

Mixed Response in Bacterial and Biochemical Variables to Simulated Sand Mining in Placer-Rich-Beach-Sediments, Ratnagiri, West Coast of India

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Abstract

We investigated the influence on bacterial community and biochemical variables through mechanical disturbance of sediment -akin to small-scale mining in Kalbadevi beach, Ratnagiri, a placer-rich beach ecosystem which is a potential mining site. Changes were investigated by comparing three periods, namely Phase-I before disturbance, Phase-II just after disturbance, and Phase-III 24 hours after disturbance as the bacterial generation time is ≤ 7 hours. Cores from dune, berm, high-, mid- and low- tide were examined for changes in distribution of total bacterial abundance, total direct viability (counts under aerobic and anaerobic conditions), culturability and biochemical parameters up to 40 cm depth. Results showed that bacterial abundance decreased by an order from 10^6 cells g^{-1} sediment, while, viability reduced marginally. Culturability on different strength nutrient broth increased by 155% during phase-II. Changes in sedimentary proteins, carbohydrates, and lipids were marked at berm and dune and masked at other levels by tidal influence. Sedimentary ATP reduced drastically. During Phase-III, Pearson's correlation between these variables evolved from non-significant to significant level. Thus, simulated disturbance had a mixed effect on bacterial and biochemical variables of the sediments. It had a negative impact on bacterial abundance, viability and ATP but positive impact on culturability. Viability, culturability and ATP could act as important indicators reflecting the disturbance in the system at short time intervals. Culturability which improved by an order could perhaps be a fraction that contributes to restoration of the system at bacterial level. This baseline information about the potential mining site could help in developing rational approach towards sustainable harnessing of resources with minimum damage to the ecosystem.

Keywords: Placer; Ratnagiri; Disturbance; Bacteria; Biochemistry

Introduction

Kalbadevi beach, in Ratnagiri on the West coast of India is a sandy beach with rich deposition of placer reserves such as ilmenite. This mineral is an ore of titanium and accounts for 52% of the total heavy minerals in the beach sediments (Siddiquie and Rajamanickam 1979). In several parts of the world, such reserves of placer deposits on beaches are extensively mined (Kudrass 1999). It is well known that sandy beaches form a major component of the coastal ecosystem and dominate temperate as well as tropical coastlines (Davies 1972). The long stressful abiotic factors such as the winds and waves make these beaches highly dynamic and ecologically sensitive (Brown and McLachlan 1990; Knox 2001). This stress is further compounded by human activities such as tourism and mining. Studies have shown that the sand mining activity causes an imbalance in the physical structure of the beach (Defeo et al. 2009). The most obvious effect of any type of mining is often the disturbance or displacement of large quantities of sediment, which in turn affects landforms and coastal processes (Hilton and Hesp 1996). Ecologically, the flora and fauna may also be affected (Lee and Correa 2005; Simmons 2005). However, little is known about the effect of mechanical disturbance on ecosystems especially at the microbial and biochemical levels in placer-rich-beach-sediment.

It is well known that the sedimentary bacteria play an important role between the non-living and living components of the beach ecosystems. The bacterial community not only depends on, but also drives the spatial distribution, metabolism, and dynamics of most benthic organisms. Further, this community is involved in the prime utilization and net mineralization of sedimentary labile organic matter, which is primarily composed of carbohydrates, lipids and proteins (Jedrzejczak 2002; 2004). Also, their ubiquity, abundance, and rapid turnover time helps this community to respond instantaneously to any environmental change. Hence, the sedimentary bacterial component is a valuable tool for monitoring changes in the environmental conditions (Danovaro et al. 1993; Fabiano and Danovaro 1994). Another parameter that perhaps could reflect a change in environmental condition is Adenosine 5'-Triphosphate (ATP) which is usually considered as a proxy for total living biomass. This parameter has been successfully tested in other environments to study the impact of disturbance on the microbial community (Bulleid 1978; Webster et al. 1984; Ciardi and Nannipieri 1990; Pangburn et al. 1994). Hence, it is hypothesized that the study of the bacterial community, ATP and sedimentary organic matter is crucial in determining the impact of any disturbance at the microbial level as any change in these components could perhaps mirror an impending imbalance in the beach ecosystem.

In this work, we investigated the impact of disturbance on the bacterial community, ATP and sedimentary labile organic matter by field experiments. Disturbance was created to simulate an artificial mining on the beach in order to comprehend the effect of mining and the changes in the bacterial and biochemical parameters were monitored over a 24 hour period.

Materials and Methods

Study area

The study was conducted at Kalbadevi beach, Ratnagiri, India (17° 04' 03.994 N, 73° 17' 17.557 E) (Fig.1). This beach is 5 km long and ~ 250 m wide with estuaries on either ends. These estuaries are responsible for the deposition of major placer minerals such as ilmenite and magnetite. Siddiquie et al. (1984) reported that heavy minerals in this area range from 7-79%. The berm region of this area has the highest accumulation of heavy minerals.

