

Total mercury and methylmercury in commercial marine species from the Goa coast: Constraints on risk assessment and environmental issues

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Mercury (Hg) is a toxic element which accumulates in fish and other aquatic organisms from various sources and poses potential risk to the consumers. Methylmercury (MeHg), the common organic species of mercury, usually forms in aquatic environments and is known for neurotoxicity. In this study, the concentration of total Hg and MeHg in 12 commercial species of fish, shellfish, prawn, and crab from coastal areas of Goa were investigated. The total Hg contents varied between 18.5 -260 $\mu\text{g}/\text{kg}$; while the concentration of MeHg had a range of 7.2 -129 $\mu\text{g}/\text{kg}$. Both the ranges were well below the values recorded at other polluted coastal sites and also meet the permissible limits for human consumption. The interspecies comparison shows species from pelagic and benthic habitats with different food habits have significant difference in body loads of total Hg and MeHg, which increases across the trophic levels. The exposure to higher level of Hg in sediment is likely responsible for enhanced bio-concentration of Hg in benthic species as compared to the values in pelagic fishes.

[Keywords: Crab; Fish; Goa coast; Mercury; Methylmercury; Prawn; Shellfish]

Introduction

Mercury (Hg) is a non-essential, toxic heavy metal and considered as one of the ten most dangerous chemicals for public health¹. In the natural environment, Hg originates from a variety of anthropogenic as well as natural sources. About one-third of the total global production of Hg is introduced through various anthropogenic activities^{2,3}. In the present century, remarkable increase in the use of Hg made it available in almost all parts of the environment⁴. Studies on global Hg-cycle show that through wet and dry depositions the naturally available Hg finds way to riverine and marine waters^{5,6}. In aquatic environments, the elemental and inorganic-Hg compounds convert into more toxic organic complexes (e.g. methyl-, ethyl- and phenyl-derivatives of Hg) by certain microbial community⁷. These organo-Hg complexes are persistent in nature and can enter into natural food chains^{5,8}. Due to lipophilic nature, these organo-Hg compounds tend to be accumulated in fishes and other aquatic organisms. The fishes and other seafoods are the major source of protein for a large fraction of the world population, therefore Hg-contamination in seafood could pose risks to human health; and which is becoming a serious global

concern⁹. Therefore, it is essential to investigate the Hg concentration in seafood on regular basis.

A study by Pacyna et al.¹⁰ indicates a considerable fraction of anthropogenic Hg (~8.93 % of total global contribution) originates from India. Mostly burning of fossil fuels and various industrial usages are the prominent sources of Hg¹⁰; which ultimately get released in different aquatic environments. In recent years, several studies addressed the Hg levels in natural seafood from a few pockets of Indian coastal waters¹¹⁻¹⁸. However, the information regarding Hg concentrations in commercial seafood from coastal waters of Goa is rarely available. Moreover, the major fraction of Hg in aquatic organisms is present as methyl-mercury (MeHg)¹⁹, which is more toxic than inorganic Hg-species. The edible marine species from Indian coastal waters are hardly investigated for MeHg contamination²⁰. In the present study, for the first time, an attempt has been made to assess the dietary risks of total mercury (THg) and MeHg in various edible species available in estuarine and coastal waters of Goa. The results are compared with the levels of Hg contamination in seafood reported in other estuaries and coastal waters around the world. The variability in abundance of MeHg in various species has also been discussed in terms of their food habits and nature of habitats.

