

## Some aspects of microbiological characteristics in the nearshore waters of Bombay

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Received 1 September 1993; revised 8 April 1994

Primary production, bacterial abundance, their generic composition, some of their biodegradative enzymes and heterotrophic uptake of labelled glucose were examined from the waters of Bombay harbour-Thana creek-Bassein creek confluence. Extremely low mineralization rate, as evidenced from poor microbial heterotrophic activity and fewer bacterial groups suggest environmental deterioration. Besides suggesting eutrophication, higher rates of primary production in the locations receiving industrial wastes were also important in discerning a nonstimulation of microbial mineralisation processes. Most microbial processes were observed to be under stress at the locations affected by effluent discharge than those relatively free of pollutants.

The confluence of Bombay harbour-Thana creek-Bassein creek (BHTCBC) was known for its high biological diversity and good nursery ground<sup>1,2</sup>. Rapid urban and industrial expansions have been the causes of environmental retardation of this complex. Several tidal inlets (Dharamtar, Nhava-Sheva and Panvel creek) and small sized rivers (Amba, Ulhas and Kalu-Waldhuni) discharge various kinds of wastes<sup>3</sup>. An estimated 350 mld of domestic and over 180 mld of industrial (comprising of fertilizer, petrochemical, automobile, thermal power station, nuclear, pharmaceutical, leather, food and chemical) wastes are discharged into this confluence<sup>3</sup>. For instance, Patel *et al.*<sup>4</sup> estimated that about 35000 tonnes of suspended solids and 1250, 35, 20, 10 and 3 kg of Zn, Cu, Hg, Pb and Cr respectively are released daily into Ulhas and Kalu-Waldhuni rivers. Though there are many planktonological and hydrological studies<sup>5-10</sup>, no study so far has examined the microbiological characteristics of these waters. In the estuaries and backwaters subjected to tidal exchanges and varying salinities, only an adaptive biota can grow and survive. Such abilities are greatly affected by various anthropogenic inputs to these ecosystems<sup>11-14</sup>. Many anthropogenic compounds adversely affect the chemistry of the seawater and are deleterious both at lethal and sublethal levels to the biotic component<sup>15-17</sup>. Scheiwer *et al.*<sup>18</sup> emphasized that primary production and microbial heterotrophic activity play very important roles in the formation and turnover of organic matter in the eutrophic estuaries. A few studies have recorded the type and abundance of indicator and general heterotrophic viable bacteria from a few of the Indian estuaries<sup>19-21</sup>. However, none of these earlier studies had looked

collectively at the microbial heterotrophic activity and primary production rates. Therefore, this study was aimed at understanding some of the important microbiological parameters and to assess their relationship with the primary productivity in the nearshore waters of Bombay.

### Materials and Methods

Seawater samples from 6 locations representing different zones of the confluence (Fig. 1) were collec-

