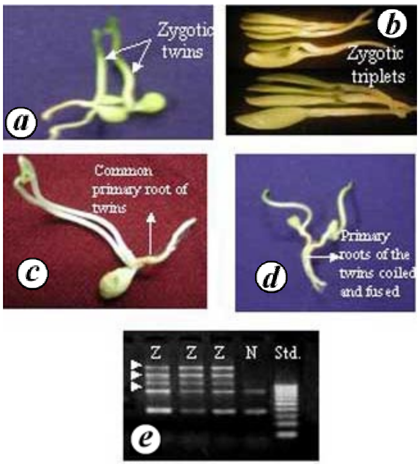

Figure 1. ***a***, Zygotic twin seedlings developing from a seed. ***b***, Zygotic triplets, division and sharing of two original cotyledons of seeds by the triplets. ***c***, Siamese twin seedlings having common radicle or primary root. ***d***, Coiling and fusion of two primary roots of closely developed twins. ***e***, RAPD profile of three zygotic triplet seedling and one nucellar seedling obtained from a single seed of a mandarin orange plant collected from Mirik, Darjeeling District, using the primer OPAL4 (ACAACGGTCC). N and Z represent nucellar and zygotic seedlings. (▶) Position of extra amplified fragments found in zygotic triplet seedlings.

In addition to the presence of normal zygotic and nucellar embryos, in 24 plants twin and triplet zygotic embryos were also observed in the seeds. In these plants up to 30% of the seedlings were recorded either as zygotic twins or triplets embryos. These embryos were developed by fission of the original zygotic embryo as the original two cotyledons of a zygotic embryo of the seed were also divided and shared among the twins or triplets during germination[3] (Figure 1 *a* and *b*). During the development of the twin embryos into seedlings, some extraordinary morphological features were observed. Some identical twin seedlings showed a common primary root (Figure 1 *c*). The twin embryos in this case germinated with common hypocotyls and primary root, but the two epicotyls were distinctly separated from the cotyledonary node. This type of development of embryos into plants was comparable with the development of Siamese twins. In other cases, during germination the two hypocotyls and sometimes also the epicotyls of the identical twins were fused. Sometimes the radicles of the twins rolled over each other from the point of transition node to ultimately fuse as the primary root common for both twin seedlings. This was interpreted as anastomosis-like development of the identical twins (Figure 1 *d*).

RAPD amplifications of genomic DNA from zygotic seedlings (twins or triplets) usually had one or more extra bands than those of the nucellar seedlings. As shown in Figure 1 *e*, all the triplet zygotic seedlings developed from a seed of a plant collected from Mirik, Darjeeling District had three extra DNA amplified fragments (above 1000 bp). This was absent in the single nucellar seedling developed from the same seed. The nature of producing a large number of zygotic twins of mandarin orange plants in this region might be one of the major causes of the wide genetic variation existing within this natural population[8].

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Bioaccumulation of heavy metals in some commercial fishes and crabs of the Gulf of Cambay, India

Accumulation of heavy metals in marine ecosystems is of global importance. Metals generally enter the aquatic environment through atmospheric deposition, erosion of geological matrix or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining wastes[1,2]. The metal contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by the organisms. The progressive and irreversible accumulation of these metals in various organs of marine creatures ultimately leads to metal-related diseases in the long run because of their toxicity, thereby endangering the aquatic biota and other organisms[3–6]. Fishes being one of the main aquatic organisms in the food chain, may often accumulate large amounts of certain metals[7,8]. Essentially, fishes assimilate these heavy metals through ingestion of suspended particulates, food materials and/or by constant ion-exchange process of dissolved metals across lipophilic membranes like the gills/adsorption of dissolved metals on tissue and membrane surfaces.

The Gulf of Cambay, India also known as the Estuarine Delta due to the convergence of many popular rivers of diverse nature like Sabarmati, Mahi, Narmada, Dhandhar, Tapti and Vishwamitri, comprises of a special ecosystem consisting of mud flats, dunes and scattered sandy beaches[9]. It receives discharges (domestic and industrial effluents) in significant quantities due to extensive industrial development and urbanization, including one of the world's largest ship-breaking yards at Alang-Sosiya, which usually make the coast rich. However, in the process its waters are being more contaminated. Heavy metals, petroleum hydrocarbons and other floating organics are some of the common contaminants which affect the quality of the environment. The geographical location of the Gulf of Cambay makes these contaminants usually undergo slower dilution and dispersion than would occur in the open marine systems. As a result, seafood, particularly fishes and crabs which have great local consumption and export value, is considerably affected. Our earlier studies on the horizontal distribution patterns of metals in the sediments of this region have suggested that the major inputs could be due to a variety of increasing anthropogenic activities with time together with the outsourcing of the ship-breaking works at Alang-Sosiya[10]. In the present correspondence, we report our preliminary findings of metal accumulation in two aquatic animals, fishes (*Harpodon nehereus*) and crabs (*Metopograpsus maculatus*).

