhganga at Bhaironghati. These variations are in concurrence with the data of Sarin *et al.*<sup>7</sup> and Bickle *et al.*<sup>8</sup> for the non-monsoon season. After HCO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> is the next dominant anion in the Alakananda and Bhagirathi river basins. The predominant carbonate lithology and also the ease of carbonate rock weathering over silicate weathering, results in making HCO<sub>3</sub><sup>-</sup> the most abundant anion. Sulphate is the next abundant anion after HCO<sub>3</sub><sup>-</sup>.

The two rocks which contain abundant sulphur are the sulphide pyrites (FeS<sub>2</sub>) and gypsum (CaSO<sub>4</sub>·H<sub>2</sub>O) and anhydrite (CaSO<sub>4</sub>). River sulphate results mainly from dissolution of gypsum or oxidation of pyrites. Gypsum weathers faster because it goes into solution quickly and also gypsum beds occur as discrete beds, thus enabling selective removal during weathering. On the other hand, pyrite occurs as

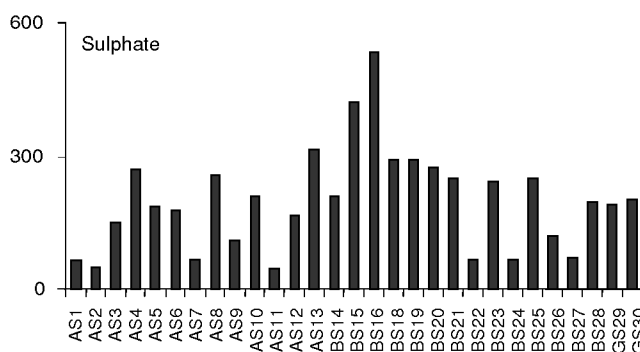
disseminated forms and requires breakdown of surrounding rocks before sulphate is released by oxidation. Berner<sup>9</sup> argues that, gypsum weathers twice as fast as pyrite. Several sources can contribute SO<sub>4</sub><sup>2-</sup> to river water. They are usually characterized by different specific sulphur isotopic compositions. These are dissolution of gypsum or anhydrite, with <sup>34</sup>S values<sup>10</sup> between 10 and 30‰ and igneous sulphide minerals such as pyrrhotite and pyrite

with <sup>34</sup>S mostly between 10 and -10‰. Oxidation of these minerals generates sulphate, which is only marginally depleted in <sup>34</sup>S compared to its precursors<sup>11</sup>. Sedimentary sulphides mostly range<sup>11</sup> between about 10 and -50‰, but river water sulphate derived from such a source is typified by negative values. Similar to the variations in total dissolved sulphate, sulphur isotopic ratios <sup>34</sup>S also show variations (Figure 3), indicating the dif-

**Table 1.** Dissolved sulphate and isotopic composition of sulphur in the samples

Sample	Location	Stream	SO <sub>4</sub> <sup>2-</sup> (µmol l <sup>-1</sup> )	<sup>34</sup> S
AS1	Mana	Saraswati	65.6	6.87 ± 0.06
AS2	Mana	Alakananda	49.8	5.17 ± 0.06
AS3	Pandukeshwar	Alakananda	149.8	4.34 ± 0.12
AS4	Vishnuprayag	Dhauliganga	269.7	0.43 ± 0.03
AS5	Birahi	Birahiganga	189.2	10.30 ± 0.08
AS6	Chamoli	Alakananda	178.8	2.65 ± 0.07
AS7	Nandprayag	Nandakini	67.8	7.73 ± 0.1
AS8	Karnaprayag	Alakananda	259.5	3.59 ± 0.05
AS9	Karnaprayag	Pinder	110.6	8.36 ± 0.09
AS10	Ratura	Alakananda	209.4	–
AS11	Rudraprayag	Mandakini	47.0	4.35 ± 0.14
AS12	Srinagar	Alakananda	166.4	2.24 ± 0.08
AS13	Kirtinagar	Alakananda	313.9	-1.67 ± 0.08
BS14	Gangotri	Bhagirathi	209.5	0.96 ± 0.09
BS15	Gangotri	Kedarganga	421.5	0.38 ± 0.05
BS16	Bhaironghati	Jadhganga	534.4	6.09 ± 0.05
BS17	Harsil	Bhagirathi	–	1.72 ± 0.09
BS18	Dabrani	Bhagirathi	292.1	1.97 ± 0.08
BS19	Malla	Bhagirathi	290.8	1.76 ± 0.06
BS20	Maneri	Bhagirathi	274.0	1.90 ± 0.09
BS21	Uttarkashi	Bhagirathi	249.0	1.91 ± 0.10
BS22	Gangori	Asiganga	68.1	–
BS23	Dunda	Bhagirathi	243.9	2.35 ± 0.05
BS24	Dunda	Dhanerigad	66.2	6.22 ± 0.07
BS25	Dharasu	Bhagirathi	250.1	2.34 ± 0.08
BS26	Ghansyali	Bhilangana	119.6	-0.56 ± 0.10
BS27	Ghansyali	Bhagirathi	69.8	–
BS28	Devprayag	Bhagirathi	196.2	1.69 ± 0.11
GS29	Shivpuri	Ganga	190.4	4.11 ± 0.07
GS30	Laxmanjhulla	Ganga	202.6	4.98 ± 0.06

–, Data not available; AS, Alakananda river or its tributaries; BS, Bhagirathi river or its tributaries.