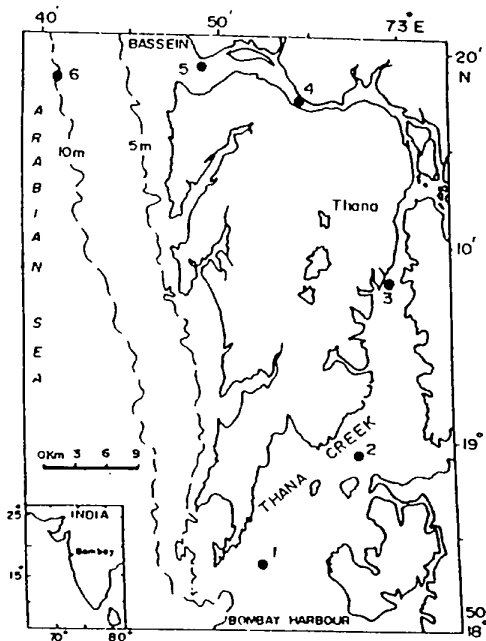


Fig. 1—Sampling locations

ted during February, May and September, 1986 and analysed for microbial heterotrophic activity, total viable counts (TVC) of bacteria and for bacterial generic distribution. Total direct counts (TDC) of bacteria were determined only from the water samples collected in June 1988 from locations closer to sts 1, 3 and 5. While TVC were determined by the standard plating method, TDC were by the epifluorescence microscopy using acridine orange<sup>22</sup>. Heterotrophic activity of the natural microbial assemblages was determined using uniformly labelled <sup>14</sup>C glucose (specific activity 53  $\mu\text{Ci.ml}^{-1}$ , BARC, Bombay) for the net uptake<sup>23</sup> and for the percentage of <sup>14</sup>C respired<sup>24</sup>. Subsamples of 50 ml and 5 different concentrations of labelled glucose were used for these determinations. For enumerating viable aerobic heterotrophs, 1 to 3 ml aliquots of water samples were filtered through membrane filters (0.22  $\mu\text{m}$ ; Millipore, USA) and the filters were placed on to the nutrient agar (Himedia, Bombay) plates prepared with half strength aged seawater (final salinity  $ca\ 17.5 \times 10^{-3}$ ). All these plates were incubated at room temperature ( $\sim 27^\circ \pm 2^\circ\text{C}$ ) for 48-72 h. After enumeration, over 60% of the total

colonies developed on the medium were randomly selected for the identification of the bacterial strains<sup>25</sup>. In addition to routine biochemical characterisation, presence of some hydrolytic enzymes (amylase, chitinase, lipase, nitrate reductase, protease and urease) was examined from all these bacterial strains. Primary production in the surface waters ( $\sim 100\%$  light intensity) was also measured<sup>22</sup>.

### Results

The TVC of bacteria did not show any major periodic or locational variations although their abundance was generally higher during premonsoon (Table 1). This was also evident from the absence of statistically significant variation in their abundances (observed  $F_{5,10}\ 1.354$  between stations and  $F_{2,10}\ 2.33$  between seasons). These numbers accounted for less than 10% of TDC. As can be seen from Table 1, the TDC ( $\text{no.ml}^{-1}$ ) were in the range of  $10^5$ - $10^6.\text{ml}^{-1}$  in the water samples collected at stations 1, 3 and 5. In general, both TDC and TVC are lower, at least by an order of magnitude when compared to those observed in the relatively nonpolluted Dona Paula bay (Ramaiah,

Table 1—Various microbiological and primary productivity parameters during February, May and September 1986 at different sampling locations

	Stations					
	1	2	3	4	5	6
<i>February 1986</i>						
TVC ( $\text{no.} \times 10^3.\text{ml}^{-1}$ )	10.6	3.7	13.5	1.1	16.3	11.1
$V_{\text{max}}$ ( $\mu\text{g.C.l}^{-1}.\text{h}^{-1}$ )	0.251	0.2627	0.3204	0.4351	0.5262	0.7351
Respiration (%)	4.62	5.3	2.47	6.	9.23	13.0
Turnover ( $T_r$ ) time (h)	134.15	65.72	42.44	217.76	176.59	114.53
$K_t + S_n$ ( $\mu\text{g.C.l}^{-1}$ )	37.37	47.4	25.9	27.4	44.61	51.01
Pr. prod. ( $\text{mgC.m}^{-3}.\text{d}^{-1}$ )	14.9	25.9	43.97	6.01	6.95	7.69
<i>May 1986</i>						
TVC ( $\text{no.} \times 10^3.\text{ml}^{-1}$ )	46.	62.5	57.6	4.0	56.0	10.
TDC ( $\text{no.} \times 10^6.\text{ml}^{-1}$ )	4.601	—	7.09	—	0.162	—
$V_{\text{max}}$ ( $\mu\text{g.C.l}^{-1}.\text{h}^{-1}$ )	0.2515	0.1972	0.299	0.3901	0.4147	0.7432
Respiration (%)	4.01	5.46	2.02	6.57	.46	13.52
Turnover ( $T_r$ ) time (h)	159.19	105.64	70.9	172.7	151.94	166.27
$K_t + S_n$ ( $\mu\text{g.C.l}^{-1}$ )	40.04	5.63	20.55	33.03	21.9	13.64
Pr. prod. ( $\text{mgC.m}^{-3}.\text{d}^{-1}$ )	6.04	9.30	29.9	6.37	5.55	7.3
<i>September 1986</i>						
TVC ( $\text{no.} \times 10^3.\text{ml}^{-1}$ )	1.0	12.7	23.3	17.6	22.4	.0
$V_{\text{max}}$ ( $\mu\text{g.C.l}^{-1}.\text{h}^{-1}$ )	0.4025	0.4296	0.3720	0.4341	0.3927	0.511
Respiration (%)	1.76	7.96	4.41	.05	9.51	14.67
Turnover ( $T_r$ ) time (h)	79.50	57.9	39.05	94.40	215.21	10.03
$K_t + S_n$ ( $\mu\text{g.C.l}^{-1}$ )	32.00	51.01	41.41	54.21	47.43	5.61
Pr. Prod. ( $\text{mgC.m}^{-3}.\text{d}^{-1}$ )	34.73	6.49	54.02	7.20	5.91	3.5

