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Research Article

CLIMATE CHANGES AND ITS IMPACT ON FISH WITH REFERENCE TO ANTIBIOTIC RESISTANT ENTERIC BACTERIA AND HEAVY METAL ACCUMULATION

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ABSTRACT

Fresh fish (*Mugil cephalus*) is a very important source of protein to the population in our country. This fish may harbor *Salmonella sp.* It may be a source of pathogen to human being. A total of 20 samples (20 muscles and 20 gills) were analyzed. The isolates were exposed to 5 different antibiotics. Most of the isolated were resistances to at least one of the antibiotic. This is a clear indication that sewage effluent causes contamination of marine wildlife. Investigation on the accumulation of heavy metals (Cd, Cu and Cr) was carried out commercially important fish (*Mugil cphalus*). The accumulation was observed in tissues of muscles and gills. The result revealed that the copper and cadmium concentration were highest in the muscle and gills. In the muscle and gill of *Mugil cephalus* the order of accumulation was Copper>Cadmium>Chromium.

Key words: *Salmonella sp*, heavy metals, fish, muscles, gills.

INTRODUCTION

Today, humankind's activities are altering the world's climate. We are increasing the atmospheric concentration of energy-trapping gases, thereby amplifying the natural "greenhouse effect" that makes the Earth habitable. These greenhouse gases (GHGs) comprise principally, carbon dioxide (mostly from fossil fuel combustion and forest burning), plus other heat-trapping gases such as methane (from irrigated agriculture, animal husbandry and oil extraction), nitrous oxide and various human-made halocarbons. Change in world climate would influence the functioning of many ecosystems and their member species. The majority of 1.3 billion annual cases of *Salmonella* – caused human gastroenteritis result from ingestion of contaminated food products such as undercooked beef, pork, eggs, milk, shell fish and fish (pang *et al*.,1995), (Gomez *et al*.,1997), (Esaki *et al*., 2004). *Salmonella* infections can also be contracted following consumption of fresh fruits or vegetables contaminated by fertilizer (Auxe, 1997). Birds and flies are important vectors for rapid widespread dissemination of *Salmonella* in the environment (Davies and Wray, 1996). *Salmonella* withstands a wider variety of stresses associated with environmental fluctuations and may persist in water environment for some time. *Salmonella* can be disseminated as a result of water currents, underground springs and rain runoff carrying contaminated material (Chao *et al*., 1987), (Abdelmonem and Dowider, 1990). Like *E.coli*, *Salmonella* is constantly released into environment from infected human, farm animals, pets and wildlife Al-Yousuf *et al.*, (2000 a). Pathogenic and potentially pathogenic bacteria associated with fish and shell fish include *Mycobacteria*, *Streptococcus iniae, Vibrio vulnificus, Vibro spp, Aeromonads*, *Salmonella spp, Shigella* and the others (Lipp and Rose 1997),(Zlotkin *et al* 1989),(Bhaftopadhyay,2003). Human infections by these fish pathogen are usually through contact with infected fish while handling them, water or other constituents of fish life environment (Acha and Szyfres, 2003). The initial microflora on the surface of fish is directly related to the water environment while the flora in the gastrointestinal tract corresponds to the type of food and condition of fish (Liton, 1980).

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Municipal untreated sewage, run off and storm water are the most important immediate microbiological pollutants (Kayambo and Sven, 2006). The low standard of health in the gulf of mannar region is caused by a general lack of awareness of good hygiene practices, direct contamination of beach waters through bathing and washing and uncontrolled waste disposal around the shoreline. Other sectors like wildlife, agriculture, forestry, urban and rural settlements have been implicated to contribute to microbiological pollution. These activities increase eutrophication process thus creating a vast conducive environment for the survival of microbes which eventually infect fish.

