# Effect of oil spill on the microbial population in Andaman Sea around Nicobar Island

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### **ABSTRACT**

The microbial studies of the follow up cruise by FORV Sagar Sampada (cruise No. 113), 9 months after the oil spill in the Andaman Sea due to accident of VLCC Maersk Navigator revealed disturbance in the natural microbial population. Higher abundance of heterotrophic microorganisms was observed at several coastal stations as well as from greater depths. Oelophilic microorganisms were fairly abundant at some of the stations especially st. 2991 at 400 m and 500 m depths. The presence of oelophilic microorganisms at different stations and greater depths could be indicative of the remanants of the oil spill.

#### INTRODUCTION

Seawater contains a range of microorganisms bacteria and fungi, which can utilize oil or a group of hydrocarbons as a source of carbon and energy. Although these microorganisms are not present in sufficient numbers in open sea, they multiply rapidly and increase in number when such substrates are available to them. Thus the presence of these organisms in an environment may indicate contamination of the environment with oil spill and they can be taken as indicator organisms (Gupta, 1990).

Microbial studies of the oil spills in the marine environment has been carried out on some of the major oil spills (Ahearn & Crow, 1980; Atlas, 1981; Atlas & Bronner, 1981). The most studied ones are of the super tanker Amoco Cadiz which grounded near Portsal, France (1978) and IXTOC I spill. In 1980 Atlas *et al.* (1980) reviewed the environmental impact of accidental oil spill in the oceans. Tanker 'Arrow' went aground and it was pronounced even after 5 years of the incident (Vandermeulan & Gordon, 1976).

Danish VLCC Maersk Navigator carrying 300000 tonne of light crude from Oman to Japan collided with Singapore registered tanker Sanko Honour on 21st January, 1993, while negotiating the narrow passage through the small Indonesian

Islands at the entry point of Malacca strait. The impact ruptured one of its tankers and an estimated 18000 tonne of oil spilled on the sea for several days. Altered by the news that the oil patch was just 16 nautical miles south of Indira Point (IP, Fig.1), survey on its environmental impact was undertaken soon, onboard *ORV Sagar Kanya* (cruise No. 81). This paper presents the results of the microbiological studies during *FORV Sagar Sampada* cruise No. 113 which was the follow up cruise of the former with the objective to examine any eventual permanent ecological damage caused to the marine environment by the above oil spill. Microbiological studies of the water samples from

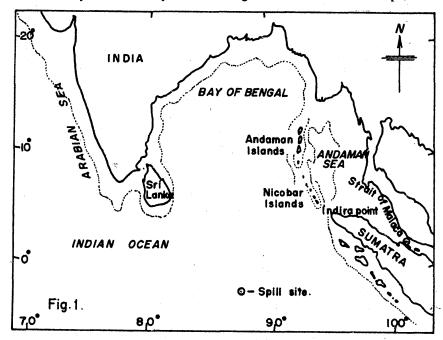


Fig.1 — Oil spill site

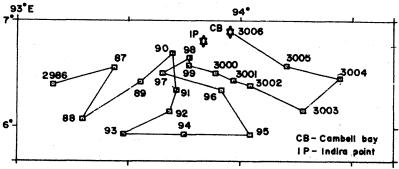


Fig.2 — Station locations in oil spill site

different depths of the water column at 9 stations of Indian EEZ (Fig. 2) in the Andaman Sea (where oil patch was sighted), south of Great Nicobar Island were done. Sediment samples from 6 shallow stations during the cruise and also from the beaches and mangrove area of the Great Nicobar Island were taken for microbiological studies, decaying pneumatophores from mangrove area were also collected. Samples were also screened for the presence of oelophilic microorganisms.

### MATERIALS AND METHODS

Water samples were collected from 9 stations during the cruise (Fig. 2) in Teflon lined well cleaned Rosette sampler from surface down to 50 m depth except at the station no. 2991, the water samples were collected from 400 m and 500 m depths where turbid bands on the CTD screen were observed. From the same station water sample from 300 m depth was collected as reference sample for the former two samples. Sediment was collected by a modified Peterson's grab. Samples from the centre of the sediment lump was taken out asceptically into presterlized plastic bags. Sediments and decaying pneumatophore samples from Magar Nala mangrove and the beaches of Great Nicobar Island were also collected in presterlized plastic bags. Beach no. 1 was 8 km from Campbell Bay (CB) of the Great Nicobar Island and Beach no. 2, 18 km from CB (Fig.2).

Immediately after collection, water samples were filtered (concentrated) on 0.22  $\mu m$  porosity filter membranes (5 ml for bacteria) and on 0.45  $\mu m$  porosity filter membranes (100 ml) for fungi. For viable plate counts of total heterotrophic bacteria each of the membrane was placed on Zo-Bells's Marine agar plates (Floodgate, 1984) while for fungi plates with nutrient agar were used with antibiotics to prevent bacterial growth (Uden & Fell, 1968).

For the study of oelophilic microbial population, the filter membranes were impregnated with 1 ml of filter sterilized hydrocarbon (Diesel or 2T super lube oil) (Sorokh *et al.* 1981). The plates were incubated at room temperature  $25^{\circ} \pm 1^{\circ}$ C, but for the samples collected at st. no. 2991 at 300 m, 400 m and 500 m depths plates were incubated at  $10^{\circ}\pm1^{\circ}$ C (*in situ* temperature).