unpublished). Mean rates of maximum uptake velocity ( $V_{\max}$ ), percentage of  $^{14}\text{C}$  glucose respired, turnover time ( $T_t$ , the time required for the complete mineralisation of the substrate) and the sum of transport constant and natural substrate concentration ( $K_t + S_n$ ), are also presented in Table 1. A point of interest is the strikingly significant (Table 2) location-wise differences in the heterotrophic uptake of labelled glucose by the natural microbial assemblages. Stations 1 and 2, closer to Bombay harbour and the

Thana industrial estate (st. 3), generally showed a lower  $V_{\max}$  and there was a gradual increase in the  $V_{\max}$  towards the northern stations during all the three observations. The  $^{14}\text{C}$  respired also followed the same trend. Higher  $^{14}\text{C}$  glucose respired commensurated generally with that of higher  $V_{\max}$  for sts 5 and 6 relatively free of pollutants. It varied from < 2% at st. 1 to over 14.5% at the coastal st. 6 off Bassein. In the south many a times, respiration was surprisingly lower. Turnover time was greatly dependent on the  $K_t + S_n$ . In general, higher the  $V_{\max}$  shorter was the  $T_t$ .

Table 2— $F$  values (two-way ANOVA) for various parameters during different months from different stations

Parameters	Stations	Months
Degrees of freedom	5	2
Viable bacterial numbers	1.354	2.33
$V_{\max}$	13.743 <sup>a</sup>	3.940
Respiration	20.443 <sup>a</sup>	0.718
$T_t$	5.168 <sup>b</sup>	0.707
Primary production	13.086 <sup>a</sup>	1.161

Calculated  $F$  significant at (a) 1% and (b) 5% level

From the biochemical characterisation of over 1000 bacterial isolates, 11 different genera were identified (Table 3). About 15% of the total isolates could not be assigned to any of the known genera. *Vibrio*, *Pseudomonas*, *Aeromonas*, *Bacillus*, *Micrococcus* and *Chromobacterium* were the most dominant genera. Seasonal variations in the generic composition were not discernible.

Proteolytic, lipolytic, chitinoclastic and nitrate reductase activities were pronounced among the strains

Table 3—Distribution (%) of different bacterial genera at different stations during February (a), May (b) and September 1986 (c). Figures in parentheses are the number of bacterial strains isolated and identified during each collection

	Bacterial genus/group*										
	Bac	Mic	Aer	Pse	Vib	Pho	Aci	Chr	Mor	Ent	Unid
St. 1											
a (167)	7.18	8.99	11.38	11.98	17.37	0.59	5.39	6.59	7.18	8.99	14.37
b (155)	5.16	10.97	14.84	9.03	16.12	0.65	5.81	7.75	6.45	9.68	13.59
c (163)	10.43	7.98	8.59	11.66	17.18	1.23	5.52	6.13	4.91	9.82	16.57
St. 2											
a (161)	6.25	5.11	10.79	15.91	18.18	0.57	4.55	5.11	6.82	10.23	14.77
b (167)	7.74	5.36	11.31	14.88	19.05	0.59	4.17	5.95	5.95	10.12	14.89
c (176)	3.73	6.83	13.04	16.15	17.39	0.62	5.59	5.59	6.83	9.32	14.91
St. 3											
a (156)	9.62	5.13	9.62	14.10	19.23	0.64	7.10	7.70	5.77	11.54	6.41
b (162)	9.88	7.41	9.26	14.19	18.52	0.62	6.17	4.93	6.79	12.96	9.25
c (178)	10.11	6.18	9.55	11.80	16.85	1.12	6.74	6.18	4.49	8.43	11.79
St. 4											
a (164)	9.76	6.71	10.36	14.02	17.07	0.61	7.32	3.66	4.88	10.37	15.85
b (158)	8.87	5.69	8.87	13.92	17.08	0.63	7.59	6.33	4.43	11.39	15.19
c (148)	8.11	6.08	10.14	13.51	15.54	0.68	6.78	4.73	4.73	12.83	16.89
St. 5											
a (147)	4.76	4.76	10.20	17.00	18.36	1.36	8.80	8.16	6.12	12.93	14.29
b (150)	4.67	4.67	9.33	15.33	18.00	1.33	8.00	8.67	4.67	12.67	12.00
c (169)	5.33	4.73	11.24	15.98	15.98	1.78	8.83	7.69	6.51	12.42	11.33
St. 6											
a (156)	8.33	5.13	12.18	10.89	19.23	2.56	5.13	5.76	7.05	8.33	15.39
b (137)	8.03	4.38	14.58	10.95	18.25	2.91	4.38	5.84	6.57	3.69	20.43
c (147)	8.84	4.76	12.93	11.57	21.09	2.72	4.76	5.44	5.44	5.44	17.00

