Environ Sci Pollut Res
DOI 10.1007/s11356-017-8555-y

RESEARCH ARTICLE

Collection of mullet fish (*Mugil cephalus*) from west coast of India: evaluation of its quality with relation to food safety

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Received: 18 July 2016 / Accepted: 2 February 2017

Abstract The present study focuses on bacterial and selected heavy metal contaminations of economically important mullet fish (*Mugil cephalus*) collected from West coast of Gujarat, India. Molecular identification using 16S rRNA sequencing revealed the presence of *Aeromonas veronii*, *A. mollusorum*, *A. cavae*, *A. bivalvum*, and *V.alginolyticus* in the gill and the intestine along with some other non-pathogenic bacteria. Pathogenicity of different *Aeromonas* species was confirmed by hemolysin assay. Apart from pathogenicity, multidrug resistance pattern was also reported against some commonly used antibiotics. Heavy metal analysis of different parts such as ventral and dorsal muscles as well as gills of *M. cephalus* revealed maximum concentration of Pb (24.08 ± 4.40 mg/kg), Cd (8.25 ± 3.04 mg/kg), and Cu (33.67 ± 5.34 mg/kg), which were higher than the permissible limit. To the best of our knowledge, this is the first study analyzing different heavy metals and associated bacteria in *M. cephalus* fish in India. Further, the distribution of heavy metals in *M. cephalus* fish from other countries was also compared.

Keywords Antibiotic resistance · Bacteria · Biochemical composition · Heavy- metals · *M. cephalus*

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (doi:10.1007/s11356-017-8555-y) contains supplementary material, which is available to authorized users.

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Introduction

Increasing human population for the last few decades has lead to the consumption of seafood by millions around the world (Hites et al. 2004). According to Kawarazuka and Béné (2011), fish is not only a good source of protein but also provides essential fats for proper growth and development of the body. It has been suggested that increasing consumption of fish dramatically increases the uptake of long-chain polyunsaturated fatty acids in the body (Surette 2008; Jenkins et al. 2009). In a case study, it was observed that the consumption of fish led to a decreased risk of anemia and common infections in children with poor nutrition (Gibson et al. 2003). However, there are many reports stating that the ingestion of both raw and cooked seafood significantly increases the risk for gastroenteritis-related illnesses as these fish are generally contaminated with pathogenic bacteria (Sobel and Painter 2005; Iwamoto et al. 2010).

Recently, there has been a considerable increase in seafood production and export from India. With the help of technological developments from the years 1951 to 2014, the export quantity fish captured from the sea using commercial equipments such as trawl net has increased from 534,000 to 3,443,000 t (HandBook on fisheries statistics 2014). Gujarat is one of the main fish-producing states with regard to capture fishery, and the annual fish production of Gujarat increased from 620,470 to 695,580 t in the period from 2001 to 2014. Among the export commodities in India, fish as the major export item secured the first position in quantity and the second position in export value (http://www.fao.org/fishery/countrysector/naso_india/en), which contributes 4.47% of the total GDP of agriculture and allied sector. In India, Andhra Pradesh ranked first, followed by West Bengal and Gujarat in total fish production.

Gujarat has developed various industries, such as textile, chemical, and fertilizer, and is one of the biggest ship-breaking yards. Tewari et al. (2001) reported increased concentrations of heavy metals near the coasts of ship-breaking yards with high levels of bacterial contaminants. Heavy metals once disposed in the sea water generally accumulate in the invertebrates, and a few of them such as cadmium and mercury ultimately biomagnify in different fish (Goodyear and McNeill 1999; Luoma and Rainbow 2005). Goodyear and McNeill (1999) tried to understand the relationship between the feeding habit of different fish and heavy metal accumulation potential. Mullet fish generally feed on microalgae, zoo plankton, and benthic and detritus sediments in different stages of life cycle, which comes under the scraper grazer category that has a moderate accumulation capacity. However, some heavy metals such as zinc, nickel, copper, etc. generally bioaccumulate rather than biomagnify in the fish and that such an accumulation is also dependent on the level of intake, followed by the loss in the fish body (Goldberg et al. 1977; Finney and Huh 1989; Larsen and Jensen 1989; Luoma and Rainbow 2005; Rainbow et al. 2009). *M. cephalus* is a plankton feeder which occupies a lower trophic level, thereby concentrating the metals along the food chain more efficiently. Among the different marine bacteria, *Aeromonas* strains are known to be associated with digestive illness in seafood consumers (Janda and Abbott 1998; Papadopoulou et al. 2007). The enterotoxicity is found to be related to the hemolytic activity in *Aeromonas* (Chopra et al. 1991; Wong et al. 1998; Li et al. 2011). Interestingly, these strains also harbor some pathogenic genes responsible for the production of enterotoxins like hemolysin (Albert et al. 2000). In aquaculture, use of antimicrobial agents is very common and approximately 80% of the antibiotics used in the aquaculture reaches the environment, leading to the development of bacterial mutant strains with antibiotic resistance (Cabello et al. 2013). According to Rizzo et al. (2013), the urban wastewater treatment plants are also a potential source for the transfer of antibiotic resistance genes into environmental strains. Sabarmati River which completely carries partially treated domestic sewage of Ahmedabad city is pouring the water into the gulf of Khambat. This water contains high bacterial and organic load (Haldar et al. 2014). Apart from this, pharmaceutical sewage from the common effluent treatment plant (CETP) also discharges the waste into this gulf. These sources are located in the upper part of the gulf which is approximately 20–25 km away from the sampling sites. However, high tidal inflow and mixing help carry these sewage materials near the sampling sites.

Fishes are considered to be the potent bioindicators of marine ecosystem because they represent a very large tropic level (Abdel-Baki et al. 2011). In general, the concentration of heavy metals in the fish tissue is important because it not only affects the fish but also the human consumers (Lee et al. 2011).

M. cephalus is a highly valued fish in the western part of India with an annual production of 4962 t in the year 2010. However, microbial and selected heavy metal contaminations frequently make this fish, along with other fish species, unacceptable for export to other countries. Therefore, in the present study, an attempt has been made to evaluate the (1) bacterial contamination and their antibiotic resistance potential in relation to safety for human consumption and (2) biomagnification of selected heavy metals such as Cd and bioaccumulation of Cu, Ni, Pb, Zn, and Cd in *M. cephalus* fish and in addition to their potential hazard to humans.

Materials and methods

Sample collection

M. cephalus fish were collected from six coastal regions of Gujarat from Diu (20°43′25.7″N, 70°59′20.5″E), Salaya (20°47′48.6″N, 70°41′49.2″E), Veraval (20°54′10.1″N, 70°22′45.7″E), Mahuva (21°05′01.2″N, 71°45′56.7″E), Jafrabad (20°52′12.6″N, 71°21′55.6″E), and Ghogha (21°41′23.2″N, 72°16′27.6″E) (Fig. 1); during the period from July to August. Fish samples used in this study were collected from the local markets or landing stations and the morphometric measurements were taken immediately (Table 1). From all the sampling stations, samples were taken in triplicate. Immediately after collection, fish samples were stored in sterile sealed polythene bags and transported in ice box to the laboratory. Although fish samples were collected from the landing center near coast, majority of them were caught from average of 5–10 km radius toward off-shore (personal communication) which is indicated in Fig. 1.

