

**Long-term changes in microbial and biochemical  
parameters in the Central Indian Basin**

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## **Abstract**

Natural kilometer-scale spatial variability and seasonal variability in deep-sea sediments at ~5000 m depth was studied at 2 reference sites in the Central Indian Basin. Biochemical parameters such as the labile organic matter (consisting of carbohydrates, protein and lipids) and total organic matter and biological parameters such as total counts of bacteria and adenosine triphosphate (ATP) were estimated at an interval of 54 months. These were compared with 9 locations (12-17 km away) where an artificial sediment disturbance was created by a hydraulic benthic disturber. Besides the above-mentioned parameters, extracellular enzymes, alkaline phosphatase, aminopeptidase and lipase were also monitored at these 9 locations. Post-disturbance (PoD1) observations were carried out immediately after the benthic disturbance experiment and compared with the pre-disturbance (PreD) pattern. The restoration process in the test site was studied after a period of 44 months (PoD2). There was a drastic reduction in total counts of bacteria, ATP, carbohydrates and lipids accompanied by a 2-4 fold increase in protein content at the undisturbed reference sites after 54 months. A similar reduction in these parameters was observed in the PoD1 compared to the PreD stage at the test site. An initial increase in total organic matter content and in the activities of three extracellular enzymes in the PoD1 stage was followed by a reduction in total organic matter, ATP and organic P content and activities of the extracellular enzymes in the PoD2 phase (i.e. after 44 months). Reduction in ATP levels suggests a negative impact of resedimentation on living biomass in the sediments. Although labile organic matter, protein concentrations and the protein/carbohydrate ratio increased in the PoD2 phase relative to the PoD1 phase, the bacterial numbers were below the concentrations in the pre-disturbance stage (PreD), indicating slow restoration of benthic conditions. Long-term analyses of the indicator parameters thus showed mixed effects of sediment disturbance. A similar pattern at the undisturbed reference location suggests that natural (seasonal) variability outweighed artificial variability caused by the benthic

disturbance in the Central Indian Basin and the former is responsible for bringing about changes in deep-sea benthic ecosystem on long-time scales.

**Key words:** Natural variability, artificial benthic disturbance, deep-sea sediments, bacterial counts, extracellular enzymes.

## **Introduction**

Sedimentation, bioturbation, physical mixing of sediments at a given site with horizontally advected material (Turnewitch et al., 2000), and resuspension by near-bottom currents (Lampitt, 1985) are natural processes that can disturb deep-sea sediments. These activities include episodic, low-impact benthic processes and unique catastrophic events. Such disturbances may result in changes in sediment distribution, biochemical composition, microbial standing stock and benthic fauna. Concentrations of labile organic fractions and extracellular enzyme activities might be expected to respond to changes in the sedimentary environment, such as those caused by bioturbation and resuspension (Thiel, 2001). The intensity of such processes and their impacts, however, may be enhanced if human disturbances are superimposed. Activities such as mining are likely to disturb benthic communities and the overlying water column on long temporal and large spatial scales (Thiel, 2001). While the resilient nature of bacteria may allow them to quickly repopulate the disturbed sediments (Alongi, 1990), long-term monitoring of the disturbance effect is necessary in order to assess the recolonization by organisms ranging from bacteria to megafauna against the background of the natural variability that affects such processes.

Several experiments have been conducted in the Pacific Ocean over periods ranging from 1-7 years to evaluate the effects of artificial disturbance in the deep sea (Thiel and Schrieffer, 1990; Thiel, 2001; Trueblood and Ozturgut, 1997; Fukushima, 1995). As a part of the environmental impact assessment studies for polymetallic nodule mining under the INdian Deep-sea Environment eXperiment (INDEX), the effect of simulated disturbance was studied in the Central Indian Basin in 1997 by estimating bacterial numbers, bulk measures of labile organic matter (consisting mainly of carbohydrates, protein and lipids), total organic matter (TOM) and ATP carbon (indicating carbon contributed by living biomass) in the pre-disturbance (May-July 1997) and post-disturbance (July-August 1997) periods (Raghukumar et al., 2001). The immediate impact of disturbance was observed on sediment characteristics (Sharma et al., 2001; Valsangkar, 2001) and macrobenthos (Ingole et al., 2001).

In the present study, the effect of benthic disturbance and then the extent of recolonization were assessed by carrying out a detailed temporal survey of a site 2 months prior to disturbance, immediately after disturbance and 44 months after the event. This paper evaluates activities of the sedimentary enzymes, alkaline phosphatase, aminopeptidase and lipase, and other microbial and biochemical indicators for short- and long-term effects of artificial deep-sea disturbance and their restoration to natural conditions. Further, we attempt to assess and compare the natural variability at an undisturbed reference site with that of the artificially disturbed site.

## **2. Material and methods**

## 2.1. Study area

An area designated A1, located between 10°01' –10°03' S and 75°59' – 76°02'E was selected (Fig. 1a) as the study area. This area includes a reference site as well as the test site (Fig. 1b) where the benthic disturbance experiment was conducted. Within the test site, a strip of seafloor, 3000 m long and 200 m wide, was selected (Fig. 1b). The test site has flat topography for easy maneuvering of the hydraulic “benthic disturber” and for easy dispersion of the sediment plume (Sharma and Nath, 2000).

## 2.2. Benthic disturbance experiment

The deep-sea sediment resuspension system (DSSRS) of Sound Ocean Inc. (SOSI, USA) was used on board the Russian Research Vessel *Yuzhmorgeologiya* for creating the disturbance. The same ‘disturber’ had been used for the deep-sea disturbance experiments conducted by NOAA (USA), MMAJ (Japan) and IOM (InterOcean Metal, a consortium of East European countries). The disturber consists of a tow frame connected to the vessel by a coaxial cable that tows as well as conducts signals from and power to the disturber unit. It has a 5 m high discharge stack and is funnel-shaped at the bottom. Sediment is sucked up from the seabed and discharged from the top by two pumps. The disturbance was caused by a combination of fluidizing pump and suction pump to dislodge and discharge the sediment into the water column 5 m above the sea floor. Sediment slurry was pumped and discharged by this system along 26 tracks for 9 days, with an estimated sediment resuspension of 6100 m<sup>3</sup> (Sharma, 2000).

A Russian-designed deep-towed photographic system Neptune was used to obtain still photographs and continuous video recording. The system consisted of a photo camera, video camera, flash unit, lights, echosounder, power module and electronic module, all of them housed in pressure casings and mounted on a steel frame. The deck control unit was connected to the tow body with an armored coaxial cable. Photographs were taken from the deck control. An echosounder mounted on the deep-tow frame was used to control the altitude of the deep-tow system (Rodrigues et al., 2001).

### *2.3. Sediment sampling*

This study started with baseline sampling in September 1996, when five box cores (BC 197, 190, 194, 193 and 188) were collected from site A1 (Fig. 1a, Table 1). Two of these (193M and 194M) were monitored 54 months later in the PoD2 stage (April 2001). This area served as the reference (undisturbed) site. Pre-disturbance (PreD) sampling (Fig. 1b) was carried out in June 1997, and the benthic disturbance experiment was conducted in July 1997. The post-disturbance sampling (PoD1) for impact assessment was carried out immediately after the experiment, during July-August 1997, and the monitoring sampling (PoD2) was carried out at the same locations 44 months later (in April 2001) at the same locations. In order to maintain accuracy of the sample locations in the pre- and post-disturbance phase 1 and 2, GPS positioning was used for primary navigation of the vessel; the GPS was coupled with an underwater hydroacoustic navigation system for position fixing of box-core samples (Sharma, 2000). For microbiological analyses, we chose three stations within the disturbed track (BC

5, 3 and 2) and three each to the south (BC 14, 7 and 16) and north (BC 13, 8 and 11) of the disturbed track (Fig. 1b, Table 1). The south and north tracks were not physically disturbed but were assumed to be passively disturbed by the redeposition of the sediment plume.

Sediment samples were collected with a USNEL- type box corer of 50 x 50 x 50 cm dimensions. Although Bett et al. (1994) and Shirayama and Fukushima (1995) have indicated the inherent problem of bow wave effects with box core sampling, we have little evidence indicating loss of the sediment surface during our sampling. The fluffy nature of surface sediments, higher organic carbon values in surface compared to sub-surface sediments (Sharma et al., 2001) and biostratigraphic zonation based on radiolarian assemblages (Gupta, 2000) indicate that the sediment-water interface was preserved. After siphoning of the overlying water, replicate sub-samples were collected in alcohol-sterilized, clean plexi-glass cylinders of 6.7 cm inner diameter. On average, 30 cm long cores were obtained. The cores were sliced at 2-cm intervals down to 10 cm depth and every 5 cm depth thereafter and extruded directly into sterile plastic bags for further biochemical and microbiological analyses. The present work reports data down to 10 cm depth, as the effects of disturbance were most noticeable in surficial sediments (Sharma, 2000).

Sediment from each sub-section (~ 10 g) was homogenized with a mortar and pestle and stored in plastic bags (4°C) for estimation of organic constituents. For direct counts of total bacteria, freshly collected wet sediment (1 g) was suspended in sterile seawater (9 mL) and vortexed (3 min). An aliquot of this

sample (1 mL) was fixed in formalin (at 5% final concentration) buffered with sodium borate buffer (0.1 M; pH 8.5) and stored in the dark (4°C).

#### *2.4 Bacterial abundance*

Aliquots from the formalin-fixed samples were sonicated (3x30 s), diluted with sterile sea water, filtered over black polycarbonate nuclepore filters (0.22 µm pore size; Millipore, USA) and stained with an aqueous solution of acridine orange (0.01 %; 3 min). Bacterial cells were counted from 10-20 microscope fields with an epifluorescence microscope (Olympus BX60, Japan). The final numbers were expressed as total counts g<sup>-1</sup> dry sediment (Hobbie et al., 1977). The bacterial numbers were converted to bacterial carbon in fg by the conversion factor of 20 (Peduzzi and Hendle, 1991). Bacterial protein was calculated from bacterial biomass to nitrogen ratios (Danovaro et al., 1998).

#### *2.5. Organic matter*

Sediments were analyzed for protein, carbohydrates and total lipids, the sum of which has been defined as the bulk labile organic matter (LOM, Danovaro et al., 1993), and total organic carbon (TOM). These were crude bulk measurements and do not provide information on molecular-level composition or, more importantly, reactivity. At deep-sea sites, a large but variable proportion of all these components will already have disappeared within the water column, and the remainder may be largely refractory. Therefore, these are not absolute values but only relative values to compare spatial and temporal effects of natural changes and those brought about by artificial disturbance.



ATP was extracted from sediments on board the research vessel in sterile, boiling Tris-HCl buffer (5 mL; pH 7.8; 5 min; Parsons et al., 1984) and the extracts were frozen immediately ( $-20^{\circ}\text{C}$ ). ATP was estimated by the luciferin-luciferase reaction, and the light generated was measured with a Turner luminometer (model TD-20E, Turner Designs, USA) in the shore laboratory. ATP (Sigma Chemicals, USA) at a concentration of  $2\text{-}12\text{ ng mL}^{-1}$  was used as the standard. Standard ATP (20 ng, Sigma Chemicals, USA) was added to three randomly chosen sediment samples and the extraction procedure was carried through to calculate R (recovery factor). Typical values of R were around 0.75. The final calculations were based on this R factor (Parsons et al., 1984).

Organic carbon was estimated by the wet oxidation method with dichromate (El Wakeel and Riley, 1957). Three replicate analyses were made for each sample, and the SD was <3-4%. A precision of 0.01% C is routinely obtained in our laboratory (Nath et al., 1997). Total organic matter (TOM) was derived by multiplication of TOC values by a factor of 1.82 (El Wakeel and Riley, 1957). We are aware that this conversion factor has a large uncertainty and can vary at different sites. Thus they are relative and not absolute values. Organic phosphate was estimated by the spectrophotometric method (Koroleff, 1983) by oxidizing organic forms of phosphate to inorganic phosphate with alkaline persulfate as the oxidizing agent. Potassium dihydrogen phosphate was used as the standard.