Materials and Methods

On the central west coast of India, Goa is a coastal state, drained by a network of nine rivers. Among these rivers, Mandovi and Zuari are regarded as the lifelines of Goa as their watershed covers a major part of the total area of the state and their waters are used by local people extensively. A large fraction of Goan population resides alongside these rivers leading to increased outfalls of wastewater in two estuaries located between 15°25' to 15°30'N and 73°45' to 73°59'E (Fig. 1). In the present study, a variety of common commercial species including fishes, prawns, shellfishes, and crab from these estuaries and near-shore areas were selected for THg and MeHg assessment. In addition, few species from the estuaries of river Chapora and Kali (Fig. 1) were also analysed. Fishes like Indian mackerel (*Rastelliger kanagartha*), sardine (*Sardinella longiceps*), bearded croaker (*Johnius amblycephalus*) and prawns (*Penaeus indicus* and *Metapenaeus dobsoni*) were collected by common fishing net with the help of local fishermen; while benthic species like oysters (*Crassostrea gigas* and *Saccostrea cucullata*), clams

(*Paphia malabarica* and *Meretrix casta*), mussel (*Perna viridis*), flat fish (*Paraplagusia bilineata*) and crab (*Scylla serrata*) were handpicked from different coastal locations.

In the laboratory, all the samples were thoroughly cleaned and the body weight was estimated. The muscle tissues from mature animals of each species were dissected out and then freeze-dried at -60 °C. Dry samples were powdered with a blender and stored in clean glass vials. The bulk concentrations of Hg (THg) in homogenized samples were estimated by using Direct Mercury Analyzer (DMA-80, Milestone Inc.) following the EPA 7473 method²¹. About 0.1 g of the homogenized dry tissue samples were weighed out into clean, muffled (at 615 °C) quartz boats and then directly analysed with Mercury Analyzer without any more processing. The average result of triplicate analyses of each sample is reported in terms of µg Hg per kg of dry tissue.

MeHg from the tissue samples were extracted by following the procedure described by Maggi et al.²². In this procedure, 1.5 g of dry powdered sample was weighed out in screw-capped polypropylene tubes. Then samples were hydrolysed with 10 ml extra pure HBr (47 – 49 %), using a vertical shaker at 50 rpm for 5.0 minutes. MeHg was extracted from the acid hydrolysed solution by shaking with 20 ml extra pure toluene. This extraction step was repeated with another 15 ml toluene and then the combined toluene phases were back extracted twice into 6 ml of 1.0 % (w/v) aqueous L-Cysteine solution to strip off the MeHg from toluene. After the addition of reagents, the contents were thoroughly mixed by using a shaker for 20 minutes and centrifuged at 2400 rpm for 20 minutes. 20 µl of L-Cysteine extract was analysed directly with DMA to estimate the MeHg present in samples.

The accuracy of analytical results was verified against the Dogfish tissue standard (DORM-4) obtained from the National Research Council, Canada. Multiple analyses of DORM-4 produced the values of THg and MeHg within the certified range and the recovery of more than 99 % was achieved (Table 1).

Results

The estimated values of THg and MeHg in selected species are summarized in Table 2 and the results showed a wide range of concentrations (Fig. 2) in the edible species from estuaries and coastal waters of Goa. Particularly, in fishes (e.g. *R. kanagartha*,