Sample processing

Instantly, the intestine and the gill of one part of the collected fish were dissected aseptically in the laboratory for microbiological studying using sterilized scalpel and scissor and the remaining part was stored at −80 °C. For each bacteriological sampling, autoclaved (121 °C temperature and 15 psi pressure) and sterilized instruments were used. For heavy metal analysis, another part of fish samples was taken out from the deep freezer, thawed, and cleaned. Ventral and dorsal tissues from three fish were taken, minced into smaller pieces, mixed, and homogenized for different analytical purposes using acid-washed plastic spatula.

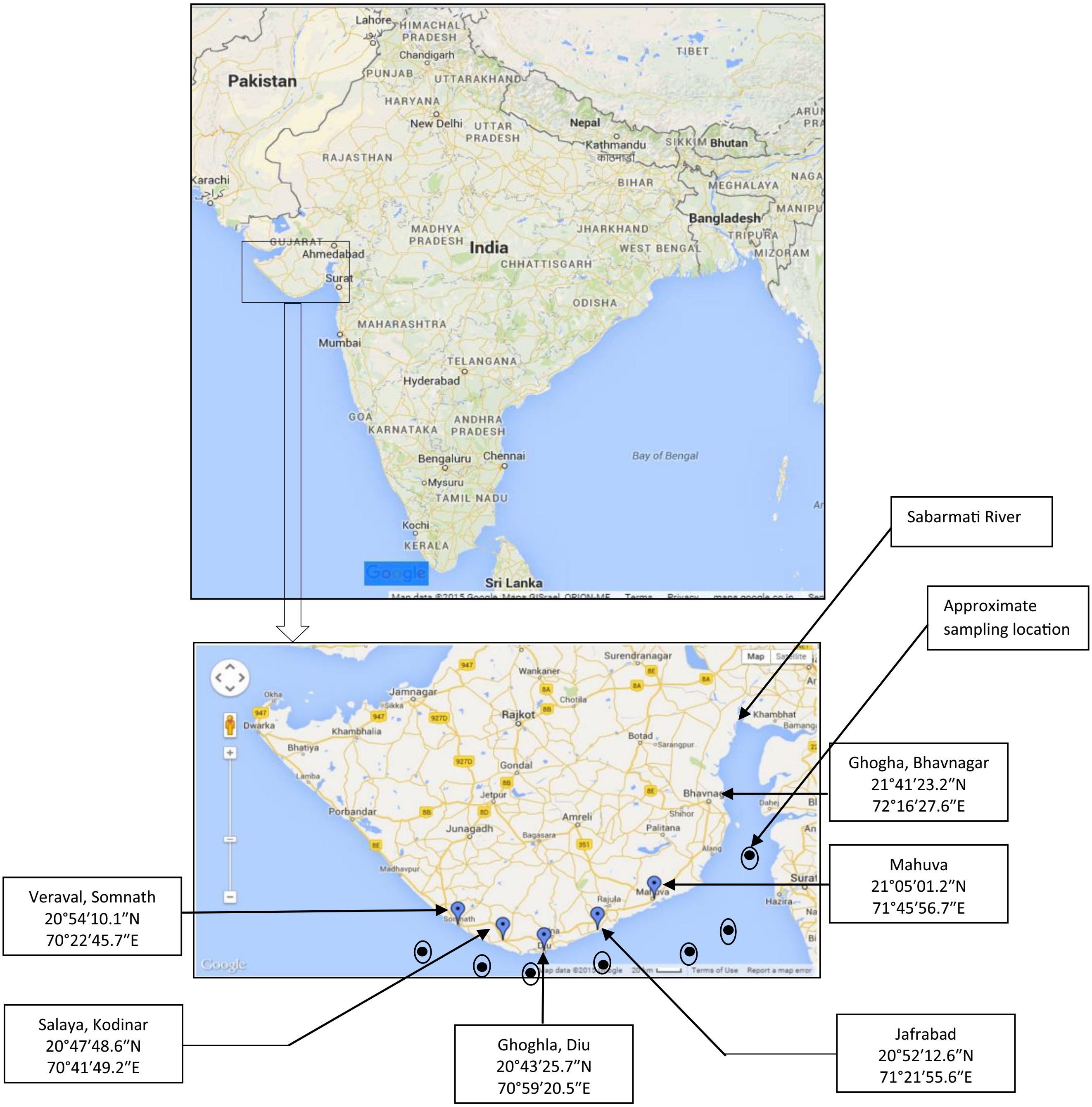


Fig. 1 Map showing the sampling sites (fish market) with approximate fish catching location in the coastal water

Isolation of bacteria

Gills and digestive tracts were taken using sterilized scissors and forceps. Then, 1 g of tissue was taken in 1 ml of normal saline after vigorous shaking on a vortex. One hundred microliters of supernatant from that was spread on the non-selective agar plates, and the plates were kept for incubation at 30 °C for 24 h. On the basis of different morphological features, colonies were isolated and single colonies were stored at −80 °C for further processing.

Identification of strains

Isolated bacterial strains were identified by partial 16S rRNA gene sequences using the universal primers 1492R and 27F (amplification of around 1400 bp) following the method described by Ben-Amor et al.

Table 1 Morphological data of collected fish

Serial no.	Place of collections	GPS	No. of fish collected	Length (cm) (mean ± SD)	Weight (gram) (mean ± SD)
1.	Diu	20°43′25.7″N , 70°59′20.5″E	3	29.43 ± 0.74	246.80 ± 10.36
2.	Salaya	20°47′48.6″N, 70°41′49.2″E	3	23.90 ± 0.46	151.47 ± 8.40
3.	Veraval	20°54′10.1″N, 70°22′45.7″E	3	26.73 ± 0.50	194.93 ± 11.52
4.	Mahuva	21°05′01.2″N, 71°45′56.7″E	3	25.07 ± 0.25	137.20 ± 8.21
5.	Jafrabad	20°52′12.6″N, 71°21′55.6″E	3	24.30 ± 0.80	131.27 ± 8.55
6.	Ghogha	21°41′23.2″N, 72°16′27.6″E	3	24.50 ± 0.50	162.27 ± 18.15

(2005). PCR conditions were kept as follows: initial denaturation at 94 °C for 7 min, denaturation at 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min for 30 cycles, followed by final extension at 72 °C for 10 min. All the PCR products were checked in 1% agarose gel and purified with QIAquick PCR purification kit (Qiagen Inc., UK). Samples were sequenced in both the directions using ABI PRISM3100 Avant 32 genetic analyzer (Applied Biosystems, M/S, Macrogen, South Korea) and a phylogenetic tree was prepared using MEGA6, ClustalW algorithm, and bootstrap methods.