Experimental design

The site (BP-2) was sampled in February 2004. Samplings were carried out on a shore-normal transect running from the dry zone to the surf zone. The beach was thus divided into five zones namely: dune, berm, high tide, mid tide and low tide level (Fig. 2). At each level, samples were collected using push cores and auger. Push cores made of plexiglass (inner diameter = 10 cm) were used to collect sediment up to a depth of 20 cm. Triplicate core samples were collected. Each core sample was divided into three layers, 0-5, 5-10, 10-20 cm. Sediment layer at 40 cm depth was collected by auger. Thus, total number of samples examined for each phase were 3 cores X 4 depths X 5 levels = 60 for each phase. In order to understand the impact of placer mining on bacterial community and biochemical variables, simulated disturbance was conducted. Excavation of pits of dimension 1x1x1 m was carried out at dune, berm, high-, mid- and low-tide level. The sediment was sieved through 250 µm mesh size to separate the major lighter sand fraction from the heavy minerals. The lighter fraction retained on the sieve was returned back to the area of collection to simulate coastal mining as the local community mining this system have been advised to return the lighter fraction to the area of collection. Thus, core samples were collected before disturbance (Phase I), just after disturbance (Phase II) and 24 hrs after disturbance (Phase III). Sub-samples were stored at 4(±2) °C and analyses were carried out with minimum delay in onshore laboratory.

Processing of samples:

Biochemical composition of sediment organic matter:

a. Protein (PROT):

Proteins were extracted by digesting 1 g of sediment with 2 ml of 1 N NaOH in a water bath at 100 °C for 5 min. The slurry was centrifuged and the clear supernatant was used for estimating PROT using Folin-phenol reagent (Lowry et al. 1951). The absorbance was determined at 750 nm. Bovine serum albumin solutions were used as standards.

b. Carbohydrates (CHO):

Carbohydrates were estimated by phenol-sulphuric acid method (Kochert, 1978) using glucose as the standard; the absorbance was measured at 480 nm.

c. Lipids (LIP):

Lipids were extracted from dried sediment samples by direct elution with chloroform and methanol (5:10). One gram of the sediment was ultra-sonicated for 1 hr in Milli-Q water to improve the extraction, followed by extraction in chloroform methanol mixture. Extractant was dried under vacuum (Bligh et al. 1959) and oxidized with 0.15% acid dichromate (Parsons et al. 1984). Stearic acid was used as a standard and absorbance was measured at 440 nm.

d. Labile Organic Matter (LOM):

Labile organic matter was calculated as the sum of sedimentary protein, carbohydrates and lipids. Quality of LOM was expressed as protein/carbohydrate ratio (PROT/CHO ratio) (Fichez 1991; Fabiano et al. 1995).

Total living microbial biomass - Adenosine triphosphate (ATP):

ATP of total living biomass in sediment was measured using luciferin-luciferase reaction (Holm-Hansen and Booth 1966). Sediment samples were extracted into 5 ml of boiling phosphate buffer, 0.1 mM, pH 7.8. Suitable aliquots of the extractant were analyzed using the firefly (Firefly lantern FLE 50; Sigma) bioluminescence assay. Counts were determined using a Luminometer (BMG Labtech, Lumistar Optima).

Bacterial parameters

A sub-sample from the sediment cores was diluted 1:10 (wet wt. vol⁻¹) with pre-filtered (0.2 µM), autoclaved natural seawater as a diluent. This sediment slurry was used for estimating bacterial parameters. Additional subsets of sediment samples were dried at 60 °C for 18 hrs to determine the dry-weight content of the sediments. All the bacterial parameters were expressed per gram dry weight sediment.

a. Enumeration of total bacterial abundance (TC):

Bacterial cells were stained using the method as described by Deming and Colwell (1982). A 5 ml aliquot of sediment slurry was fixed using pre-filtered formaldehyde (2.5% final concentration). The fixed sample was stored at 4(±2) °C in the dark until processed for counting, with minimum delay. In the laboratory, one ml of the fixed slurry was stained with acridine orange (AO; Sigma) at a concentration of 0.01% (wt. vol⁻¹) for 5 min and

filtered onto 0.22 μm Nuclepore black polycarbonate filters (Millipore). The filter papers were examined by epifluorescence microscopy (Nikon 50i), and bacterial counts were enumerated according to Hobbie et al. (1977).

b. Enumeration of total direct viable counts (TVC):

The percentage of the total direct viable number (TVC) responsive to nutrient enrichment was determined by the method of Kogure et al. (1979) and Joux and LeBaron (1997).

To enumerate responsive cells under aerobic conditions (TVCa), yeast extract (0.01% final concentration) and an antibiotic cocktail was added to 5 ml of the sediment slurry. After incubation period of seven hrs (pre-standardized), the slurry was fixed, stained and counted as described by Deming and Colwell (1982) and Hobbie et al. (1977). Similarly, to enumerate responsive cells under anaerobic conditions (TVC_{an}), in addition to yeast extract and antibiotic cocktail, a reductant, $\text{Na}_2\text{S}\cdot\text{H}_2\text{O}$ (0.0125% final concentration) was added to induce anaerobic conditions (Loka Bharathi et al. 1999). Elongated and enlarged cells with clearly visible invagination were counted as viable cells (Roszak and Colwell 1987a).

c. Retrievable numbers of heterotrophs (RC):

Retrievable heterotrophic population was assessed after further diluting the sediment slurry at 1:1000 and spread plating on medium prepared using various nutrient strengths of 10, 0.01 and 0.001% nutrient broth (HiMedia Laboratories Pvt. Ltd., Bombay, India; 100% = 1.3 g) in seawater and 1.5% agar. RC was determined in terms of colony forming units (CFU) after a 2-day incubation period at $28(\pm 1)$ °C. Counts on nutrient strengths of 10, 0.01 and 0.001% nutrient broth were denoted as $\text{RC}_{10\%}$, $\text{RC}_{0.01\%}$, $\text{RC}_{0.001\%}$ respectively.