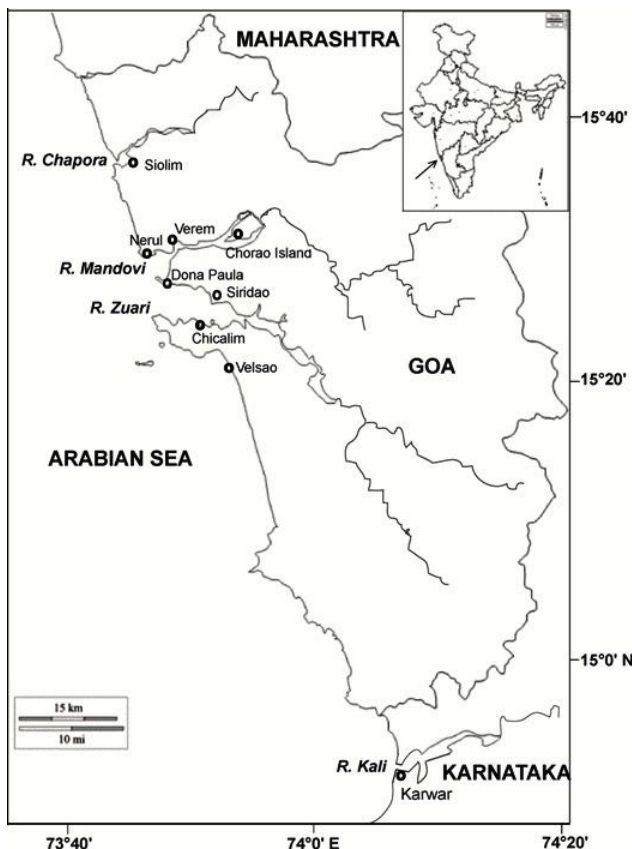


Fig. 1 — Map displaying the sampling locations along the estuarine and coastal areas of Goa

S. longiceps, *J. amblycephalus*, *P. billineata*) the variation of THg was maximum (18.5 –260 µg/kg) as compared to values found in prawns (45 -53 µg/kg in *P. indicus* and *M. dobsoni*); shellfishes (20 -75 µg/kg in

M. casta, *P. malabarica*, *P. viridis*, *C. Gigas*, and *S. cucullata*) and edible crab (105 µg/kg in *S. serrata*). Similarly, these groups also had a significant variation of MeHg contents in their body tissue; which ranged from 12.6 -130 µg/kg in fishes; 7-44 µg/kg in shellfishes; 14.8 µg/kg in penaeid prawns and 60.5 µg/kg in crab (Fig. 2; Table 2). The estimated range of MeHg in these commercial species represents ~ 24 to 84 % of THg available in their soft body tissue (Table 2). Results also showed that there were marginal differences in THg and MeHg contents in mussel and clams (e.g. *P. viridis*, *P. Malabarica*, and *M. casta*) collected from different locations (Table 2). The comparison among species from different habitats shows benthic animals have wider ranges of Hg concentration (19-261 µg/kg) as compared to the values found in pelagic fishes (18-155 µg/kg). Irrespective of types of habitat, typical predatory species like *J. amblycephalus* and *P. billineata* had

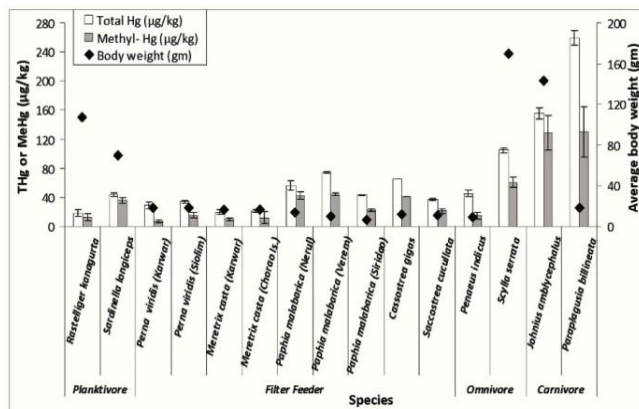


Fig. 2 — The abundance of mercury and methylmercury in comparison to body weight of different commercial species from estuarine and coastal areas of Goa

Table 1 — Estimated concentrations of THg and MeHg in biological reference material.

Certified Reference Material	Analytical parameters	Number of analyses	Certified value (µg/kg)	Estimated value (µg/kg)	Percentage recovery (%)
Dogfish tissue (DORM-4)	Total mercury	12	410 ±55	407 ±11.7	99.21
	Methylmercury	08	354 ±31	352 ±23.2	99.40

Table 2 — Total and methylmercury contents in the tissues of different edible marine species collected from pelagic and benthic environments in coastal areas of Goa.

