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Original Research

Effect of Mercury Transfer from Producer to Consumer in a Marine Environment

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Abstract

Biomonitoring a stressed Kuwait Bay environment revealed a differential and collective transfer of mercury (Hg) between the primary producer and primary and secondary consumers, in addition to possible Hg bioconcentrations and bioaccumulation in such marine organisms that attributed harmful effects to humans at the tertiary level of the food chain. Selected live samples were collected from five bay sites and exposed for 96 h and 30 d in aquarium tanks. Samples analyzed by direct mercury analyzer (detection limit of 0.0015 $ng \cdot g^{-1}$) revealed Hg concentrations in the sequence of *Barbatia helblingii* >*Acanthopagrus berda*>phytoplankton>zooplankton at sites IV>V>III>I>II during the summer and winter seasons, respectively. Bioaccumulation factor (BAF) was >1 in most of their trophic transfer, although Hg-BAF was <1 in a few trophic levels. Seasonal variations, anthropogenic sources, vestiges of Hg from the shut-down chlor-alkali plant, urbanization, slow water current, and nutrient upwelling attributed to the persistent Hg accumulation in the marine ecosystem. Since Hg is a ubiquitous pollutant in the bay, their transfer through the medium, diet, and net accumulation in higher predators is of importance to marine life and is a concern to tertiary consumers, including humans.

Keywords: bioconcentration, bioaccumulation, food chain, mercury

Introduction

Globally, the marine environment is facing tremendous ecological stress due to the influence of organic and inorganic pollutants that are dispersed in the aquatic system. Heavy metals contaminants are not completely biodegradable and have lengthy biological half-lives [1-2]. Many species in the marine environment are stressed due to diverse inputs of industrial, domestic, recreational, and agricultural pollutants, plus the effect of Gulf Wars oil spills in the past [3-4]. The seafood catch from the Arabian Gulf is of commercial importance to the locals and export requisites. Specifically, Kuwait Bay caters 50-60% of the commercial seafood catch to the residents of Kuwait. Therefore, maintaining the quality of the marine environment is crucial for economic reasons [4].

Kuwait Bay is a semi-enclosed area that extends from the Arabian Gulf to the west. This bay has a shallow northern part inundated by mud flats, a central region with high nutrients, and sandy beaches in the southern shoreline region. Mercury pollution in Kuwait increased when the Salt Chlor-alkali plant (SCP) used mercury electrolytes and contaminated the marine ecosystem in the recent past. Mercury levels in the Kuwait marine ecosystem declined after the shutdown of the plant. However, rapid urbanization made it necessary to investigate total mercury in the

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marine environment. Mercury contamination was found to adversely affect organisms through absorption from the seawater, sediment, metal uptake, and bioaccumulation in the aquatic chain [5-9]. The finfish and shellfish provide useful tools for monitoring mercury concentrations and their impact on the aquatic environment [1, 8, 10-11]. Earlier researchers [10-12] used atomic absorption spectrophotometry to detect mercury with sensitivity at ±0.01 ppm. The precision of their results was limited due to interference by organic constituents. Based on these factors, the present study overcame the shortfalls of instrumental detection limits by using a direct mercury analyzer (DMA-80, Milestone, Italy) that could detect Hg from 0.0015 ng·g^{-1} onwards in seawater and selected marine samples. This study determined the transfer of Hg from the primary producer (phytoplankton) to selected primary, secondary, and tertiary consumers, namely, zooplankton, shellfish (*Barbatia helblingii*), and finfish (*Acanthopagrus berda*) to validate the Hg bioconcentration (BCF) and bioaccumulation factors (BAF) in the food chain. BCF and BAF factors were determined by evaluating the initial Hg concentration in seawater to the mean Hg in the primary producer and the mean Hg concentrations between the secondary and primary consumers at the final exposure time. Furthermore, this study ensures that tertiary consumer-humans undertake precautionary measures regarding seafood consumption.