Antibiotic resistance test

All the strains were inoculated in 1 ml liquid nutrient broth (Himedia, India) for 18 h up to OD_{660} that reached around 1. Subsequently, culture was spread on the Muller-Hington Agar and ICOSA Universal disks (Himedia. India) were used for antibiotic resistance test against 20 antibiotics. Plates were incubated overnight at 30 °C and zone of inhibition was observed for testing resistance pattern.

Hemolysis test

Hemolytic activity was observed in 5% sheep blood agar (Himedia, India) plates for all the *Vibrio* and *Aeromonas* strains isolated in this study. The test was done as described by Radu et al. (2003). A clear yellow zone was the indicator of α-hemolytic activity while a hazy zone displayed β-hemolytic activity, and white colonies were the signs of γ-hemolytic activity.

Heavy metal analysis

Heavy metal analysis was done by ICP-OES (Perkin Elmer, Waltham, MA; Optima 2000 DV). Samples were digested with assistance from a microwave oven. Acidification of samples was done with concentrated nitric acid (Upadhyaya et al. 2014). ICP multielement standard solution (MERCK, Germany) was used as standard.

Statistical analysis

Prism 7.02 (www.graphpad.com) software was used for all statistical analyses. Results were compared using two-way ANOVA to find out the significant difference between the means of the concentrations obtained and between those of various stations.

Hazard quotient analysis

Hazard quotient for each sample was calculated using hazard quotient risk calculation tool (http://www.popstoolkit.com/tools/HHRA/NonCarcinogen.aspx). All the standards were followed as per the USEPA guidelines. Fish consumption was assumed to be 0.30 kg/day (Ravikanth and Kumar 2015). Limits for receptor body weight and daily intake were considered as per USEPA guidelines. All the other parameters were kept zero to find out influence by fish consumption only.

$$HQ = \frac{\left(\mathrm{Dose}_{\mathrm{soilingestion}} + \mathrm{Dose}_{\mathrm{Wateringestion}} + \mathrm{Dose}_{\mathrm{soilingestion}} + \mathrm{Dose}_{\mathrm{foodingestion}} + \mathrm{Dose}_{\mathrm{particle\ \ inhalation}} + \mathrm{Dose}_{\mathrm{dermalcontact}}\right)}{\mathrm{Tolarable\ \ daily\ \ intake}}$$

Results

Average body weight and length of the fish were found to be 170.65 ± 10.86 g and 25.65 ± 0.54 cm, respectively. Summary is provided for all stations in Table 1.

Identification, antibiotic resistance, and hemolysis test

On the basis of different morphological characters, 34 different strains were isolated from all the fish samples. Distribution of bacterial isolates in each station was depicted in Fig. 2. The phylogenetic analysis showed *Aeromonas* forming two major clusters and *Vibrio* forming three major clusters (Fig. 3).

All the sequences were compared using NCBI BLAST, and the percentage homology, query coverage, and other detailed information were depicted in supplementary materials (ST1).

Out of 34 strains, 50% strains were resistant to amoxicillin; 52.9% against ampicillin; 41.1% against penicillin; 11.7% against erythromycin; 8.8% against vancomycin, cefadroxil, and cefoperazone; and 2.9% against chloramphenicol, co-trimoxazole, ceftazidime, netilin, and nitrofurantoin (Fig. 4). Antibiotic resistance patterns of each bacterium against all the 20 antibiotics tested were also evaluated (Fig. 5). And, the number of strains resistant to each antibiotic can be seen in Fig. 6.

All strains of *Aeromonas* were able to grow on blood agar. *Aeromonas veronii* (Strain No. 26), *A. caviae* (Strain No. 11), and all the remaining *Aeromonas* strains displayed β-hemolytic activity, α-hemolytic activity (Fig. 7), and γ-hemolytic activity, respectively, of which the latter were generally considered to be non-pathogenic.

Heavy metal analysis

Heavy metal analysis with two-way ANOVA uncovered some interesting facts about the concentrations of heavy metals in the present study. Amounts of Cd are almost the same in all the stations and surprisingly, Cd was not detected in Ghogha samples although it is situated near the ship-breaking yard Alang. The highest amount of Cd was detected in gill tissue of Salaya (8.25 ± 3.04 mg/kg) and Veraval (8.25 ± 0.25). Amount of Cu was found to be the highest in the gill tissues of fish from the Mahuva site (33.67 ± 5.34 mg/kg) that was significantly higher from the other stations. Ni was found to be the highest in the gills of fish from the Ghogha (14.33 ± 1.18 mg/kg) which was significantly higher than the other stations. Pb was almost similar in all locations except Veraval where the fish gills especially contained the highest amount (24.08 ± 4.40 mg/kg) that was significantly higher than Diu Salaya and Ghogha. Zn was found to be the highest in the gills of fish samples from Ghogha station (135.83 ± 1.76 mg/kg) which was significantly higher in comparison to other stations. A trend was observed that the gills accumulated more heavy metals then did the ventral and dorsal tissues (Fig. 8) (Table 2).