Statistical analyses:

The data was subjected to statistical analyses using Statistica version 6 to test the correlation among bacterial and biochemical variables during the different phases. Bacterial data were $\log(x+1)$ transformed before analyses. The number of samples for each core was 12 (3 cores X 4 depths).

Result:

The results of the present studies are restricted to three phases, pre-disturbance (phase I, PI), immediately after disturbance (phase II, PII) and 24 hours after disturbance (phase III, PIII). Results presented here are averaged for the 40 cm core at each tidal level.

Total counts (TC):

The distribution of TC along the transect is shown in figure 3a. The abundance within the sediment ranged over an order of magnitude (10^5 to 10^6 cells g^{-1} sediment) during the baseline observations. On an average, the berm sediment recorded the highest abundance ($2.90 \pm 1.83 \times 10^6$ cells g^{-1} sediment) while the mid tide recorded an order less. The population declined immediately after disturbance by nearly an order from 1.99 to 0.45×10^6 cells g^{-1} sediment. This decreasing trend in abundance did not seem to revert to original numbers even after 24 hrs and continued to be 85% lower than the baseline values.

Total Direct Viable counts (TVC)

Both types of viability yielded 10^5 cells g^{-1} sediment and followed the trend of total bacterial abundance (Fig. 3b and 3c). Overall, TVCa ranged from $0.18 \pm 0.21 \times 10^6$ cells g^{-1} sediment at high tide to $0.99 \pm 1.03 \times 10^6$ cells g^{-1} sediment at low tide. Likewise, higher TVCan population of $1.00 \pm 1.02 \times 10^6$ cells g^{-1} sediment was recorded from low tide sediments. With disturbance, the average viabilities of the 40 cm cores decreased by 69% with the anaerobic viability decreasing by 79% after 24 hrs.

Retrievable counts (RC):

Maximum culturability was found to be in the dunes at 10^5 CFU g^{-1} sediment. Generally, bacteria forming the culturable fraction retrieved on the three nutrient broth concentrations (NB) of 10, 0.01 and 0.001% also followed the trend of TC (Fig. 3d, 3e, 3f). Prior to disturbance, the average culturability was $0.42 \pm 0.28 \times 10^4$ CFU g^{-1} sediment on RC_{10%}, $4.73 \pm 1.87 \times 10^4$ CFU g^{-1} sediment on RC_{0.01%} and $7.99 \pm 4.60 \times 10^4$ CFU g^{-1} sediment on RC_{0.001%}. This fraction of culturability accounted for 0.2 to 4% of TC. However, with disturbance there is an improvement in culturability. The retrievable fraction increased by 66, 34 and 47% on 10, 0.01 and 0.001% NB respectively (Table 1).

Biochemical parameters of the sediment:

The PROT content ranged between $39 \pm 26 \mu g g^{-1}$ sediment at mid tide level to $438 \pm 352 \mu g g^{-1}$ sediment at berm. The concentration in the dune and the berm sediments were higher than at the other tidal levels (Fig. 4a). The PROT concentration decreased by 60% at high tide level and increased by 90% at the dune after 24 hrs. Like PROT, CHO generally decreased towards the surf zone and its concentration in the sediment varied from $230 \pm 164 \mu g g^{-1}$ sediment at high tide to $917 \pm 660 \mu g g^{-1}$ sediment at dune (Fig. 4b). The immediate response to disturbance was an increase by 123% at high tide and a decrease by 66% at the low tide. The 24 hrs response was mixed, with the concentration increasing by 37% at the berm and decreasing by 42% at the mid tide suggesting variable rates of replenishment and degradation. Unlike the distribution in PROT and CHO, the LIP concentration

varied irregularly. The mid tide had the lowest sediment LIP concentration of $44 \pm 15 \mu\text{g g}^{-1}$ sediment while the highest of $93 \pm 43 \mu\text{g g}^{-1}$ sediment was found at dune (Fig. 4c). Like the other constituents of LOM, LIP also exhibited a varied response after 24 hrs of disturbance. At the dune it decreased by 8% while at the low tide level it nearly increased by 139%. During phase I, CHO contributed 62%, PROT 24% and LIP 14% to sedimentary LOM. Major changes in LOM are not discernible immediately after the disturbance. However, after 24 hrs, PROT and LIP contribution increased to 25 and 24% while CHO decreased to 52%. In the present study, the ATP values were generally elevated at the dune and berm, with still higher values in the high tide sediments ($222 \pm 60 \text{ ng g}^{-1}$ sediment). Immediately after disturbance, these ATP values tend to increase by about 45% at dune, berm and mid tide region and fall drastically by 90% after 24 hrs (Fig. 4d).

Statistical analyses

According to Pearson correlation coefficients and probability, bacterial abundance decreased with increasing depth ($p \leq 0.05$) during phase I. During this phase, bacterial parameters did not show significant correlation ($p > 0.05$) with biochemical variables. However, during phase III there was a significant positive correlation between most of the parameters (Table 2).

