

Fig. 1—Uptake of copper by *M. viridis* in relation to copper concentration in the medium

water at Velsao during the season of spatfall of mussels was low and nondetectable. Uptake levels of copper from the medium at 24, 48, 72 and 96 hr (Fig. 1) revealed that the rate of intake of copper is faster in the medium containing concentrations higher than 0.1 ppm. Levels of copper in the tissues were related to their concentrations in the medium and also with time (Fig. 1). At low concentrations (0.05 ppm) the uptake was lower (7.50 ppm) and took more time (72 hr) than at a higher concentration (1.0 ppm) when it was greater (30 ppm) and took a shorter time (48 hr). In the 96 hr experiments, maximum uptake (47 ppm) was recorded when a 100% mortality occurred (Table 1).

From these experiments it is evident that time is a major factor in the uptake of copper leading to the death of mussels. At lower (background) concentrations of copper, shell fishes are known to accumulate the metal in their tissues, though not up to the level of mortality<sup>3</sup>. Galstoff<sup>5</sup> reported that oysters contain from 1.24 to 5.12 mg/100 g of copper in their body. These concentrations are not toxic to the animals though they attain levels of about 100 times greater than what is found in normal sea water. McLusky and Philips<sup>6</sup>, while experimenting on the effects of copper on the polychaete *Phyllodoce maculata*, have found that copper concentrations reach to 567.8 ppm dry weight of animal tissue when the organisms were exposed to 0.06 ppm concentration for 3 weeks without any adverse effects. It is clear from the present study that greater the concentration of copper in the experimental medium, the faster will be the rate of uptake in terms of time.

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#### Microbial Flora & Dehydrogenase Activity of Beaches

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Heterotrophic bacterial flora of beaches, viz. Velsao (open sea beach receiving nitrogenous wastes from a fertilizer factory) and Dias (estuarine beach free from contamination) were studied. At Velsao, the organic carbon content in the sand was lower (0.18%) than at Dias (0.45%). Nitrate nitrogen content of the former was very high (619.36 µg at N/litre), as compared to the latter (4.52 µg at N/litre). Though Dias beach was rich in both terrestrial and marine flora (12.8 and 8.6 × 10<sup>3</sup>/g dry wt of sediment respectively), the percentage of proteolytic bacteria and nitrate reducers were more at Velsao. This indicates that the latter has a low organic carbon and high nitrogen content. Microbial flora was correlated with the dehydrogenase activity so as to determine the use of dehydrogenase activity as an index of beach pollution. Dehydrogenase activity at Dias was twice that of Velsao. The peak value was attained on the 5th day of incubation in samples from Dias (0.54 mg/g dry wt sediment) and on the 3rd day in samples from Velsao (0.26 mg/g dry wt sediment).

INDUSTRIAL wastes discharged into the sea induces changes in the ecosystem by changing its chemical and physical properties and its microbial flora, which then react with the pollutants either favourably or adversely. To understand this phenomenon, bacterial population as physiological groups has been studied in relation to ecological factors in a beach at Velsao which has a fertilizer factory in its vicinity and in another beach, Dias near NIO which is free from such pollution. The role of dehydrogenase in a beach ecosystem has not been reported so far. In the present study an attempt has been made to compare bacterial flora with dehydrogenase activity in these two beaches with a view to finding out whether this activity can be an indicator of beach pollution.

Random sediment samples were aseptically collected (June 1975) from the beaches and transferred to the laboratory in ice.

*Isolation of bacteria*—The samples were mixed and subsamples were taken for studying the bacterial population. Serial dilution method was followed to plate bacteria employing nutrient agar and Zobell 2216 E. Plates were incubated at room temperature (26°C ± 2) and bacterial counts recorded after 48 hr. Morphologically different colonies were isolated, purified and physiologically grouped<sup>1</sup>.

Organic carbon of the sediment was estimated using the method described by Wakeel and Riley<sup>2</sup>. Particle size analysis was made following wet sieving technique<sup>3</sup>. Interstitial waters collected from the sampling areas were used for the determination<sup>4</sup> of salinity, oxygen, nitrite-N and nitrate-N.