Experimental

Seawater

Using a Van Dorn water sampler, seawater replicates collected in sterile polystyrene containers in an icebox from five sites of the Kuwait Bay (Fig. 1) were transported to the laboratory. Total mercury (Hg) in seawater during 2016-17 was tested in the direct mercury analyzer (DMA-80, Milestone, Italy).

Fig. 1. Sampling sites of Kuwait Bay, Kuwait.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

Trophic Level I: Phytoplankton

Phytoplankton was collected twice every month during 2016-17 using a plankton net (size 20 µm and 30 µm) towed by a boat at 0.2 knot speed from five Kuwait Bay sites. Storage, preservation, identification, and segregation of culture facilities were carried out in the laboratory following the standard methods [13-16]. The seasonal Hg concentrations variation in most common phytoplankton (dinoflagellates and diatoms) from five Kuwait Bay sites were initially determined. Three batches of phytoplankton replicates were categorized in this study to determine:

a. Hg concentrations in phytoplankton from five Kuwait bay sites.
b. The toxicity of Hg concentrations (0.5, 1.0, 1.5 ng·l^{-1}) at LC_5, LC_{15}, LC_{50} in the laboratory to validate 96 h BCF and BAF exposure.
c. BAF at 30 d exposure to test their effective accumulation in the marine food chain [17-18].

Details are schematically represented (Fig. 2). The criterion continuous concentration (CCC) given by the national recommended ambient water quality criteria is 0.94 µg·l^{-1} [15]. Phytoplankton concentrations exposure was equivalent to 2.46 to 3.39 times the CCC values. Phytoplankton was grown in F-medium following the standard method [16]. Phytoplankton exposed for 96 h toxicity and 30 d bioaccumulation tests followed the standard methods [19].

Test Samples: *B. helblingii*, Ark Clam; *A. arabicus*, Fish

TEST 1: Hg CONCENTRATIONS (96h@ ng. l^{-1})

SITES	I			II			III			IV			V		
Conc.	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
No. Fishes*:	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
No. Ark clam*:	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Zooplankton*:	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Phytoplankton*:	@			@			@			@			@		

TEST 2: CONTROL (no Hg inclusion) (96h)

SITES	I	II	III	IV	V
	10	10	10	10	10

No. Fishes, Ark clam*, zooplankton* and phytoplankton@

Test 3: BIOACCUMULATION (Hg inclusion) (30 d)

SITES	I	II	III	IV	V
No. samples*:	10	10	10	10	10
Phytoplankton fed	1st 5d	1st 5d	1st 5d	1st 5d	1st 5d
Zooplankton fed	5th-10thd	5th-10thd	5th-10thd	5th-10thd	5th-10thd
Ark Clam fed	10th -20thd	10th -20thd	10th -20thd	10th -20thd	10th -20thd
Fish Fed	20th -30thd	20th -30thd	20th -30thd	20th -30thd	20th -30thd

*Experiments conducted twice for summer and winter seasons (total nos./species = 500)

@: (2-4 × 10^6 cells l^{-1})

Fig. 2. Schematic representation of Hg toxicity and bioaccumulation tests.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

Trophic Level II: Zooplankton

Zooplankton was collected from five Kuwaiti marine sites using a nitex net (100 µm). The density of zooplankton was constantly maintained. The cultured zooplankton were fed with Hg-exposed phytoplankton ($2\text{-}4 \times 10^6$ cells· L^{-1}) two times per day for 96 h and days 5-10 during the 30 d bioaccumulation tests, respectively (Fig. 2).

Trophic Level III: Mollusc-Barbatia helblingii (Ark Clam)

This study collected replicates of *Barbatia helblingii* (Ark clam), from five Kuwait Bay sites, each measuring 20.0 g ± 2 g and 35 mm ± 5 mm, weight and length, respectively. A toxicity test was modified to determine site- and seasonal-wise analyses [20-21]. The different Hg concentrations (as in trophic level I) and ethically compliant by the local statutory bodies elicited responses to this species at LC_5, LC_{15}, and LC_{50}. Biometric characteristics of *B. helblingii* were monitored and Hg concentrations determined by the DMA-80 analyzer. Workflow determining the 96 h and 30 d exposure following the earlier methods [22-23] are schematically represented (Fig. 2).