Habitat	Species (common name)	Sampling location	No. of animal (N)	Average weight (gm)	Total-Hg (µg/kg)	Methyl-Hg (µg/kg)	% of MeHg
Pelagic	<i>Rastelligerkanagurta</i> (Indian Mackerel)	Goa coast	03	107.1	18.5 ±4.3	12.6 ±5.0	67.9
	<i>Sardinella longiceps</i> (Indian oil sardine)	Goa coast	03	70.2	44.2 ±2.6	36.1 ±3.9	81.6
	<i>Johniusamblycephalus</i> (Bearded croaker)	Goa coast	05	143.2	155 ±7.8	129 ± 24	83.2
Demersal or Benthic	<i>Paraplagusiabillineata</i> (Tonguesole)	Mandovi estuary	03	18.3	260 ±10	129.9 ±35	49.7
Benthic	<i>Meretrix casta</i> (Yellow clam)	Karwar, Kali estuary	14	16.2	19.9 ±2.9	9.7 ±2.0	48.7
		Chorao Island, Mandovi estuary	10	16.5	21.1 ±1.6	11.9 ±8.3	56.3
	<i>Paphiamalabarica</i> (Shortneck clam)	Nerul, Mandovi estuary	12	14.1	56.6 ±6.3	42.6 ±5.4	75.3
		Verem, Mandovi estuary	36	9.7	74.8 ±1.2	44.3 ±1.5	59.3
	<i>Pernaviridis</i> (Green mussel)	Siridao, Zuari estuary	28	6.2	43.4 ±0.9	22.7 ±2.2	52.4
		Karwar, Kali estuary	09	17.9	29.1 ±4.5	7.18 ±2.0	24.7
	<i>Cassostrea gigas</i> (Pacific Oyster)	Siolim, Chapora estuary	04	18.0	34.2 ±1.6	15.4 ±4.1	44.9
		Dona Paula, Zuari estuary	34	12.3	65.7 ±0.2	40.9 ±0.4	62.4
	<i>Saccostrea cucullata</i> (Rock oyster)	Velsao, Zuari estuary	23	10.6	36.9 ±1.2	21.5 ±3.1	58.2
	<i>Penaeus indicus</i> (Indian white prawn)	Goa coast	03	8.9	45.5 ±4.2	14.8 ±4.5	32.4
<i>Metapenaeusdobsoni</i> (Kadal shrimp)	Goa coast	03	6.1	53.1 ±5.3	--	--	
<i>Scylla serrata</i> (Mud crab)	Chicalim, Zuari estuary	03	169.6	105 ±3.1	60.5 ±7.0	57.6	

higher THg and MeHg concentrations relative to filter feeders or omnivorous organisms (Table 2).

Discussion

Total mercury and methylmercury in commercial species: Assessment of dietary risk

The common fishes like mackerel, sardine, and edible bivalves including clam, green mussels, and oysters which are largely consumed by local people have very low ranges of THg and MeHg. Even the highest observed concentration of THg (261 µg/kg) in fish like tonguesole (*P. billineata*) was lower than values reported in fishes from several polluted marine environments within or outside of India²³⁻²⁶. Similarly, the concentrations of MeHg in edible species from Goa (7 -129 µg/kg) were also quite less than the values recorded elsewhere (Table 3). The low levels of THg in seawater (0.03 -1.5 µg/L)¹³ and surface sediment (40- 200 µg/kg)¹³ in coastal areas of Goa restrict Hg-contamination in biological species. Less availability of THg also reduces the chance of MeHg production in the ambient environment, which limits the bioavailability of MeHg for aquatic organisms. Besides the reduced loads of Hg, other environmental factors like oxic and alkaline conditions of the water column also would not favor MeHg generation.