## *2.6. Extracellular enzyme activities*

Alkaline phosphatase activity in the sediment was measured with the substrate p-nitrophenyl phosphate (Taga and Kabori, 1978). One mole of p-nitrophenol liberated from the substrate is equivalent to 1 mole of phosphorus. The standard was prepared with p-nitrophenol for estimating concentrations of the enzyme. The activity is expressed as U, which is equivalent to  $\mu\text{mol}$  of phosphate phosphorus released  $\text{h}^{-1} \text{g}^{-1}$  dry sediment. The blanks contained a) sediment without substrate, b) substrate without sediment and c) sediment suspension boiled for 25 min and treated as above. All the enzyme estimations were carried out at  $4^{\circ} \text{C}$ .

Lipase activity was measured with methylumbelliferyl (MUF)-butyrate as substrate (Hoppe,1983). A calibration curve was prepared with fluorescent methylumbelliferon mixed with sediment slurry (to correct for adsorption onto sediments) and centrifuged at  $2^{\circ}\text{C}$ , 5000 rpm for 10 min. Fluorescence intensity was measured at 455 nm with excitation at 365 nm with a spectrofluorometer (Hitachi F-2000, Japan). Activity is expressed as  $\text{U h}^{-1} \text{g}^{-1}$  dry sediment, where U is equivalent to  $\mu\text{mol}$  MUF released. Blanks contained a) sediment without MUF substrate, b) MUF substrate alone without sediment and c) sediment suspension boiled for 25 min and treated as above. Fluorescence readings of the appropriate blanks were subtracted from the sample readings.

Aminoamidase (protease) activity was measured with the model substrate L-Leucine-4-methylcoumarinyl-7-amide (Leu-MCA, Sigma Chemicals, USA). A calibration curve was prepared with the fluorescent MCA mixed with sediment slurry (to correct for adsorption onto sediments) and centrifuged at  $2^{\circ}\text{C}$ ,

5000 rpm for 10 min. Fluorescence intensity was measured at 440 nm with excitation at 380 nm with a spectrofluorometer (Hitachi, F-2000, Japan). Activity is expressed as  $U\ h^{-1}\ g^{-1}$  dry sediment, where U represents  $\mu\text{mol}$  MCA released (Hoppe, 1983). All the substrates and standards used were from Sigma Chemicals (USA).

For all the assays, sediment sub-samples (0.2-0.5 g) were slurried in 15-mL tubes containing filtered seawater and vortexed. Assays were initiated by adding appropriate substrates, vortexing and incubating at 4°C in the dark. All the enzyme assays were carried out at 5-6 substrate concentrations and from 2-10 h in the dark at 4°C to calculate the optimum parameters for estimations. Boiling of sediment samples did not cause detectable hydrolysis of the enzyme substrates.

### *2.7. Statistical analyses*

Multiple regression, and 2-way Analysis of Variance between various parameters for PreD, PoD1 and PoD2 phases were calculated with Minitab version 8.3 (Minitab, 1991). Thus, for 9 box cores, each divided into five depth horizons (0 to 10 cm), there were 45 values for each parameter ( $n=45$ ) during each sampling stage. Each value was an average of 3 replicates, and the standard deviations were better than  $\pm 10\%$ . The spatial and temporal variations in the various replicated parameters ( $n=3$ ) measured from 3 stages (PreD, PoD1 and PoD2), 3 sectors (center, north and south) and 3 box cores in each sector at five depths were assessed by 3-way nested ANOVA. We regarded the temporal (sampling stages) and spatial (sectors such as north and south of disturbance and the disturbance track in the centre) variations as fixed factors. Cores were

nested within sectors and were treated as random factors. Homogeneity of variance was checked by Cochran's test. The relative contribution of each factor to the total variance in the ANOVA was also calculated. These contributions were extracted from the mean-square estimates and expressed as proportions of the sum of all variances.

### **3. Results**

#### *3.1. Physical impact of disturbance*

Photographs taken with the deep-towed camera system typically showed undisturbed conditions before the disturbance, with extensive biological activity in the form of benthic organisms, their fecal casts, burrows, tracks and trails. In contrast, immediate post-disturbance observations showed disturber sled marks, a reduction in benthic fauna and lumps of sediment that had been redeposited from the center of the disturbed area. The areas just outside the disturbance site, up to 250 m on either side, showed a very fine layer of resedimentation with few burrows, fecal casts or animal trails. Areas beyond 250 m from the tow zone did not show any effects of disturbance and had fecal casts, burrows, trails and megabenthic organisms (Sharma et al., 2001; Rodrigues et al., 2001).

In deep-tow photographs taken in and around the disturbance area during the monitoring phase (PoD2), the appearance of the seafloor was similar to that observed during the pre-disturbance phase (PreD) with no significant disturbance features. A few photographs showed disturber track marks with rounded edges and megafauna, indicating physical restoration of the seafloor and re-colonisation (Sharma et al., 2005).

### 3.2. *Bacterial abundance and organic matter*

Total counts of bacteria decreased from  $2.0 \times 10^9 \text{ g}^{-1}$  dry sediment in PreD to  $4.3 \times 10^7 \text{ g}^{-1}$  in the PoD1 phase but showed an increase of 1-2 orders of magnitude ( $1.5 \times 10^8 \text{ g}^{-1}$ ) in the PoD2 stage, 44 months later (Table 2; Fig. 2).

The ATP values as indicators of living biomass decreased in several cores in the north and south sectors and increased in the disturbed track during the PoD1 sampling (Fig. 3). The values in the PoD2 phase were uniformly lower by several fold than the PoD1 values at all locations.

Sediment protein decreased 5-fold in PoD1, whereas carbohydrate concentration remained unchanged after disturbance. In the PoD2 phase protein and carbohydrate values increased by 6- and 2-fold respectively (Figs. 4, 5; Table 2). The lipid concentrations decreased after disturbance (PoD1) and remained unchanged in the PoD2 phase (Fig. 6 & Table 2). The concentrations of bulk labile organic matter (LOM; cumulative values of lipids, protein and carbohydrate) decreased in the PoD 1 phase but increased 7-fold during the PoD2 phase (Fig. 7; Table 2). In contrast, the total organic matter (TOM) concentrations increased in the PoD1 phase but decreased in all the cores in the PoD2 stage (Fig. 8; Table 2).

The quality of LOM also showed variability over time; the percentage of carbohydrate and lipids increased in the PoD1 stage and decreased in the PoD2 period (Table 3). On the other hand, the percentage of protein and the protein to carbohydrate ratio in the LOM decreased in the PoD1 samples by ~50% and increased by ~50% in the PoD2 stage (Table 3). Bacterial carbon and protein

and ATP contribution to the LOM decreased in the PoD1 and the former two decreased further in the PoD2 phase.

### *3.3. Extracellular enzymes*

Aminopeptidase (protease) activity was saturated at a substrate concentration of 0.5  $\mu$ M Leu-MCA with 0.5 g sediment incubated for 3 h in the dark. Lipase activity showed  $V_{\max}$  at a substrate concentration of 4  $\mu$ M MUF-butyrate with 2 mg sediment incubated for 30 min in the dark. Alkaline phosphatase activity showed  $V_{\max}$  at 0.01% concentration of p-nitrophenyl phosphate with 0.5 g sediment sample.

In general, protease activity decreased from the surface to 10 cm depth in all the cores except in some cases where a subsurface increase was observed at 4 to 8 cm depth. In 5 out of 9 cores, protease activity decreased after disturbance and in the others it increased by 2-3 fold after disturbance (Fig. 9). On the other hand, phosphatase activity increased in 5 out of 9 cores after the benthic disturbance (Fig. 10). Lipase activity decreased in most of the cores after the disturbance. Lipase activity remained almost constant from the surface down to 10 cm depth (Fig. 11). Activity of all the three enzymes was below one unit in several of the cores during the PoD2 phase.

### *3.4. Reference location*

Total counts of bacteria, ATP, CHO and lipid concentrations in the 2 cores taken at the undisturbed reference location (ca. 12-17 Km away) also decreased in the PoD2 phase, measured 54 months after (BC 193M and 194M) the baseline measurements (BC 193 and BC 194; Figs. 12 & 13). Only the concentration of

protein and LOM increased in both the cores during the monitoring phase (Fig. 13).

### *3.4. Statistical analyses*

Results of two-way ANOVA showed that there was no statistically significant difference in concentration of most of the parameters within different depth horizons in all the cores during all the sampling stages. On the other hand, there was a highly significant difference between the 9 cores in all the 3 samplings stages (data not shown). For each parameter, analyses of data from 3 cores in each sector, namely north, centre and south, by one-way ANOVA showed significant differences in one or two sectors only (Table 4). Most of the differences in values in the southern track appeared to be highly significant ( $P=,0.01$ ; Table 4). To evaluate the different levels of temporal and spatial variability at the sampling site, a three level-nested ANOVA was performed for all the parameters measured. Results showed (Table 5) the percent contribution to total variability by individual parameters in the sampling stages, sampling tracks (sectors) and individual box cores. ATP, lipids, bacterial counts, carbohydrate, lipase and phosphatase activities contributed to >70% of temporal variability, whereas protein, TOM content and protease activity contributed to <40 % of the variability (Table 5). Concentrations of TOM showed higher spatial (32 %) than temporal (12 %) variation. One-way ANOVA showed significant difference in some tracks for some of the parameters during some stage of sampling (Table 4), However, percentage variation contributed by bacterial numbers, ATP and

lipid contents, lipase and phosphatase activities between cores was less than 5%, but was highly significant between the stages (Table 5).

Two-way analysis of variance performed for distribution of enzymes within various depth horizons of cores and between cores showed that only protease activity exhibited significant differences at different depths (data not shown). The differences in concentrations of all three enzymes were statistically significant between cores during all three sampling stages. This variance was reflected in the significant variation seen in the north, center and south sectors during PoD1 sampling (Table 6) and in only one or two sectors during the PreD and PoD2 stages. The average values for all the depths and cores together in different stages showed that activities of the three enzymes increased in the PoD1 stage, whereas they decreased by >90% during the PoD2 stage (Table 2).

Multiple regression analyses were performed for bacterial abundance and sediment organic matter. Results indicated significant correlations between bacterial numbers and TOM content ( $r=0.32$ ;  $P<0.05$ ;  $n=44$ ) and concentrations of LOM and TOM ( $r=0.32$ ;  $P<0.05$ ;  $n=44$ ) in the PreD sediments. In the PoD1 sediments, total bacterial counts and ATP ( $r=0.39$ ;  $P<0.01$ ;  $n=44$ ) and LOM and ATP ( $r=0.43$ ;  $P<0.1$ ;  $n=44$ ) showed significant correlations. In the PoD2 phase, ATP and LOM concentrations correlated with TOM concentrations ( $r=0.35$  and  $0.32$  respectively;  $P<0.05$ ;  $n=44$ ). Correlation between LOM and TOM alone remained significant in the PreD as well as in the PoD2 phase.

There was a significant correlation between aminopeptidase activity and bacterial numbers ( $r=0.35$ ;  $P<0.05$ ;  $n=44$ ) and protein concentration ( $r=0.47$ ;



P<0.001; n=44) in the PreD sediments. Phosphatase and lipase activity correlated most closely with ATP concentrations. Phosphatase activity also correlated with P content in PreD and PoD1 stages (r=0.38, P=<0.05, n=40 and r=0.36, P=<0.05, n=40 respectively). Activities of lipase correlated with lipid concentrations only during the PoD2 stage (r=0.40; P<0.01; n=44).