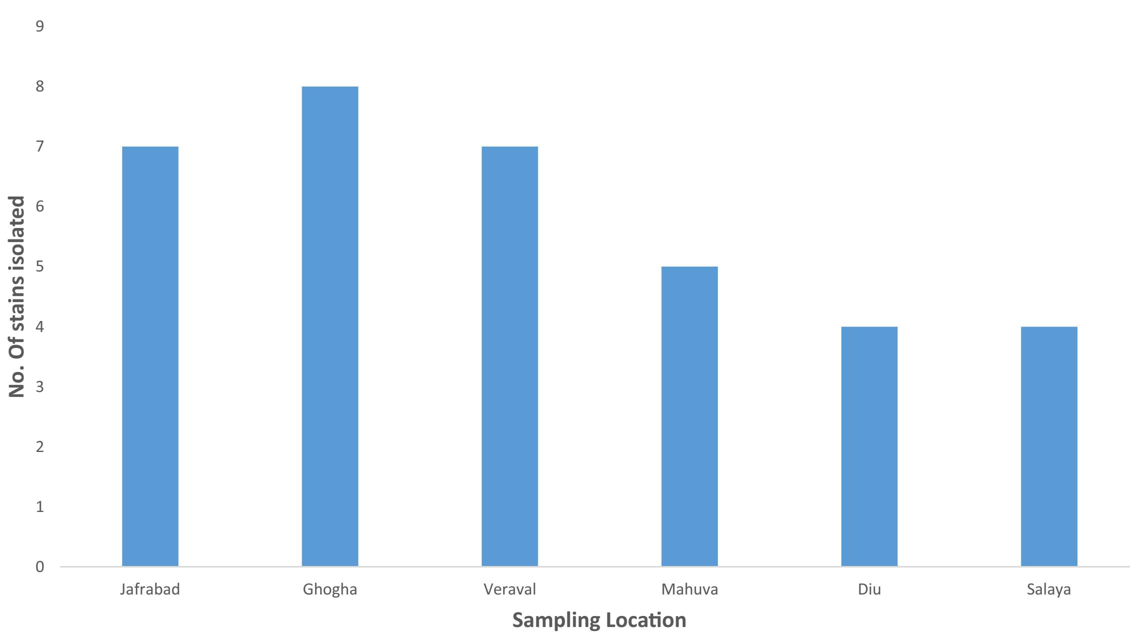


Fig. 2 No. of stains isolated from different sampling locations

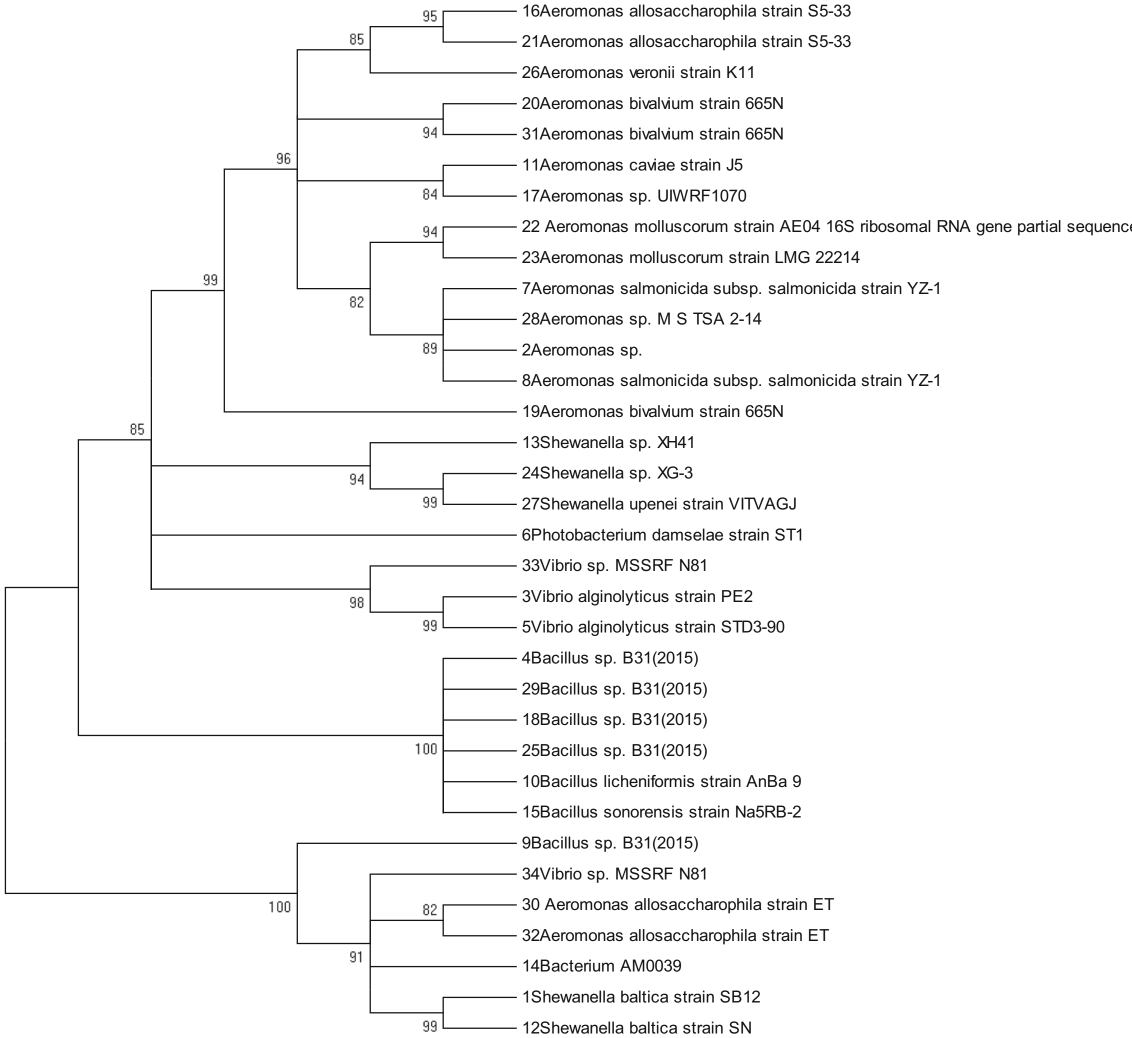


Fig. 3 The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 2.09753074 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 34 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + non-coding. All positions containing gaps and missing data were eliminated. There were a total of 798 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013)

