pollution and pathogens are believed to be some of the causal factors. It will be worth examining the relationship between the extreme incidence of bleaching observed by us in July 1998 in Andaman and the SST during the summer of this year. Similar high incidences of bleaching have been reported this year from Seychelles, Maldives, and Sri Lanka reefs (Jason Rubens and Thomas Goreau, pers. commun.). A species of *Vibrio* has been described recently to be causing bleaching of the coral *Oculina patagonica* in the coast of Israel[13]. Disturbances in biodiversity and the removal of predatory animals may result in increase in population of *Acanthaster planci*, the predator of corals[14]. Corals grazed by the star fish could be distinguished from bleached colonies. While the former had the trails of predation and a bare skeleton without polyps, the bleached colonies did not show any trails of predation and the corals still retained their polyps. The star fish was always associated with the grazed colonies.

Silt deposition was observed to cause a high incidence of coral mortality in the Gulf of Kutch, Gujarat. Increased construction activities and altered tidal currents in the areas could be a major cause for this. The deleterious effects of quarrying and mining on Indian coral reefs have been described by Patel[15], Rashid[16], and Wafar[17]. Most corals in this area were almost buried under silt. Intercolonial space between coral colonies in Paga reef revealed silt deposition of few mm to two feet in thickness.

Thus, during our survey of two years, we observed various factors which result in the different disease symptoms of corals. Table 2 summarizes the causes of coral mortality in different locations.

Global reef monitoring in the name of 'Reef check 97' (ref. 6) was carried out in 31 countries during the Year of Coral Reefs in 1997. India was not a participant in the event. Such monitoring programmes should be given priority as these help in assessing reef health and evolve long-term strategies to preserve and protect them.

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Copper resistance in *Candida guilliermondii* strain DS31

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***Candida guilliermondii* strain DS31 could resist copper (Cu) up to 25 mM on a complex medium. Cells turned bright blue on Cu(II)-amended medium and accumulated 5.5% Cu(II) on dry weight basis. While in the resistant strain DS31 most of the Cu(II) could be extracted by simple EDTA treatment, a Cu(II)-sensitive derivative CS2 retained significant amount of Cu(II) even after extraction with EDTA. Ultrastructural studies indicated deposition of electron-dense granules in the cell wall. Cu(II) also induced plasmolysis and vacuole formation. We report here morphological and ultrastructural changes induced by Cu(II) in the yeast strain. A possible mechanism of copper resistance has also been discussed.**

CONSIDERABLE amounts of Cu(II) are also released into the atmosphere through mining and other industrial processes as well as agricultural applications. Although Cu(II) is essential for synthesis of a number of enzymes and electron transfer proteins[1], high levels of it are toxic to micro-organisms. Microbes have therefore evolved

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several strategies to cope with toxic action of Cu(II)[2,3]. These mechanisms include efflux[4–6], sequestration[7,8], and extracellular complexation[9]. Copper-resistance has been demonstrated in a number of micro-organisms, including *Neurospora crassa*, *Saccharomyces cerevisiae*, *Candida glabrata*[3,10] and several others[2,4,5,8,11]. Yeast and fungi, upon exposure to metals are known to synthesize metallothionein and phytochelatins[10,12]. We report here, high level of Cu(II)-resistance in a clinically important strain of yeast. In this report, we focus on morphological and ultrastructural alterations in *Candida guilliermondii* strain DS31 induced by Cu(II); a possible mechanism of Cu(II)-resistance in this organism is also discussed.

A yeast strain DS31, procured amongst the laboratory isolates could resist high levels of copper (25 mM), zinc (Zn 20 mM), and cadmium (Cd 2 mM) on a nutritionally-rich medium. This strain was identified as *Candida guilliermondii* by Microcheck Inc., USA. Strain DS31 could grow well on a nutritionally-defined unbuffered minimal medium, MM[12], routinely used in our laboratory for bacterial strains, but only when glucose (0.4% w/v final concentration) was used as a carbon source. Media were supplemented with required concentration of metals from 1 M stocks of $CuCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 2H_2O$ and $CdCl_2 \cdot 2H_2O$. The colonies of the strain DS31 turned bright blue when cultured on Luria–Bertani agar (LB Agar) plates supplemented with high concentrations of Cu(II), i.e. 15 to 25 mM (Figure 1).