Discussion:

Naturally, higher sedimentary protein concentration in the berm region could not only be due to the greater abundance of bacteria but also due to the proteinaceous exudates that could emanate from the roots of the existing vegetation (Hertenberger et al. 2002). A similar reason could also be attributed to the maximum carbohydrate concentration at the dune. Vascular plants are known to contribute to sedimentary carbohydrates (Bhosle and Dhople 1988). Following disturbance, the sedimentary protein (PROT), lipid (LIP) and carbohydrates (CHO) patterns are clearly disturbed. Change in PROT, CHO and LIP concentration at the dune and the berm may be due to the redistribution from the deeper layers to the surface sediments. The increased sedimentary PROT concentration during phase III suggests increased bacterial activity due to death or decay of other organisms. The increase at high tide is attributed to higher concentrations of CHO, which surface due to mechanical disturbance. Disturbance improved the sediment LIP concentration. However, the homogenous distribution even after disturbance in the intertidal area may be due to the masking effect by the tidal and wave action. It is well known that strong hydrodynamism permits deposition of coarser sediments through which water runs easily preventing the accumulation of organic matter (Langezaal et al. 2003).

In this study, there is a general decrease in labile organic matter with depth and from the dune to the low tide level. Labile organic matter (LOM) which is derived from the sum of protein, carbohydrates and lipids gives the holistic status of the nutrient of the sediment. During phase I, CHO, PROT and LIP contributed significantly to

LOM. Percentage contribution was 62% CHO ($r = 0.969$, $p \leq 0.001$) > 24% PROT ($r = 0.864$, $p \leq 0.001$) > 14% LIP ($r = 0.667$, $p \leq 0.001$). Thus, most of the LOM was dominated by CHO through the whole transect. The high background CHO contribution of 62% ($r = 0.969$, $p \leq 0.001$) to LOM could be attributed to the sand dune vegetation and it is also suggested that the input rate of this substrate is greater than its degradation rate. During phase III, protein contribution increased to 25% while carbohydrates decreased to 51% indicating an increase in the newly generated organic matter. This was corroborated by the increase in PROT/CHO ratio. PROT/CHO ratio also increased from 0.39 to 0.48 within 24 hrs of disturbance suggesting availability of newly generated detritus (Dell'Anno et al. 2000). As PROT are more readily utilized by bacteria than CHO, a high PROT/CHO ratio indicates the generation of recently produced organic matter. Further, the background ratio of 0.39 also indicated that the ecosystem continuously generated detritus. Interestingly, the lability of the organic matter indicated by PROT:CHO ratio increased with depth suggesting mechanical exchange.

Biochemical characteristics like ATP is a proxy for total living biomass and is a central compound in the energy metabolism of all living organisms. It is generated by energy-yielding reactions and is subsequently consumed in energy-requiring reactions in the cell. After cell death, production gets arrested and the ATP gets rapidly degraded (Vosjan et al. 1987). ATP measurements have therefore been used in various marine environments (Holm-Hansen and Booth 1966), including sediments (Karl and LaRock 1975; Stoeck et al. 2000) to understand its distribution and dynamics. In the present study, the ATP values were generally elevated at the dune and berm, with still higher values in the high tide sediments ($222 \pm 60 \text{ ng g}^{-1}$ sediment). This could be because of the entrapment of small meiobenthic/zooplankton organisms at this level. Reichgott and Stevenson (1978) have shown that copepods are major contributors to sediment ATP. Immediately after disturbance, these values tend to increase by about 45% at dune, berm and mid tide region and fall drastically by 75% after 24 hrs. Immediate rise in ATP could be due to instantaneous stimulation only to be followed by drastic inhibition in 24 hrs. In the present study, LOM influenced ATP and was responsible for 63% ($p < 0.01$, $n=60$) variation during phase II.

The study of benthic communities is an useful and sensitive tool for identifying sediment-related stress (Alongi 1990). The analysis of changes in benthic community structure has now become one of the main methods of detecting and monitoring the biological effects of marine disturbance (Chou et al. 2004). Bacteria could perhaps act as one of the component to identify this stress. Bacteria are ubiquitous in their distribution and have short generation times. In addition, they are sensitive to changes in the physical and chemical environment. Hence, they are likely to be the first component of the marine habitat to be affected by any disturbance. The natural abundance of sedimentary bacteria of the Kalbadevi beach was generally 10^6 cells g^{-1} sediment and decreased with depth ($p \leq 0.05$). This abundance is generally an order less than reported elsewhere (Luna et al. 2002). The total bacterial load did not show any relationship to the biochemical variables and LOM, thus suggesting that these are not the only influencing factors. Similar results were also obtained in deep-sea sediments of Central Indian Basin (CIB)

(Nair et al. 2000). Simulated disturbance in the study site generally decreased the bacterial population immediately after disturbance. In addition, the depth wise distribution patterns were also disturbed ($p > 0.05$). This decrease could perhaps be due to the mechanical displacement of sediments where manual sieving and mixing of the sediment, homogenized regions harboring lower and higher bacterial abundance. Kaneko et al. (1995) also observed a decrease in the Pacific Ocean sediments while conducting such artificial disturbance experiment. In the Indian Ocean, INDEX (Indian Ocean Deep-sea Experiment) results are also in accordance with the above findings (Nair et al. 2000; Fernandes et al. 2005; Loka Bharathi and Nair 2005). Earlier, laboratory scale experiments conducted by Findlay et al. (1990) with intertidal sediments of Florida also strengthen our observations. However, in this study the depth wise distribution perhaps restored to original trend within 24 hr ($p \leq 0.05$). It is known that bacteria have rapid growth rates and individual species within functional groups often have differing responses to changing environments (Torsvick et al. 1996). Thus, any change in viability or culturability could be an indicator of this response.