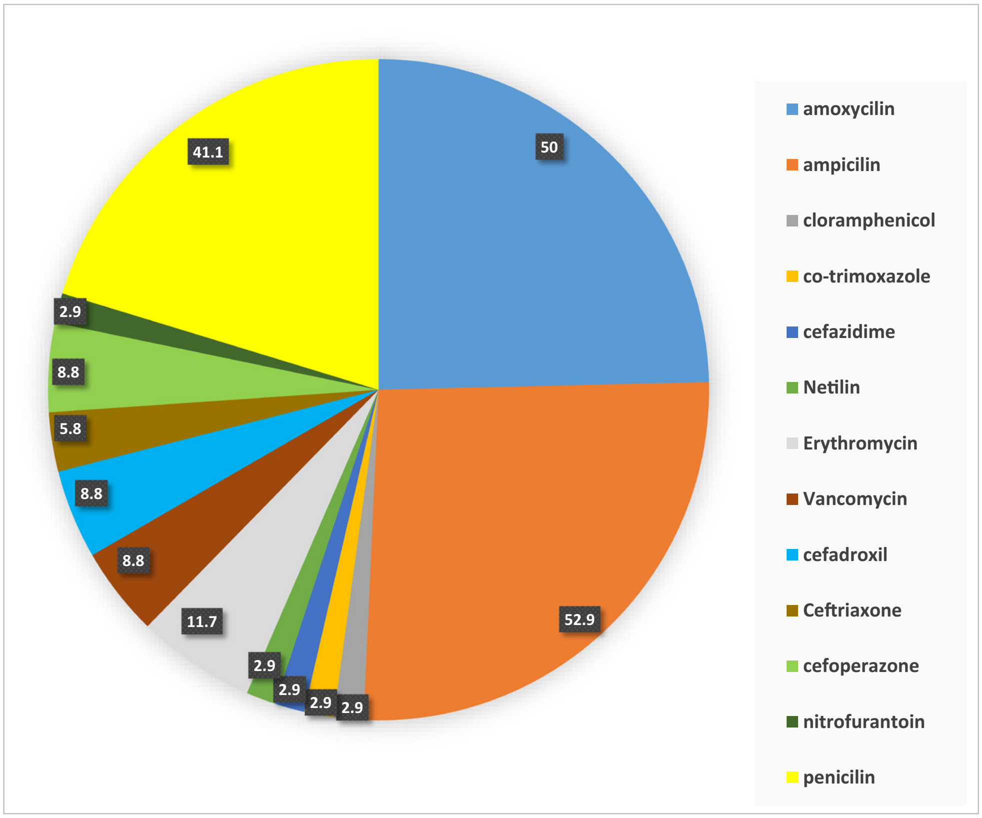
Hazard quotient analysis

According to USEPA limits, the hazard quotient value of more than 0.2 indicates that there is a potential risk to the human health. In the present study, the hazard quotient for Cd was higher in more than 0.2 in all the stations except Ghogha. Hazard quotient for Pb was very much higher in every station than the permitted limit, i.e., 0.2. Apart from these, the hazard quotient for Cu was high in the gill tissues of fish from Ghogha and Mahuva and in the ventral tissues of fish from Jafrabad. In the case of Ni, the hazard quotient was less than 0.2 except for gill tissues of fish from Ghogha station. Hazard quotient for Zn was below the permitted limit in all the stations. Summary of hazard quotient is shown in Table 3.

Discussion

Among a total of 34 bacteria identified and characterized, seven to eight were isolated from the upper part of the gulf. Chances for mixing of domestic sewage both from Sabarmati river and Bhavnagar city are comparatively more for these sites, which might result in the isolation of more bacteria. 16S rRNA sequencing revealed that the majority of the strains identified in the present study belonged to different species of *Aeromonas* such as *A. veronii*, *A. mollusorum*, *A. cavae*, and *A. bivalvum.* Different *Aeromonas* species are generally considered to be responsible for different gastrointestinal diseases in humans. For instance, *A. veronii* is known to

Fig. 4 Distribution of antibiotic resistance in each bacterium



be associated with the epizootic condition in fish and diarrheal condition in humans (Rahman et al. 2002). It has also been reported earlier that *A. cavae* is involved in cystitis (Al-Benwan et al. 2007). There is some controversy over the involvement of *Aeromonas* in food poisoning. However, the research in the past years