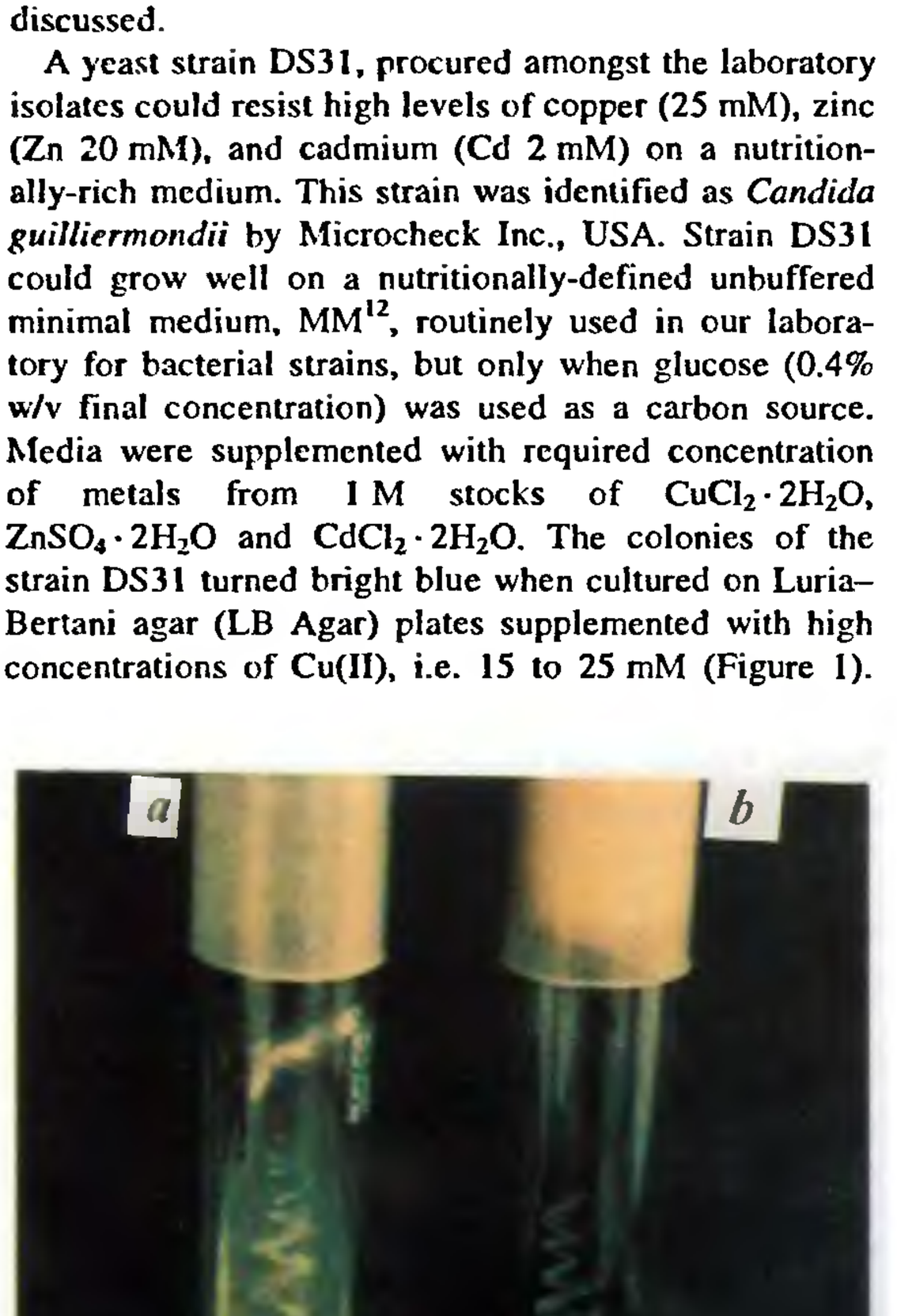

Figure 1. *Candida guilliermondii* DS31 grown on *a*, LB, and *b*, LB containing 25 mM Cu(II). Note the blue colour of the cells in presence of Cu(II).