Trophic Level IV: Fish-Acanthopagrus berda

Acanthopagrus berda fish caught from five Kuwait Bay sites were immediately transported to the laboratory. *A. berda* fish representing the five sites each, with similar total length and weight (18 cm ±2 cm and 55 g ±5 g, respectively) were acclimated for 72 h in five aquarium tanks (1,000 l) containing filtered seawater (27°C, salinity 39‰, pH 8.0, and dissolved oxygen >7 mg·l^{-1}) following the earlier methods [24-26]. The fish were fed daily to satiation with brine shrimp without Hg concentrations [25]. After 72 h, the fish were not fed and gut depurated before the feeding experiments. Toxicity and bioaccumulation tests on these fish followed the earlier methods [27-30], and as described for *B. helblingii* 96 h and 30 d.

Hg Analysis

Mercury in seawater, phytoplankton cultured cells (96^{th} h), zooplankton (w/v), mollusc (body tissues), and fish (10 each) homogenized whole body parts (unlike the normal procedure in using the gills, liver, and muscle tissues), were analyzed from the Hg-exposed (0.5, 1.0, 1.5 ng. g^{-1}) concentrations as well, plus bioaccumulation studies (30 d) using the DMA-80 [31-34]. Quality control was assured following the use of blanks, controls, and standard reference materials from BCR, IAEA, NIST, and NRC (CRM-414 phytoplankton powder), (MA-A-1/TM copepod), (SRM:1566b oyster tissue), and (CRM: DORM-2 dogfish muscle) [35-36]. Sample recovery (>95 %) to that of the standard reference materials were alone considered as the benchmark for quality assurance tests. Bioconcentration (BCF) and bioaccumulation (BAF) evaluated from the whole-body burden yielded the trophic transfer of Hg in the marine food chain [18]. BCF and BAF were determined by the following equation [18]:

$$BCF = \frac{\text{Hg concentration in phytoplankton}}{\text{Hg concentration in seawater}} \quad (1)$$

$$BAF = \frac{\text{Hg concentration in secondary consumers}}{\text{Hg concentration in primary consumer}} \quad (2)$$

Results and Discussion

Hg Concentrations in Seawater

Total Hg concentrations in seawater from the five sites of Kuwait Bay varied from 0.13 ng·l^{-1} to 0.32 ng· l^{-1} with a mean concentration of Hg (0.22 ng. l^{-1}) during 2016-17. This was within the concentrations compared to the Hg levels observed by earlier investigators [23, 31-32]. This was also lower than 0.94 µg·l^{-1} – the Hg criterion continuous concentration (CCC) in natural seawater [35] (Fig. 3). This study on three experimental Hg concentrations (Fig. 2) in the laboratory revealed a mean Hg loss of 82% in the control seawater without phytoplankton, which was attributed to the organic complexion or precipitation process. Site-wise observations revealed Hg concentrations in seawater in the order of magnitude collected from sites V>IV>III>I>II (Fig. 3). The high Hg concentrations in the sequence of sites V and IV indicated the past effect of salt chlor-alkali plants besides the present influence of discharges from desalination, thermal, power plants, industrial and domestic wastes, sedimentation, stagnation, and single-flow direction of Shatt Al-Arab River water mixing from the north to the south of Kuwait Bay. As the Bay water is characterized by restricted exchange with