**Figure 2.** Variation in dissolved sulphate concentration in the samples.

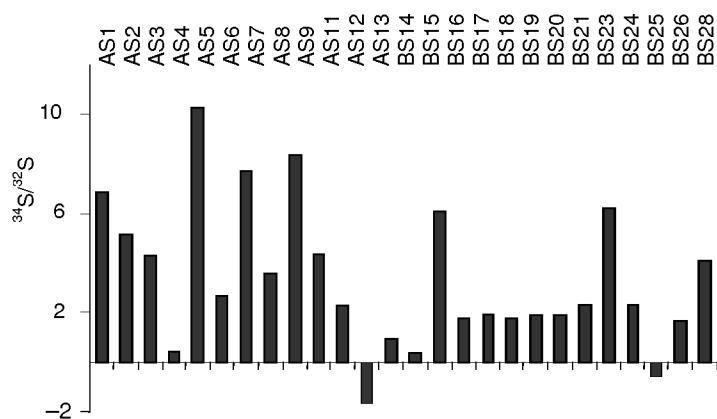


Figure 3. Variation of  $\delta^{34}\text{S}$  in river samples.

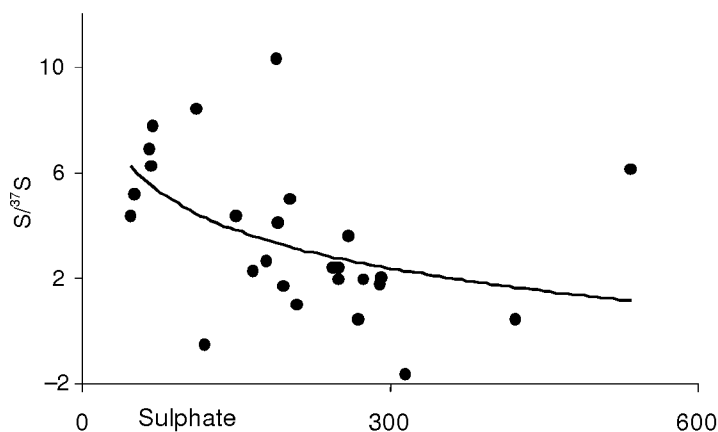


Figure 4. Relationship between total dissolved sulphate ( $\mu\text{mol l}^{-1}$ ) and sulphur isotopic composition.

ferent sources.  $\delta^{34}\text{S}$  varies from  $-1.67\text{‰}$  (AS13) to  $+10.30\text{‰}$  (AS5). In general, samples from Alakananda river are more enriched compared to those from Bhagirathi river. Although there are no published values of dissolved  $\delta^{34}\text{S}$  for rivers draining the Himalayas in India, data of Karim and Veizer<sup>12</sup> for Indus river in Pakistan also show variable  $\delta^{34}\text{S}$  values. In general, samples in the present study show slightly higher positive enrichment of  $\delta^{34}\text{S}$  (mostly around  $5 \pm 1\text{‰}$ ) for dissolved sulphate concentrations below  $200 \mu\text{mol l}^{-1}$ , whereas those with dissolved sulphate concentrations above  $>200 \mu\text{mol l}^{-1}$  have slightly lower  $\delta^{34}\text{S}$  enrichments (mostly around  $2 \pm 1\text{‰}$ ), indicating that the lower  $\delta^{34}\text{S}$  values were obtained in samples where dissolved  $\text{SO}_4^{2-}$  originated from oxidation of sedimentary sulphides and the higher  $\delta^{34}\text{S}$  values originated from

dissolution of gypsum. In general, low dissolved sulphate concentrations resulted in enriched  $\delta^{34}\text{S}$  (Figure 4). A comparison of the values of  $\delta^{34}\text{S}$  of our samples shows that of 27 samples whose  $\delta^{34}\text{S}$  values were determined, six show  $\delta^{34}\text{S}$  values of  $6\text{--}11\text{‰}$  which might have originated from sulphate deposits, ten samples show  $\delta^{34}\text{S}$  values of  $2\text{--}6\text{‰}$  which might have originated from the oxidation of sulphides present in igneous and metamorphic rocks and 11 samples have  $\delta^{34}\text{S}$  values of  $<2\text{‰}$ , indicating that the dissolved sulphate originated from the oxidation of sulphides associated with sedimentary rocks such as shales and limestones. Rainwater collected on 24 May 2004 show dissolved  $\text{SO}_4^{2-}$  concentration of  $36 \mu\text{mol l}^{-1}$ . The concentration is not high; however, at locations where dissolved sulphate concentration is less, the

role of rainwater contribution to sulphate could be significant.

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