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Arylsulfatase Activity in the Gut Microflora of Fish and Shellfish of Veli Lake, Kerala

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Moraxella sp., *Vibrio* sp. and *Micrococcus* sp. were isolated from representative cultures derived from *Villorita cyprinoides, Therapon jarbua* and *Penaeus indicus* caught from Veli lake in Kerala (India). pH optimum for *Moraxella* sp. was at 5.6 whereas it was 7 for *Vibrio* sp. and *Micrococcus* sp. Maximum growth of *Moraxella* sp. and *Micrococcus* sp. was at pH 5.6 and that of *Vibrio* sp., at pH 7.0. Optimum temperature for *Micrococcus* sp. was 29°C and for both *Vibrio* sp. and *Moraxella* sp., 37°C. The elevated arylsulfatase activity in these strains was found to vary with the addition of amino acids, organic and inorganic salts. Of these, phosphate was the key factor determining the type of arylsulfatase and inhibited the enzyme activity at 0.05M concentration in *Moraxella* sp. and *Vibrio* sp. and at 0.2M concentration in *Micrococcus* sp. Optimum buffer molarity was 0.1M when nitrocatechol sulfate was used as the substrate. The growth and enzyme activity of gut microflora were inhibited under growing conditions by the addition of heavy metals. Observations suggest that gut flora decompose esters of sulfuric acid thereby assisting digestion and metabolism of host fish and shellfish.

Key words: Arylsulfatase activity, gut microflora, *Moraxella* sp., *Vibrio* sp., *Micrococcus* sp., Veli lake.

In recent years, considerable interest has been focused on the enzymes of fish, shellfish and the microbes associated with them. Arylsulfatase is one such enzyme and its activity in different parts of marine gastropods, polychaetes, microbes and sediments of Indian coastal waters have been investigated (Dhevendaran, 1984; Dhevendaran *et al.* 1980; Dhevendaran *et al.* 1985; Dhevendaran *et al.* 1987) Further, microbes in the primary film in gut flora of foulers exhibited arylsulfatase activity (Dhevendaran *et al.* 1986; Maya *et al.*, 1990; 1995) and Dhevendaran & Maya (1998) established the association of arylsulfatase producing bacteria with the gut of *Therapon jarbua* and *Villorita cyprinoides*. Little attention has so far been paid to understand the factors controlling the activity and growth of gut microflora. The effect of cultural conditions on arylsulfatase activity of human pathogen, *Proteus rettgeri* was studied by Milazzo & Fitzgerald (1967). The present study aims to investigate the effect of cultural conditions on arylsulfatase activity of the gut microflora of fish and shellfish of Veli lake, Kerala.

Materials and Methods

Arylsulfatase producing bacteria (APB) were isolated using chromogenic substrate, tripotassium phenolphthalein disulfate (PDS) (Dhevendaran *et al.*, 1985) from representative cultures derived from *V. cyprinoides, T. jarbua* and *Penaeus indicus* caught from Veli lake (Kerala, India). It was incorporated into the Zobells 2216e marine agar medium at 60 mg. $100ml^{-1}$. After 12 days of incubation at room temperature (28±2°C), bacterial colonies were exposed to ammonium hydroxide solution. Colonies with pink halos around them were considered as APB. Isolated bacteria were identified upto generic level following the method of Simidu & Aiso (1962) and of Staley *et al.* (1989). Growth was determined by estimating the protein values of bacterial samples according to the method adopted by Lowrey *et al.* (1951). The standard curve was prepared using bovine albumin serum.

Arylsulfatase activity in growing cultures was studied using nutrient broth and nitrocatechol sulfate (NCS) as the substrate. The broth was prepared with appropriate buffer at specific pH ranging from 4.5 to 10.6. 18 h old culture was inoculated into the 10 ml medium with 0.002M concentration of NCS substrate. The culture mixture was incubated for 96 h. After the incubation period was over, 1 ml of culture broth was taken for protein estimation. The remaining culture mixture was treated with 1N NaOH to terminate the enzyme activity. After centrifugation at 10000 RPM, absorbance of the supernatant was measured at 515 nm in spectrophotometer. Standard curve was prepared using nitrocatechol (NC).

The influence of temperature on the hydrolysis of NCS was measured by incubating the reaction mixture at temperature ranging from 4 to 45°C. The enzyme activity and growth of bacteria were estimated as described earlier. The influence of various inorganic compounds such as sodium chloride, magnesium sulfate, sodium phosphate, heavy metals and amino acids such as methionine, theonine, cystein, tryptophan and L-glutamic acid and carbon sources such as starch, sucrose and lactose were studied using NCS as the substrate. These compounds were added to enzyme reaction mixture individually at different concentrations and activity and growth were measured. The composition of the reaction mixture and assay methods were similar as above.