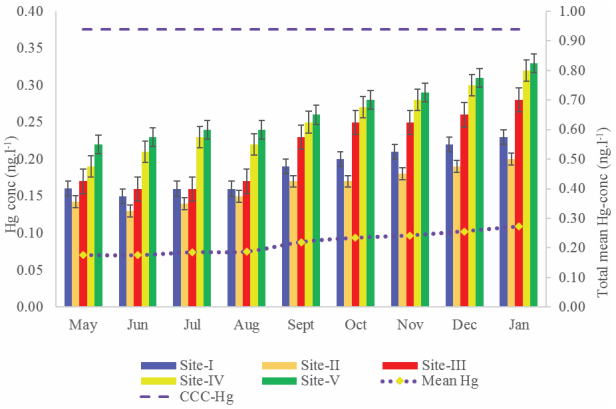

Fig. 3. Mercury concentrations in seawater from Kuwait Bay. CCC-Hg: Criterion Continuous Concentration for Hg (EPA, 2004). Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

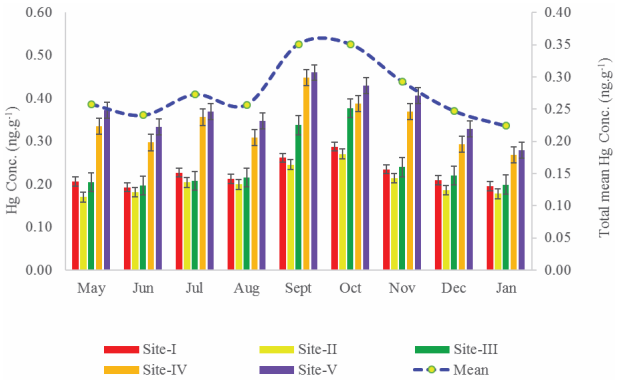

Fig. 4. Mercury concentrations in phytoplankton from Kuwait Bay.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

the open sea, the chances of coastal and catchment habitat destruction and biodiversity loss was observed with significant environmental damage from pollution, thus supporting the views of [20-21, 23]. A gradual increase of Hg concentrations in seawater was observed during the winter compared to summer (Fig. 3). This is attributed to the low absorption of Hg concentrations by phytoplankton due to the slow photosynthesis process, precipitation, and low water current mixing in the winter. The above factors were validated statistically by ANOVA, indicating a significant difference between site-wise and season-wise Hg concentrations in seawater (Table 1). Such factors agreed with earlier studies [28].

Hg Concentrations in Phytoplankton (Level I)

The mean Hg concentrations in phytoplankton collected from the five sites of Kuwait Bay ranged

Table 1. Site- and season-wise ANOVA of Hg concentrations in seawater for the primary producer and consumers.

Source of Variation	SS	df	F	P-value	F crit
Hg in seawater from nature					
Site-wise	0.067	4	113.93	0.0001	2.66
Season-wise	0.052	8	44.77	0.0001	2.24
Error	0.005	32			
Total	0.125	44			
Hg in phytoplankton from nature					
Site-wise	0.193565	4	88.85759	7.36×10^{-17}	2.66
Season-wise	0.084487	8	19.39219	3.06×10^{-10}	2.24
Error	0.017427	32			
Total	0.295479	44			
Hg in zooplankton from nature					
Site-wise	0.027	4	24.42	2.46×10^{-09}	2.66
Season-wise	0.111	8	50.05	6.3×10^{-16}	2.24
Error	0.009	32			
Total	0.147	44			
Hg in ark clam mollusc from nature					
Site-wise	0.181	4	10.07	2.15×10^{-05}	2.66
Season-wise	0.426	8	11.82	1.19×10^{-07}	2.24
Error	0.144	32			
Total	0.751	44			
Hg in seabream fish from nature					
Site-wise	0.247	4	51.51	1.69×10^{-13}	2.66
Season-wise	0.272	8	28.42	1.92×10^{-12}	2.24
Error	0.038	32			
Total	0.558	44			

Table 2. BCF and BAF in nature and the experimental marine food chain.