## **4. Discussion**

### *4.1. Physical impact of the disturbance*

Sediment redistribution showed a 3-fold increase in particle flux during the disturbance experiment, which reduced to 2-fold immediately after the experiment (Parthiban, 2000). A directional movement of the sediment flux, organic carbon and sediment size fractions were observed around the disturbed area immediately after the experiment (Sharma et al., 2001, Valsangkar, 2001). However, the observations during the PoD2 phase, 44 months after the disturbance, showed a multi-directional increase in the clay content of the sediment compared to the PoD1 phase. This reflected a uniform natural flux in the area, indicating restoration of conditions similar to those of the PreD1 stage. Murty et al. (1999) have shown that currents close to the seafloor in CIB change their directions frequently. These currents had average velocity of 2 cm min<sup>-1</sup> at 50 m above bottom, resulting in tranquil and gentle movement and deposition of sediment, without bringing about drastic compositional changes.

### *4.2. Comparison of natural versus artificial changes*

From the data presented here the following picture seems to emerge. There was a decrease in bacterial numbers, ATP, carbohydrate and lipid and an

increase in protein concentration in the two box-core samples taken at the reference site 54 months after the initial baseline sampling (Figs. 12 & 13) and in the nine cores from the disturbance site in the PoD2 phase. These results indicate that the seasonal changes, apparently natural, far outweighed (in many cases by orders of magnitude) anything caused by the disturbance. Alternatively, it can be postulated that the changes seen at the reference site were due to redeposition of the sediment plume from the disturbed test site. However, the presence at the experimental site of weak abyssal currents of 1-2 cm s<sup>-1</sup> directed towards the southeast in November-April and northwest during the remaining period (Murty et al., 1999; 2001), and movement of clay particles to the north west (Valsangkar, 2001), do not support the hypothesis that the plume movement was directed towards the undisturbed reference site located northeast (BC 194M) and southeast (BC 193M) of the disturbance site (see Fig. 1a).

The sediment trap data (Sharma et al., 2001) have also shown that the sediment trap closest to the disturbance site (~100 m) recorded very high fluxes, and the sediment fluxes decreased with distance from the disturbance site. Lack of change in the sand-silt-clay content ratios at the reference site in the PoD2 stage suggested that the area was not affected by a sediment plume or by any natural processes (Valsangkar, 2005). Thus this evidence leads to the hypothesis that the changes observed at the reference site 54 months after initial sampling, were natural. Such natural changes masked the changes made by the artificial disturbance at the test site. While the changes caused by the benthic disturbance (evident from decreased bacterial numbers and increased TOM

content) were distinct from PreD to PoD1, the changes in the PoD2 phase seem to be influenced by natural processes. Long-term monitoring of the benthic disturbance and redeposition of sediments did not reveal any of the predicted catastrophic effects.

Short-term monitoring (9 months) during the Benthic Impact Experiment in the Pacific showed that the heaviest resedimentation occurred within 50 m of the tow zone (Trueblood and Ozturgurt, 1997), with most of the discharged sediment settling quickly. These observations further indicated that the local changes around the tow zone most likely limited the dispersal distance of the discharged plume and thus did not cause any adverse effects. On the other hand, during a similar, large-scale, long-term disturbance experiment (DISCOL) in the deep Peru Basin, it was demonstrated that even 7 years after the benthic impact, megafauna had not returned to their original density (Bluhm, 2001). Thus, it can be concluded that the local conditions, such as the prevailing currents and the topography, in addition to the seasonality, make the impact of benthic disturbance very site-specific.

#### *4.3. Downcore uniformity at the disturbed (test) and undisturbed (reference) sites*

The absence of statistically significant changes down to 10 cm depth in nearly all the parameters during 3 stages of sampling at the disturbance site and the reference site could be indicative of bioturbation resulting in intense mixing of sediments, irrespective of the disturbance experiment. Bottom photographs revealed well-developed surficial biogenic structures in the form of tubes, tracks,

mounds and burrows associated with epifaunal and infaunal activity in the area (Sharma and Rao, 1992; Sharma et al., 2001).

As the sediments did not show any downcore variation at the PreD stage, disturbance did not change this pattern, and the status was maintained even after 44 months at the PoD2 phase. Only TOM content showed distinct downcore variation during all the sampling stages. In addition, the deep-sea sediments of the Central Indian Basin, an area with low surface primary productivity (Matondkar et al, 2005) and sedimentation rates of about 1 cm per 1000 years (Borole, 1993; Gupta, 1999), may not experience dynamic changes, in contrast to heterogeneity of the bacterial abundance and associated biochemical changes seen in areas with high sedimentation rates such as the Arabian Sea (Boetius et al., 2000). In addition, the percentage of silt and clay in these cores showed similar downcore homogeneity (Valsangkar, 2001, 2005). A similar downcore compositional uniformity in lipids and protein concentration was reported in 10 cores (sediment sections from 0-10 cm) taken in the deep eastern Mediterranean Sea (Danovaro et al., 1993). The maintenance over time of uniform downcore biochemical profiles in the CIB is remarkable. Previous reports of uniform downcore distribution of labile OM (Danovaro et al., 1993) and hydrolytic enzymatic activity (Poremba and Hoppe, 1995) in deep-sea sediments were limited to one time point.

Using laboratory-based simulation of sediment suspension and resettling behaviour of deep-sea sediments disturbed by nodule mining, Becker et al. (2001) demonstrated that only 16% of the sediment is resuspended and about

80% remains on the sea floor in the form of large and small lumps. These observations help to explain the results of benthic impact experiments in the Peru Basin, during which only relatively small amounts of particulate material were suspended following an experimental *in situ* disturbance (Oebius et al., 2001). These observations may partly explain the downcore uniformity of almost all the parameters even after the benthic disturbance. Extremely low sedimentation rates in the Central Indian Basin may account for a similar situation at the reference site. However, more detailed seasonal and spatial studies are required to understand the complexity of deep-sea sediment processes.

#### *4.4. Spatial and temporal variability*

Despite the apparent vertical uniformity within cores, there was a significant difference between cores for all the parameters during the 3 sampling periods at the disturbed and reference sites. Statistically significant vertical uniformity “within cores” might be related to large standard deviations in the data which are normal in such environmental studies. “Between box cores” and “between-sectors” (disturbed, north and south of the disturbed sector) together contributed to the spatial variability of <0.5-56% when tested by 3-way-nested ANOVA (Table 5). Temporal change was the most important source of variability in all the cases. The variation of 12-98 % contributed by sampling stages (Table 5) indicated significant seasonal influence. Bacterial numbers, ATP and lipid concentrations and lipase and phosphatase activities contributed to this high percentage variation (Table 5), suggesting that they are suitable indicators of benthic impact over a time period. Spatial variability contributes more than

temporal variation only in the case of TOM content and therefore could interfere with seasonal studies. At the HEBBLE site (~4800 m water depth) on the Nova Scotia Rise, which experiences benthic storms, “within box core” variations were very significant (Baird et al., 1985). On the other hand Lavigne et al. (1997) reported a predominance of “between-box core” variability in microbial abundance and enzyme activity in the Laurentian Trough in the Atlantic (350 m water depth) due to spatial irregularities in organic matter supply to the seafloor. Uniformity in distribution of meiofauna among sampling stations in a large area (ca. 8000 km<sup>2</sup>) of the Venezuela Basin at a depth of 4800 m (Woods and Tietjen, 1985) and in the deep sea off North Carolina, USA (Coull et al., 1977) has also been observed. Therefore, homogeneity of meiofauna in a small area (10 km<sup>2</sup>) at abyssal depths (5000-5300 m) in the CIB was not unexpected.

#### *4.5. Spatial and seasonal variations in bacterial abundance and organic matter at the disturbance site*

For all parameters, there was little consistent difference between directly disturbed sites and those north and south of the disturbance track, and there was a considerable (often dramatic) spatial variability within a given set of sites. Thus, if there was a disturbance effect, either it was small or the process of physical ploughing had basically the same effect as sediment redeposition at more remote sites. Natural temporal changes could also have been a factor.

The total counts of bacteria in the PoD2 stage were higher by 1-2 orders of magnitude than in the PoD1 stage, but were below the counts (by 2 orders of magnitude) obtained during the PreD period (Fig. 2). During the Japan deep-sea

impact experiment in the eastern equatorial Pacific, Kaneko and Maejima (1997) reported bacterial numbers to be much higher one year after the benthic disturbance than in pre-disturbance samples, suggesting that recolonization after a disturbance may vary from site to site. ATP values, which reflect living biomass (bacteria and meiobenthos, the two groups measured by us), decreased significantly in the PoD2 samples taken more than 3 years after the disturbance. Bacterial counts and ATP values at the reference site also showed a marked decrease 54 months after the initial sampling. These results indicate that although natural changes are often very different from the artificial changes (PreD versus PoD1), and generally much greater, these cannot readily be distinguished.

There was a significant increase in the carbohydrate, protein and LOM concentrations in PoD2 stages of sampling to support the growth of bacteria and meiofauna. Although the percentage of protein in the total LOM increased considerably, there was a substantial reduction in percentage contribution by carbohydrate and lipid (Table 3). The low protein to carbohydrate ratio before the disturbance may indicate the presence of aged organic matter (Danovaro et al., 1993). The high protein to carbohydrate ratio in the PoD3 stage reflects “newly generated” detritus (Danovaro *et al.*, 1993). Bacterial protein *per se* did not contribute to this increase (Table 2) nor did it contribute to the increase in LOM during this stage (Table 3). A similar increase in protein and LOM concentrations at the reference location 54 months after the initial sampling (Figs. 12 and 13), suggests that natural variations have masked the effects of artificial disturbance.

The decrease in TOM at PoD2 probably indicates a change in bottom-water redox conditions, the bottom waters becoming more oxic and decomposing the organic carbon either arriving from surface waters or derived from dead carcasses during the disturbance. Nath et al. (2005) reported increased Fe and Mn contents during the PoD2 phase, indicating increased bottom-water oxygenation. Pore-water nitrate, sedimentary phosphate and organic C/P ratios during the PoD2 phase also indicate more oxygenated conditions. Although, the deep water at this site is already highly oxygenated, renewal of bottom water through Antarctic Bottom Water flow must result in changes in bottom-water oxygenation at the disturbance site and possibly also at the reference site. Thus, changes in oxygenation may be a natural regional phenomenon, rather than a local feature.

The contribution of LOM to TOM (average 9.75%) ranged between 2.7 and 40%, and consequently a large fraction of TOM is represented by refractory material (Sharma and Nath, 2002) or is uncharacterized. This is consistent with the general notion that most of the organic matter in the deep-sea abyssal regions is uncharacterized. LOM values in sediments collected during the PoD1 phase were lower than those from the PreD phase. In contrast, sediments collected during the PoD2 period showed an increase in LOM concentrations above those of the PreD and PoD1 phases (Fig. 7). This increase in LOM, however did not support higher bacterial numbers. The low sediment enzyme activities during the PoD1 phase might be the limiting factor in making the substrates available to bacteria.



#### 4.6. Seasonal and spatial variations in enzyme activities at the disturbance site

The activities of aminopeptidase and lipase in the deep-sea sediments of the Central Indian Basin were much lower than those reported from the deep-sea sediments (4500 m depth) of the NE Atlantic (Boetius and Lochte, 1994) or proteolytic activity in the sediments of the subantarctic Indian Ocean sector at 4200-5000 m depth (Talbot and Bianchi, 1997). However, they were similar to those reported from deepsea sediments of the Arabian Sea (Boetius et al., 2000). We used similar techniques of sampling and enzyme estimations using MUF and MCA substrates and incubation without hydrostatic pressure as reported by these workers. Decompression of deep-sea samples might result in a reduction in the activities of the various enzymes tested (Bianchi and Garcin, 1994). However, this may not be a major problem, since Lochte (1993) reported these enzymes to be barotolerant and their activity at 1 atm similar to that at *in situ* pressures in the abyssal NE Atlantic (4500 m depth). Furthermore, as the same methods of sampling and estimation of enzyme activities were used in each phase, the changes observed during the 3 phases are presumably not “decompression” related.

Extremely low enzyme activity in several sediment cores (usually <1 Unit) in the PoD2 stage was a very intriguing phenomenon, as bacteria and ATP were present in these samples. Either the enzyme concentrations were in reality below the detection limit because of low substrate availability, or they may have been regulated by some other factors (Meyer-Reil, 1991).