Analysis of fish tissue slurry indicated that fish harvested from landing beaches along gulf are infested with; *Salmonella*, *Shigella* and *E. coli* (Onyango *et al*., 2008b). Given the prevalence of water and food borne disease; Salmonellosis in marine fishes, it was important that all possible infection routes of the pathogens be investigated and prevention measures recommended. Fishes are major part of the human diet and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish. Predominantly, fish toxicological and environmental studies have prompted interest in the determination of toxic elements in seafood.

This study aimed to isolate and characterize *Salmonella* from marine water and fish muscle and gills and to determine the concentrations of heavy metals such as Cadmium, Chromium and Copper in fish muscle, collected from Rameswaram and Tuticorin. It is expected that the results of this research will assist in acquiring information about the level of toxic metals in this region.

MATERIALS AND METHODS

1. Collection of sample

Marine fish samples (*Mugil cephalus*) were collected from markets located in two different cities (Rameswaran and Tuticorin).The samples were collected in the month (batch wise) of January-March-I, April-June-II, July-September-III, October -December-IV. An average of 5 fishes was bought and transported to laboratory in plastic container within 4 hours. Then marine water samples were also collected in the same area.

2. Sample processing for microbial analysis

With gloved hands and sterilized knife, the fish was severed into parts (Gill and whole body). 20 grams of each part was grinded with 225ml Buffered Peptone Water (BPW) for 3min. Pellet were obtained by centrifugation at 20^0C, 10,000 x g RPM, for 15minutes for fish sample. The pellet was then dissolved into 10ml of BPW.

The inoculums were later streaked on to *Salmonella Shigella Agar* (SSA Difco), Xylose-Lysine Deoxycholate (XLD Difico) and Bismuth sulfite Agar (BSA – Difco) and were incubated at 37^0C for 48hrs. In SSA *Salmonella spp.* were seen as white or yellow with black spot centrally, in XLD, *Salmonella spp.* grew as pink color with black centre while in BSA salmonella colony grew as grey black with metallic sheen color.

3. Antimicrobial sensitivity test

The agar diffusion method according to Kirby Bauer guidelines was applied for antimicrobial susceptibility testing of *Salmonella* isolates. Mueller Hinton Agar was used in order to perform this test. Suspension of 0.5ml density was used for inoculation. Total of eight isolates from Rameswaram and Tuticorin fishes were tested for antimicrobial susceptibility. The following antimicrobial agents were used: Amoxycillin, Tetracycline, Penicillin, Streptomycin and Chloramphenicol. The results were tabulated.

4. Quantitative analysis of heavy metals

Sample preparation for heavy metal analysis: About 20.0 g of fresh fish samples were weighed accurately and homogenized then poured in an iodine flask separately; 25 ml of concentrated HNO3 was added into each flask. The iodine flasks were refluxed for 1 hr. at 95°C ± 5°C. The sample solutions were cooled and 10 ml of Concentrated HNO3 was added into each flask. The flasks were again were refluxed for about 1 hr. at 95°C ± 5°C. Repeated the process until the digestion was completed. Evaporated the solution to 5 ml. Solutions were cooled and 10 ml of Concentrated HCl was added into each flasks. The solution was kept for refluxed for about 15 min to remove the nitrous fumes. Cooled the digested sample solutions, 20 ml of HPLC grade water was added into each flask. Filtered the digested solution through Whatman filter paper no. 41 into 50 ml volumetric flask and made up to the volume using HPLC grade water. Recovery study was carried out by fortifying known concentration of standards into pre analyzed sample.

To determine the metals (cadmium, chromium and copper) concentration in the samples, a atomic absorption spectrometer (AAS) was used. All chemical regents were from analytical reagent grade (Merck). All solutions were prepared in deionized water. Calibration standards of each heavy metal were prepared by appropriate dilution of the stock solutions (1000 ppm, Merck). The glassware and plastic containers were acid washed with nitric acid 10% for 24 h and rinsed with double distilled water before use.