*Dehydrogenase*—Dehydrogenase activity was determined by following the reduction of triphenyl-tetrazolium chloride (TTC) to triphenyl formazan.

CaCO<sub>3</sub> (100 mg) was added to several flasks containing 20 g of air dried sediment. To this was added 0.1% of glucose (1 ml) and 0.025% of TTC (0.5 ml) with respect to soil. Moisture was maintained at 60% of water holding capacity of the sediment. The flasks were incubated in the dark at room temperature. Similarly, sets of flasks were also incubated with varying concentrations of NaNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub>. Suitable controls for glucose TTC, sediment and NO<sub>3</sub> were incubated. Formazan was extracted with methanol at 24 hr intervals and the intensity of the red colour was measured colorimetrically (Spekol) at 520 nm against methanol blank.

Particle size analyses showed that the sediment of Dias beach was finer than that of Velsao and organic carbon was 2½ times greater at Dias than at Velsao (Table 1). Jacob and Qasim<sup>5</sup> have studied the relation between grain size and organic carbon and have shown that organic carbon was more in the finer sediment. A low level of organic carbon ranging between 0.05 and 0.21% through the year has been reported from the same beach earlier<sup>6</sup>. Salinity at Dias beach was less than at Velsao. This low value was due to the estuarine influence.

Nitrite-N and nitrate-N of the interstitial waters of the 2 beaches varied considerably (Table 1). Nitrate-N content at Velsao was abnormally high as compared to Dias beach. This could be due to influence of nitrogenous effluents discharged by the fertilizer factory at Velsao.

Total population of heterotrophic bacteria was much higher at Dias beach than at Velsao both in nutrient as well as in Zobell 2216 E media. (12.8 and 8.6 × 10<sup>8</sup> /g dry wt in the former and 4.1 and 2.4 × 10<sup>8</sup> /g dry wt in the latter). The high bacterial load at Dias might be because of smaller grain size and high organic carbon<sup>7</sup>.

Biochemical properties of the bacteria isolated from the beaches were studied (Fig. 1). The isolated strains failed to exhibit cellulose degradation and indole production. It was thus assumed that the cellulose decomposers were absent and that both the beaches were almost devoid of any faecal contamination.

Starch hydrolysers at Dias were abundant both in nutrient agar and Zobell media which might explain to some extent the high organic carbon at this beach. At Velsao these were mostly marine in origin as they were present only in Zobell medium.

Fat hydrolysers were generally of marine origin. At Velsao beach they were exclusively marine.

Both marine and terrestrial fat hydrolysers were present at the Dias beach and the former outnumbered the latter.

Percentages of casein hydrolysers and gelatin liquefiers at both the beaches were almost equal in nutrient agar whereas, the former were much higher at Velsao in Zobell medium. H<sub>2</sub>S producers on the other hand were very less at Velsao in Zobell medium. Even then, the overall proteolytic activity was found to be slightly higher at Velsao. This being a nitrogen rich and carbon poor ecosystem, higher proteolytic activity could be attributed to the breaking down of proteinaceous carbon.

Nitrate reducers were more at Velsao beach than at Dias and these were more pronounced in Zobell medium (96%) suggesting their marine origin. Higher percentage of nitrate reducers further establishes the previous finding (Table 1) that this beach has a high level of nitrogen which may be due to effluent of the fertilizer factory. Nitrate reducers tend to lower the level of nitrate-N and thus help in bringing about a balance between carbon and nitrogen ratio. Deleterious effect which the excess of nitrogenous chemicals has on productivity has been discussed<sup>8</sup>. As could be expected in an ecosystem polluted with nitrogenous waste both density and physiological variety of bacterial flora were low. Among these groups one or two dominated at Velsao.

Low level of organic carbon at Velsao could be explained on the basis of high nitrogen content<sup>6</sup>, in addition to its relation with the sand grain size. Nitrogen which is usually the limiting factor in an ecosystem is abundantly available at this beach as a result of the effluent from the fertilizer factory and because of this the organic carbon gets immobilized<sup>9</sup>.