No.	Relationships	Mean-Hg Kuwait Bay sites†	BCF/BAF (96 h) nature; experimental (0.5,1.0,1.5 ng.l^{-1}) Hg exposure	BCF/BAF (30 d) nature†; experimental (0.5,1.0,1.5 ng.l^{-1}) Hg exposure
1.	**Seawater	0.22± 0.01	0.25† 0.24,0.27,0.31	Not Applicable
2.	**Seawater → Phytoplankton	0.28 ± 0.06	1.29†, 0.65,0.69,0.91	2.74, 2.63, 3.09
3.	*Phytoplankton → Zooplankton	0.24 ± 0.02	0.74†, 0.58,0.79,1.03	0.81, 1.33, 1.30
4.	*Zooplankton → Ark clam (Phytoplankton→ Ark clam)	0.38 ± 0.03	1.92†,0.97, 1.60, 2.13 (1.42†)	1.94, 1.95, 2.08
5.	*Ark clam → Fish *A. berda* (*Zooplankton → Fish) (*phytoplankton → Fish)	0.33± 0.04	0.88† 1.67†0.86,1.54, 2.21 1.18†	0.89, 1.02, 1.10 1.72, 1.75, 1.98
	*Entire Food Chain (Nos. 2-4) *Phytoplankton* to *A. berda*	0.29 ± 0.02	0.44†	1.43

**BCF: bioconcentration factor- initial Hg concentration in seawater to the mean Hg in phytoplankton, †: samples analyzed in natural environment, *BAF: bioaccumulation factor- mean Hg concentrations at the final exposure time in the organisms

between 0.17 ng·g-^{1} and 0.46 ng·g^{-1} (Fig. 4). Phytoplankton cells exposed to Hg doses (0.5, 1.0, 1.5 ng·l^{-1}) revealed 2.46 to 3.35 times higher concentrations than the Hg-exposed phytoplankton that ranged from 0.45 ng·g^{-1} to 1.51 ng•g-^{1}. Hg concentration recovery was higher at 0.5 ng•l^{-1} (55%) compared to the Hg recovery at 1.0 ng•l^{-1} (27%) and 1.5 ng•l^{-1} (18%) exposure concentrations. In other words, phytoplankton exposed for 96 h at 1.5 ng•l^{-1} revealed the maximum Hg concentrations loss (82 %) at 1.5 ng·l^{-1} test dose from that of the Hg concentrations in the naturally collected phytoplankton. Thus, low assimilation of Hg concentrations in the phytoplankton was observed and possibly found in line with the earlier observations of [29, 33]. Site-wise Hg concentrations in phytoplankton were in the sequence such as the observations of Hg levels in seawater. However, the season-wise Hg concentrations in phytoplankton were high during the summer, which is attributed to the necessity of sunlight and temperature in the photosynthetic process in relation to their abundance in the five respective sites. This was also statistically validated (Table 1). High Hg accumulation in the phytoplankton was confirmed by a high bioconcentration factor (BCF). The mean BCF in phytoplankton were higher in the three Hg-exposed test concentrations when compared to the Hg-BCF collected from nature (Table 2).

The mean 96 h uptake rate at 1.5 ng•l$^{-1,\ 1.0}$ ng•l^{-1}, and 0.5 ng•l^{-1} of Hg exposure concentrations in phytoplankton was 1.4 x 10^{-2} ng h^{-1}•cell^{-1}, 7 x 10^{-3} ng•h-^{1}•cell^{-1} and 3.0 × 10^{-3} g•h^{-1}•cell^{-1}, respectively. However, phytoplankton revealed an uptake of 2.9 x 10^{-3} ng•h^{-1}•cell^{-1} Hg concentrations in the natural environment that were considerably lower than the phytoplankton subjected to Hg exposure in the laboratory, indicating the low assimilation of Hg *by phytoplankton in nature.* These results were in line with earlier studies [12, 23, 31].