Blue colour of the mycelia as well as bacterial colonies have been previously reported[7,8,13], but, the same is being reported for the first time in case of yeast. However, no colour was observed when the cells were streaked on Cu(II)-containing MM. The tolerance level for Cu(II) was also reduced on this medium to 2 mM. When MM was amended with each component of LB, i.e. yeast extract (0.5%), tryptone (1%), and NaCl (1%), separately, the blue coloured colonies appeared only when the yeast extract was added. This suggested that the Cu(II) is taken up in a complexed form and is thus less toxic to the cells. Components of complex media are known to chelate metal ions[6]. When present in complexed form, toxicity of metal is reduced, as indicated by higher tolerable concentration of Cu(II) on LB as compared to MM. Yeast extract is known to have very high chelation capacity[6]. Gradual decolourization of the medium was also observed with the growth of the organism, indicating that the cells could remove Cu(II). All these findings led to the suggestion that sequestration might be the possible mechanism of resistance. To confirm Cu(II) accumulation by the strain DS31, exponentially growing cells were transferred to MM supplemented with 2 mM Cu(II) and incubated in a New Brunswick Scientific Shaker Incubator (Edison NJ, USA), at 200 rpm, 30°C for 24 h. Cells were harvested at 5000 *g* for 10 min, washed with 0.85% saline to remove non-specifically bound Cu(II), and dried at 80°C in a hot air oven for 48 h. Pre-weighed cells were digested with 2 ml of nitric acid-perchloric acid (6:1, v/v), diluted with deionized distilled water, and Cu(II) was estimated by Atomic Absorption Spectrophotometer (Perkin–Elmer model 3110) at 324.8 nm[11]. Such an analysis showed that strain DS31 could accumulate Cu(II) to 5.5% of its dry weight (Table 1). All the experiments were conducted in three independent sets and a mean is always represented.

In order to assess the morphological and ultrastructural alterations induced by Cu(II), cells were grown on LB-agar plates supplemented with 15 mM Cu(II) for 7 days (when they attained blue colour) at 30°C. Cells were then scraped off, washed twice with 0.85% saline and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h, at 4°C. After 2 h, pellets were

Table 1. Cu(II)-uptake by wild-type (DS31) and copper-sensitive (CS2) strains of *Candida guilliermondii* in MM supplemented with 2 mM Cu(II), and Cu(II)-retention by the same after 4 mM EDTA treatment (designated as residual Cu(II)). Each value represents the mean of three independent sets

Strain cells	Cu(II) uptake (μg/mg dry weight)	Residual Cu (II)	Percentage of Cu (II) retained by the *C. guilliermondii* strain after 4 mM EDTA treatment
DS31	55.0 ± 2.0	0.6 ± 0.10	1.09
CS2	48.0 ± 3.0	17.1 ± 1.0	35.63

washed thrice with the same buffer. Samples were post-fixed in 1% OsO_4 at 4°C, and then washed with water. Dehydration was carried out in an ascending series of acetone followed by toluene. Specimens infiltrated with a 3:1 (v/v) mixture of toluene and araldite for 2 h and finally embedded in beam capsule with araldite + accelerator (trimethyl aminomethyl phenol) were polymerized at 50°C for 24 h, followed by 60°C for 48 h. Ultrathin sections were cut with a Reichert Jung Ultracut Microtome E and stained with uranyl acetate and lead citrate. Sections were examined in a transmission electron microscope (CM-10 Philips). Control sets were grown without Cu(II) and treated in the same way.