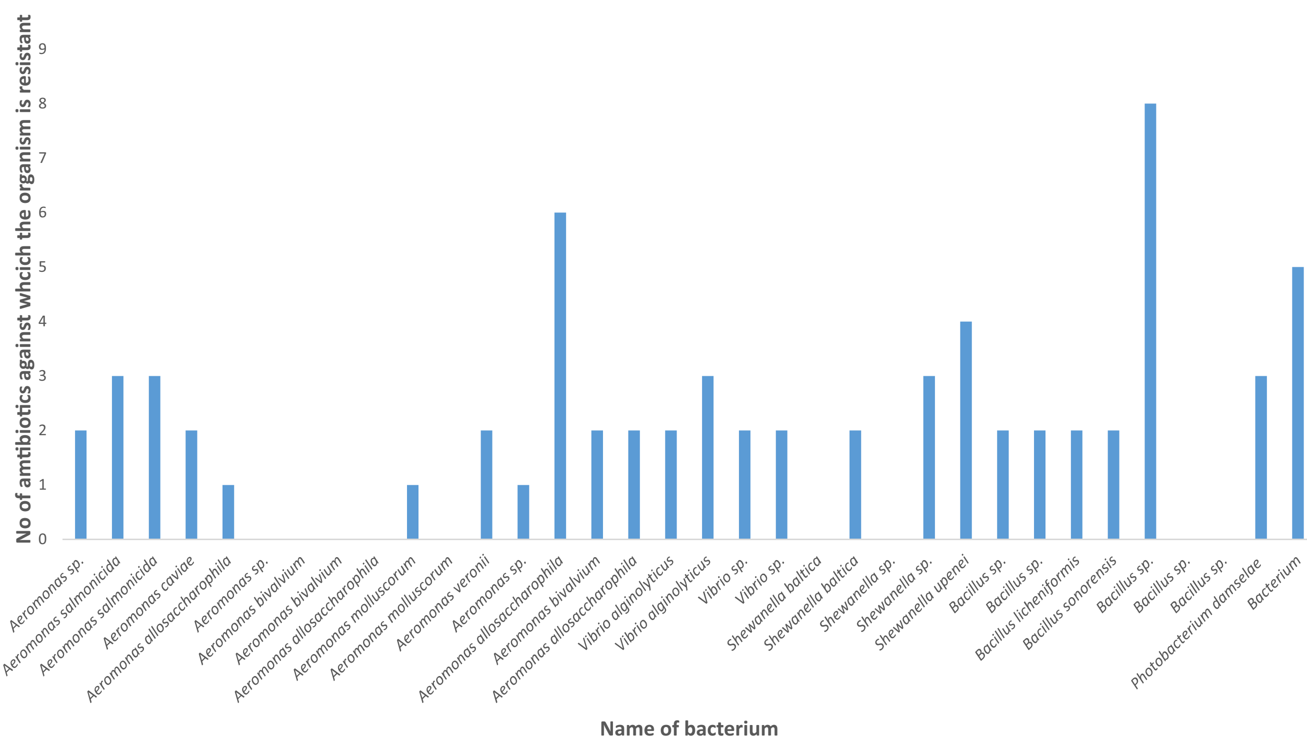


Fig. 5 Antibiotic resistance profile of each bacterium

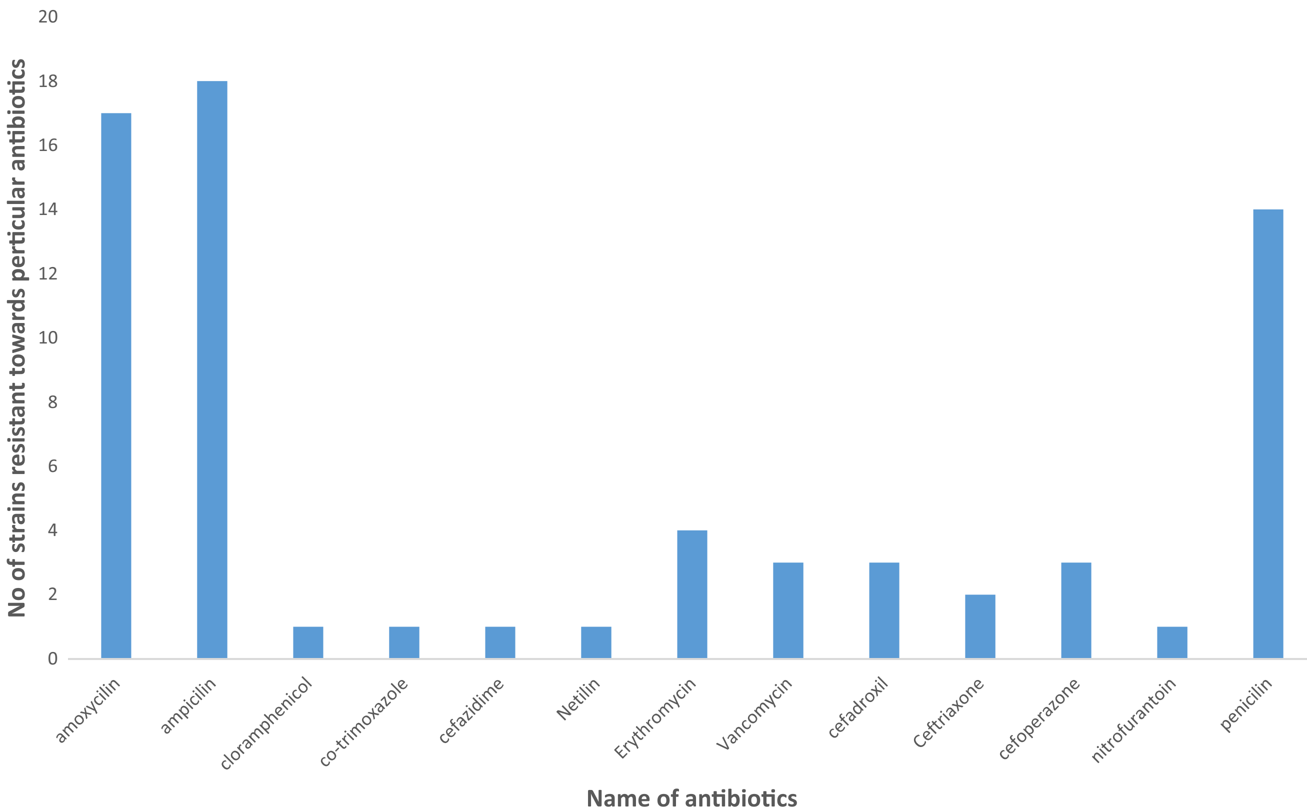


Fig. 6 No. of strain resistant to each antibiotic

revealed the presence of various toxins, which suggests that the mechanism of infection involves multiple factors and is so complex (Granum et al. 1998; Chopra and Houston 1999; Kirov et al. 2000). In the past years, *Aeromonas* species were known to have hemolytic potential. However, it is not necessary that all the pathogenic

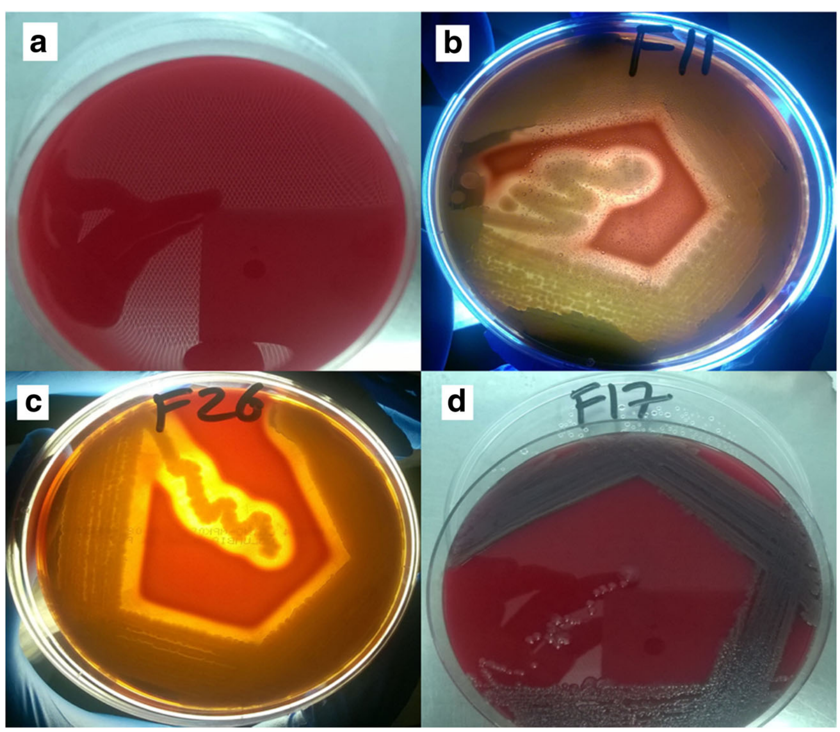


Fig. 7 Hemolytic activity by different strains of *Aeromonas* **a** uninoculated plate (blank). **b** *Aeromonas caviae* α-hemolytic activity. **c** *Aeromonas* veronii β-hemolytic activity. **d** γ-hemolytic activity of *Aeromonas* sp.