The results show that all the commercial species used in this study contain detectable amounts of Hg and MeHg in tissue samples. But the observed ranges of THg (18 -261 µg/kg) and MeHg (7 -129 µg/kg) have not exceeded the limits for human consumption, recommended by several international and national agencies like WHO (500 µg/kg of Hg and 300 µg/kg of MeHg)⁹, EPA (0.5 ppm or 500 µg/kg of Hg)²⁷, US-Food and Drug Administration (1000 µg/kg of Hg)²⁸, European Commission (0.5 ppm or 500 µg/kg Hg)²⁹ and Food Safety and Standard Authority of India (0.5 ppm or 500 µg/kg of Hg and 0.25 ppm or 250 µg/kg of MeHg)³⁰. Therefore, the consumption of these species from coastal waters of Goa is quite safe for public health. Even though these species are safe; according to Hg-loads, there should be optimum limits in the consumption of each species. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the “Provisional Tolerable Weekly Intake” (PTWI) limits for inorganic Hg and MeHg at 4.0 and 1.6 µg/kg of body weight per week respectively³¹. According to this PTWI recommendation, the dietary limits for adults with an average body weight of 60 kg should be maintained at

~0.73 kg dry weight of tonguesole (contents highest MeHg of 129 µg/kg) to ~7.6 kg of mackerel (contents least MeHg of 12.6 µg/kg) in a week.

Interspecies variation of total mercury: Influences of trophic position and habitat

Even though the levels of THg in all the edible species were within the safety limit, a wide range of Hg concentration has been noticed in different species. Among the bottom-dwelling species, the highest Hg concentration (260 ±10.2 µg/kg) was observed in benthic tonguesole (*P. billineata*). This typical carnivorous fish of *Cynoglossidae* family commonly lives in muddy shelf and slope areas and feeds on small benthic invertebrates including various worms, crustaceans, and molluscan species. In contrast, the body load of Hg in filter-feeding benthic bivalves like mussel, clams, and oysters (e.g.

Table 3 — Total and methylmercury in various edible species from aquatic environments recorded in previous studies.

Area	Organism	THg (µg/kg)	MeHg (µg/kg)	Reference
Kuwait Bay	Fish	10 – 3923	<1.0 - 3270	16
Tagus River, Spain	Fish	38 – 1335	31 - 1158	19
Florida coast, USA	Fish	110 – 4980	60 – 4500	20
Ria de Aveiro lagoon, Portugal	Fish	63 - 1100	70 - 250	35
Elbe River, Czech Republic	Fish	62 – 225	ND	36
Scheldt Estuary, North Sea	Fish	22 –613 (wet wt.)	7 -598 (wet wt.)	37
Crete Island, Mediterranean Sea	Fish	0 - 5790	ND	38
New Zealand coast	Fish	20 - 2480	40 - 1970	39
Persian Gulf	Fish	12 – 87 (wet wt.)	11 – 100 (wet wt.)	40
Caspian Sea	Fish	20 – 108 (wet wt.)	10 -107 (wet wt.)	
Taranto Gulf, Mediterranean Sea	Fish	324 -1740	190 - 1040	41
English Channel	Shell fish	0 - 1870	0 - 1321	
Sundarban Wetland, India	Fish	40 -1630	ND	42
Tuticorin Coast, India	Fish	*BDL- 40	ND	14
Thane Creek, India	Fish	34 – 54	31- 51	15
Ulhas Estuary, India	Fish	40 -1118	ND	16
Mumbai Port area, India	Fish	10 – 230	ND	17
Cochin backwaters, India	Fish	62 - 7966	ND	18
Goa Coast, India	Shell fish	20- 75	7 – 44	Present study
	Fish	18 -260	12 - 130	
	Prawn	45 -53	15	
	Crab	105	61	

*BDL = Below detection level; ND= Not determined

P. viridis, *M. casta*, *P. malabarica*, *C. gigas*, *S. cucullata*) of the same estuarine environment of River Zuari was significantly low (19 -74 $\mu\text{g}/\text{kg}$). The omnivorous giant crab (*S. serrata*), collected from estuarine mud-flats; mostly feeds on a variety of detritus materials settling from the water column as well as small benthic species available on the sediment surface, has an intermediate range of Hg concentration of $105 \pm 3.0 \mu\text{g}/\text{kg}$. Thus, bio-concentrations of Hg showed an increasing trend across the trophic structure (Fig. 2) that follows the pattern: planktivore < omnivore < carnivore species.