High viability under both aerobic and anaerobic conditions was encountered at the berm, dune and the low tide region. This high numbers could be attributed to the influence of the plant exudates while that recorded at low tide may be due to continuous nutrient replenishment from the surf waters. In addition, there was a strong relationship ($r = 0.72$, $p \leq 0.001$) between TVCa and TVCan suggesting that there is higher abundance of facultative anaerobes in the bacterial community. However, with disturbance this interrelationship reduced marginally ($r = 0.54$, $p \leq 0.001$). In addition, there was a general reduction in viable forms encountered. Viability under aerobic conditions decreased by 69%. Further, the interrelationship between viable aerobic forms and total bacterial abundance increased from 0.32 ($p \leq 0.05$) during phase I to 0.59 ($p \leq 0.01$) during phase III. This increase suggests that the viable aerobic forms could have increased their role in controlling the total abundance of bacteria. The effect of disturbance was more prominent after 24 hrs on direct viability under anaerobic conditions, which decreased by 79%. This effect could possibly be attributed to higher stress on the viability of anaerobic population brought about by aeration due to mixing of sediment. Instantaneous response by decrease in viability could be related to stress on the system by disturbance. The incubations lasting up to 7 hours would not take into account the recuperation that is required by the organisms. Stevenson (1978) has stated that the bacterial cells respond to stress by decrease in size and activity. It is possible that in the present study, that the decrease in viability could be a response to stress imposed by mechanical disturbance. However, in the absence of disturbance, bacteria can resume their normal development over time. In the dormant state, the bacteria function at very low metabolic rates and do not undergo cell division. When the stress is released, they tend to become more viable and resume cell division (Roszak and Colwell 1987b). This perhaps could be the reason why bacteria respond by higher culturability. Plating and incubation gives sufficient time for bacteria to recuperate, thus becoming more viable to form colonies. In environmental samples, culturable counts usually represent only a

small fraction ($0.1\% \pm 10\%$) of the active microbial community (White et al. 1998) as most organisms are in the viable but not in the cultivable state (Xu et al. 1982; McCarthy and Murray 1996). However, with disturbance there is an improvement in culturability. The culturable fraction increased by 127 and 91% on $RC_{0.01\%}$ and $RC_{0.001\%}$ respectively. Improved culturability after disturbance could be either due to increased availability of nutrient brought to the surface or due to mixing and aeration of sediment. Thus, the effect is more positive on the culturability of the microbes. This may be because this parameter could enable to measure the state of the bacteria after the effect of mechanical stress decreases. Under the culture conditions, there is enough time for bacterial revival and growth. Similar results were obtained with deep-sea bacteria in the INDEX experiment. There was an increase by two orders of magnitude of culturability after the simulated disturbance (Loka Bharathi and Nair 2005).

Positive correlation between culturability (RC) and TC increased from non-significant levels to significant levels at 0.44 ($p \leq 0.001$) for $RC_{0.001\%}$, 0.398 ($p \leq 0.001$) for $RC_{0.01\%}$ and 0.58 ($p \leq 0.001$) for $RC_{10\%}$. Thus, the variation of $RC_{10\%}$ had a higher influence on the variation of TC after disturbance. This fraction of bacteria retrieved on higher nutrients was also capable of bringing a significant variation of about 28% ($p \leq 0.001$) in proteins. On the other hand, $RC_{0.001\%}$ which caused a significant 8% variation in TC during phase I was capable of bringing about 20% variation in TC and 21% variation in the aerobic viable forms during phase III. These forms also contributed to 14 and 16% variation in carbohydrates and protein respectively. The variation in aerobic viable forms accounted for 31% of the variation in protein during phase III. It is suggested that relatively more oligotrophic forms were able to proliferate and improve the viability of the community after disturbance. This was further corroborated by LOM which had a greater influence on $RC_{0.001\%}$ (19%, $p \leq 0.001$) than on culturability at other concentrations perhaps suggesting the prevalence of substrate at these concentrations in this system. In addition, during phase I, ATP was also markedly influenced (0.44, $p \leq 0.001$) by culturable bacteria retrieved on $RC_{0.001\%}$. The influence was nearly 29% at $p \leq 0.001$ before disturbance and diminished after disturbance. During phase II, ATP brought about 37 and 7% variation in $RC_{0.01\%}$ and $RC_{10\%}$ respectively. This increased relationship could be partly due to higher number of retrievable bacteria during phase II of the experiment. There could be other factors involved because this relationship diminishes at the end of 24 hrs when total ATP values decrease. In fact, after 24 hrs, all variables related negatively to ATP. This could be mainly due to the destruction of the living biomass (i.e. microbial biomass other than bacteria) as evidenced by low values of ATP.

Thus, when the whole beach was examined, the interrelationship between the variables was most prominent in phase three. The relationship of culturability and viability with biochemical parameters were more significant during this phase. Relationship between ATP and biochemical parameters suggest that the living microbial biomass was more dependent on LOM, which possibly could be plant derived. Alternatively both ATP and LOM values could be from a common source namely dune vegetation.