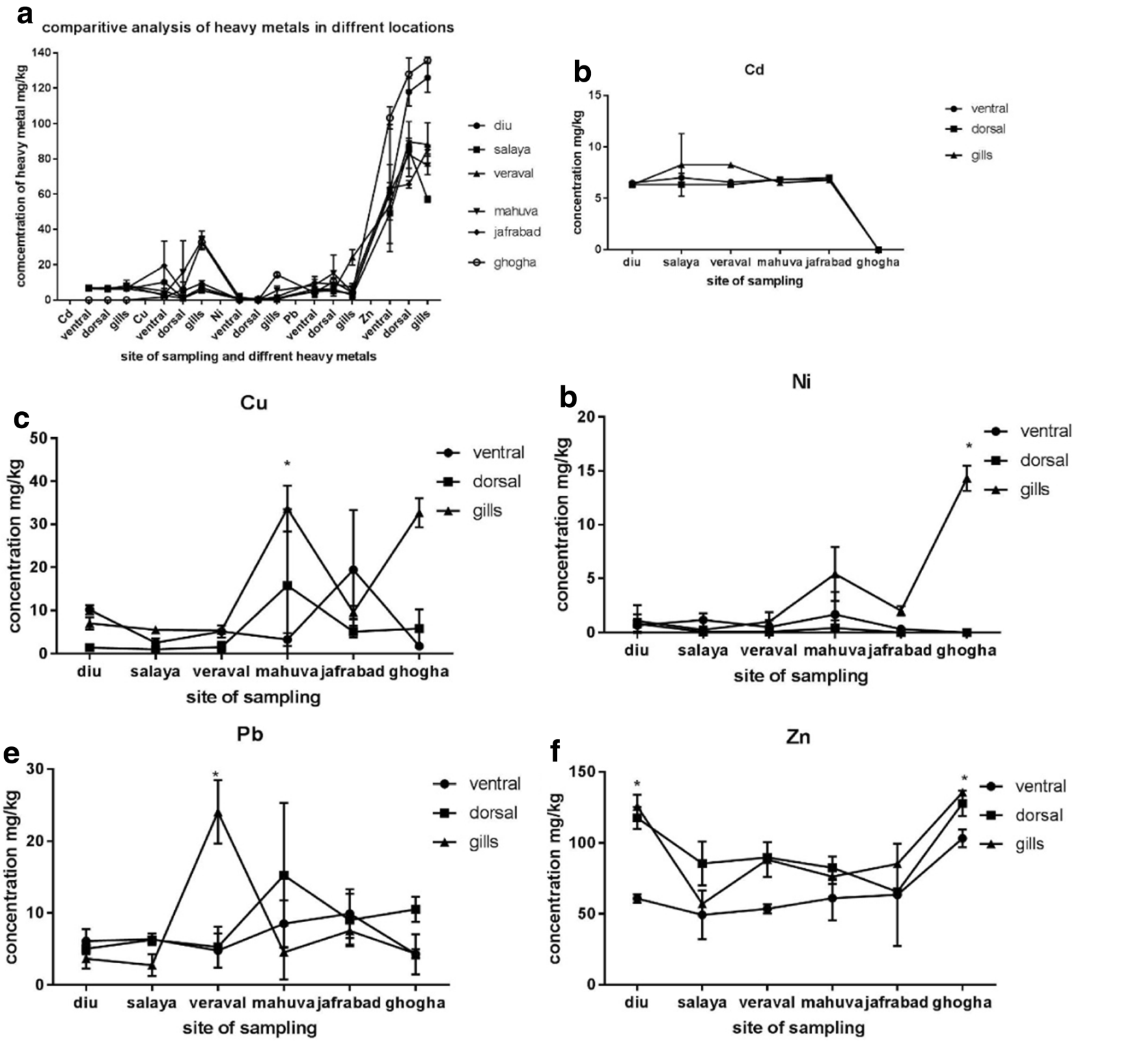


Fig. 8 Heavy metal analysis of the different sampling sites. **a** Comparative concentrations of all heavy metals in all the stations. **b** Comparison of Cd concentration in different stations. **c** Comparison of Cu concentration in different stations. **d** Comparison of Ni concentration in different stations. **e** Comparison of Pb concentration in different stations. **f** Comparison of Zn concentration in different stations. *Asterisk* indicates significance difference at 95% confidence level using two-way ANOVA

Aeromonas must be hemolytic (Namdari and Bottone 1990). In the present investigation, we found *A. caveii* and *A. veronii* having α- and β- hemolytic activities, while all other strains were found to have γ-hemolytic activity, being generally considered as potential pathogenic organisms (Sha et al. 2002). Apart from different species of *Aeromonas*, other identified bacteria such as *V. alginolyticus* are found to be associated with sporadic diarrhea and septicemia in humans (Schmidt et al. 1979; Caccamese and Rastegar 1999). Among other bacteria, *Shewanella baltica* which is already reported to be involved in the fish spoilage (Gram and Dalgaard 2002) was also isolated and identified in the present study. *Shewanella spp.* were also reported from clinical samples with food poisoning with an ability to produce teradotoxin (Wang et al. 2013).

In the current study, out of 34 bacteria, more than 50% bacteria was found to be resistant against the common antibiotics, which is a serious concern because majority of strains were resistant against broad-spectrum antibiotics like ampicillin (52.9%), amoxicillin (50%), and penicillin (41.1%), and this indicates indiscriminate use of broad spectrum antibiotics in the coastal area studied. This kind of resistance will make the infections difficult to be treated. There are so many sources for the antibiotic resistance development in marine bacteria. Some environmental strains which produce the antibiotics may transfer the genes horizontally to the other strains and also eventually develop the antibiotic resistance (Muniesa et al. 2013). In the present study, the discharge from common effluent treatment plant (CETP) near the study site might help multi-antibiotic resistance trait among the isolates. In general,

Table 2 Concentration of heavy metals in wet fish tissues with comparison to other references

Serial no.	Sampling locations	Body parts of fish	Concentration of heavy metals (mg/kg dry wet of fish tissues)						
			Mean ± SD						
			As	Cd	Cu	Hg	Ni	Pb	Zn
1	Diu	Ventral	ND	6.50 ± 0.25	10.17 ± 1.04	ND	0.67 ± 0.58	6.08 ± 1.67	60.75 ± 3.03
		Dorsal	ND	6.33 ± 0.14	1.42 ± 0.80	ND	0.83 ± 0.88	5.00 ± 0.75	117.83 ± 7.71
		Gill	ND	6.33 ± 0.14	7.08 ± 1.44	ND	1.08 ± 1.47	3.67 ± 1.38	125.92 ± 8.31
2	Salaya	Ventral	ND	7.00 ± 0.43	2.58 ± 1.04	ND	1.17 ± 0.63	6.33 ± 0.80	49.25 ± 17.24
		Dorsal	ND	6.33 ± 0.14	1.00 ± 0.90	ND	0.08 ± 0.14	6.25 ± 0.43	85.58 ± 15.58
		Gill	ND	*8.25 ± 3.04*	5.58 ± 0.95	ND	0.25 ± 0.25	2.75 ± 1.52	57.08 ± 1.67
3	Veraval	Ventral	ND	6.58 ± 0.14	5.17 ± 1.42	ND	0.50 ± 0.66	4.75 ± 2.39	53.50 ± 3.31
		Dorsal	ND	6.33 ± 0.14	1.58 ± 1.23	ND	0.08 ± 0.14	5.25 ± 2.84	89.75 ± 2.00
		Gill	ND	8.25 ± 0.25	5.42 ± 0.72	ND	1.00 ± 0.87	*24.08 ± 4.40*	88.25 ± 12.28
4	Mahuva	Ventral	ND	6.83 ± 0.14	3.33 ± 1.53	ND	1.67 ± 2.08	8.50 ± 3.25	61.08 ± 15.69
		Dorsal	ND	6.83 ± 0.14	15.75 ± 17.78	ND	0.42 ± 0.72	15.25 ± 10.11	82.50 ± 7.89
		Gill	ND	6.50 ± 0.25	*33.67 ± 5.34*	ND	5.42 ± 2.50	4.50 ± 3.73	76.33 ± 5.20
5	Jafrabad	Ventral	ND	6.83 ± 0.29	19.42 ± 13.90	ND	0.33 ± 0.38	9.92 ± 3.40	63.42 ± 36.17
		Dorsal	ND	7.00 ± 0.00	5.13 ± 1.35	ND	ND	9.00 ± 3.68	65.58 ± 2.18
		Gill	ND	6.75 ± 0.00	9.58 ± 1.51	ND	2.00 ± 0.43	7.50 ± 1.95	85.25 ± 2.39
6	Ghogha	Ventral	ND	ND	1.75 ± 0.50	ND	ND	4.25 ± 2.78	103.33 ± 6.29
		Dorsal	ND	ND	5.84 ± 4.40	ND	ND	10.50 ± 1.75	128.00 ± 9.09
		Gill	ND	ND	32.67 ± 3.39	ND	*14.33 ± 1.18*	4.33 ± 0.63	*135.83 ± 1.76*
Std. values from different agencies	FDA, 2001		–				70–80		
	EC, 2001		–	0.05		0.5–1.0		0.2	
	FAO, 1983		–		30				40

Italics indicates highest concentration of particular metal found in the present study

an administration of two narrow-spectrum antibiotics will lead to a better activity with less chances of resistance (Bonhoeffer et al. 1997).