Like bottom-dwellers, the Hg concentration is also varied in pelagic fishes. The predatory fish, *J. amblycephalus* has higher THg content (>188 $\mu\text{g}/\text{kg}$) and which differs by an order from the values ($18.5 \pm 4.3 \mu\text{g}/\text{kg}$) found in typical filter-feeding epi-pelagic carp, *R. kannagurta*. A similar observation of higher Hg contents in carnivores in comparison to plankton feeders was also reported in other studies^{13,23}. Therefore, the inter-species variability of Hg concentrations in organisms of both pelagic and benthic habitats is mainly due to their diverse food habits.

Results also showed that typical benthic predatory fish (*P. bilineata*) accumulates more Hg relative to pelagic predatory fish, *J. amblycephalus* (Table 1). Of course, the topmost pelagic predators like sharks, swordfish have not been included in the present study and cannot be discussed regarding their Hg contamination. The higher Hg concentration in demersal species probably suggests besides trophic transfer, the higher load of Hg in sediments is also responsible to make Hg more bio-available in benthic environments. Studies showed that Hg available in ambient nature can also diffuse through gills of aquatic organisms and get absorbed into the blood stream³². Therefore, direct inputs from sediment, which have higher Hg contents ($40 - >150 \mu\text{g}/\text{kg}$)¹³ than in local seawater ($0.03 - 1.5 \mu\text{g}/\text{L}$) cannot be ruled out for higher bioaccumulation of Hg in benthic fauna as compared to pelagic species. Thus, bio-accumulation of Hg may also differ depending on the availability of Hg in natural habitat. Moreover, THg and MeHg contents in green mussels and clams (e.g. *M. casta* and *P. malabarica*) from these estuaries were slightly different from each other (Table 1) due to minor differences in availability of Hg at different locations.

Interspecies variation of methylmercury: The environmental and physiological factors

The concentration of MeHg in pelagic fishes ($12.5 - 130 \mu\text{g}/\text{kg}$) was largely comparable to the

ranges found in demersal species ($9 - 130 \mu\text{g}/\text{kg}$). Like THg, the MeHg contents in these species showed increasing trends along the trophic structure with the minimum values in filter-feeders (*P. viridis*, *P. malabarica*, *M. casta*, *C. gigas*, *S. cucullata*, *R. kannagurta*, *S. longiceps*) followed by omnivores (*S. serrata*, *P. indicus*, *M. dobsoni*) and predators (*J. amblycephalus*, *P. bilineata*) (Fig. 2). Compared to planktivorous ($9 - 45 \mu\text{g}/\text{kg}$ MeHg) and omnivorous organisms ($13 - 68 \mu\text{g}/\text{kg}$ MeHg), predators (e.g. *J. amblycephalus* and *P. bilineata*) have higher loads of MeHg ($\sim 130 \mu\text{g}/\text{kg}$) in their body tissues. Such disparity of MeHg concentration in species belongs to different trophic levels suggests MeHg can easily bio-magnify across the marine food chains.

The average MeHg concentration in all the selected species was highly correlated ($R^2 > 0.8$) to the body-load of THg (Fig. 3A). This observed correlation suggests irrespective of the type of animal, MeHg contamination in marine organisms would increase with the concentration of THg in their body. This is in agreement with earlier studies which suggest the concentration of THg as the proxy for MeHg contamination in fish³³. In spite of such correlation, it is interesting to note that even though two predators, *J. amblycephalus*, and *P. bilineata* have distinct values of THg (155 and 260 $\mu\text{g}/\text{kg}$ respectively); but they have very similar MeHg concentrations ($\sim 129 \mu\text{g}/\text{kg}$) (Table 1). Therefore, this result apparently suggests the extents of methylation are different in these two species and that probably controlled by the physiology of particular species. Earlier studies showed that discrepancy in physiological controls over reaction mechanism including the bio-dynamics of organic and inorganic forms of Hg can affect methylation in different species³⁴. Moreover, the estimated relative enrichment of MeHg in most of the species varied between 24 and 85 % (Table 1) and such wide ranges of % MeHg also confirm that the rates of methylation in all species are not uniform. The average ratios of MeHg to THg in typical pelagic fishes (average ~ 77 % MeHg) were higher than those found in benthic organisms (average ~ 53 % MeHg). A similar observation with a higher percentage of MeHg in pelagic carps relative to benthic crabs was also reported by Luo *et al.*²³. Therefore, besides environmental conditions, other biological factors also could be responsible for variable methylation of Hg in different organisms. Kannan *et al.*²⁶ also suggested that factors such as age, sex and feeding habits of animals control the concentration of MeHg

