Original Article

Mercury Biomagnification between Two Trophic Levels of a Grazing Food Chain (Plankton and Planktivorous Fish) in a Fresh Water Ecosystem

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ABSTRACT

Background: The Present study was carried out to track and calculate Biomagnification Factor (BMF) of total mercury (T-Hg) between two different trophic levels (i.e., plankton and a planktivorous fish) in a fresh water grazing food chain.

Methods: Experimental organisms were planktonic biomass and silver carp (*Hypophthalmichthys molitrix*) as a planktivorous fish. Silver carp samples were obtained from randomly selected points from different sampling stations. The concentrations of T-Hg in collected samples were determined by Advanced Mercury Analyzer.

Results: Means of T-Hg in planktonic biomass and muscle tissue of silver carp were 78.21 ± 3.13 and 367.12 ± 26.43 ng g^{-1} dry weights, respectively. Mean T-Hg in plankton, sampled fish during the study months and amongst the sampling stations did not show significant differences. The $BMF_{Hg(plankton-fish)}$ was differ among months; moreover, calculated BMF was greater than 1 during study months, which means biomagnification was occurring in SGR. The concentration of T-Hg in the muscle tissue of all fish samples that weighed more than 850 gr was higher than the acceptable limits based on EPA (300 ng g^{-1}) and WHO (500 ng g^{-1}) standards. The highest BMF_{Hg} was observed in August

Conclusion: It seems that mercury pollution of SGR has a natural source. The calculated BMF_s were greater than 1 and the concentrations of T-Hg in muscle tissues of those samples weighing more than 850 gr were higher than FAO and WHO standards. Therefore, consumption of the SGR's silver carp must be accompanied by serious health considerations.

Keywords: Biomagnification, Grazing Food Chain, Mercury, Plankton, Silver Carp.

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INTRODUCTION

Mercury (Hg) in freshwater ecosystems is more common pollutant in comparison with other heavy metals due to its higher toxicity, bioaccumulation susceptibility in living organism's tissues and biomagnification throughout the natural food chains [1, 2]. Mercury naturally exists in both organic and inorganic forms [3-6]. Inorganic Hg could convert to methylmercury, a highly toxic compound, by anaerobic microorganisms in the sediments of aquatic ecosystems and planktons [7, 8].

Due to the high binding capability of methylmercury with sulfhydryl proteins, it could be accumulated in living organism's tissues at large amounts [2, 9]. Methylmercury is extremely poisonous, non-biodegradable, accumulates in living organism's tissues, biomagnifies in food chains with long biological half-life and is able to be transmitted over natural food webs [1, 9, 10].

Planktons [8, 11], aquatic plants, fish tissues and organisms in higher trophic levels of a natural food chain [10, 12] can absorb methylmercury at the higher levels easily. Hence, the highest concentrations of methylmercury would be accumulated in the upper predatory fishes such as sharks and tuna [2]. There are no significant known biological rules for Hg and its compounds. However, all forms of Hg contaminations should be considered as undesirable and potentially harmful [6, 7, 13-15].

Mercury bioaccumulation in aquatic food chains often starts from planktonic trophic level [16, 17]. Mercury accumulates in phytoplankton tissues in both organic and inorganic forms, while zooplanktons only absorb the organic compounds

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of Hg [16]. The sum of organic and inorganic Hg components called total Hg (T-Hg). Mercury speciation studies reported that 80 to 90 percent of T-Hg is methylmercury [2]. Assessing and determining Hg contamination in basic trophic levels could provide a comprehensive description of pollution at higher trophic levels (especially in the food chains that i involves humans) [17].

Mercury biomagnification is a prevalent phenomenon in aquatic environments particularly in fresh water ecosystems [2, 17, 18]. Biomanification refers to a condition that concentration of a pollutant in living organism's tissues increases from one trophic level to the next one [2]. Biomagnification factor (BMF) conveys the quantity of this transmission [2, 19].

Silver carp (*Hypophthalmichthys molitrix*) is a fish that feeds mainly in surface as well as median layer of water on phytoplanktons [20]. Due to the biomagnification process, it is expected that the concentration of T-Hg in the muscle tissue of this fish be higher than the concentration of T-Hg in plankton's biomasses [2].

The Sanandaj Gheshlagh Reservoir (SGR) is the most inmportant water source to supply drinkable water and fishery products in the region (Fig. 1). According to the field observations in the SGR watershed district, industrial activity was not developed in the region at all and the conventional agronomic practice was rain depending type, mostly without using chemical fertilizer and herbicides. Moreover, there are considerable amounts of mineral Hg components in the soil texture and bedrocks of the study site [21]. As a result, we assumed that the SGR is a typical freshwater ecosystem, naturally polluted by Hg [22].

The objective of this study was to determine the concentrations of T-Hg in plankton's biomasses and muscle tissue of silver carp fish (as a plankton consumer) in SGR, to calculate biomagnification factor of Hg (BMF_{Hg}) between two trophic levels of plankton and silver carp (related to a grazing food chain in a freshwater ecosystem). In addition, this is important to know whether in a naturally polluted water reservoir the concentration of Hg in its organisms (i.e. plankton and fish) changes or not? Since Hg pollution in this reservoir has negative effects on water quality for human consumption, fisheries, agriculture and so on, this information could help the managers and decision-makers to make an informed decision towards solving the problems that are related to pollution.