For each sediment sample 1 g of the sample was asceptically taken out from the plastic bag inside a sterile chamber and shaken well with 100 ml presterlized (autoclaved) seawater and allowed to stand for 10 minutes to let the sediment particles settle down. The supernatant was then concentrated on millipore filter membranes of 0.22  $\mu$ m porosity for bacteria and 0.45  $\mu$ m for fungi. These filter membranes were placed in plates on nutrient media for bacteria and fungi respectively as stated earlier. All the plates were incubated at room temperature, for 4 days for bacteria and 7 days for fungi. Pneumatophore pieces were washed well with presterlized seawater and planted on nutrient media and incubated at room temperature. Each sample was run in triplicate and for each set blank plates were prepared and incubated in triplicate as controls. The results are the mean of the triplicate samples. Microbial counts are expressed as colony forming units (cfu)/litre of seawater for water samples and cfu/g of sediment samples.

## RESULTS AND DISCUSSION

The nearshore sts. 2986, 2997, 2998, 3004 and 3005 had higher microbial population (Table 1). There were a few oelophilic bacterial and fungal patches at sts. 2986, 2987, 2997, 2998 and 3005 but they showed in high numbers at all the three depths of st. 3004.

Table 1 - Microbial cell density (cfu/litre) of water samples at different stations of FORV Sagar Sampada cruise 113

	Position		Depth	Bacteria	Fungi	Oelophilic	
St. no.	Lat.(N)	Long.(E)	(m)	(cfu)	(cfu)	Bacteria (cfu)	Fungi (cfu)
2985	05°45'	92°00'	1	2500	10	-	- <u>-</u>
		. •	25	100	110	-	
			50	500	20		• -
2986	06°24'	93°06'	1	4000	250	50	120
			40	500	190	60	<b>-</b> ,
2987	06°34.2'	93°26.25'	1	2600	1000	-	-
			25	6400	5000	20	10
			50	4280	50	· -	-
2988	06°05'	93°18'	. 1	3000	30	-	-
			25	2000	50	-	-
			50	1800	90	-	-
2991	06°41.9'	93°42.76'	300	420	- '	-	-
			400	5000	-	1120	<b>.</b>
			500	6000	-	2020	
2997	05°56.41'	94°3.58'	1	10000	2000	70	-
			25	8000	330	-	-
			50	5000	60	70	-
2998	06°21'	93°55.6'	1	10000	160	-	•.
			25	5000	1500	150	-
			50	6000	220	160	60
3004	06°28.3'	94°30.15'	1	10000	1060	1000	200
			25	400	800	6000	360
			50	1700	250	3000	300
3005	06°34'	94°12'	1	8200	160	60	-
			25	7000	480	150	50
			. 50	7000	60	60	70

At st. 2991 high turbidity was observed on CTD screen at 400 m and 500 m depths but sonar system could not detect anything, thus, as they were expected, bands were of bacterioplankton with 5000 cfu and 6000 cfu and a good number of them (1120 and 2020 cfu) proved oelophilic in nature, while the samples from the reference depth 300 m at the same station had 420 cfu of bacteria and no fungus nor oelophilic microorganism.

Sediment samples were mostly coralline sand. At st. 2999 the samples were only big pieces of dead corals and had no sediments, so they were not processed. At sts. 2990, 2992, 3000, 3001 and 3006 (samples collected near the Campbell Bay) were full of corals but fine coralline sand was also present. These samples yielded up to 35 cfu of bacteria and up to 13 cfu of fungi per gram of sediment sample of which some were oelophilic too (Table 2).

Table 2 - Microbial cell density (cfu/g) of sediment samples at different stations of Sagar Sampada cruise 113

	Position		Bacteria	Fungi	Oelophilic	
St. no.	Lat.(N)	Long.(E)	(cfu)	(cfu)	Bacteria (cfu)	Fungi (cfu)
2990	06°42′	93°43′	25	5	3	1
2992	06°42′	93°42′	15	2	-	-
3000	06°38′	93°47′	35	13	6	4
3001	06°36′	93°47′	3	6	-	-
3006	opp: C.B		18	8	2	-
	<del> </del>	C.B - 0	Campbell Bay	(Fig. 2)		

Table 3 - Microbial cell density (cfu/g) of sediment samples of Great Nicobar Island

	Bacteria (cfu)	Fungi (cfu)	Oelophilic	
			Bacteria (cfu)	Fungi (cfu)
Mangrove				
Sample 1	150	30	-	-
Sample 2	80	15	-	•
Beach no. 1	26	15	-	-
Beach no. 2	150	6	100	8

Sample nos. 1 and 2 are from near the Magar Nala bridge Beach no.1 is 8 km from Campbell Bay from the Campbell Bay

Sediment samples from the mangrove area were clayey and provided high bacterial (up to 30 cfu/g) count but one was oelophilic. Beach no.1 was full of coralline sand and yielded some (26 cfu/g) of bacterial and (15 cfu/g) fungal but none oelophilic (Table 3). At beach no. 2 below the coralline sand was fine sand, this yielded good bacterial (150 cfu/g) population and a few fungal (6 cfu/g) with reasonable number of oelophilic (100 cfu/g) ones. Pneumatophores yielded heavy growth of celluloytic filamentous fungi, *Dendryphiella salina*, *Curvularia* sp. and *Fusarium* sp.

The appearance of oelophilic microbial population at particular stations and at different depths could well be indicative of the remnants of the oil patch transformed into tar balls, sinking at different rates in the water column (Sebastian, 1981). This may be taken as a change or disturbance in their natural population pattern due to the effect of the oil spill.

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