Summary and Conclusion

Thus, the study on simulated sand mining in a beach ecosystem assesses the short-term effect on bacterial and biochemical parameters over a span of 24 hrs. It showed that bacterial responses could be varied. While total counts and viability decreased, culturability increased and improved the lability of the available substrates. Bacteria are instantaneous indicators of disturbance and they are also highly resilient and therefore capable of restoring ecosystem to the nearly original state at the bacterial level. ATP could be a much more sensitive factor that could act as a proxy for impact on not only bacteria but also other microbes at higher level, instantaneously. Perhaps it could be used as a proxy to decipher the time frame required for other microbes in the system to get restored to the original state. Thus, any mechanical stress in the ecosystem is reflected in the distributory pattern of this parameter and its relationship with other physico-chemical and biological parameters. Examination of interrelationships between bacterial and biochemical parameters suggested the extent of interdependence between them. Though these relationships were few and weak before disturbance, they were more significant after 24 hrs when transect was examined as a whole.

In conclusion, though the disturbance had a negative impact on the total bacterial counts, total viable counts, and ATP, the simulated mining experiment improved the culturability of bacteria by an order. It is perhaps this fraction that would contribute to restoration of the system through self-regulation primarily at the bacterial level.

Further studies on sedimentary microbes other than bacteria like the protozoans on a longer time scale could throw more light on the influence of simulated sand mining on a larger microbial community and its long-term impact. These observations at higher trophic levels for a longer period would complement our observations and give an overview of the response of the whole ecosystem. Repeating these observations over definite cycles of mining would also elucidate the responses when mining is done continuously.

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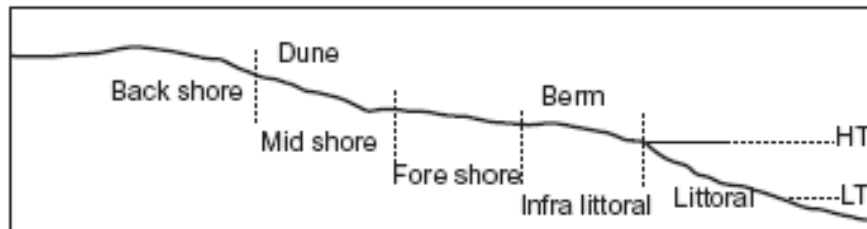
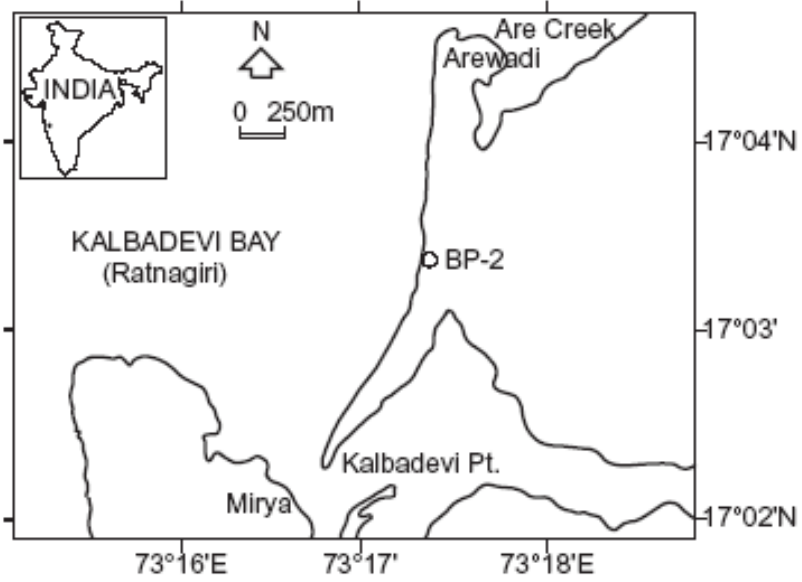
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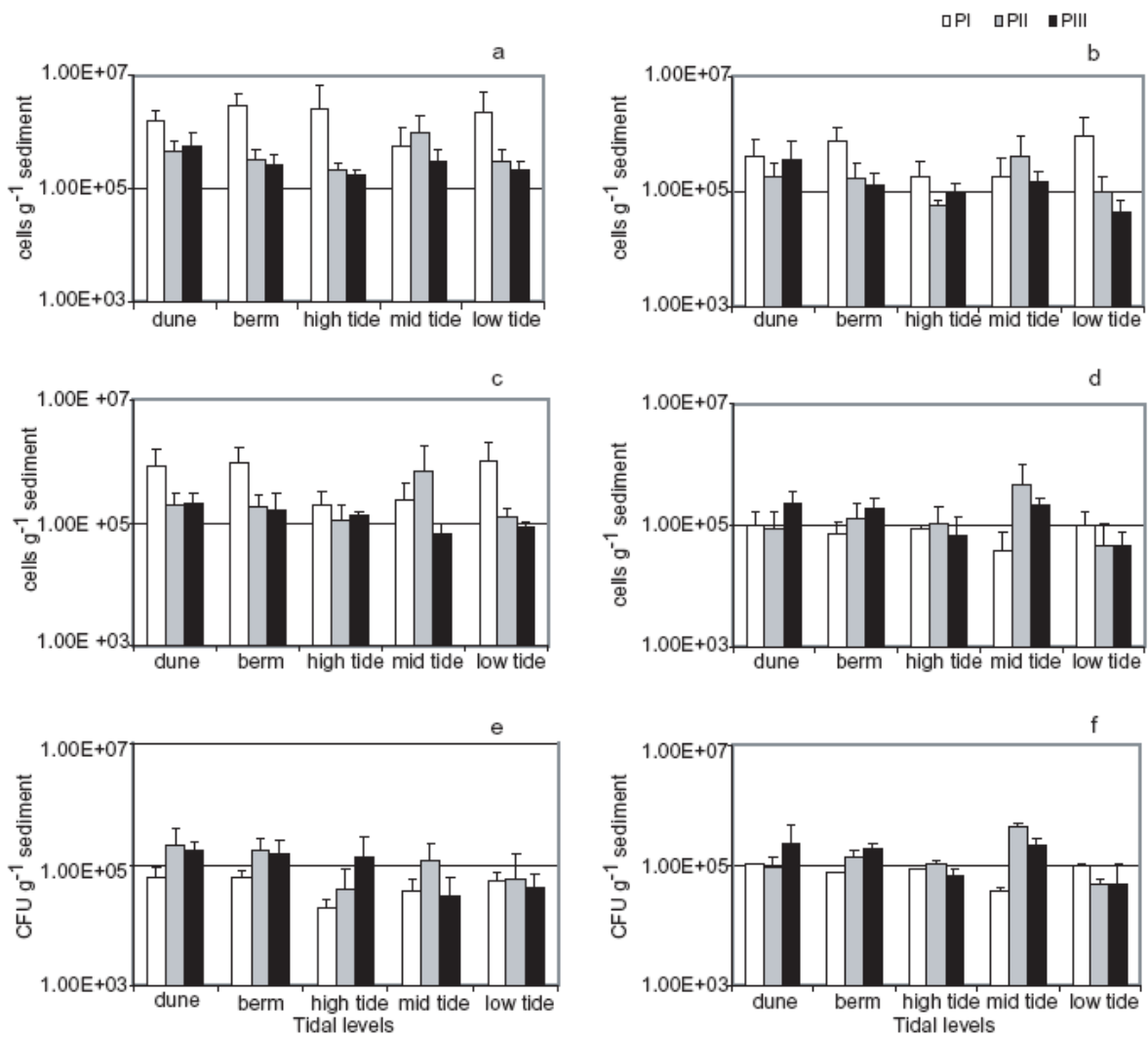
Fig 1 Location of the sampling area, Kalbadevi Beach, Ratnagiri on the West Coast of India