MATERIALS AND METHODS

Study Area

Sanandaj Gheshlagh Reservoir (SGR) (35° 25' – 35° 30' N & 46° 57' – 47° 30' E) is located in the Northeast of Sanandaj City, western Iran. The SGR covers an area of approximately 8.5 km^2 with a capacity of 224 million m^3 water (Fig. 1).

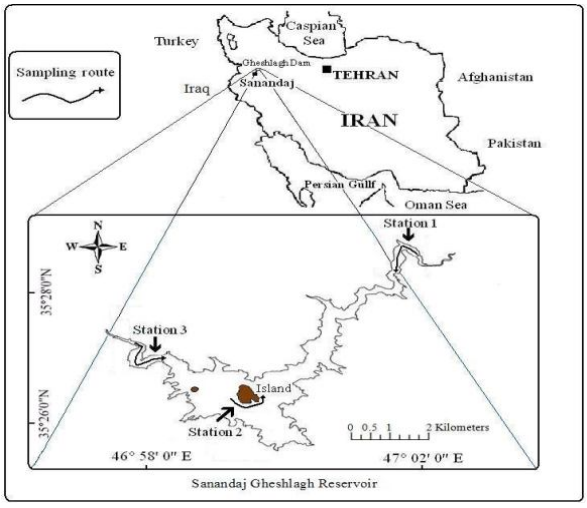

Figure 1. Sananadaj Gheshlagh Reservoir (SGR) map and plankton sampling stations.

Samples Collection

Plankton biomasses were collected monthly, from three different stations, during July to December 2010 (18 samples in total) using a motorboat and plankton net, with 25 cm and mesh size 20 µ (we used plankton net with 20 µ mesh size to catch all existing planktonic creatures in the SGR). Stations 1 and 3 were located at the end of the two main river branches entering the SGR (Gheshlagh and Chehel Gazzi rivers) and station 2 was located at the center of the SGR (Fig. 1). The suitable sampling depths were determined using Secchi disk index, (a standard tool used to measure water clarity), so the sampling depth would not be more than the depth of light penetration [23]. The plankton samples were collected by pulling plankton net through water in each station over a distance of 1.5 – 3 km using a motorboat with 25 horsepower (model; YAMAHA).

Twenty-four silver carp fish were caught in the same period (4 samples per month). Sampling was done randomly from different parts of the

SGR using a 50 × 6 m gill net (with 5×5 cm mesh size), because silver carp is a mobile animal and we assumed that total Hg distribution in SGR is not even. The caught fish samples were taken alive to the fish biology laboratory in University of Kurdistan for biometric study, including total length, total weight, determination of sex through observation of sexual gonads and tissue sampling.

Preparation of Samples

The plankton samples were transferred to mercury-free screw cap bottles, all used bottles had been immersed in cleaning liquid (sodium hypochlorite) for 24 h and then washed by 10% nitric acid (Merck Company) and deionized water. Except for planktons, all suspended particulates were separated under stereomicroscope and fixed with 4% grade A buffered formalin to prevent samples degradation prior to measurement of T-Hg concentration. Then fixed plankton samples were centrifuged (Model; Centurion, 2000 series) at 3200 rpm, for 20 min [23]. When fish biometric procedure was done, 10 gr of muscle tissue was separated from each fish and samples were frozen at -20 $^{\circ}$C in small plastic bags until measurement of T-Hg [24]. Methylmercury is a volatile component, therefore, to prevent methylmercury evaporation, all samples were dried out using a freeze-dryer (OPERON, Model; FDCF – 12012) in -52 ^{0}C. Freeze-drying was preformed until constant weight obtained [18].

Determination of Total Mercury

After samples freeze drying, homogenized solid tissue, with a weight of 50-100 ± 0.01 mg, was separated into the pre-cleaned combustion boats from the original dried tissue samples and T-Hg concentrations were determined by Advanced Mercury Analyzer (Model; LECO AMA 254, USA), [18] in the environmental laboratory of Tarbiat Modares University, with ASTM D-6722 standard on the basis of ng g^{-1} dry weight (dry wt).

Statistical Analysis

SPSS software, *ver*. *16* (Chicago, IL, USA) was used for statistical analyses. Data were tested for goodness of fit to a normal distribution using Kolmogorov-Smirnov's test and homogeneity of variances, using Bartlett's procedure. Mean T-Hg in the muscle tissue of silver carps and plankton masses during the studying months were compared using one-way analysis of variance (ANOVA). ANOVA test was also used to compare T-Hg in plankton masses among three different sampling stations. Pearson's correlation test was used to determine the correlation between T-Hg in muscle tissue of silver carp and its total length and weight. Finally, for comparing T-Hg concentrations between male and female silver carps, Student's *t*-test was employed.