The highest activities of aminopeptidase, lipase and alkaline phosphatase were found in the upper layer of the sediment, which receives a supply of fresh organic matter from the water column. The significant correlation between LOM concentration and phosphatase and lipase activities in the PoD1 sediment samples indicates the important contribution from sinking particles to the labile organic matter pool of the ocean floor (Talbot and Bianchi, 1997). In deep-sea sediments, a part of the organic material can be permanently buried because of slow and low microbial activity. Sub-surface sediments with unutilized organic matter exposed during the artificial benthic disturbance may have induced higher enzymatic activity. Findlay et al (1985) suggested that elevated microbial activity in the burrow walls of invertebrates could be attributed to physical disturbance of the sediment as well as to infaunal metabolism. Reichardt (1988) speculated that the excretion of protease through the guts of lungworms in intertidal sediments of the North Sea could be a mechanism by which an essential biocatalyst is transported from subsurface to surface sediments, which are the major sites for decay of organic material. It is likely that mechanical disturbance destroyed small infauna and their carcasses contributed to the increased organic matter after the disturbance. Consequently, extracellular enzymes released during cell lysis might have contributed to the increased degradative enzyme activities (Meyer-Reil, 1986) in the PoD1 phase.

## **5. Conclusion**

Considering only the PreD to PoD1 data from the 3 sites within the direct disturbance track (Station 5, 3 and 2), and thus disregarding long-term and

natural changes as well as the sites to the north and south of this track and the reference sites, the following changes were observed: 1) bacterial numbers decreased by 2 orders of magnitude, 2) ATP decreased by 1 order of magnitude at one site and increased slightly at two sites, 3) protein concentration decreased at all the sites, 4) carbohydrate concentrations remained unchanged largely, 5) lipids showed a decline at two sites and a marginal increase at one site, 6) labile organic matter content decreased marginally at all the sites, 7) total organic matter content increased and 8) enzyme activities were highly variable without showing a consistent pattern. These results indicate that direct short-term effects of disturbance at these 3 sites were a) general homogenization of sediment, b) physical loss or subsequent decay of LOM components and c) heavy negative impacts on bacterial numbers while small organisms (as indicated by ATP content and enzyme activities) remained active at the test site, perhaps stimulated by reworking/exposure of sediments.

Spatial variability at the test site was limited except in the case of TOM, protein and protease, while temporal changes explained 70-98% of variance in bacterial counts, ATP, lipid, carbohydrate content and lipase and phosphatase activities as indicated by 3-way nested ANOVA. The activities of the extracellular enzymes in the sediment were induced by the disturbance that released the substrates buried in the deeper layers. As the activities of these enzymes respond quickly to the available substrates and are short-lived, they are not suitable for monitoring long-term disturbance effects. Values for

biological and biochemical parameters remained uniform downcore despite large differences between seasons.

A similar reduction in bacterial abundance and organic matter concentrations at the undisturbed reference site 54 months later supports the hypothesis that natural processes cause long-term variations in the deep-sea sediments of the Central Indian Basin. Our results further suggest that natural changes related to seasonality are indistinguishable from the changes brought about by the artificial disturbance over long-time scales. Thus, although certain changes occur in microbial and biochemical parameters immediately after the disturbance, these effects do not persist, and natural processes predominate over longer time scales.

### **Acknowledgements**

This study was carried out as a part of ‘Environmental impact assessment for nodule mining in the Central Indian Basin’ funded by the Department of Ocean Development (Government of India). We acknowledge the technical assistance rendered by Ms. Sonali Naik, Ms. Sapana Nabar and Ms. Salita Benaulikar. We thank the captain and the crew members of the RV *Yuzmorgeologiya* for their assistance on board the research vessel. We are thankful to Dr. A. Gooday and the other 2 anonymous reviewers for improving our manuscript. This is NIO contribution No. 4169.

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## CAPTION OF FIGURES

Fig.1. a) Study area A1 with reference and test sites. Stations designated BC 194 and BC 193 during the baseline sampling phase (PreD) were designated 194 M and 193 M during the monitoring cruise PoD2. b) An enlargement of the test site with disturbance zone and sampling locations. The centre disturbed track with 3 stations (BC 5, 3 and 2), the northern track (BC 13, 8 and 11) and the southern track (BC 14, 7 and 16).

Fig. 2. Total counts (TC) of bacteria  $g^{-1}$  dry sediment in PreD (o), PoD1 (●) and PoD2 (Δ) phases at a depth of 0-10 cm. The three rows correspond to cores taken to the north (top row) and south (bottom row) of the disturbance track and from the disturbance track itself (centre row). The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 3. The living biomass represented as sediment ATP ( $ng\ g^{-1}$  dry sediment). Symbols, sampled depths and locations of cores are the same as in Fig. 2. The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 4. Protein concentrations ( $mg\ g^{-1}$  dry sediment). Symbols, sampled depths and locations of cores are the same as in Fig. 2. The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 5. Carbohydrate (CHO) concentrations ( $mg\ g^{-1}$  dry sediment). Symbols, sampled depths and locations of cores are the same as in Fig. 2. The

horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 6. Lipid concentrations in  $\text{mg g}^{-1}$  dry sediment. Symbols, sampled depths and locations of cores are the same as in the Fig. 2. The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 7. Labile Organic Matter (LOM) as  $\text{mg g}^{-1}$  dry sediment. Symbols, sampled depths and locations of cores are the same as in Fig. 2. The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 8 Total organic matter (TOM) as  $\text{mg g}^{-1}$  dry sediment. Symbols, sampled depths and locations of cores are the same as in Fig. 2. The SD values were extremely low in some cases and therefore the SD bars are not evident. The sediment sample from core # 13 of the PreD was not analyzed for TOM, and core #11 of the PoD1 stage was lost on retrieval.

Fig.9. Aminopeptidase activity in Units, where one U represents release of  $1\mu\text{mol MCA h}^{-1} \text{g}^{-1}$  dry sediment at  $4^{\circ} \text{C}$ . Symbols, sampled depths and locations of cores are the same as in Fig. 2. The horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 10. Alkaline phosphatase activity in Units, where 1U is equivalent to  $\mu\text{mol}$  of phosphate phosphorus released  $\text{h}^{-1} \text{g}^{-1}$  dry sediment at  $4^{\circ} \text{C}$ . Symbols, sampled depths and locations of cores are the same as in Fig. 2. The

horizontal bars represent SD values. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 11. Lipase activity in Units, where one Unit is equivalent to release of 1  $\mu\text{mol}$  MUF released  $\text{h}^{-1} \text{g}^{-1}$  dry sediment at  $4^{\circ} \text{C}$ . Symbols, sampled depths and locations of cores are the same as in Fig. 2. The horizontal bars represent SD values. As the enzyme values showed a large variation between different stages of sampling, the x-axis scale is different for some of the core stations. The sediment sample of core #11 of the PoD1 stage was lost on retrieval.

Fig. 12. Comparison of the bacterial numbers, ATP and LOM concentrations at the undisturbed reference site. The left-hand column (a) refers to 193 (o) and 193M ( $\Delta$ ), and the right-hand column (b) refers to 194 (o) and 194M ( $\Delta$ ). The box-core samples BC 193 and BC 194 were collected during baseline studies, whereas 193M and 194M were collected during the PoD2 phase. Because of the extremely low concentrations of ATP, the values on the x axis could not be plotted to the same scale as those in Fig. 3.

Fig. 13. Comparing the protein, carbohydrate and lipid values at the undisturbed reference site. The left-hand column (a) refers to 193 (o) and 193M ( $\Delta$ ), and the right-hand column (b) refers to 194 (o) and 194M ( $\Delta$ ). The box core samples BC 193 and BC 194 were collected during the baseline studies, whereas 193M and 194M were collected during the PoD2 phase.

**Table 1.** Details of the box corer sampling and locations during various stages in the Central Indian Basin.

<b>Sampling stage</b>	<b>Box core #</b>	<b>Latitude (S)</b>	<b>Longitude (E)</b>	<b>Depth (m)</b>
<b>Baseline sampling at the reference location (September, 1996)</b>	BC 194	10°00'.00"	76°04'.72"	5316
	BC 193	10°10'.11"	76°05'.00"	5290
	BC 197	10°00'.00"	75°54'.95"	5350
	BC 190	10°10'.28"	75°55'.35"	5453
	BC 188	10°04'.50"	76°00'.04"	5435
<b>Monitoring (April 2001) at the reference location (PoD2)</b>	BC 194M	10°00'.00"	76°04'.72"	5316
	BC 193M	10°10'.11"	76°05'.00"	5290
<b>PreD (June '97), PoD1 (Aug '97) &amp; PoD2 (April 2001)</b>				
<b>North of the disturbed track</b>	BC 13	10°01' 522"	76°00' 341"	5320
	BC 8	10°01' 795"	76°00' 753"	5320
	BC11	10°02' 105"	76°01' 236"	5330
<b>Disturbed track in the centre</b>	BC 5	10°01' 787"	76°00' 122"	5320
	BC 3	10°02' 056"	76°00' 569"	5320
	BC 2	10°02' 313"	76°01' 067"	5320
<b>South of the disturbed track</b>	BC 14	10°02' 172"	76°00' 120"	5330
	BC 7	10°02' 313"	76°00' 470"	5330
	BC 16	10°02' 606"	76°00' 947"	5330

PreD=pre-disturbance; PoD1=post-disturbance phase 1; PoD2= post-disturbance phase 2; BC=box corer



**Table 2.** Average values of the various measured parameters from all nine cores down to 10 cm depth normalized to g<sup>-1</sup> dry sediment.

<b>Variables</b>	<b>Pre-disturbance</b>	<b>Post-disturbance 1</b>	<b>Post-disturbance 2</b>
<b>TC (Nos)</b> <b>SD</b>	2.0x10 <sup>9</sup> ± 2.2x 10 <sup>7</sup>	4.3x10 <sup>7</sup> ± 2.2 x 10 <sup>6</sup>	1.5x10 <sup>8</sup> ± 2.2x 10 <sup>6</sup>
<b>ATP (ng)</b> <b>SD</b>	55.1 ± 1.2	3.8 ± 0.1	0.1 ± 0.009
<b>LOM (mg)</b> <b>SD</b>	0.7 ± 0.006	0.3 ± 0.003	2.2 ± 0.02
<b>Carbohydrates (mg)</b> <b>SD</b>	0.15 ± 0.002	0.12 ± 0.002	0.29 ± 0.005
<b>Proteins (mg)</b> <b>SD</b>	0.33 ± 0.002	0.07 ± 0.001	0.43 ± 0.016
<b>Lipids (mg)</b> <b>SD</b>	0.26 ± 0.004	0.16 ± 0.002	0.16 ± 0.09
<b>TOM (mg)</b> <b>SD</b>	5.5 ± 0.6	6.7 ± 0.07	4.5 ± 0.03
<b>Bacterial protein (mg)</b> <b>SD</b>	0.06 ± 0.0	0.001 ± 0.0	0.005 ± 0.0
<b>Bacterial carbon (mg)</b> <b>SD</b>	0.04 ± 0.0	0.001 ± 0.0	0.003 ± 0.0
<b>Protease (μM g<sup>-1</sup> h<sup>-1</sup>)</b> <b>SD</b>	0.03 ± 0.001	0.05 ± 0.001	0.003 ± 0.0
<b>Phosphatase (μM g<sup>-1</sup> h<sup>-1</sup>)</b> <b>SD</b>	4.3 ± 0.06	7.8 ± 0.16	0.06 ± 0.02
<b>Lipase (μM g<sup>-1</sup> h<sup>-1</sup>)</b> <b>SD</b>	5.0 ± 0.16	5.8 ± 0.16	0.02 ± 0.0
<b>Organic P (%)</b> <b>SD</b>	0.007 ± 0.0	0.0073 ± 0.0	0.011 ± 0.0

TC= total bacterial counts; SD= standard deviation; ATP=adenosine triphosphate; LOM=labile organic matter; TOM=total organic matter

**Table 3.** Percentages of individual components contributing to labile organic matter during the three stages of sampling. The values from all nine cores (5 subsections each) were added to arrive at their concentrations in the upper 10 cm.