As seen in transmission electron micrographs, a number of structural alterations were induced by Cu(II). Plasmolysis was very prominent in case of cells grown on Cu(II) containing medium (Figure 2 *b* and *c*). In case of control, however, no such shrinkage of cytoplasm was observed (Figure 2 *a*). Unlike controls, Cu(II) grown cells also revealed electron-dense deposits in the cell wall (Figure 2 *c*). Metal-induced morphological as well as ultrastructural changes have been reported earlier also[13]. Copper-induced plasmolysis is reported in the case of *Pseudomonas syringae*[14]. Other heavy metals, e.g. nickel (Ni) and mercury (Hg) also induce plasmolysis[15,16]. Similarly formation of electron-dense particles was observed when *Enterobacter* was grown in the presence of Hg[16]. Cells of *P. aeruginosa* treated with Cd and those of *P. stutzeri* RS34 exposed to Zn also showed deposition of electron-dense particles[17,18]. Vacuole formation was also observed in Cu(II)-treated cells of *C. guilliermondii* (Figure 1 *b*). A similar response has been reported in *P. stutzeri* in the presence of Zn (ref. 17). In order to decipher the mechanism of Cu(II)-resistance, a Cu(II)-sensitive mutant, CS2, was procured upon *N*-methyl-*N*'nitro-*N*-nitrosoguanidine (MNNG) treatment. Exponentially growing cells were treated with 10 µg/ml of MNNG for 30 min, and the colonies were checked for copper sensitivity. One mutant strain CS2, could resist only upto 0.75 mM of Cu(II). When uptake of Cu(II) was compared with DS31, both the strains accumulated almost similar amounts of Cu(II) on the whole cell basis as shown in Table 1.

To correlate the morphological and ultrastructural changes described above, with the localization of Cu(II), cells were treated with ethylenediaminetetraacetic acid (EDTA). For this, Cu(II)-loaded cells were suspended in 4 mM EDTA (pH 8.0) for 30 min, collected by centrifugation at 5000 *g*, and washed with 0.85% saline. Cells were procesed in the similar way as described earlier, and Cu(II) was estimated.

As represented in Table 1, EDTA treatment could remove 99% of the cell associated Cu(II) from the strain DS31 indicating that the same was localized extracellularly, i.e. in the cell wall and extracellular space. However, in case of CS2, a significant amount of Cu(II), i.e. ~ 35.6% was retained by the cells even after EDTA