RESULT AND DISCUSSION

The method of isolation and media used contributed to the effect of *Salmonella sp* significance. This proves that different media produce different results and performance. The result obtained of the gills and whole body fish differ because of the sensitivity of different media that was used. From this study, SSA media was more sensitive than BSA (Plate 1). *Salmonella spp.* was present in all parts of the fish especially the whole body, gills and marine water represented in Table 1 and 2. In the above study, SSA gave more bacterial isolates than the other two XLD and BSA. Dutch *et al*., (1995) reported that the sensitivity of SSA and BSA were 76.6% and 50.0% respectively. (Michael *et al*., 2003) showed that SSA presented better conditions for isolation of *salmonella* sp. colonies, hence eliminating the volume of false positives.

As a result, the better selectivity of the media is responsible for the greater detection of *Salmonella sp:* majorly when streaked from a selective enrichment that eliminates overgrowth of competitors the method of isolation was largely responsible for the significant difference on *Salmonella sp.* From this study, *Salmonella sp.* was found to contaminate different parts of the body. This was supported by the finding (Haltha *et al*., 1997). That these bacteria would exist on fish's skin, gills and intestine and the most potential reservoir of *salmonella* spp. was the intestine. Hence, it is highly recommended that cross – contamination of other tissue notably digestive tract during handling or preparation be avoided. This is important for future study in order to know the route of salmonella species transmission from pond to the next food chain supply. The study showed that salmonella was more on the gills than whole body of fish. *Salmonella shigella agar* proved to be a better selective media than the other two media.

Determinations of *Salmonellae* by specific media (SSA) in water sample were detected. The highest numbers during one year (January 2012-December 2012) was 11 x 10^2in Rameswaram in Table 1. The highest *Salmonella* sp was detected in muscle and gills of Rameswarm fishes 3.0 x 10^2 and 4.3X10^2 respectively. The maximum number of *Salmonella* sp occurred in Tuticorin fish muscle and gills region were 5.2X10^2 and 7.2X10^3 respectively. From this result we concluded that the density of *Salmonellae* sp the minimum value was recorded in month of January to march (Table 3). Similar results were obtained by Hunter and McDonald number was significantly higher in STE and polluted (1991), Tian *et al.* (2002) and Hyland *et al.* (2003) who stated that seawater over unpolluted. *Enterobacteriaceae* were the faecal indicator bacteria populations normally peak in isolated from gills, skin, and muscles.

In our study, the isolated *Salmonella sp* are more sensitivity to Sreptomycin, Amoxycillin and Pencillin. But highly resistant pattern was observed in Chloramphenicol and Tetracycline drugs (Table 4, 5 and Plate 2). Antimicrobial agents and their metabolites entering the aquatic environment become highly diluted and therefore detection of these compounds becomes extremely difficult (Kümmerer, 2009). Overuse of antibiotics has led to the emergence of resistant bacteria and consequently caused an imbalance between susceptible and resistant bacteria. This eventually has sub-grouped them into susceptible and resistant variants (Levy, 1994). In addition, the potent killing and growth inhibition of bacteria have increased the number of resistant strains which have ultimately evolved into prominent populations of the microbial flora (Levy, 1992).

Hence, the presence of antibiotic resistant bacteria have been used as bio-indicators of polluted effluents since resistant bacteria can be easily isolated and detected (Al-Bahry *et al*., 2009). Based on the present results, the coastal area of Gulf of Mannar and Tuticorin its fish are continuously exposed to contaminated discharge. Various types of plastics ,bottles, waste clothes, papers, rusted materials and wasted or unused pharmaceutical compounds were dumped at the area of Gulf of mannar through the ship from other countries (Srilanka, Malaysia and Myanmar).The microbes of human origin are affecting the marine environment and antibiotic resistant determinants are being transferred to other bacteria in the area. Further studies on the effects of sewage discharge and human contamination and bacterial contamination on the environment and public health are urgently needed in different regions of Indian coastal.