Results and Discussion

Optimum pH for arylsulfatase activity of *Moraxella* sp. was 5.6 and it was 7.0 for both *Vibrio* sp. and *Micrococcus* sp. (Fig. 1). But growth was found to be maximum at pH 5.6 for *Moraxella* sp. and *Micrococcus* sp. and 7.0 for *Vibrio* sp., thereby indicating that arylsulfatase enzyme synthesis was influenced by pH of the media but not by the growth of the organisms. Similar observations have been reported by Milazzo & Fitzgerald (1967) and by Dhevendaran *et al.* (1986) in *Proteus rettgeri* and in *E. coli*, where most rapid rate of growth occurred at pH 6.6 and 5.6, respectively, and the highest level of arylsulfatase activity was found at pH 7.0

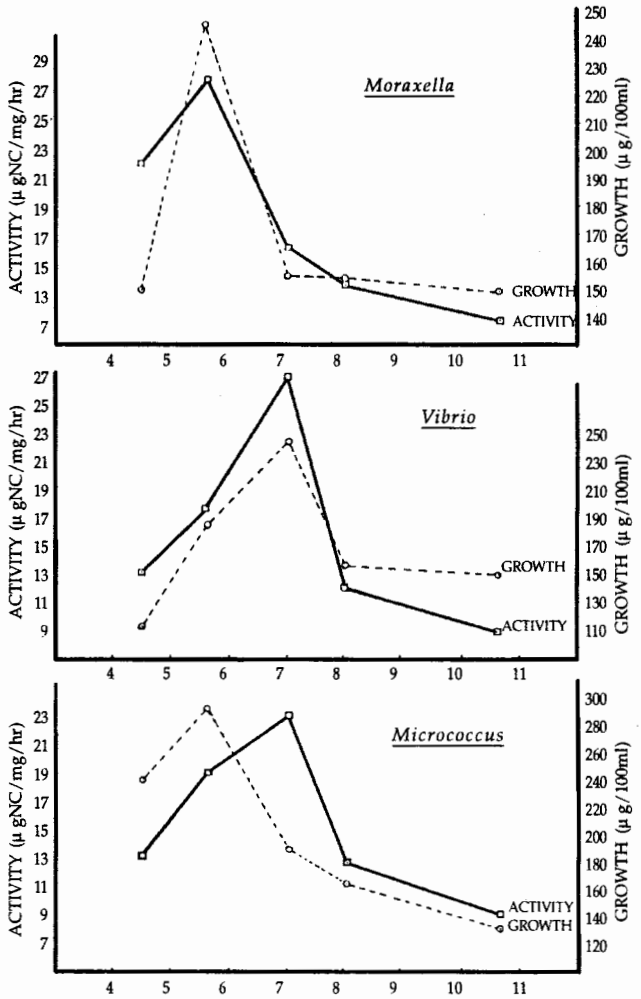

Fig. 1. Effect of pH on arylsulfatase activity and growth of bacteria

and 6.6, respectively. When the pH of the medium was raised to 8, arylsulfatase activity reduced by 70 to 75% approximately. The pH of Veli lake water ranged from 6.0-7.2 normally throughout the year (Dhevendaran *et al.*, 1987; Maya *et al.*, 1990) and this favours arylsulfatase activity.

Although growth and enzymes synthesis could be noticed in the temperature ranging from 4 to 40°C, there were differences in the rate of growth and enzyme activity (Fig. 2). In all the three cultures, maximum growth was observed at 29°C. Optimum enzyme activity in *Micrococuus* sp. was at 29°C whereas in *Vibrio* sp. and *Moraxella* sp. the maximum arylsulfatase activity was at 37°C. Milazzo & Fitzgerald (1967) observed that maximum growth and highest arylsulfatase activity was