\*Bac—*Bacillus*, Mic—*Micrococcus*, Aer—*Aeromonas*, Pse—*Pseudomonas*, Vib—*Vibrio*, Pho—*Photobacterium*, Aci—*Acinetobacter*, Chr—*Chromobacterium*, Mor—*Moraxella*, Ent—*Enterobacteriaceae*, Unid—Unidentifiable

of *Pseudomonas* (minimum 58%), *Vibrio* (72%) and *Chromobacterium* (69%). Strains of *Moraxella* (75%), *Acinetobacter* and Enterobacteriaceae (59%) showed urease, nitrate reductase and amylase activity. *Micrococcus* (75%) and *Bacillus* (> 80%) showed pronounced amylase activity. Nearly 80% of the unidentifiable strains elaborated urease and over 50% of them were positive for chitinase and lipases. At none of the stations during any of the three collections was any one enzyme present in > 30% of the strains (Fig. 2). In addition, in terms of different sampling months also, variations in the percentages of bacterial strains producing any of these enzymes were not at all marked.

Surface primary production showed wide fluctuations at different stations. However, it was relatively higher at st. 3 during all the three collections (Table 1). Variations in southern stations were well-defined with maximum production during May followed by September and February. In general, lower rates of primary productivity were recorded at st. 5 during all the collections.

Regression analyses between the heterotrophic uptake of labelled glucose and autotrophic production showed inverse correlations (Fig. 3). Whereas,  $V_{max}$  was found to be positively correlated with all other parameters of heterotrophic activity (Fig. 4) reflecting a total dependence among net uptake and other mineralisation processes. Analysis of variance revealed that the heterotrophic activity of the natural microbial assemblages and primary production showed significant locational variations but were not affected by the change in sampling months (Table 2).

## Discussion

Estuaries and backwaters are among the most productive zones of the marine environs. Nutrient inputs from freshwater drainage as well as industrial establi-

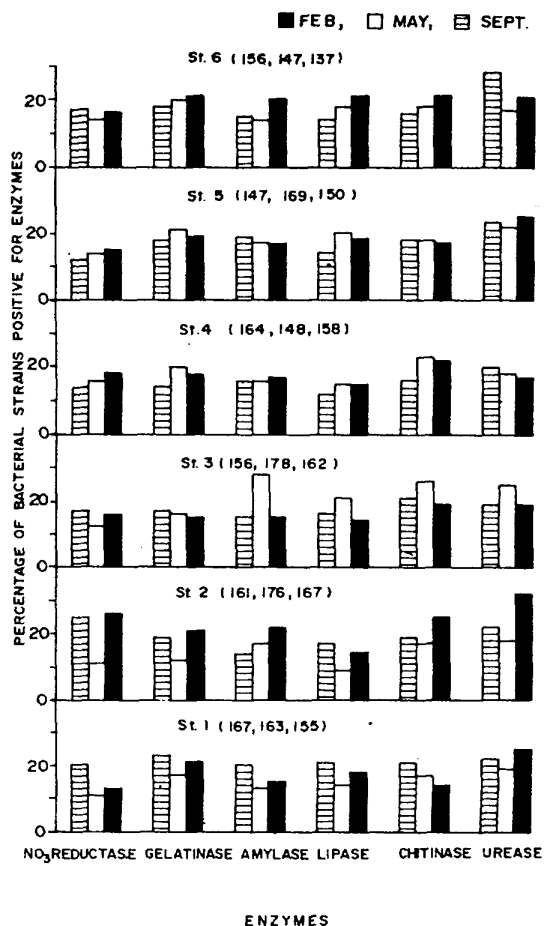


Fig. 2—Distribution and different months in the bacterial strains isolated during different months (Figures in parentheses are the number of isolates made during February, May and September 1986)