<b>Variables</b>	<b>Pre- disturbance (PreD)</b>	<b>Post- disturbance 1 (PoD1)</b>	<b>Post- disturbance 2 (PoD2)</b>
<b>Carbohydrates</b>	20.2	33.5	13.3
<b>Protein</b>	44.4	20.0	78.0
<b>Lipids</b>	35.0	45.0	7.3
<b>Protein/Carbohydrate ratio</b>	2.2	0.59	5.9
<b>ATP</b>	7.6	0.01	6.04
<b>Bacterial protein</b>	8.6	0.4	0.21
<b>Bacterial carbon</b>	5.5	0.3	0.13

**Table 4.** Results of one-way ANOVA between 3 cores each in the north, center and south of the disturbance. F-critical value is 3.9. Values above the F-critical values are considered statistically significant.

Variable	North (Core # 13, 8,11)		Centre (Core # 5, 3, 2)		South (core # 14, 7,16)	
	F	P-value	F	P-value	F	P-value
<b>TC</b>						
PreD	1.9	0.2	0.6	0.6	<b>7.8</b>	<b>0.007</b>
PoD1	<b>22.2</b>	<b>9.2E-05</b>	2.2	0.2	<b>15.6</b>	<b>0.0005</b>
PoD2	2.2	0.2	<b>10.8</b>	<b>0.002</b>	1.5	0.3
<b>ATP</b>						
PreD	<b>24.8</b>	<b>5.5E-05</b>	<b>16.6</b>	<b>0.0003</b>	1.6	0.01
PoD1	0.3	0.8	0.2	0.8	<b>6.9</b>	<b>0.01</b>
PoD2	3.1	0.08	<b>5.3</b>	<b>0.02</b>	<b>36.4</b>	<b>8E-06</b>
<b>LOM</b>						
PreD	0.8	0.5	<b>14.9</b>	<b>0.0006</b>	<b>16.7</b>	<b>0.0003</b>
PoD1	0.4	0.7	2.1	0.2	<b>220</b>	<b>3.5E-10</b>
PoD2	2.4	0.1	<b>5.2</b>	<b>0.02</b>	<b>4.2</b>	<b>0.04</b>
<b>Lipids</b>						
PreD	0.8	0.5	<b>17.4</b>	<b>0.0003</b>	<b>6.8</b>	<b>0.01</b>
PoD1	<b>4.1</b>	<b>0.04</b>	<b>4.8</b>	<b>0.03</b>	<b>86.7</b>	<b>7.4E-08</b>
PoD2	<b>40.8</b>	<b>4.5E-06</b>	1.8	0.2	<b>5.4</b>	<b>0.02</b>
<b>Carbohydrates</b>						
PreD	<b>11.1</b>	<b>0.002</b>	1.6	0.3	<b>74.7</b>	<b>1.7E-07</b>
PoD1	1.3	0.3	<b>4.6</b>	<b>0.03</b>	<b>17.1</b>	<b>0.0003</b>
PoD2	3.7	0.06	1.4	0.3	0.8	0.5
<b>Protein</b>						
PreD	1.5	0.3	<b>4.6</b>	<b>0.03</b>	1.5	0.3
PoD1	3.4	0.07	0.3	0.8	<b>55.7</b>	<b>8.5E-07</b>
PoD2	3.2	0.02	<b>4.9</b>	<b>0.03</b>	<b>11.9</b>	<b>0.001</b>
<b>TOM</b>						
PreD	<b>77.2</b>	<b>1.4E07</b>	<b>7.3</b>	<b>0.008</b>	0.4	0.7
PoD1	<b>82.2</b>	<b>9.9E-08</b>	1	0.4	3.6	0.06
PoD2	1.1	0.4	0.6	0.6	<b>7.3</b>	<b>0.008</b>
<b>P</b>						
PreD	<b>7.25</b>	<b>0.027</b>	2.53	0.12	0.083	0.78
PoD1	0.4	0.54	<b>8.65</b>	<b>0.005</b>	<b>107.27</b>	<b>6.5E-06</b>
PoD2	<b>11.58</b>	<b>0.009</b>	<b>5.48</b>	<b>0.02</b>	0.95	0.36

Values in the bold denote statistically significant spatial variations at the experimental site.

**Table 5.** Percentage variations contributed by each term (stage, sector and core) in the 3-Way- nested ANOVA carried out for 9 different parameters.

<b>Variables</b>	<b>Stage (Temporal)</b>	<b>Sector (Spatial)</b>	<b>Core</b>
<b>TC</b>	95	4.5	<0.5
<b>ATP</b>	98	<0.1	1.9
<b>Lipids</b>	97	<0.5	2.6
<b>Carbohydrates</b>	72	<0.5	28
<b>Protein</b>	40	12	48
<b>TOM</b>	12	32	56
<b>Protease</b>	36	22	42
<b>Lipase</b>	86	10	4
<b>Phosphatase</b>	96	3	1

Stage refers to the three sampling phases, sectors to center

(the disturbed track), north and south of disturbance.

**Table 6.** Results of two-way ANOVA to test the difference between the cores in different tracks (spatial variation within each stage) for the extracellular enzymes measured in the sediments collected during pre-disturbance (PreD), post-disturbance 1 (PoD1) and post-disturbance 2 (PoD2) stages of sampling. As there was no significant difference between depths in the cores, these values are not shown here. F-values more than the F-critical values denote significance and are shown in bold here.

	North (Core # 13,8,11)			Centre (Core # 5,3,2)			South (Core # 14,7,16)		
	F-value	F-crit	P-value	F-value	F-crit	P-value	F-value	F-crit	P-value
<b>Protease</b>									
PreD	1.2	4.5	0.35	1.7	4.5	0.2	1.5	4.5	0.29
PoD1	<b>6.5</b>	4.5	<b>0.02</b>	<b>13.4</b>	4.5	<b>0.003</b>	<b>5.3</b>	4.5	<b>0.03</b>
PoD2	<b>7.1</b>	4.5	<b>0.02</b>	1.2	4.5	0.35	<b>114.3</b>	4.5	<b>1.3E.06</b>
<b>Phosphatase</b>									
PreD	2.1	4.5	0.02	<b>5.6</b>	4.5	<b>0.03</b>	<b>16.4</b>	4.5	<b>0.001</b>
PoD1	<b>38.0</b>	4.5	<b>8.2E.05</b>	<b>41.2</b>	4.5	<b>6.2E.05</b>	<b>11.7</b>	4.5	<b>0.004</b>
PoD2	<b>6.8</b>	4.5	<b>0.02</b>	0.96	4.6	0.4	<b>10.9</b>	4.5	<b>0.005</b>
<b>Lipase</b>									
PreD	<b>313</b>	4.5	<b>2.5E.08</b>	1.5	4.5	0.3	<b>6.9</b>	4.5	<b>0.02</b>
PoD1	<b>623</b>	4.5	<b>1.7E.09</b>	<b>978</b>	4.5	<b>2.8E.10</b>	<b>61.2</b>	4.5	<b>1.4E.05</b>
PoD2	<b>18.5</b>	4.5	<b>0.001</b>	<b>5.2</b>	4.5	<b>0.04</b>	<b>6.5</b>	4.5	<b>0.02</b>