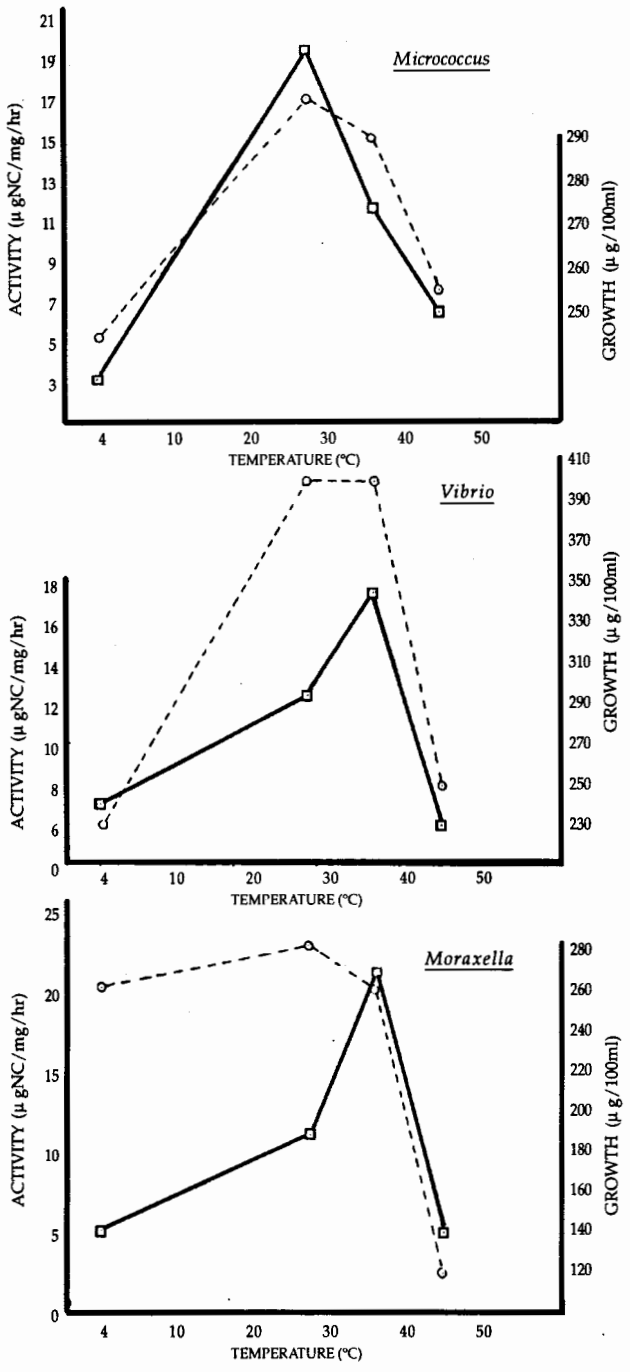

Fig. 2. Effect of temperature on arylsulfatase activity and growth of bacteria

at 28°C. In the shallow Veli lake region from where the fish and shellfish were collected, the temperature ranged from 29 to 30°C throughout the year. Further, the temperature of the gut of the fish and shellfish was 28±2°C at the time of microbial isolation. This suggests that natural environmental temperature was conducive for maximum growth of all the three species and for maximum enzyme activity in *Micrococcus* sp.

The addition of sodium chloride influenced growth as well as enzyme activity (Fig. 3). The maximum enzyme activity was recorded at 1% sodium chloride concentration for *Moraxella* sp. and *Vibrio* sp. and at 0.5% for *Micrococcus* sp., whereas the growth was optimum at 0.5, 1 and 2% for *Moraxella* sp., *Micrococcus* sp. and *Vibrio* sp., respectively. In the absence of sodium chloride, *Moraxella* sp. and *Vibrio* sp. exhibited feeble growth and reduced enzyme activity, thereby confirming their estuarine origin. Chloride ions have been reported to activate arylsulfatase activity in *E. coli* (Dhevendaran *et al.*, 1986) and in the gastropod, *Patella vulgate* (Dodegson *et al.*, 1953).