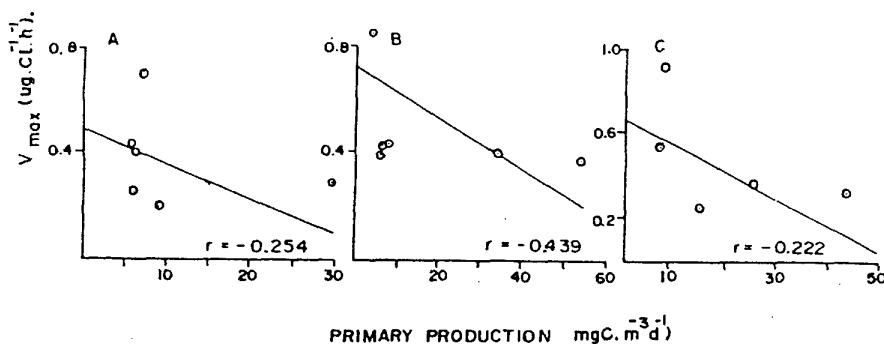


Fig. 3—Relationship between Pr. prod. and  $V_{max}$  during (A) May, (B) September and (C) February 1986 ( $n=6$ , observed  $r$  is significant at 1% when it is  $\geq 0.75$ ; at 5% when it is  $\geq 0.755$  and at 10% when it is  $\geq 0.699$ )

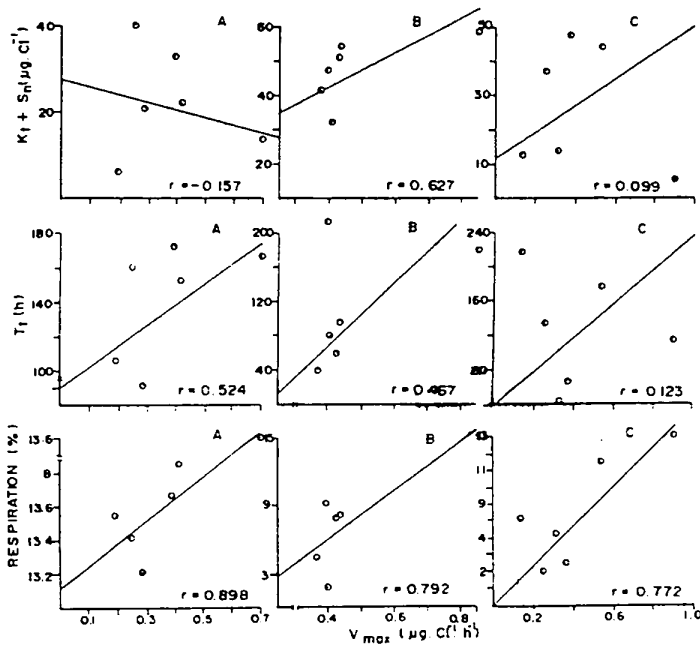


Fig. 4— Relationship between  $V_{max}$  and other kinetic parameters during (A) May, (B) September and (C) February 1966 ( $n = 6$ , observed  $r$  is significant at 1% when it is  $\geq 0.75$ ; at 5% when it is  $\geq 0.755$  and at 10% when it is  $\geq 0.699$ )

shments affect their productivity either adversely or favourably. Although, inflow of river discharges has been ascribed as the stimulant of higher rates of microbial heterotrophic activity<sup>26</sup>, in the present study however, neither the substantial quantities of organic wastes<sup>3</sup> continuously discharged near st. 3 nor the riverine discharges, seemed to stimulate heterotrophic activity. Not only were the  $V_{max}$  and percentage of glucose respired far lower at this station but they were also the lowest during the study period. There are many evidences<sup>12, 14, 17, 18</sup> which show that heterotrophic activity is mainly dependent on the availability of organic carbon produced by autotrophs. The observed values of <sup>14</sup>C glucose uptake are far lower than those reported for other tropical regions<sup>14</sup> and coral reef ecosystems<sup>27, 28</sup>. It was surprising to note the lower rates of heterotrophic activity since the numbers of viable bacteria recorded were more or less in the similar range from the entire area. That very low  $V_{max}$  rates were recorded from places of high primary production might suggest the possible quick response of the *in situ* microflora to the toxic discharges. From these results it is possible to suggest that the *in situ* heterotrophic activity in the Bombay environs appears to be affected adversely by the input of industrial effluents. These effluents may bring about metabolic stress and induce a non-growing state. This could be