Fig 2 Beach profile showing the sampling sites, Dune, Berm, High tide, Mid tide and Low tide along a transect (BP-2) perpendicular to the shore

Fig 3 Variation in the a) total counts, b) direct viable counts of aerobes c) direct viable counts of anaerobes d) retrievable counts on 0.001% NB e) retrievable counts on 0.01% NB f) retrievable counts on 10% NB, at different tidal levels during phase-I (P1), phase-II (P2) and phase-III (P3)

Fig 4 Variation in a) protein b) carbohydrate c) lipid d) ATP concentration at different tidal levels during phase-I (P1), phase-II (P2) and phase-III (P3)





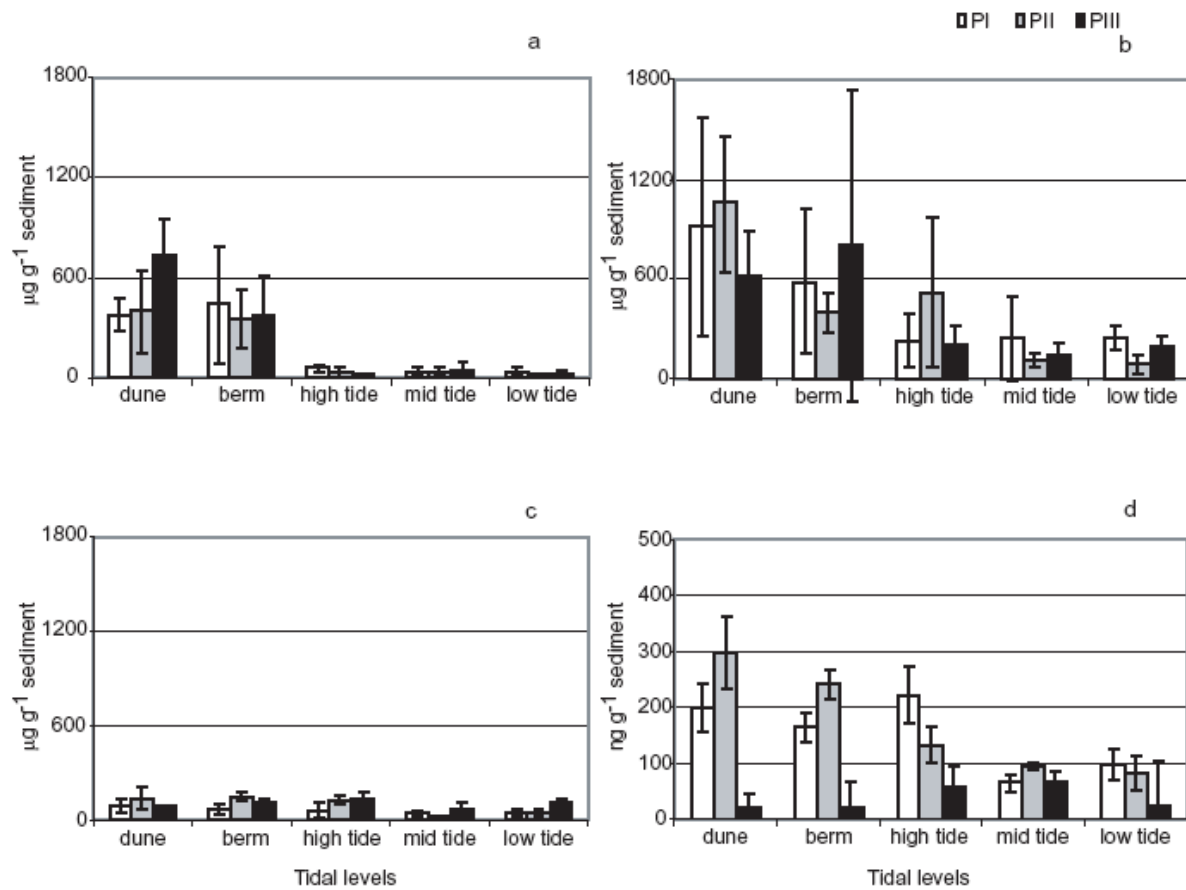


Table 1: Percentage contribution of different bacterial parameters to total bacterial abundance during phase-I (P1), phase-II (P2) and phase-III (P3)

Phase	x 10 ⁵ cells g ⁻¹ sediment			x 10 ⁵ CFU g ⁻¹ sediment		
	TC	TVCa	TVCAn	RC _{0.001%}	RC _{0.01%}	RC _{10%}
PI	19.9	4.93	6.33	0.79	0.47	0.04
Contribution to TC		25%	32%	4.0%	2.4%	0.2%
P2	4.53	1.8	2.58	1.64	1.20	0.62
Contribution to TC		40%	57%	36%	27%	14%
P3	2.97	1.55	1.31	1.52	1.07	1.96
Contribution to TC		52%	44%	51%	36%	66%

TC (Total counts), TVCa (Total direct viable counts under aerobic conditions), TVCAn (Total direct viable counts under anaerobic conditions), RC_{0.001%}

(Retrievable counts on 0.001%NB), RC_{0.01%} (Retrievable counts on 0.01%NB), RC_{10%} (Retrievable counts on 10%NB),

Table 2: Pearson correlation analysis between bacterial and biochemical parameters during phase-I (P1), phase-II (PII) and phase-III (PIII)

Interrelated parameters	Phase		
	I	II	III
TC/Depth		-0.334*	--
TC/TVCa		0.323	0.579
TC/TVCan		0.458	0.428
TC/ATP		0.295	--
TC/ RC _{0.001%}		0.287	0.435