Five samples of each species, fishes and crabs of equal size were collected directly from fishermen near Alang-Sosiya and Mahuva coasts during September 2004 (Figure 1). The samples were washed with clean sea water, brought to the laboratory in an icebox and then frozen to −20°C. The samples were measured (size ~1 mm and weight ~1 g), dissected with clean equipment and then freeze-dried for 48 h. The muscles of the all fishes and soft tissues of all crabs (excluding legs) were pooled and placed in 30 g small plastic vial separately. A 3.5 cm diameter teflon ball was added to the vial containing dried tissues. The vials were tightly closed and placed in a shaker to pulverize and homogenize the samples. Next 4 ml of 30% perchloric acid and 2 ml of 60% nitric acid were sequentially added to 1–8 g of the homogenized powder in a 250 ml teflon beaker and digested on a sand bath. The residue left after digestion was dissolved in 50 ml of 0.1 N nitric acid and analysed for As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn by Inductively Coupled Plasma Atomic Emission Spectrometer (Perkin Elmer Optical Emission Spectrometer, Optima 2000 DV). All digested samples were analysed three times for metals. Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Accuracy and precision of the results were checked and compared with standard reference material (SRM, Dorm-2). All metal concentrations were quoted as mg/kg dry wt unless otherwise stated. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. Double-distilled water was used throughout the study. All glassware and other containers were thoroughly cleaned, finally rinsed with double-distilled water several times and air-dried prior to use.

The concentration of metals (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in fish and crabs is presented in Table 1 and Figure 2. The data show that iron has the highest concentration, followed by zinc and manganese in fish, while copper showed the highest concentration followed by iron, zinc and manganese in crabs. Metal accumulation levels in crabs were high in all cases, except arsenic. Nickel and arsenic were in nondetectable level in all samples of fish and crab respectively. Metal accumulation follows the order Fe > Zn > Mn > Cu > As > Pb > Hg > Cr > Co > Cd in fish and Cu > Fe > Zn > Mn > Hg > Ni > Pb > Cr > Cd > Co in crab. The highest mean Fe concentration in fish was 94.35, while Cu in crab was 175.45 mg/kg dry wt. The mean concentration of the essential elements Zn and Mn ranged from 36.8 to 39.5 and 11.7 to 12.8 mg/kg dry wt of fish and from 43.2 to 45.4 and 11.65 to 12.90 mg/kg dry wt of crabs. Crabs exhibited slightly higher mean concentration of both Zn (44.22) and Mn (12.27) than that of fishes. Overall, the concentration of Cd (8 times), Co (2 times), Cr (3 times), Cu (180 times), Fe (2 times), Hg (4 times), Ni (3 times) and Pb (2 times) was found to be more in crabs compared to fishes, which may be accounted for due to the major functional differences in their body. The study indicates that the accumulation of metals is relatively more in crabs than in fishes.

The concentration of some of highly toxic metals (Cd, Hg and Pb) detected in fish was compared with reported values for other regions (Table 2) in an effort to determine the degree of contamination in the study area. The mean Hg concentration found in fish of the Gulf of Cambay is high, about three times to that of the Manila Bay, two times that of Calcasieu River and Lake, and five times that of the Arabian Sea (Pakistan). On the other hand, Pb accumulation is about nine times that of Manila Bay, two times that of Calcasieu River and Lake, and twenty times more than that of Osaka, but about two times, two to six times and three times less compared to Ikenderun Bay, Mediterranean Sea and Arabian Sea (Pakistan) respectively. Similarly, Cd accumulation in fish in the Gulf of Cambay is about ten times more than that of Manila Bay and Osaka, two times more that of Calcasieu River and Lake, but four times less and

Table 1. Mean metal concentration (mg/kg dry wt) in commercial fishes and crabs, Gulf of Cambay, India

Heavy metal	Fish	Crab
As	1.74 ± 0.856	ND
Cd	0.23 ± 0.029	1.600 ± 0.566
Co	0.24 ± 0.057	0.425 ± 0.177
Cr	0.77 ± 0.054	2.075 ± 0.389
Cu	2.37 ± 0.451	175.45 ± 2.45
Fe	94.35 ± 2.64	155.85 ± 5.10
Hg	0.97 ± 0.332	4.000 ± 0.023
Mn	12.14 ± 0.72	12.270 ± 0.884
Ni	ND	3.150 ± 0.041
Pb	1.09 ± 0.071	2.775 ± 0.177
Zn	38.24 ± 1.641	44.22 ± 1.21

Concentration of all metals is in mg/kg. ND, Not detectable.