The results of heavy metals in fish sample from Rameswaram and Tuticorin are presented in Figure 1 and 2. The concentration of cadmium was found to be higher in fish gills (1.32µg/g) least in the muscles (0.09µg/g). Chromium had an overall mean value of (1.04 µg/g) in the fish parts. Copper also had its highest value (5.22µg/g) in muscles at Rm3. It however had its lowest concentration in the fish gills (0.09µg/g) followed by in the fish muscles (0.13µg/g). It was generally observed that amongst the fish parts, gills have the highest concentration of heavy metals while the muscles had the lowest concentrations. Heavy metals entering the fish have a possibility to get accumulated in different parts of the body and the residual amount can build up to a toxic level. The fish, *Mugil cephalus* is economically important and they form a large part of the fish catch in the study area.

Metal elimination routes are more than uptake routes, however metal accumulation is more rapid than metal elimination probably due to the presence of metal binding proteins in tissues (Kendrick *et al*., 1992). The accumulation of the metals in liver could be based on the greater tendency of the elements to react with the oxygen, carboxylate, amino group, nitrogen or sulphur of the mercapto group in the metallothionein protein, which was at highest concentration in the liver (Kendrick *et al*., 1992). These complexes are slowly redistributed to the renal cortex. Liver has also an important role in contaminant storage, redistribution, detoxification or transformation and also serve as an active site of pathological effects induced by contaminants (El-Shahawi, 1996). This study revealed that metal accumulation in gills and liver occurs in higher magnitude than what appeared in the muscle. This is a common finding that is also reported by several investigations (Usero *et al*., 2003; Dural *et al*., 2007; Al-Yousuf *et al*., 2000 b). Because of the presence of high levels of metallothionein protein, liver tissue acts as a target organ for heavy metal detoxification (Yilmaz, 2003; Kraemer *et al*., 2006; Canli and Atli, 2003; Al-Yousuf *et al*., 2000 b; Romeo *et al*., 1999). Gills act as the main site for entry of different kinds of contaminants such as heavy metals due to its continuous contact with the external medium. This organ serves a variety of physiological functions such as respiratory gas exchange, osmoregulation and nitrogen excretion (Hoar and Randall, 1984; Altindag and Yigit, 2005). Therefore, heavy metals may appear in a high level in liver and gill tissues compared to what occurs in muscle. Muscle tissue has lower tendency to accumulate heavy metals (Huang, 2003, Al-Saleh, and N. Shinwari, 2002, Altındag, and S. Yig, 2005, Aucoin *et al*., 1996). Altindag and Yigit, (2005), Romeo *et al*., (1999) and Huang, (2003) found higher concentration of heavy metals in liver and gill than in muscle.

Table 1. The number of *salmonella spp*. Occurred in marine water sample.

Water sample	No. of CFU/mL
R1	11 $X10^{2}$
R2	29 $X10^{1}$
R3	15 $X10^{1}$
R4	12 $X10^{1}$
T1	21 $X10^{1}$
T2	19 $X10^{1}$
T3	5 $X10^{1}$
T4	9 $X10^{1}$

R1, R2, R3, R4-Rameswaram water sample

T1, T2, T3, T4-Tuticorin water sample.

Table 2. Antibiotic sensitivity (mm) patterns of *Salmonella spp*. isolated from water sample.

Antibiotic Compound	R1	R2	R3	R4	T1	T2	T3	T4
Amoxycillin	5.0	-	5.0	6.1	12	5.0	8.2	-
Pencilline	7.4	15.2	11.2	-	-	-	-	-
Tetracycline	-	12.4	8.4	12	10.2	-	10.8	8.4
Streptomycin	-	10.2	7.2	9.4	-	9.5	-	12.8
Chloramphenicol	5.2	-	8.4	-	-	-	-	7.6

Table 3. The number of *Salmonella* bacteria present in marine fish sample.