Hg in Zooplankton (Level II)

The most common zooplankton collected throughout the year was considered for this study to determine unbiased Hg concentrations. The mean Hg concentrations in zooplankton collected from the five Kuwait Bay sites were between 0.08 ng· g^{-1} and 0.32 ng· g^{-1}. Exposure of (96 h) Hg concentrations at 1.5 ng· g^{-1} revealed 84% loss, indicating a poor transfer of Hg in the zooplankton. This agreed with earlier studies [25, 33-34].

Site-wise observations revealed peak Hg concentrations in zooplankton in the sequence of sites V>IV>III>I>II (Fig. 5). Seasonally, the mean Hg concentrations in zooplankton were observed high during the peak summer season (August-October) compared to the onset of winter (November-January). Reasons may be attributed to the influence of the metabolic rate and the assimilation of Hg from phytoplankton by zooplankton during the summer. This agreed with earlier findings [2, 9, 33]. During the

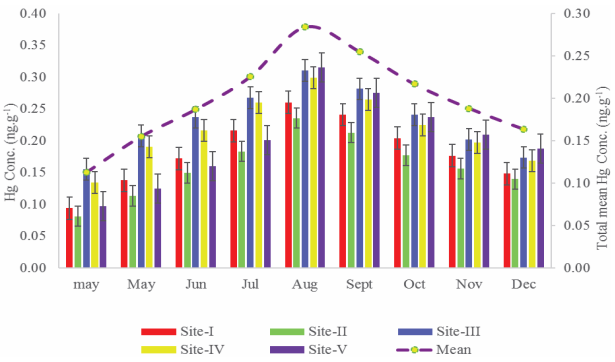

Fig. 5. Mercury concentrations in zooplankton from Kuwait Bay.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

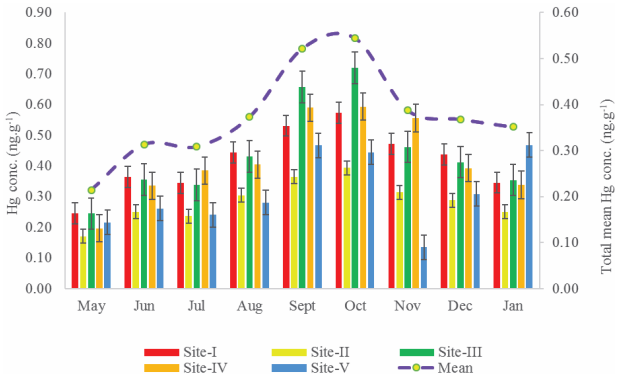

Fig. 6. Mercury concentrations in *B. helblingii* ark clam from Kuwait Bay.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

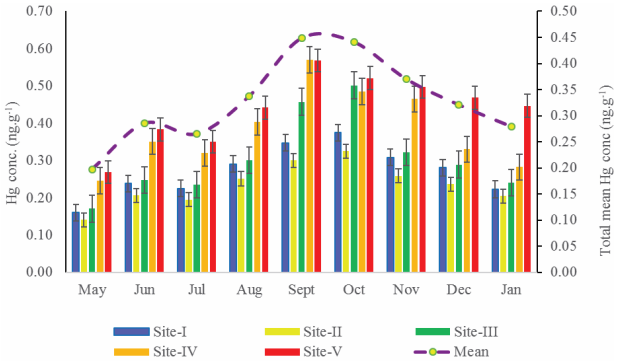

Fig. 7. Mercury concentrations in *A. berda* fish from Kuwait Bay.
Site I: Subiyah, Site II: Khazma, Site III: Doha, Site IV: Kuwait Towers, Site V: Salmiya

onset of winter (November-January), low water current, low phytoplankton abundance, and the photosynthesis process in Kuwait Bay was found to elevate the overall Hg accumulation in the zooplankton compared to the Hg concentration during the onset of the mid-summer season (May-July) (Fig. 5). The 96 h test of Hg-BAF at 0.5 and 1.0 ng·l^{-1} exposure indicated a low bioaccumulation ability or effective filtration and excretion by the zooplankton (Table 2). However, at 1.0 and 1.5 ng·l^{-1} Hg exposure, the BAF in *zooplankton* was observed to be higher than the Hg-BAF in p*hytoplankton,* indicating a positive bioaccumulation process over a 30 d period of time (Table 2).