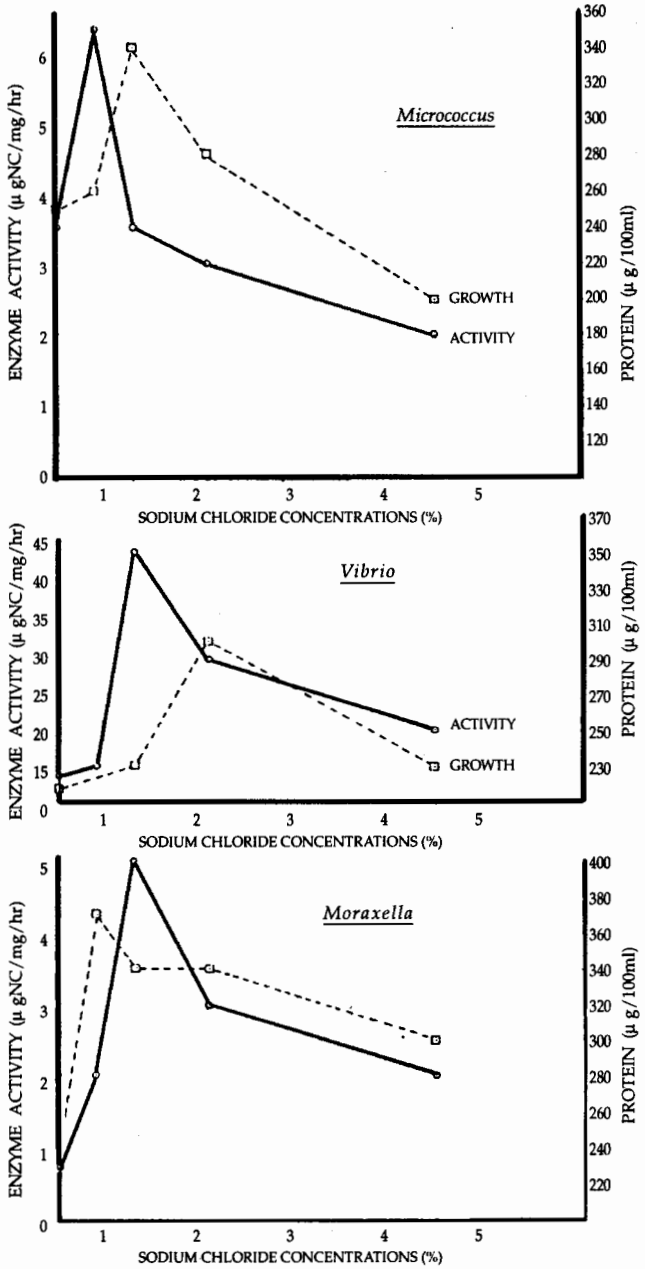

Fig. 3. Effect of sodium chloride on arylsulfatase activity and growth of bacteria