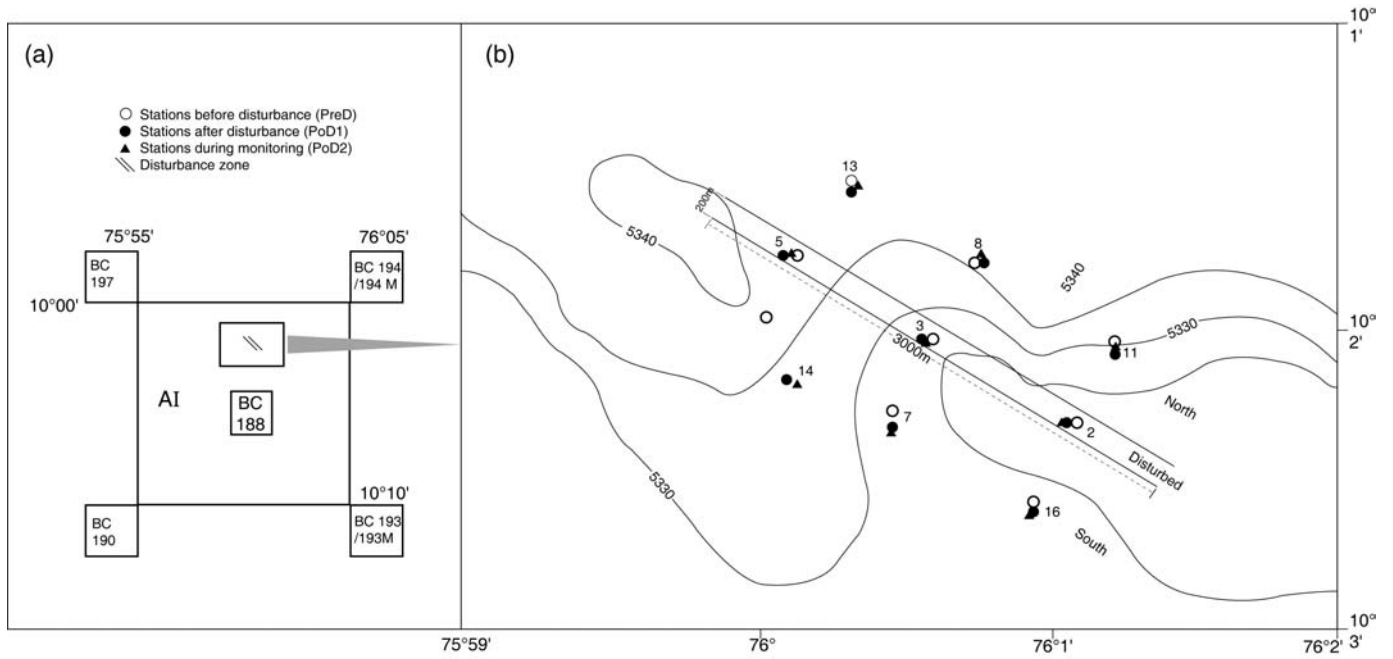
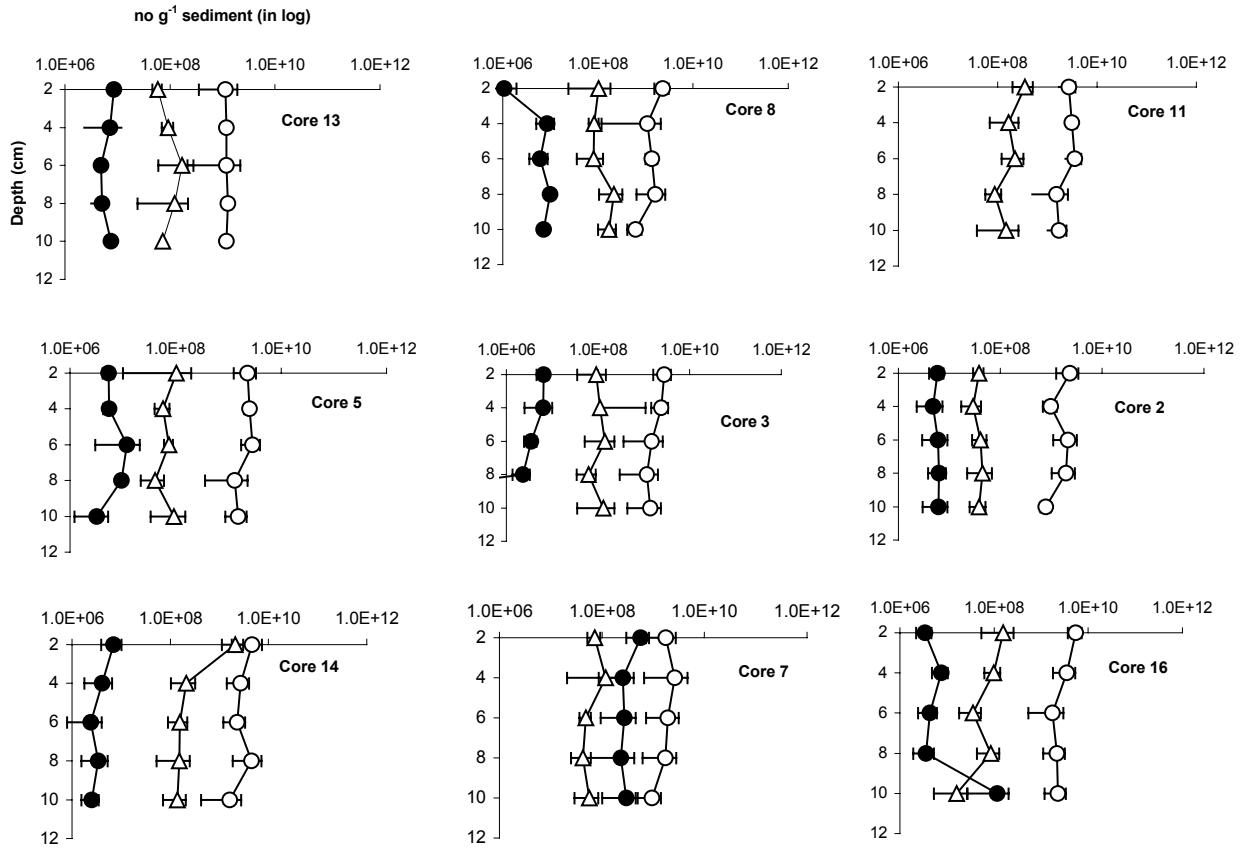
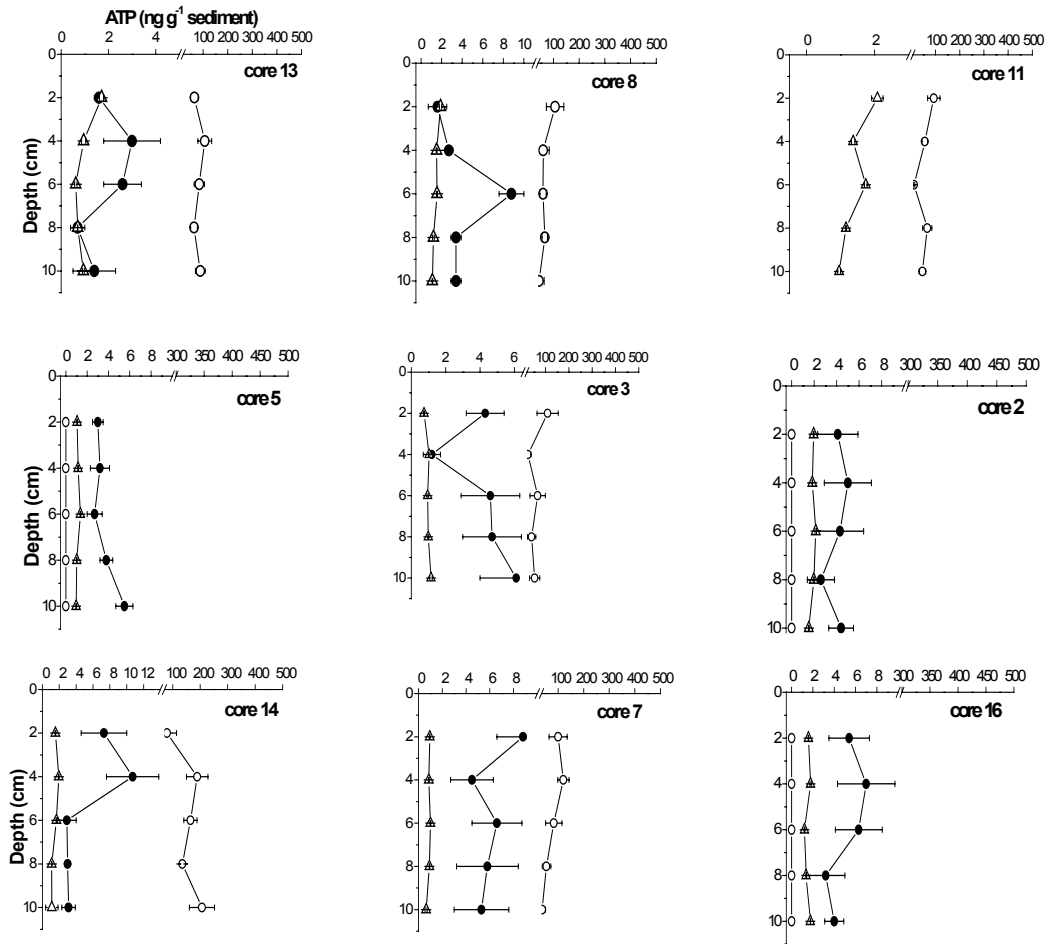


Fig.1:



**Fig.2**



**Fig.3**



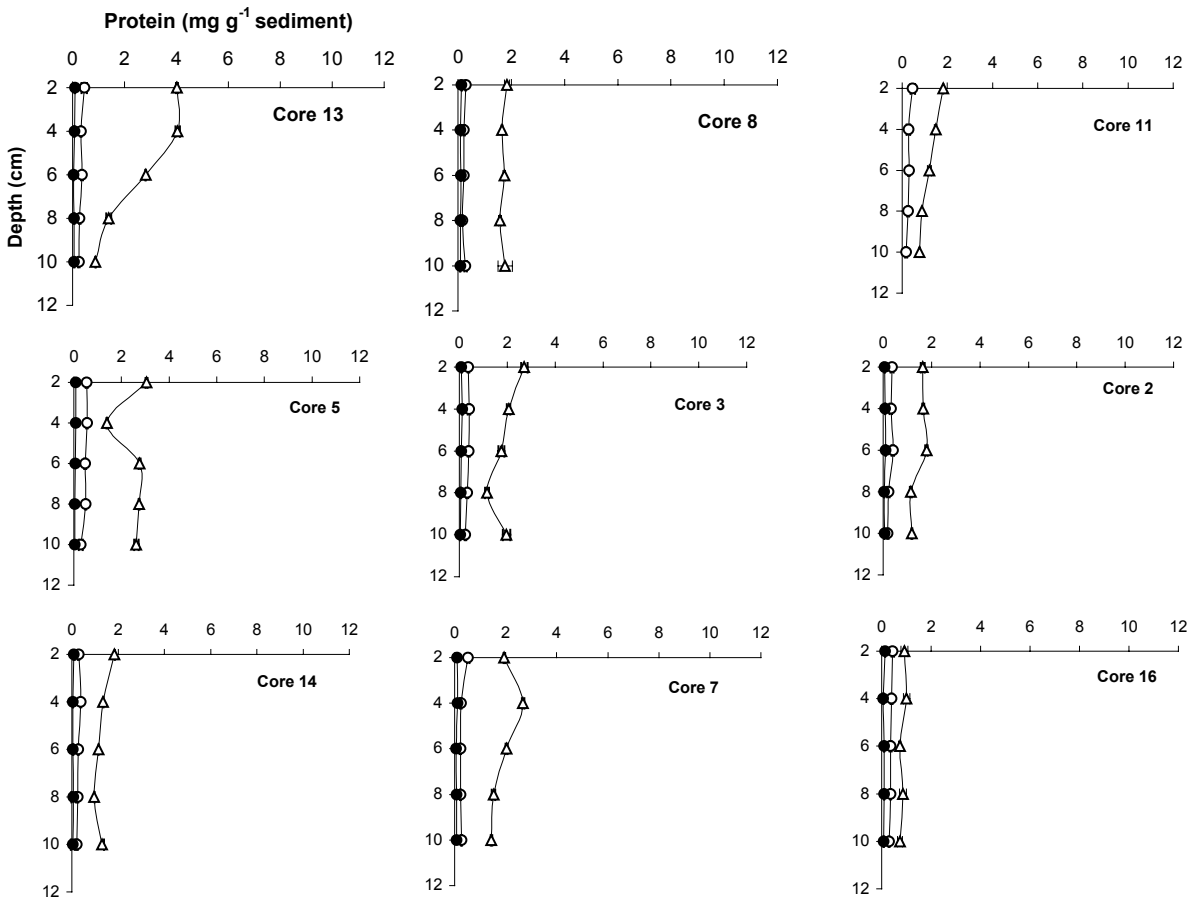
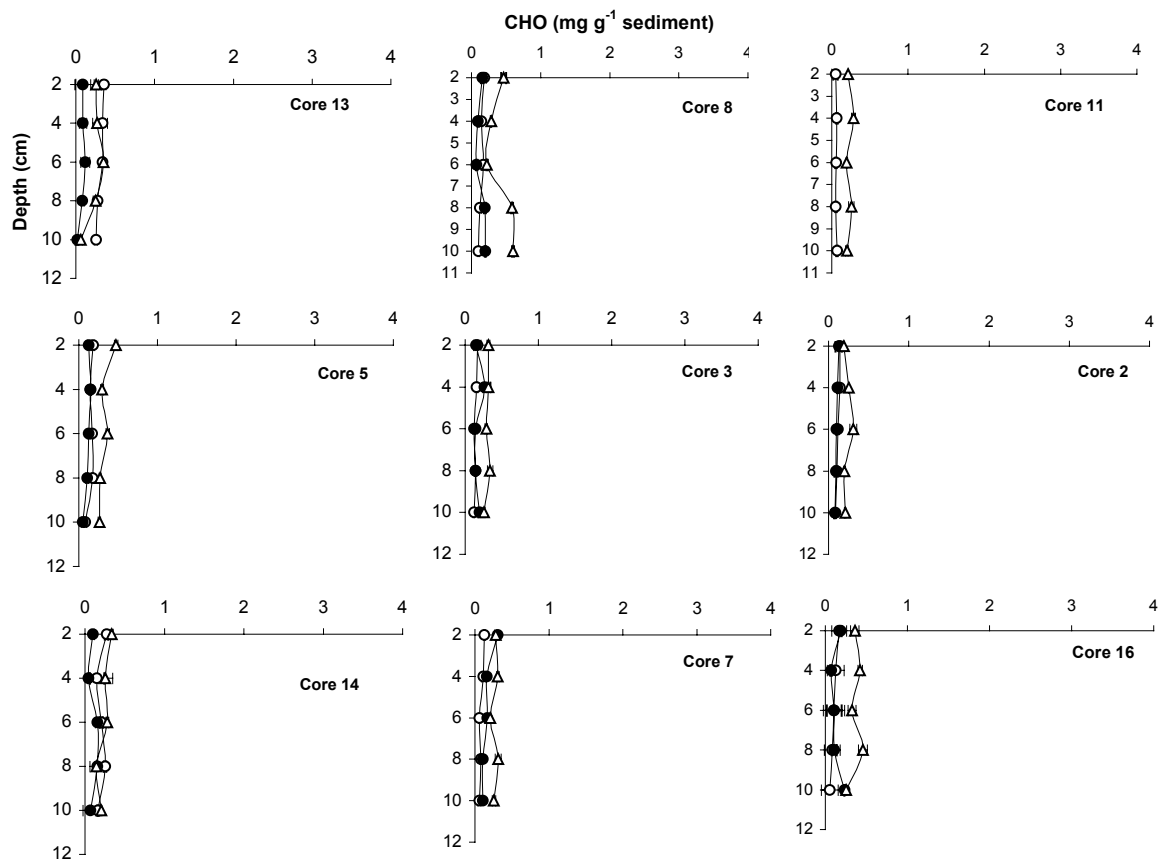
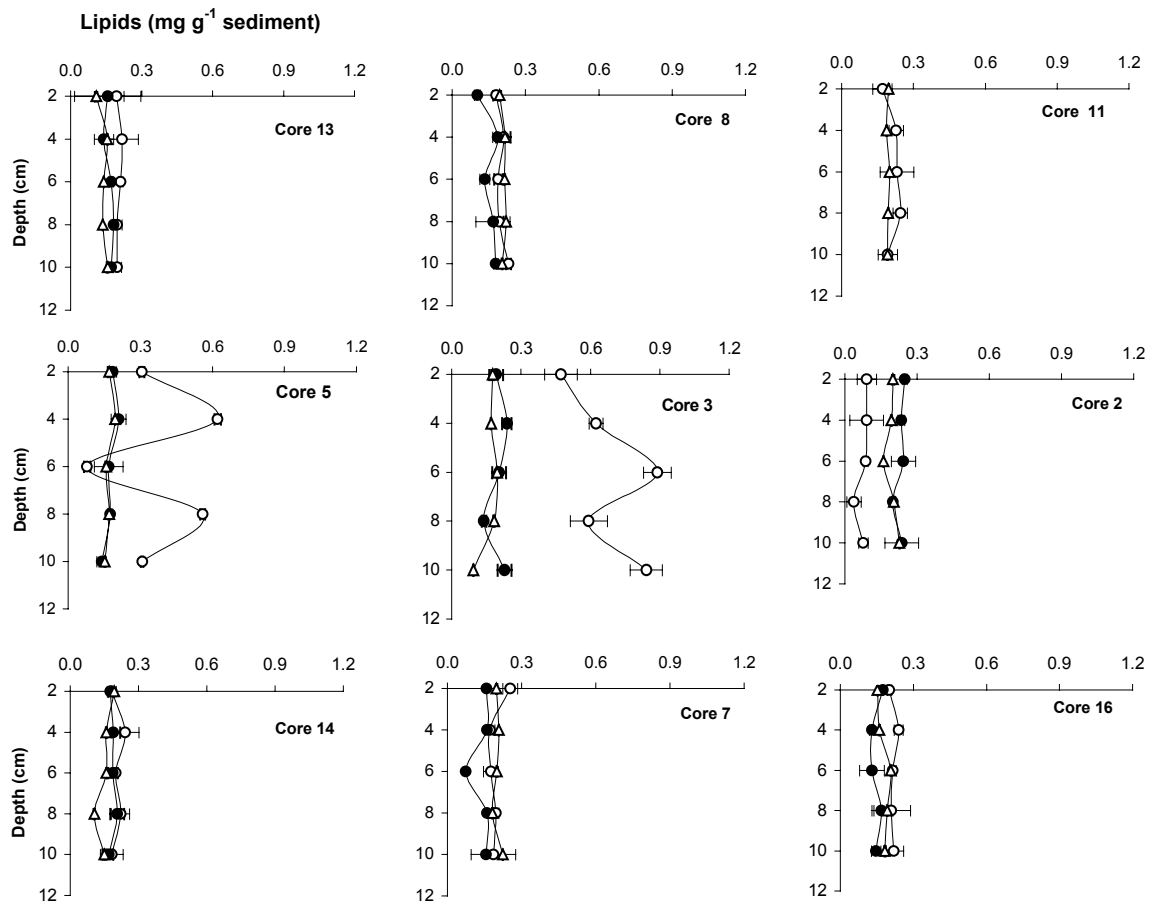