The highest concentration of Cd in our study was found at Salaya and Veraval, which is non-significantly higher than at the other stations, and the hazard quotient was also higher than the permissible limits for these two stations, Apart from that, the concentration was higher than the 3.900 ± 0.984 mg/kg reported earlier from other fish (Bahnasawy et al. 2009). Cd enters in the marine environment with sewage, which is mainly stored in the liver and the kidney with half-life of approximately 17–30 years in the body (Castro-González and Méndez-Armenta 2008). Cd has an ability to affect DNA repair mechanism by generating free radicals, and it is also known to interfere with energy metabolism of the body (Müller 1986; Hartwig et al. 2002).

The highest concentration of Cu was found at Mahuva station which is significantly higher than the other stations, being also higher in comparison to 13.320 ± 0.850 mg/kg (Bahnasawy et al. 2009). High accumulation of Cu may lead to anemia, liver and kidney damage, stomach and intestinal irritation, etc. (Singh et al. 2011). Hazard quotient was higher than the permissible limits at Mahuva, Jafrabad, and Ghogha stations.

Previous reports suggest permissible limits of Ni concentration which can be up to 17.52 ± 1.02 mg/kg (Safahieh et al. 2011). In the present study, its concentration was found to be 14.33 ± 1.18 mg/kg in the fish from Ghogha. It may be due to the oil spillage contamination because Alang is nearest to Ghogha where the world's biggest ship-breaking yard is situated. So, it may be possible that the high concentration of Ni is due to pollution from ship-breaking yard. Usually Ni is found at very low levels. However, it has an ability to cause pulmonary diseases like tumors (Forti et al. 2011). Ni is an essential element for phospholipid metabolism in the body (Stangl and Kirchgessner 1996). This observation is also supported by hazard quotient calculation that the amount of Ni was higher than the permissible limits in Ghogha. Although its concentration was higher in other stations, it was not reported to be hazardous, according to USEPA guidelines.

Pb concentrations found at each station were concerning as each sample analyzed was found to have a hazard quotient higher than the permissible limits if the comparison between the stations is taken into account. The Veraval samples had

Table 3 Hazard quotient of heavy metals in wet fish tissues

Serial no.	Sampling locations	Body parts of fish	Hazard quotient						
			If the calculated hazard quotient (HQ) is greater than 0.2 which indicates that a risk to human health potentially exists						
			As	Cd	Cu	Hg	Ni	Pb	Zn
1	Diu	Ventral	ND	*2.758132*	0.107885	ND	0.014214	*25,799.151343*	0.085926
		Dorsal	ND	*2.685997*	0.015063	ND	0.017609	*21,216.407355*	0.166661
		Gill	ND	*2.685997*	0.075106	ND	0.022913	*15,572.842998*	0.178104
2	Salaya	Ventral	ND	*2.970297*	0.027369	ND	0.024823	*26,859.971711*	0.06966
		Dorsal	ND	*2.685997*	0.010608	ND	0.001697	*26,520.509193*	0.121046
		Gill	ND	*3.500707*	0.059193	ND	0.005304	*11,669.024045*	0.080735
3	Veraval	Ventral	ND	*2.792079*	0.054844	ND	0.010608	*20,155.586987*	0.075671
		Dorsal	ND	*2.685997*	0.01676	ND	0.001697	*22,277.227722*	0.126944
		Gill	ND	*3.500707*	0.057496	ND	0.021216	*102,178.217821*	0.086393
4	Mahuva	Ventral	ND	*2.898161*	0.035325	ND	0.035431	*36,067.892503*	0.086393
		Dorsal	ND	*2.898161*	0.167079	ND	0.00891	*64,710.042432*	0.11669
		Gill	ND	*2.758132*	*0.357178*	ND	0.114992	*19,094.766619*	0.107963
5	Jafrabad	Ventral	ND	*2.898161*	*0.206011*	ND	0.007001	*42,093.352192*	0.089702
		Dorsal	ND	*2.970297*	0.05442	ND	ND	*38,189.533239*	0.092758
		Gill	ND	*2.864214*	0.101626	ND	0.042432	*31,824.611032*	0.092758
6	Ghogha	Ventral	ND	ND	0.018564	ND	ND	*18,033.946251*	0.146152
		Dorsal	ND	ND	0.061951	ND	ND	*44,554.455445*	0.181046
		Gill	ND	ND	*0.34657*	ND	*0.304031*	*18,373.408769*	0.192121

significantly higher concentrations than the others, which is well-supported by the fact that because there are large numbers of fishing boats in the area, which use industrial paints that contain Pb (Gondal and Hussain 2007). Average half-life of Pb in soft tissues is around 40 days. However, if it reaches the bones, it can stay there for 20–30 years (Papanikolaou et al. 2005). Toxic effects of the lead include dizziness and nervous damage, apart from which Pb is a known agonist of calcium in the body, having the ability to bind with to calmodulin and other calcium channels (Gwalteney-Brant 2002; Simons 1986; Goyer 1997; Bridges and Zalups 2005).

The highest concentration of Zn was found at Ghogha, which is higher than the previously reported concentration of 127.67 ± 3.08 mg/kg (Omar et al. 2013). However, Zn is a low-to-moderate kind of toxic metal because it is an essential element for some body functions. Nevertheless, a chronic exposure to high doses of zinc may lead to nausea, headache, skin irritation, and deficiencies of calcium and copper. Zn, being a divalent ion, may cause agonistic effects to such metals as Ca and Cu (Fosmire 1990). Present samples, however, proved to be safe in risk assessment by hazard quotient study that all the stations were found to have such low concentration of Zn whose potential risk is lower in comparison to other metals.

This is a comprehensive study to understand the pollution level of fish captured from an important natural habitat called Gulf of Khambat. As the export of fishery product is one of the major sources of foreign exchange in this state, understanding the quality of fish with respect to different contaminations is very important. Hazard quotient calculation from heavy metal data and presence of pathogenic bacteria in the study indicates a potential risk to human health. On the basis of this and other related studies, appropriate measures can be taken to check the quality of fish before consumption both in domestic and international markets.

Acknowledgements Financial support for this research for IR was given by DST-INSPIRE fellowship of the Government of India. We thank anonymous reviewers whose comments were very helpful to improve the manuscript.

The Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre), the Department of Science and Technology (DST), and Ministry of Science and Technology, Government of India, are acknowledged for providing the research training fellowship for developing country scientists (RTF-DCS) to carry out this research. We sincerely acknowledge GAP 2031 project funded by the Ministry of Earth Sciences for financial support. Sincere thanks are also extended to AD and CIF of CSIR-CSMCRI for having provided all the instrumental facilities.

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