Sample month/Number	Rameswaram		Tuticorin	
	Muscle	Gills	Muscle	Gills
Jan-Mar-I	3.0×10^2	2.8×10^3	4.6×10^2	7.2×10^3
Apr-Jun-II	2.5×10^2	3.1×10^2	5.0×10^2	6.4×10^3
Jul-Sep-III	2.0×10^2	3.6×10^2	5.2×10^2	5.9×10^3
Oct-Dec-IV	2.8×10^2	4.3×10^2	4.0×10^2	6.0×10^3

Table 4. Antibiotic sensitivity (mm) patterns of *Salmonella spp*. Isolated from Rameswaram fish (Muscle and gills) sample.

Antibiotic compound	Rm1	Rm2	Rm3	Rm4	RG1	RG2	RG3	RG4
Amoxycillin	-	8.5	8.0	-	7.0	6.3	-	12.2
Pencillin	10	-	-	8.4	-	6.8	-	11.0
Tetracycline	8.3	6.5	-	5.3	-	-	12.5	9.0
Streptomycin	9.0	4.8	-	8.0	11	-	-	8.8
Chloramphenicol	8.0	-	6.5	-	-	-	-	-

Rm1, Rm2, Rm3, Rm4- Fish muscle of Rameswaram.

RG1, RG2, RG3, RG4-Fish gills of Rameswaram.

Table 5. Antibiotic sensitivity (mm) patterns of *Salmonella* sp isolated from Tutucorin fish (Muscle and gills) sample.

Antibiotic compound	Tm1	Tm2	Tm3	Tm4	TG1	TG2	TG3	TG4
Amoxycillin	-	-	-	6.7	8.7	-	-	5.4
Pencilline	6.9	-	4.0	7.3	-	4.2	-	-
Tetracycline	7.8	-	10.0	-	-	-	-	-
Streptomycin	12.5	8.5	-	9.3	9.2	6.2	-	8.4
Chloramphenicol	-	-	-	12.6	4.0	-	8.8	7.9

Tm, Tm2, Tm3, Tm4-Fish muscle of Tuticorin.

TG1, TG2, TG3, TG4-Fish gills of Tuticorin.

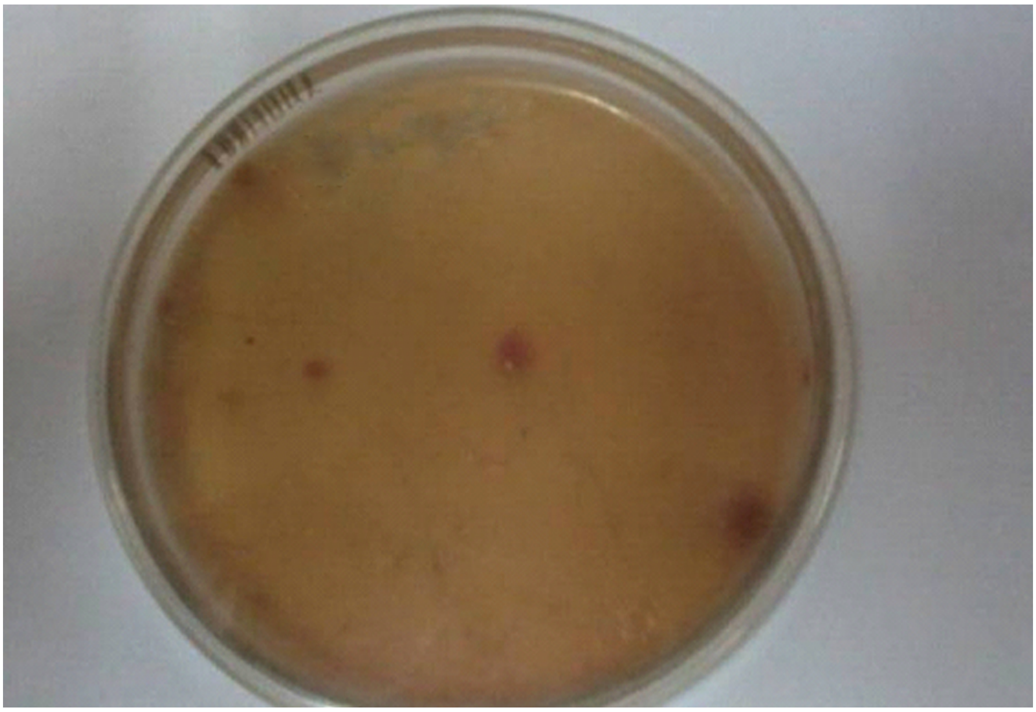

Plate 1: Isolation of *Salmonella sp* from fish by using SS agar medium.