TC/ RC _{0.01%}		--	--
TC/ RC _{10%}		--	0.717
TC/PROT		--	--
TC/CHO		--	--
TC/LIP		--	-0.27
TC/LOM		--	--
TVCa/Depth		--	-0.28
TVCa/TVCa		0.722	0.83
TVCa/ RC _{0.001%}		0.573	0.846
TVCa/ RC _{0.01%}		0.286	
TVCa/ RC _{10%}		0.262	0.696
TVCa/PROT		--	--
TVCa/LOM		--	--
TVCa/ATP		0.255	--
TVCa/ RC _{0.001%}		0.540	0.932
TVCa/ RC _{0.01%}		0.37*	--
TVCa/ RC _{10%}			0.563
TVCa/PROT		--	--
TVCa/CHO		--	--
TVCa/LIP		--	--
TVCa/LOM		--	--
ATP/ RC _{0.001%}		0.437	--
ATP/ RC _{0.01%}		--	0.609
ATP/ RC _{10%}		-0.32	0.271
ATP/PROT		--	0.625

ATP/LIP	--	0.708	--
ATP/CHO	--	0.74	-0.339*
ATP/LOM	--	0.792	-0.415
RC _{0.001%} /RC _{0.01%}	0.499	0.254	--
RC _{0.001%} /RC _{10%}	--	0.54	0.709
RC _{0.001%} /PROT	--	--	0.402
RC _{0.001%} /CHO	--	--	0.378
RC _{0.001%} /LIP	0.267	-0.263	--
RC _{0.001%} /LOM	0.263	--	0.438
RC _{0.01%} /CHO	0.369*	0.384*	0.355*
RC _{0.01%} /PROT	0.429	0.516	0.364*
RC _{0.01%} /LIP	--	0.332*	--
RC _{0.01%} /LOM	0.413	0.47	0.419
RC _{10%} /PROT	0.312	--	0.523
RC _{10%} /LIP	--	--	--
RC _{10%} /LOM	--	--	0.387*
PROT /CHO	0.719	0.61	0.468
PROT/LIP	0.465	0.448	--
PROT/LOM	0.864	0.798	0.778
CHO/LIP	0.659	0.584	--
CHO/LOM	0.969	0.961	0.918
LIP/LOM	0.667	0.653	--

TC (Total counts), TVCa (Total direct viable counts-aerobic), TVCan (Total direct viable counts-anaerobic), RC_{0.001%} (Retrievable counts on 0.001%NB), RC_{0.01%} (Retrievable counts on 0.01%NB), RC_{10%} (Retrievable counts on 10%NB), ATP (Adenosine triphosphate), PROT (Protein), CHO (Carbohydrate), LIP (Lipid), LOM (Labile organic matter). N=60 ~ (3 cores X 4 depths X 5 levels), df=59, Significance p≤0.05 is reported in bold and italic, p≤0.01 is reported in bold and *, p≤0.001 is reported in regular