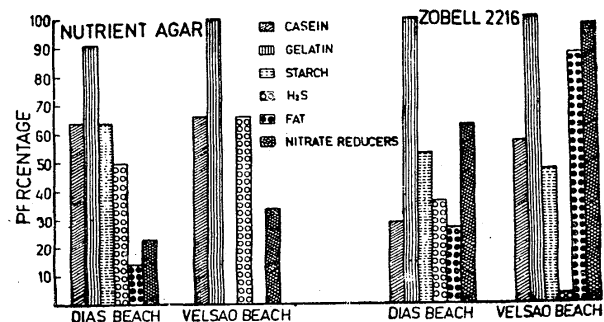


Fig. 1 — Per cent distribution of bacteria based on the biochemical properties

TABLE 1 — CHEMICAL PROPERTIES AND PARTICLE SIZE OF SEDIMENTS OF DIAS AND VELSÃO BEACHES

Chemical properties	Chemical properties		Particle size analysis		
	Dias beach	Velsao beach	Size (mm)	Dias beach %	Velsao beach %
Organic carbon (%)	0.45	0.18	2.0-1.0	nil	1.54
Nitrite (µg atoms N/litre)	0.19	5.44	1.0-0.5	nil	1.04
Nitrate (µg atoms N/litre)	4.52	619.56	0.5-0.25	0.96	12.2
pH	7.5	7.3	0.25-0.125	37.62	72.46
Salinity (%)	15.9	23.0	>0.125	61.42	12.76
Oxygen (mg/litre)	2.26	2.58	—	—	—

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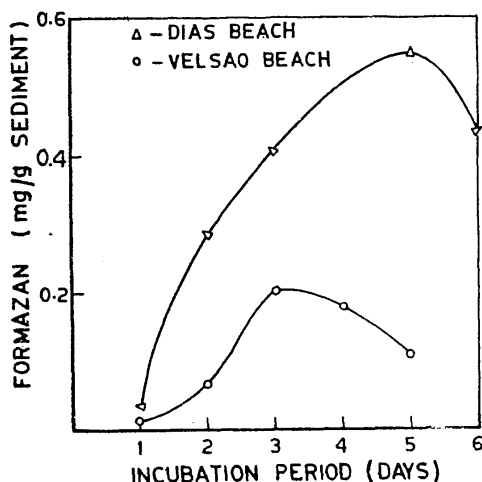


Fig. 2 — Dehydrogenase activity in sediments of Dias and Velsao beaches

Dehydrogenase activity at Velsao is relatively low (Fig. 2). The peak value at Dias (0.54 mg/g dry wt sediment) was attained on the 5th day of incubation, and was almost double as compared to Velsao where the peak was attained on the 3rd day. At Dias the curve was similar to what is normally expected in a balanced system, whereas at Velsao the activity was low and the fall was abrupt, which is indicative of an unbalanced system.

Low activity of dehydrogenase at Velsao could be due either to decrease in population having low dehydrogenase activity or inhibitory effect of the excess  $\text{NO}_3^-$  or both. In order to determine how these factors operate, Dias beach samples were incubated for a period of 12 days with  $\text{NaNO}_3$  and  $\text{NH}_4\text{NO}_3$  at various concentrations. Since organic carbon is not a limiting factor at Dias beach, greater concentrations of nitrate were used. Dehydrogenase activity (Fig. 3) and bacterial load (Table 2) showed the following trend.