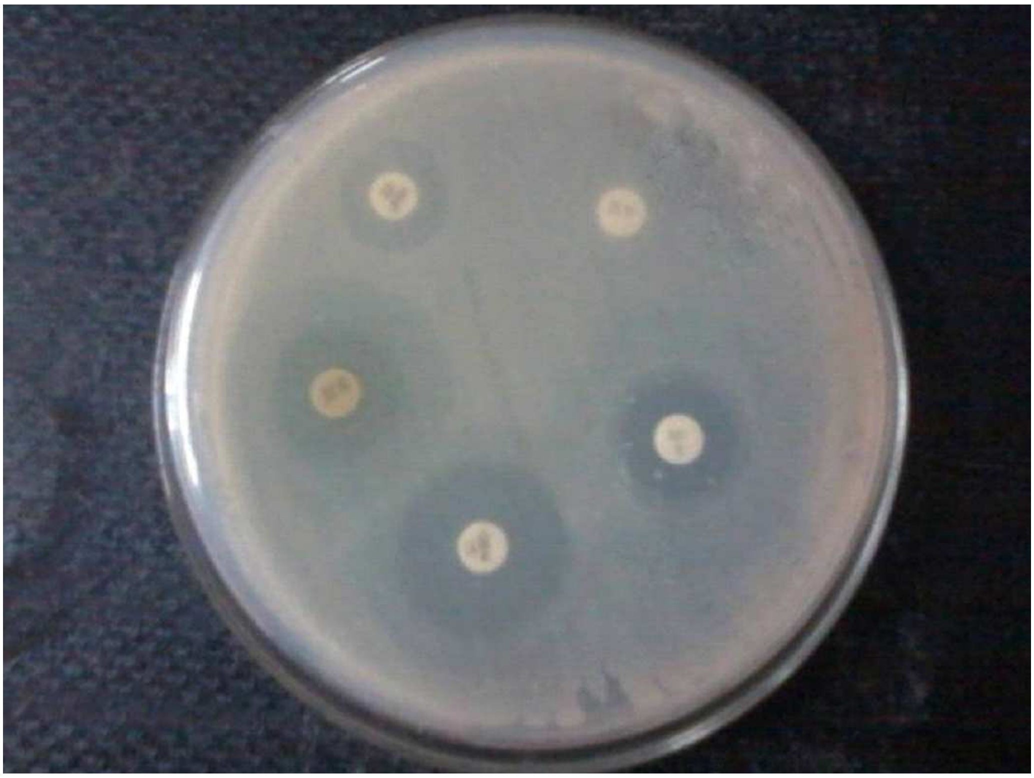

Plate 2: Antibiotic sensitivity (mm) patterns of *Salmonella sp* isolated from fish (Muscle and gills) sample.