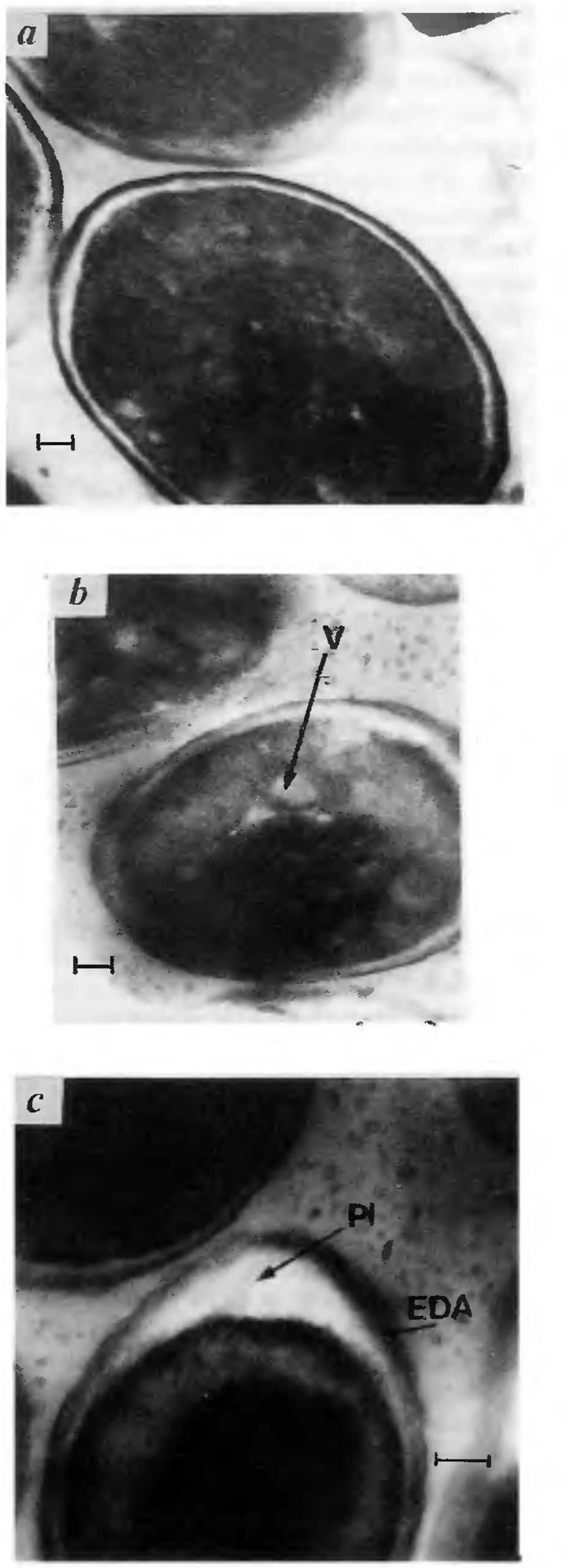

Figure 2. Transmission electron micrographs of *Candida guilliermondii* DS31 control cells *a*, and Cu(II)-treated (15 mM) cells; *b*, *c*, Plasmolysis (Pl), electron-dense aggregates (EDA), and vacuolization (V) are seen in Cu(II)-treated cells. Bar = 0.1 µm.

treatment, indicating internal localization of the same. This finding explains the Cu(II)-sensitivity in the strain CS2.

In conclusion, we can say that Cu(II) is toxic to the system and induces several ultrastructural alterations. Being an essential ion, its entry into the cell cannot be prevented. The resistant cells must therefore encode some mechanism to avoid the toxic action. *Candida guilliermondii* strain DS31 has adapted a strategy to accumulate Cu(II) extracellularly so that it could survive under high levels of this metal.

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The meandering Indus channels: Study in a small area by the multibeam swath bathymetry system – Hydrosweep

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The discharge of sediments by the river Indus has accumulated into a 2500 m thick pile, forming one of the largest deep sea fans in the world. Though there are many reports on channels in different regions of the fan, we report for the first time the presence of distinct channels far from the mouth of the fan. A multibeam seafloor mapping system, Hydrosweep has been used to trace the channels and determine related physical parameters. The channels are largely comparable in size and shape to some of the world's largest fluvial systems.

LITHOGENIC influx due to erosion of the Himalaya has resulted in formation of the Bengal and the Indus Fans[1]. Both have formed predominantly due to turbidites originating from the mouths of the Indus and the Ganges–Brahmaputra river systems[2]. Most of the turbidity flows have taken place during the Pleistocene and earlier times of lowered sea level, and are thought to have been inactive during the Holocene[3].

In the past the Indus river discharged 200 cubic km of water annually, and carried to the head of the Arabian Sea some 450 million tonnes of suspended sediment[4]. Today, the sediment discharge has been reduced to 45 million tonnes due to the construction of dams and barrages. The piling of sediments has created the Indus cone, a 2500 m thick pile of loose sediments on the floor of the Arabian Sea, 1500 to 2500 km away from the mouth of the river. Some of the sediments settled immediately giving rise to the Indus delta. The rest have been carried to deeper areas of the Arabian Sea by turbidity currents as a dense sediment suspension through the Indus Canyon[4].

The bathymetry and internal structure of these fans, deltas and cones have been studied by numerous investigators[5,6]. Sedimentation and mechanism of sediment transport in the entire fan has also been studied[7,8]. The available data on bathymetry, shallow acoustic character of the sea floor, seismic stratigraphy, internal structure and sediment distribution of the entire Indus fan have been compiled[9]. However, the area undertaken for the present study has not been studied.

The 'GLORIA' side scan sonar and seabeam swath bathymetry system allows individual channels to be

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