With an increase in the concentration of  $\text{NH}_4\text{NO}_3$  there was an increase in the population and a decrease in dehydrogenase activity with prolongation in the peak attainment. With the increase in the concentration of  $\text{NaNO}_3$ , there was an increase in the population and increase in dehydrogenase activity with an earlier peak attainment. The population was generally greater in the former. Ready availability of  $\text{NH}_4^+$  for assimilation would have led to bacterial proliferation, and the less utilized  $\text{NO}_3^-$  would have inhibited the dehydrogenase activity. In the  $\text{NaNO}_3$  added sediment, nitrogen was not in a readily assimilable form. This might account for the low population in this system. With a part of the  $\text{NO}_3^-$  being utilized for assimilation by reduction, only a reduced net quantity of  $\text{NO}_3^-$  would have been left behind, and this might explain the relatively greater dehydrogenase activity. However, the peak value and activity of dehydrogenase in the control (0.274 mg formazan/g dry wt sediment) was far more than with  $\text{NaNO}_3$  or  $\text{NH}_4\text{NO}_3$  (0.06 and 0.006 mg formazan/g dry wt sediment respectively).

It is inferred that the low dehydrogenase activity at Velsao could have been mainly because of the

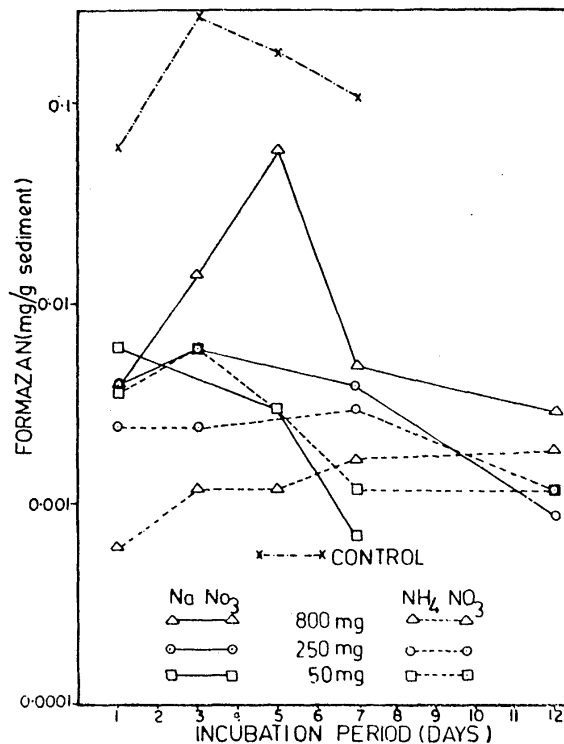


Fig. 3 — Dehydrogenase activity in the presence of nitrogenous compounds

TABLE 2 — HETEROTROPHIC BACTERIA OF  $\text{NO}_3^-$  ADDED DIAS SEDIMENT IN ZOBELL 2216E MEDIUM  
(Values,  $\times 10^6$ /g dry-sediment)

	$\text{NO}_3^-$ , mg		
	800	250	50
$\text{NH}_4\text{NO}_3$	75636.36	36571.43	22400.00
$\text{NaNO}_3$	25641.03	14883.72	7890.41
Control	—	14354.29	—

direct inhibitory influence of  $\text{NO}_3^-$ . Nonetheless, the possibility of small population due to limited organic carbon reducing the dehydrogenase activity cannot be totally ruled out.

Efficiency of TTC in the estimation of dehydrogenase activity would be reduced in aerobic condition in the presence of  $\text{NO}_3^-$  as it prefers  $\text{NO}_3^-$  respiration because the redox potential (Eh) of  $\text{NO}_3^-$  is lower than that of TTC<sup>10</sup>. To minimize error, the period of incubation was extended in the present study so that a major fraction of TTC was reduced to formazan.

It is therefore evident that dehydrogenase activity can be used as a criterion for the determination of pollution in a given system<sup>11</sup>.

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### Studies on Photosynthetic Bacteria Isolated from an Estuarine Beach of Goa

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A bacterial strain capable of fixing CO<sub>2</sub> was isolated from an estuarine beach of Goa. The strain grew in sea water containing a piece of boiled egg under anaerobic condition and in the presence of subdued sunlight or fluorescent illumination. The strain was gram negative and varied from spiral to Y shape. It had no sulphur deposit either inside or outside the cells and produced purple pigment. Based on these studies, it was inferred that the isolate belongs to the family Athiorhodaceae (non-sulphur purple bacteria). It had a characteristic absorption maximum at 850 nm which is a general feature of purple bacteria. The optimum pH for this strain was 7. Effect of different organic substances, nitrogenous compounds, and pollutants on CO<sub>2</sub> fixation by this isolate was also studied.