the principal reason for the lower  $V_{max}$  and associated respiration rates particularly in the interior and Bombay harbour stations. It might also be due to the prevalence of only those microbial groups that can withstand the pollution stress. From a recent study it was possible to pinpoint the adverse effects of pollutants present in this complex<sup>29</sup>. Besides highlighting the selection pressure on the microbial communities, present study is helpful in recognising the sublethal effects of heavy metal and other organic toxicants being continuously discharged into this confluence.

The patterns of enzyme distribution in the bacterial isolates observed during this study would imply the differential utilization and breakdown of available organic matter so that there is a close coupling of utilization of different nutrients by different microbial types<sup>30, 31</sup>. In general, such pattern implies that the whole of the microbial community is involved in the organic material biotransformation process. This pattern is very interesting in the sense that, it suggests the ability of tolerant microbial types to carry on with their primary role of biodegradation even in the presence of such large concentrations of toxicants<sup>3</sup>. This fact is further substantiated by the least or no variations in the percentage of isolates showing different enzyme activities. In addition, the types of bacterial genera recorded from this confluence although not

very different from most other locations<sup>19</sup>, the preponderance of *Vibrio* and *Pseudomonas* probably indicate the adaptation and tolerance of these communities to various allochthonous inputs. Mean rates of primary production recorded off Bombay are about 106 mgC.m<sup>-3</sup> and these rates are far higher than the present ones. Varshney and Abidi<sup>9</sup>, and Varshney *et al.*<sup>10</sup> observed very high rates of production (474 to 533 mgC.m<sup>-2</sup>.d<sup>-1</sup>) from polluted regions (closer to sts 2 and 3 of this study) than in the unpolluted nearshore (off Versova) waters around Bombay (106 to 332 mgC.m<sup>-2</sup>.d<sup>-1</sup>). The overall rates of primary production from both the polluted as well as non-polluted regions are lower than those reported earlier. Higher primary production in the Thana creek may be ascribed to the increased allochthonous input of inorganic and organic matter. It appears that phytoplankton productivity is limited by the nutrient availability particularly of phosphate in the northern stations<sup>7</sup>. Nonetheless, these rates are similar to those seen in other coastal regions<sup>32,33</sup>. It was seen from the heterotrophic activity measurements that the V<sub>max</sub> and respiration rates were far higher in the northern stations. Both these observations suggest that the northern stations experience low pollution stress.

Detailed analyses of various physico-chemical parameters carried out during this study period are published elsewhere<sup>6-8</sup>. In short, many of the examined water quality parameters did not indicate any specific or drastic seasonal changes in this estuarine complex which has a peculiar local geography, substantial discharges through the rivers and enormous quantities of effluents besides a constant influence of semidiurnal tides. Because of the pronounced tidal exchange, the sewage and other industrial effluents discharged into it are flushed out rather continuously resulting in rapid dilution of the contaminants and their adverse effects. Except for occasional higher nitrite (st. 3) and nitrate (sts 2-4) concentrations at some locations, all other physico-chemical parameters monitored during this period were found to show normal variations as is true for many other Indian estuaries<sup>34,35</sup>.

From this study, it is highlighted that higher primary production was not supporting higher heterotrophic activity which is a deviation from the usual process. This is suggestive of the metabolic stress on natural microbial assemblages owing to the fact that the input of toxic effluents adversely affect their mineralisation processes. There were higher rates of primary production at places receiving effluents, suggesting a eutrophicated condition. Predominance of few communities of bacteria and the patterns of possessing of biodegradative enzymes among these microbial communities clearly suggest a continuous sublethal toxic-

ity (due to heavy metals, toxic inorganics as well as organics) on the basic heterotrophic processes.

#### Acknowledgements

Author is thankful to Drs A.H. Parulekar, D. Chandramohan and M.D. Zingde for encouragements and for the help of Drs V.R. Nair and T. Govindan during various collections.

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