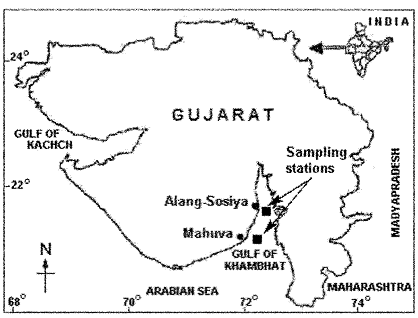

Figure 1. Location map of the study area.

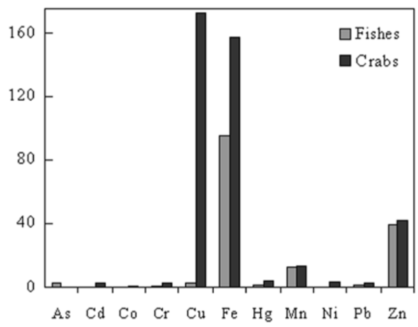

Figure 2. Bar diagram showing bioaccumulation of heavy metals in fishes and crabs.

Table 2. Comparison of metal accumulation in fishes from different regions vs present study

Location	Hg (mg/kg)	Pb (mg/kg)	Cd (mg/kg)
Gulf of Cambay, India[a]	0.970 ± 0.332	1.090 ± 0.071	0.230 ± 0.029
Manila Bay, the Philippines[a,13]	0.289 (0.049–1.400)	0.135 (0.038–0.300)	0.0243 (0.006–0.071)
Iskenderun Bay[a,14]	–	2.320	0.95
Calcasieu River and Lake, Louisiana, USA[a,15]	0.400 (0.030–0.670)	0.400 (0.060–1.600)	0.1 (0.003–0.12)
Mediterranean Sea[a,16]	–	2.980–6.120	0.37–0.79
Arabian Sea, Pakistan[a,17]	0.133 (0.510–2.400)	4.120 (2.400–16.000)	0.32 (0.19–0.73)
Osaka, Japan[b,18]	–	0.048 (0.030–0.070)	0.027 (0.017–0.045)

[a]Dry weight basis; [b]Wet weight basis; Values shown in parentheses are normal ranges reported.

one to three times less compared to Ikenderun Bay and Mediterranean Sea respectively. Interestingly, from the data in Table 2, it is noted that the accumulation of Pb and Cd in the Gulf of Cambay is comparatively less than that reported in Ikenderun Bay, Mediterranean Sea and Arabian Sea (Pakistan). Tariq *et al.*[11] found that Hg contamination in fishes from the Indian Ocean was 0.09–0.21 mg/kg wet wt[11]. These values are about ten times lower compared to those observed in the present study. Although the sample sizes and species in the current study are limited, comparison of metal concentrations in Table 2, except at Ikenderun Bay and Mediterranean Sea reveals that the anthropogenic loading of Hg, Pb and Cd in the Gulf of Cambay region is relatively high. It shows that fish from the Gulf of Cambay are being affected by effluents from metallurgical, ship-breaking, chloro-alkali industries.

Investigations regarding the red king crabs (mined and unmined) of northeastern Bering Sea, Arctic Alaska[12] revealed that the crabs contain 15–16 mg As, 0.15–0.19 mg Cd, 75–78 mg Cu, 0.10–0.14 mg Hg, 1.80–2.1 mg Ni, 0.23–0.26 mg Pb, 147–159 mg Zn per kg dry wt. These metal concentrations are relatively high for Zn (about four times) and comparable to Ni, but less (about 10 times) for Cd, two times for Cu, 20 times for Hg and 10 times for Pb compared to those in the Gulf of Cambay.

Accumulation of heavy metals in two common aquatic food species in the Gulf of Cambay has been investigated in the present study. The data show accumulation of heavy metals (Hg, Pb and Cd) in fishes and crabs to a considerable extent, and relatively more than that reported from other regions in the literature. The high bioaccumulation of these metals is believed to be occurring due to rigorous anthropogenic activities.

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