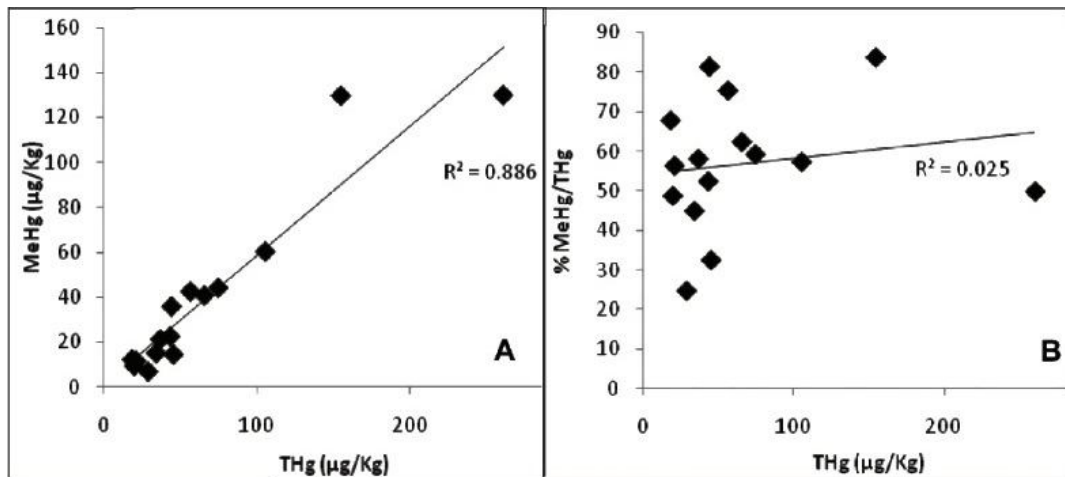


Fig. 3 — Relationship between total mercury and methylmercury in the body tissues of edible marine species from Goa coast. Plots showing (A) MeHg vs. THg and (B) Percentage ratio of MeHg/THg vs. THg in those species

in the particular organism irrespective of THg in their body. The poor correlation ($R^2 < 0.1$) between MeHg to THg ratios and total Hg (Fig. 3B) also suggests besides the body-load of THg, methylation may also be controlled by other biological factors.

Conclusion

This study provides information on Hg contamination in most common edible fishes and other seafood available in the estuaries and coastal areas of Goa. Assessments of THg and MeHg in selected species did not exceed the permissible limits and cannot produce any risk to the consumers. As this study included a very limited number of commercial species; therefore, it can be suggested that to get more detailed information regarding Hg contamination in seafood's similar studies should be carried out in more variety of species. In particular, some rare but commercially available species should also be assessed for Hg contents. The bio-concentrations of THg and MeHg in both pelagic and benthic species showed the increasing trends along the trophic structure; that follows the pattern: planktivore (filter-feeder) < omnivore < carnivore or predator species. The body load of MeHg in these species is related to physiological factors as well as environmental conditions. Low levels of MeHg in fish and other aquatic animals also indirectly suggest that the level of Hg contamination and rate of methylation of Hg in coastal water and sediment off the Goa coast is certainly less.

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