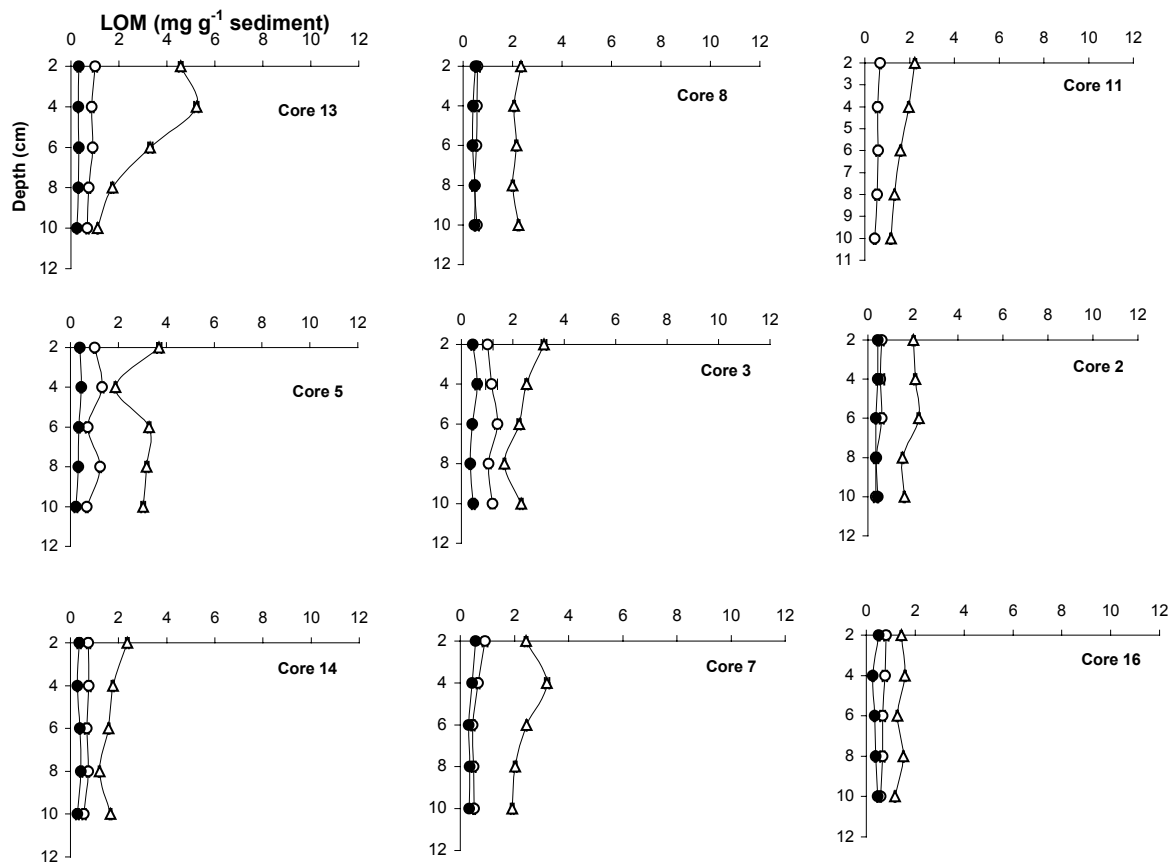
Fig.4



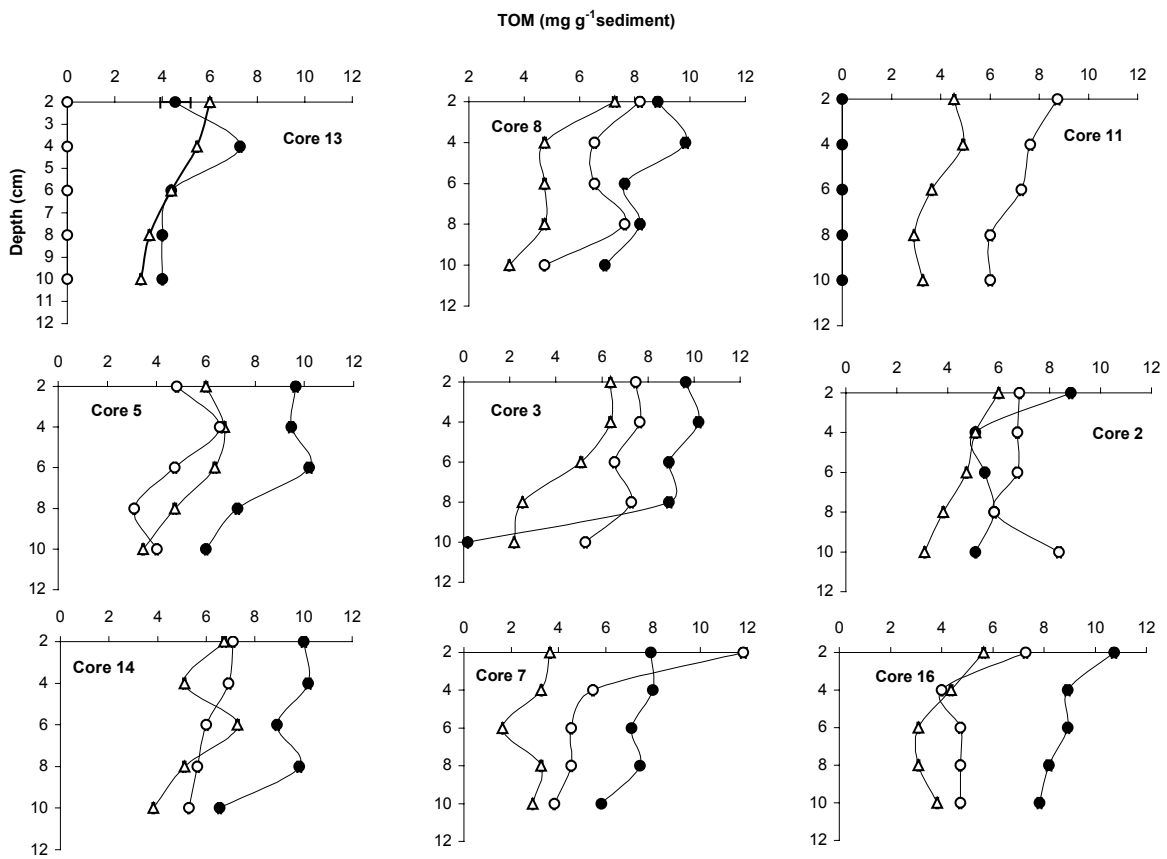
**Fig.5**



**Fig.6**



**Fig.7**



**Fig.8**

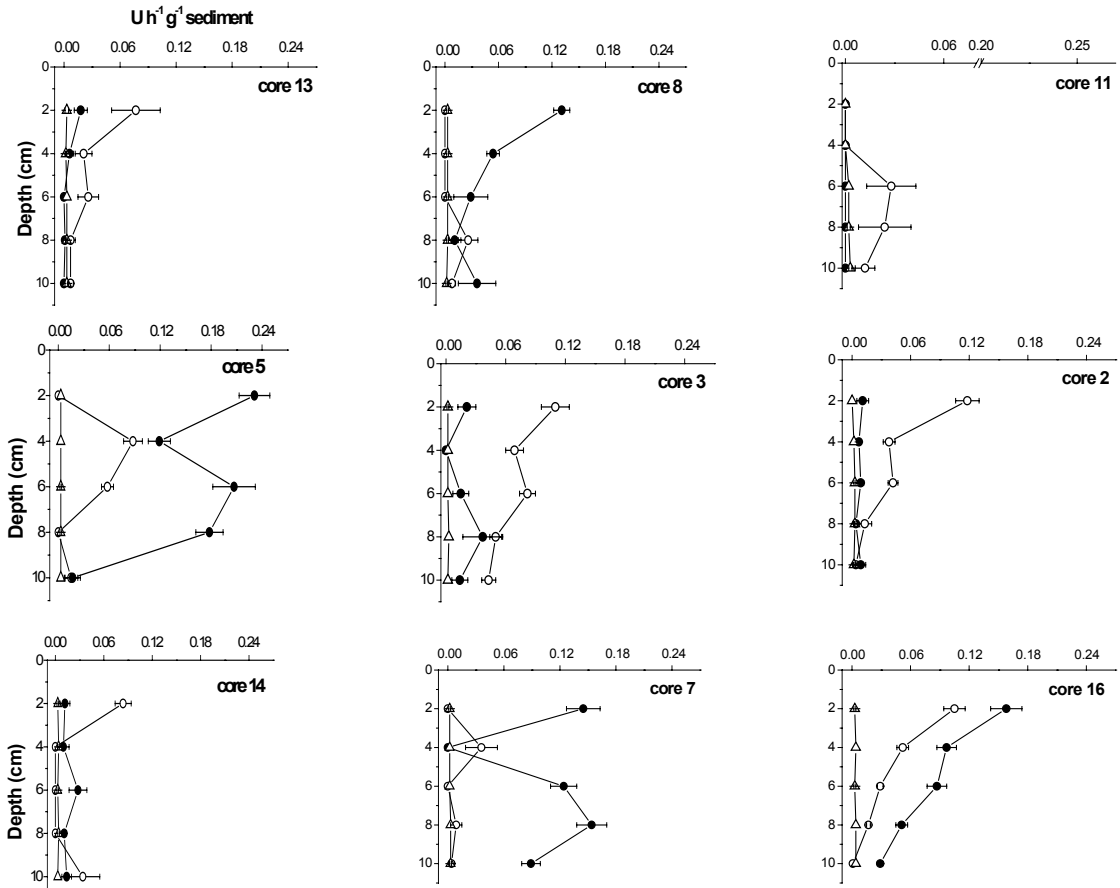
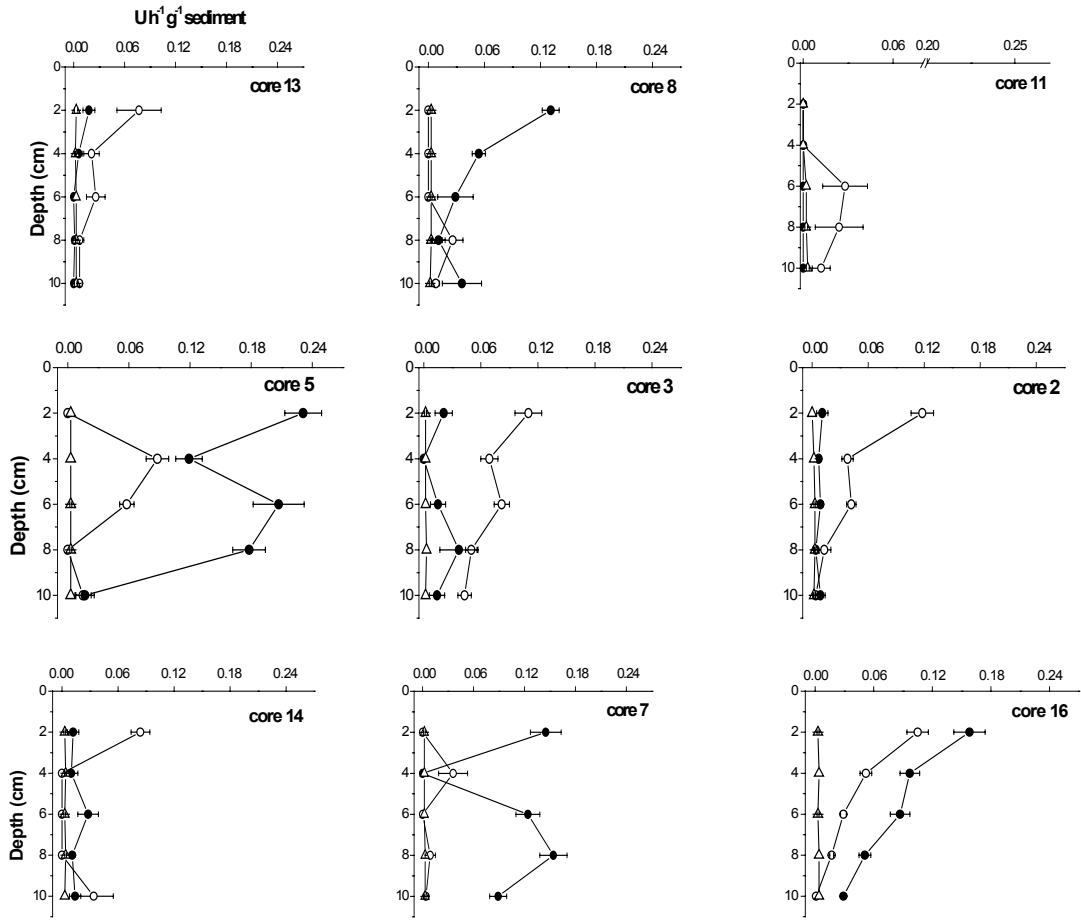


Fig.9



**Fig.10**

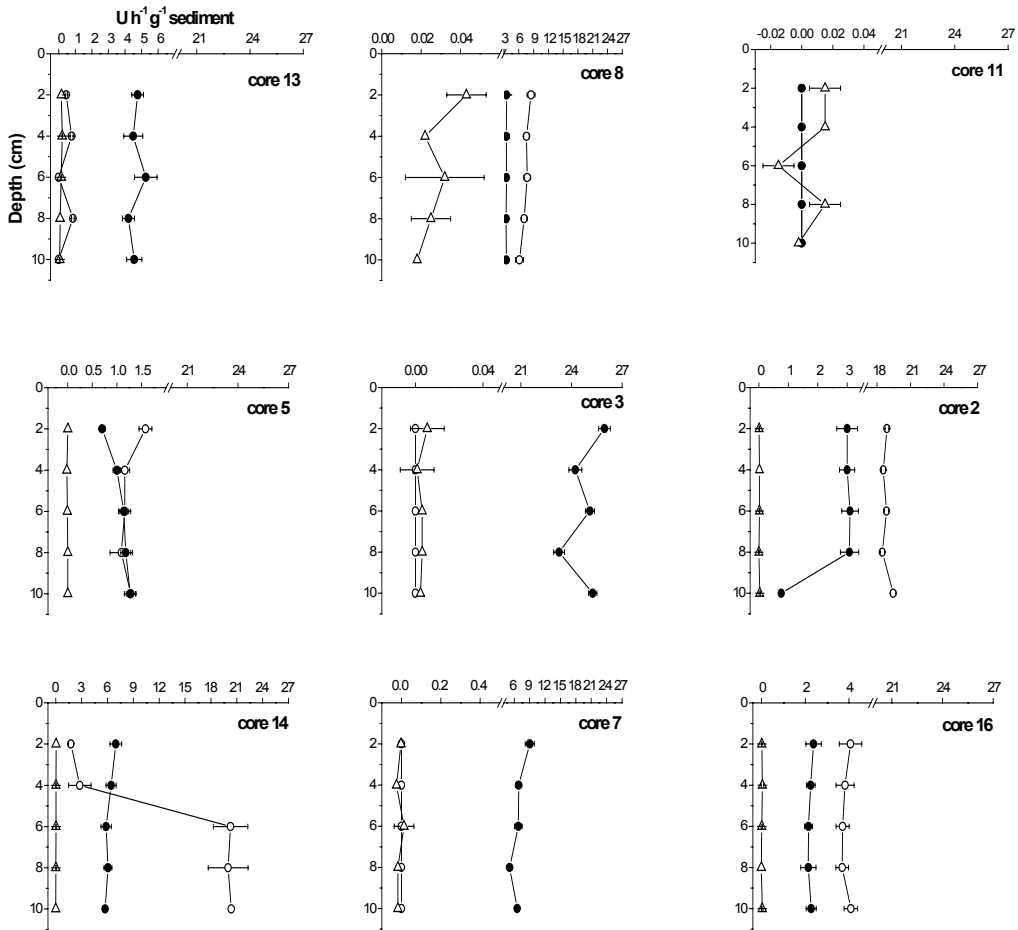
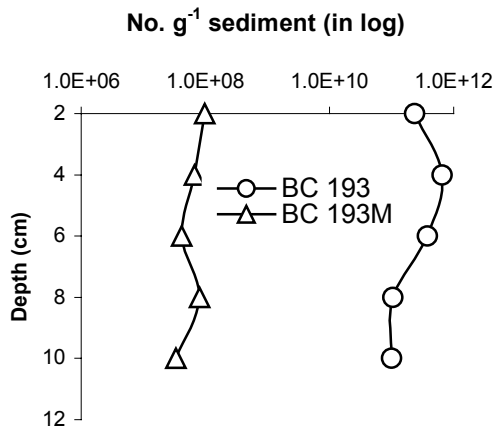