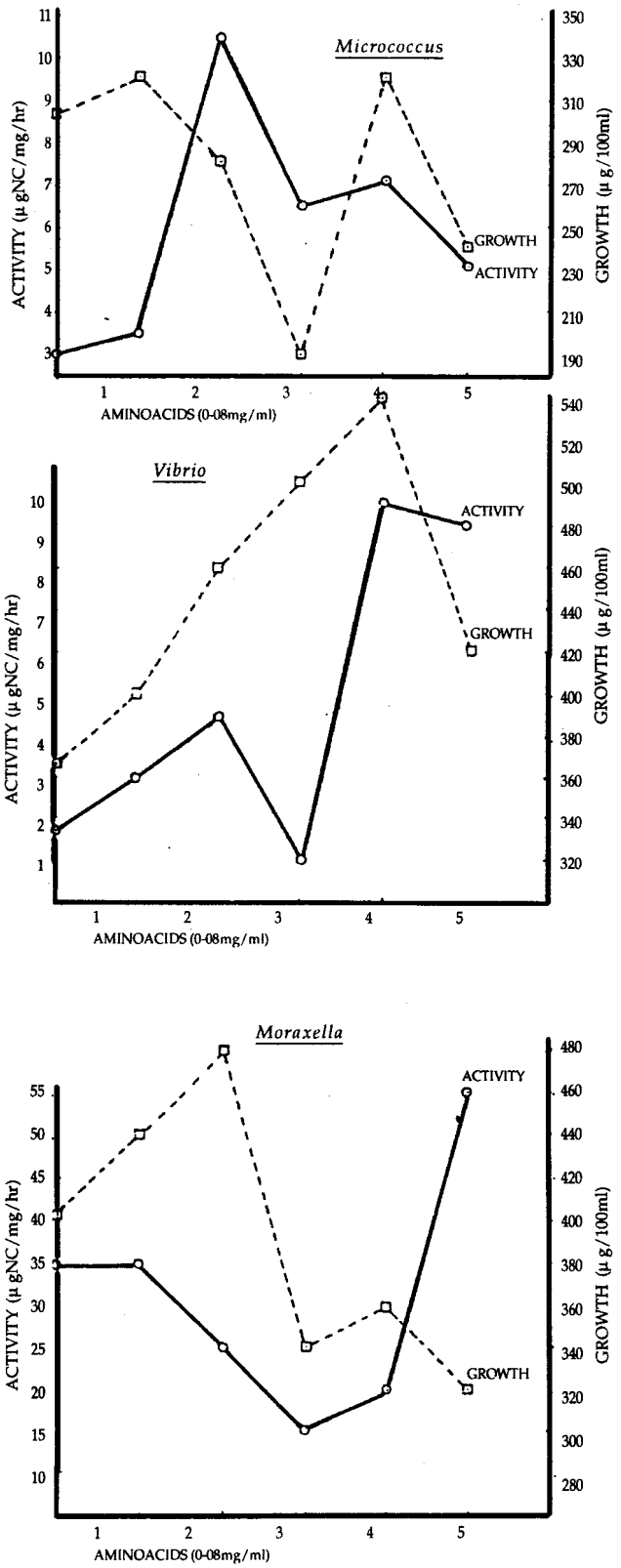

Fig. 4. Effect of aminoacids on arylsulfatase activity and growth of bacteria

It is clear that inorganic salt concentrations in the environment were sufficient enough to induce arylsulfatase activity (Dhevendaran & Maya, 1998).

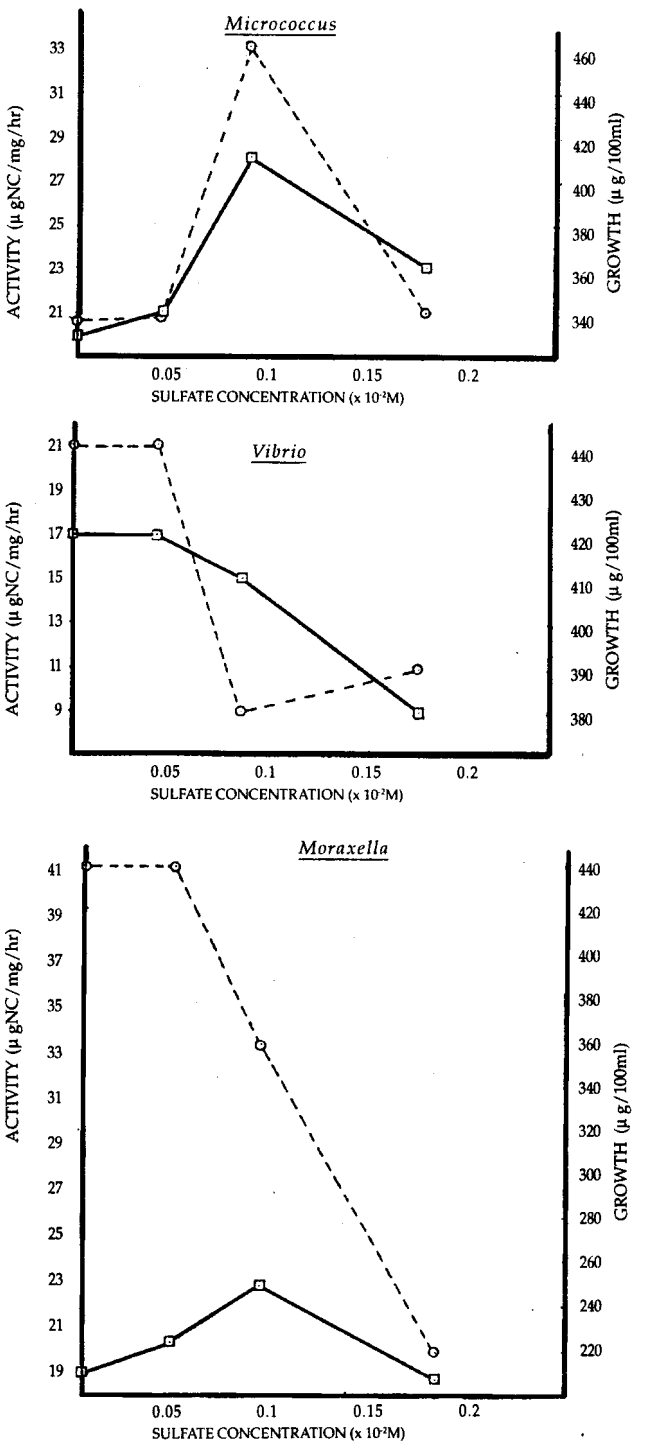

Fig. 5. Effect of sulfate concentration on arylsulfatase activity and growth of bacteria

Glutamic acid induced enzyme activity and threonine enhanced growth in *Moraxella* sp. Tryptophan activated both growth and activity of *Vibrio* sp. (Fig. 4). Threonine stimulated arylsulfatase activity and the methionine enhanced growth in *Micrococcus* sp. Availability of assimilable amino acids in host organisms for the growth and