**R**OLE of photosynthetic bacteria in primary production and nitrogen fixation in the coastal ecosystem especially in nutrient deficient regions has been reported<sup>1</sup>. Distribution of these bacteria in anaerobic aquatic habitats such as salt marshes, estuarine muds and tidal pools has also been reported<sup>2</sup>. Although these groups of bacteria have been of considerable interest very little is known about their characteristic features in the Indian region except for a brief report on the green photosynthetic bacteria by Gore<sup>3</sup>. Therefore, an attempt has been made to study the purple non-sulphur photosynthetic bacteria isolated from an estuarine beach of Goa.

*Isolation* — Small amount of the sediment, collected from an estuarine beach near NIO, was added to a flask containing sterile sea water and a piece of boiled egg. The flask was sealed air tight and incubated in natural day light at room temperature<sup>4</sup>. After a week, bacterial growth was indicated by the development of purple colour. It was subsequently

sub-cultured once in 15 days under similar conditions. On examination, all the cells in the sub-cultures were found to be alike, thus indicating that the cultures were pure.

A loop of growing culture from the 5th sub-culture was smeared on a slide and observed under a microscope after gram staining. Also hanging drop preparation was made to examine the culture directly.

*Effect of light and air on CO<sub>2</sub> fixation* — To study the optimal condition for CO<sub>2</sub> fixation, the culture was grown using the same medium in test tubes in 10 ml quantities. Several test tubes, each containing 8 ml sea water were sterilized at 121°C for 20 min. After cooling, 1 ml of H<sup>14</sup>CO<sub>3</sub> was added (0.8 μCi obtained from BARC, Bombay) and inoculated with 1 ml of actively growing culture. These tubes were immediately sealed using cotton-wool, liquid paraffin and a rubber bung. The other set of tubes were kept for aeration and half the number of tubes were covered with blue cloth. All the tubes were kept in a container, maintained on a magnetic stirrer, and illuminated by 100 w lamp. The culture was allowed to grow at room temperature (28°C ± 2) for 48 hr. Duplicate samples were used for each treatment.

*Pigment measurement* — At the end of 48 hr growth, the culture was mixed well for 2 min using a vortex mixer. From this, 5 ml was pipetted out and centrifuged for 10 min at 10000 × g using a Serwall RC 2B centrifuge and the remaining part was used for taking the counts. To the pellet, after washing twice with cold sea water, 2 ml acetone (90%) was added and kept at 5°C for 12 hr. The supernatant was removed and the cells were extracted with 2 ml acetone and the supernatant was pooled. The cells were white at this stage and were discarded. The supernatant was made to 5 ml and its colour was read in Bausch and Lomb colorimeter at 490 nm against acetone blank. The optical density was corrected to 10 ml.

*Measurement of growth* — <sup>14</sup>C uptake into the cells was used as an index of growth of the culture. Culture (5 ml) was centrifuged and washed twice with cold sea water. To the pellet, 5 ml of 75% ethanol was added and kept in boiling water bath for 10 min, after which the cells were centrifuged at 10000 × g for 10 min. The supernatant was transferred to a test tube. The cells were again extracted twice with ethanol as above. The alcohol extract was pooled, made to a known volume (henceforth known as soluble fraction), from which 0.5 ml aliquot was pipetted out carefully on to a planchet, dried at 90°C in a hot air oven and counted using a Gas Flow Proportional Counter having 5% efficiency. From the counts, per cent incorporation was calculated.

*Effect of organic substrates on CO<sub>2</sub> fixation* — The culture technique was same as earlier, except that the egg was replaced by organic substrate (1%) and growth was measured following tracer technique. In addition to alcohol-soluble fraction, the incorporation of CO<sub>2</sub> was measured in alcohol insoluble fraction also.

*Activity in alcohol insoluble fraction* — After alcohol extraction, the pellet was digested overnight in