Fig.11

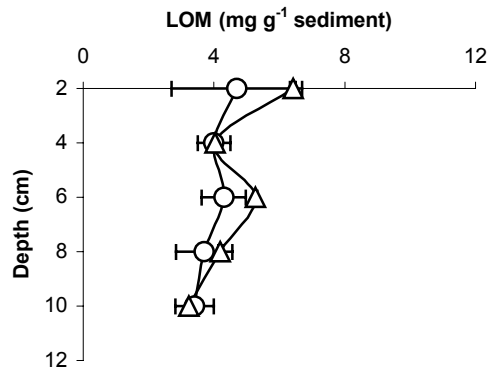
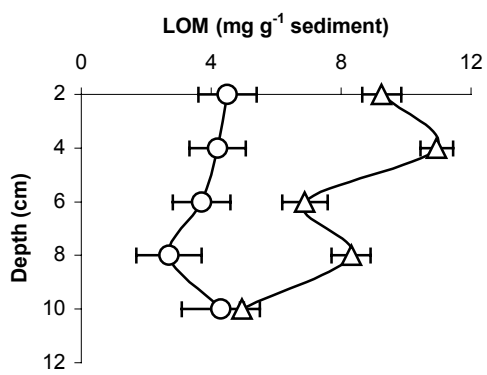
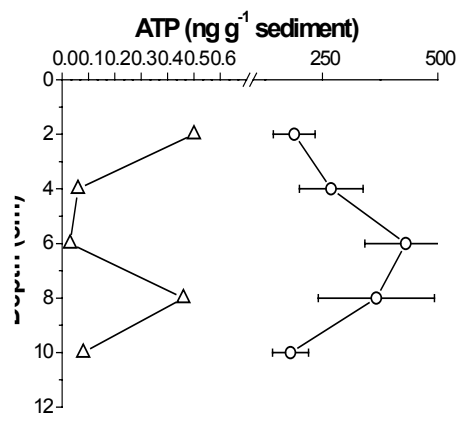
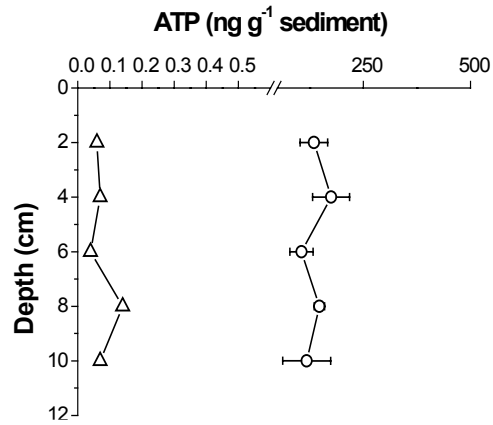
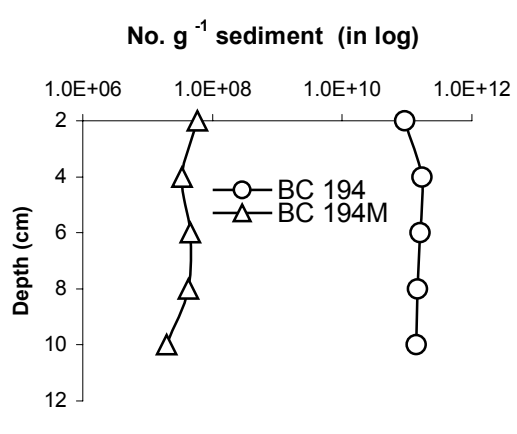


Fig.12

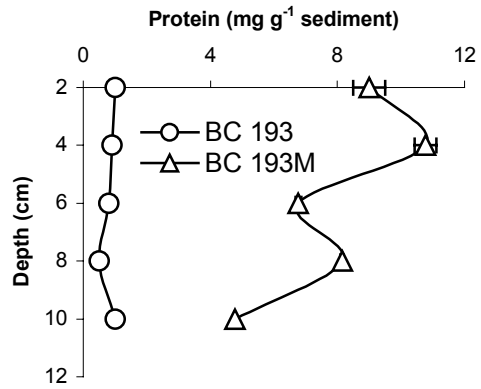
(a)



(b)



**Fig.13 (a)**



**(b)**