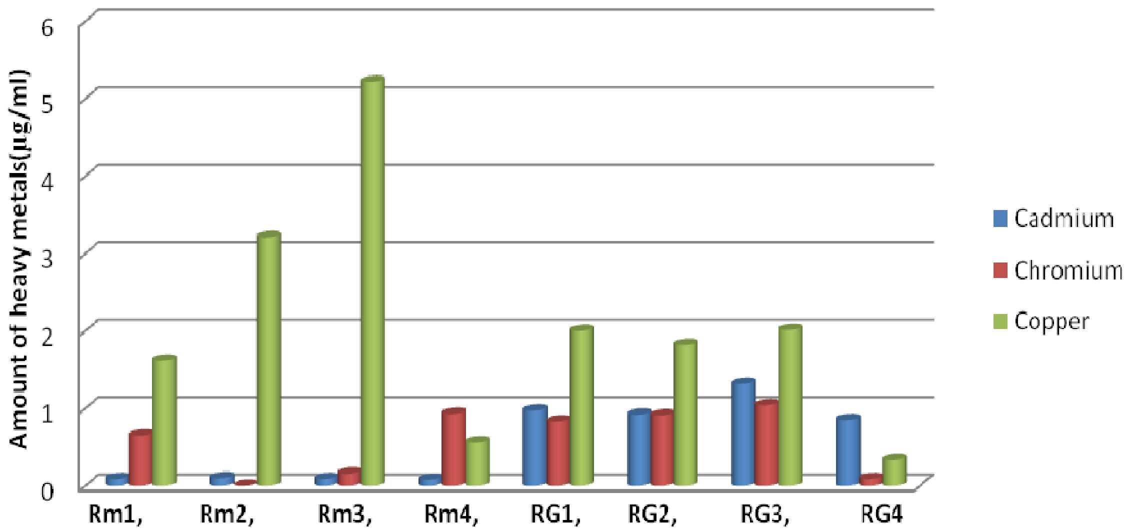

Figure 2. Heavy metals concentration in the muscles and gills region of Rameswaram fish.

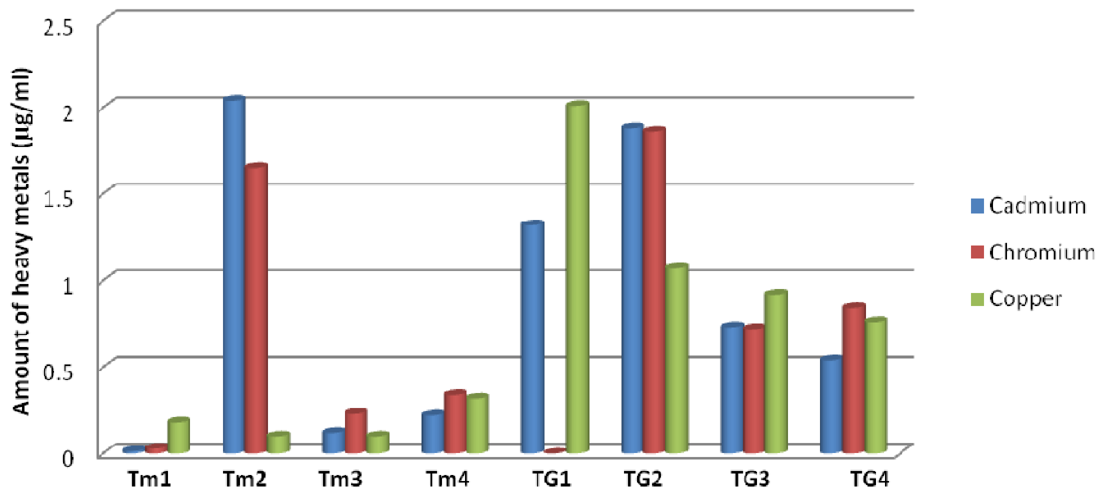

Figure 2. Heavy metals concentration in the muscles and gills region of Tuticorin fish.

CONCLUSION

In the present study, the microbes of human origin are affecting the marine environment and antibiotic resistant determinants are being transferred to other bacteria and human being. Immunosuppressive person and old age people are easily affect the salmonellasis diseases. In our study, provides new information on the concentration of heavy metals in the fish from Rameswaram and Tuticorin Harbor Area. Heavy metal concentrations in coastal waters as well as fish tissues have been found variable. The public health implication of the research seems to show no possibility of acute toxicity of heavy metals (Cu, Cd, Cr,) of edible fishes consumed. Nonetheless, continuous monitoring of biodata in these areas should continue while government should enforce existing pollution control laws, so that the metal concentrations do not get to critical levels but the fish adopted to that metal concentration then it will accumulate more metals in body that will threaten human health.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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