Hg in Barbatia helblingii (Level III)

The mean Hg concentrations in the whole tissue of *Barbatia helblingii* (ark clam) varied from 0.17 ng·g^{-1} to 0.72 ng·g^{-1} (Fig. 6). This was higher than the concentrations reported earlier in other species [5, 8-10, 20, 26]. Seasonal and site-wise results showed a similar pattern of Hg concentrations as observed in zooplankton. *B. helblingii* exposed for 96 h revealed a mean of 0.97 ng·g^{-1}, 1.60 ng·g^{-1}, and 2.13 ng·g^{-1} at 0.5, 1.0, and 1.5 ng·l^{-1} Hg concentrations, respectively, in their whole-body tissues. The mean Hg-BAF was found to increase from phytoplankton and zooplankton in *B. helblingii* in the natural environment (Table 2), evidencing the bioaccumulation process. Low BAF at 0.5 ng·g^{-1} Hg exposure suggests *B. helblingii* species have mechanisms to detoxify and self-regulate uptake metal over a short exposure period (96 h) (Table 2). These mechanisms support the earlier views of metallothioneins and phosphate granule production [22, 30].

Hg in Acanthopagrus berda (Level IV)

The mean Hg concentrations in *Acanthopagrus berda* fish ranged 0.2-0.45ng·g^{-1} (Fig. 7), which was in line with earlier studies [3, 6, 11]. Fish exposed to three different concentrations showed an increasing trend of Hg concentrations with 96 h exposure. The transfer of Hg concentrations between *A. berda* (predator) and molluscs, zooplankton (prey), and phytoplankton (diet) evaluated by regression analysis (r^2 = 0.95, 0.91, and 0.66 $p<0.05$), validated the strongly dependent Hg concentrations in the predator-prey relationship. The regression analysis (r^2 = 0.66) evaluated between *A. berda* and phytoplankton indicated their weak dependency and the least Hg concentration uptake by the fish. Hg levels in the different prey decreased the availability with a bio-diminution effect of accumulated Hg to upper trophic levels [25, 30, 36]. Seasonal and site-wise analysis in *A. berda* showed a similar trend of Hg concentrations as observed in the zooplankton (Fig. 7). The mean Hg-BAF transfer between *A. berda* and zooplankton in nature was high compared to the Hg-BAF in the phytoplankton and ark clam (Table 2), indicating a high Hg accumulation and low Hg assimilation efficiency in the former and later species, respectively. Hg-BAF was high in 30 d tests, indicating a positive bioaccumulation process in *A. berda*. Studies evidenced Hg concentrations distribution in the gills and liver of fish as the main organ for the detoxification and elimination of metals in fish, although different body parts reported high Hg concentrations by earlier investigators [26-27]. This study analyzed the whole-body parts of the *A. berda* fish to maintain the evenness and uniform distribution of samples. Thus differential Hg concentrations in the body tissues and organs were not investigated.

Conclusions

In an overall view, this study revealed a significant trophic transfer of Hg concentrations from the primary producer (phytoplankton) to the secondary (zooplankton) and tertiary consumers (mollusc and fish), although the percentage of Hg transfer between the secondary and tertiary consumer at certain trophic levels was diminutive because of bioaccumulation, the environment, and ocean dynamics. However, in light of commercial seafood resources, the high Hg concentrations in seawater,

residues from the chlor-alkali plant in the past, and the discharge of domestic and industrial wastes into the marine environment in the present represent a risk to human health. Because Hg is not an essential trace element for humans, their presence in the human body, even at low concentrations, attributes deleterious effects. Thus, this study recommends consumption of marine organisms with precautionary measures.

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