Table 1. Effect of carbon sources on arylsulfatase activity and growth of bacteria

	Activity ($\mu g.NC.mg^{-1}.h^{-1}$)			Growth (μg protein.$100ml^{-1}$)		
	Starch (1%)	Sucrose (1%)	Lactose (1%)	Starch (1%)	Sucrose (1%)	Lactose (1%)
Moraxella sp.	2		4	390	260	350
Vibrio sp.	3.5	1.0	5.1	254	210	400
Micrococcus sp.	5.5	4.0	6.0	450	290	354

Table 2. Effect of phosphate on arylsulfatase activity and growth of bacteria

	Activity ($\mu g.NC.mg^{-1}.h^{-1}$)			Growth (μg protein.$100ml^{-1}$)		
	Sodium phosphate concentration			Sodium phosphate concentration		
	0.05 M	0.1 M	0.2 M	0.05 M	0.1 M	0.2 M
Moraxella sp.	–	–	–	560	460	400
Vibrio sp.	–	–	–	260	350	310
Micrococcus sp.	5.6	4	1.5	400	390	380

metabolism of gut microflora varies. However, the amino acid concentrations in host organisms have not been analysed. It was found that cystein inhibited both growth and activity in all the three bacterial strains as in the case of *Pseudomonas* $C_{12}B$ (Fitzgerald & Ash, 1982).

Influence of carbon sources had varying effects on growing cultures and enzyme activity (Table 1). When sucrose was utilized, the pH of the medium was lowered from 7.0 to 4.5. That might also be a probable cause for the inhibition of growth as well as arylsulfatase activity. This observation confirms the earlier reports by Whitehead *et al.* (1952), Milazzo & Fitzgerald (1967) and Dhevendaran *et al.* (1986).

It was observed that phosphate at 0.05M concentration retarded arylsulfatase activity in *Moraxella* sp. and *Vibrio* sp. (Table 2), whereas in *Micrococcus* sp. the activity was inhibited at 0.2M concentration. However, the growth was not affected in any of the three strains at the above concentrations. Arylsulfatase activity in *Vibrio* sp. (Fig. 5) was inhibited by addition of sulfate at $1x10^{-3}$ M concentration, whereas in *Moraxella* sp. and *Micrococcus* sp. it was inhibited at $2x10^{-3}$ M. It was found that $2x10^{-3}$ M sulfate repressed arylsulfatase activity in *P. rettgeri* (Milazzo & Fitzgerald, 1966) with NCS as the substrate. The endogenous phosphate values were 2.4, 5.3 and 2.6 $mg.mg^{-1}$ protein in the gut of *T. jarbua, V. cyprinoides* and in *P. indicus,* respectively.

The effect of heavy metal toxicity on growth and enzyme activity of the three selected bacterial strains is given in Table 3. At 2 ppm concentration, all the metals inhibited enzyme activity except zinc which enhanced the growth of all the three bacterial strains. Maya *et al.* (1990) observed mercury toxicity on arylsulfatase activity and growth of APB in the gut of *T. jarbua.* Veli lake region is often polluted by effluents containing heavy metals (Nair *et al.*, 1986). Arylsulfatase inhibition by copper, nickel and zinc have been studied in soils and lake sediments (Tabatabai & Bremner, 1970).

The association of arylsulfatase producing bacteria in fish and shellfish plays a significant role in the breakdown of conjugated sulfuric acid esters of aromatic compounds present in their diet. However, the fluctuations in abiotic and biotic factors in the external environment may influence the optimum arylsulfatase activity and growth of gut microflora. This in turn may lead to variations in the enzyme activity in fish, shellfish and other aquatic organisms.

Table 3. Effect of heavy metal toxicity on arylsulfatase activity and growth of bacteria

	Activity (µg.NC.mg^{-1}.h^{-1})					Growth (µg protein.100ml^{-1})				
	Control	Mercuric chloride (2 ppm)	Copper sulphate (2 ppm)	Zinc sulphate (2 ppm)	Sodium arsenate (2 ppm)	Control	Mercuric chloride (2 ppm)	Copper sulphate (2 ppm)	Zinc sulphate (2 ppm)	Sodium arsenate (2 ppm)
Moraxella sp.	28	–	–	–	0.04	245	150	222	270	210
Vibrio sp.	23	3.0	1.50	2.00	–	285	230	270	344	186
Micrococcus sp.	27	0.05	0.09	0.05	–	245	210	220	270	210

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