RESULTS

The total weight and total length of silver carps varied between 300 to 1020 gr (average (± Standard Error) 665.77 ± 36.94 gr) and 32 to 49 cm (average (± Standard Error) 40.64 ± 0.82 cm), respectively (Table 1). The mean (± S. E.) of T-Hg concentration in total mass of planktons and muscle tissue of silver carps was 78.21 ± 3.13, and 367.12 ± 26.43 ng g^{-1} dry weight, respectively (Table 1). During the summer months (except for September), the concentration of T-Hg in plankton biomass were higher than the autumn samples (Table 1).

The highest mean (± S.E.) of T-Hg in plankton biomass was observed in station 2 (85.02 ± 11.83 ng g^{-1} dry wt). Means T-Hg (± S.E.) in plankton biomass at stations 1 and 3 were 74.51 ± 5.67 and 75.11 ± 5.60, respectively (Fig. 2). Averages T-Hg in plankton biomasses during the study months (P = 0.49) and among the sampling stations (P = 0.34) showed no significant differences.

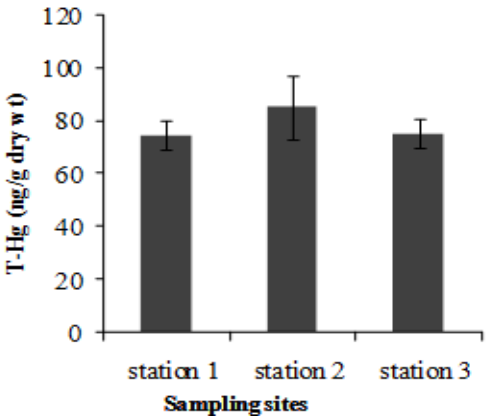

Figure 2. Means (± S.E.) T-Hg (ng g^{-1} dry wt) in plankton's biomasses from sampling sites of Sanandaj Gheshlagh Reservoir.

In silver carp muscle tissue samples, the highest and lowest T-Hg (± S.E.), (ng g^{-1} dry wt) were observed in August (501.75 ± 95) and November (297.50 ± 54), respectively (Table 1),

although a significant difference among monthly means of T-Hg in the muscle tissues of silver carps (P= 0.23) was not seen. In addition, sex had no influence on T-Hg levels in the fish samples (P = 0.27). In order to demonstrate the relationship between T-Hg in the muscle tissue of silver carp and its morphometric variables (e.g., total weight and total length) we used Pearson's correlation test, and the result are shown in Figure 3.

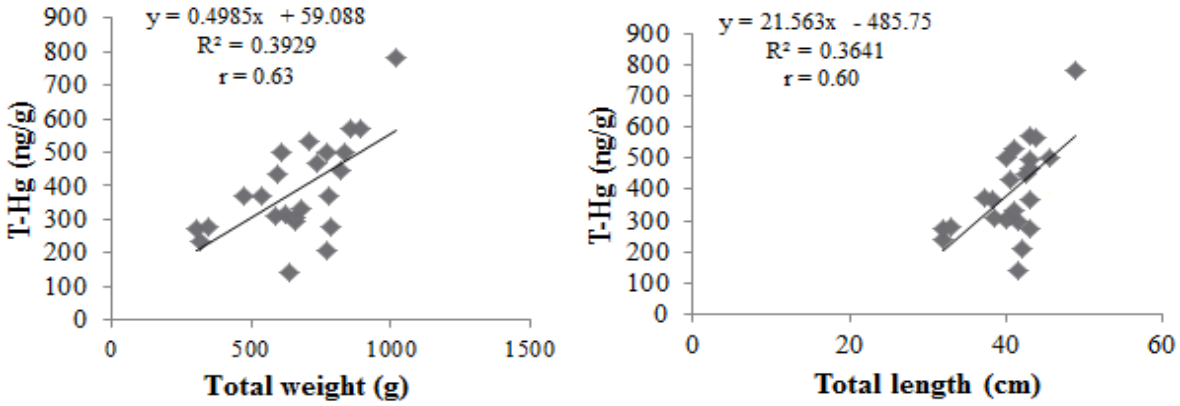

Figure 3. The relationship of T-Hg (ng g^{-1} dry wt) in muscle tissue of silver carp from Sanandaj Gheshlagh Reservoir with total weight and total length by Pearson's correlation test ($P < 0.05$).

Table 1. Monthly means (± S.E.) of total Hg (T-Hg) concentrations (ng g^{-1} dry wt) in planktons and muscle tissue of silver carps (*Hypophthalmichthys molitrix*) collected from Sanandaj Gheshlagh Reservoir, from July to December 2010 (and the means (± S.E.) of total weight and length of silver carps during the study period).

Sampling months	T-Hg in plankton	T-Hg in silver carp	Total weight of silver carp	Total length of silver carp
July	86.60 ± 7.32	370.25 ± 64	620.8 ± 113.77	39.02 ± 2.42
August	86.81 ± 10.9	501.75 ± 95	881.5 ± 52.07	45.42 ± 1.31
September	68.00 ± 2.52	306.25 ± 50.5	758.9 ± 35.03	42.30 ± 0.14
October	73.51 ± 7.90	332.50 ± 35	542.77 ± 81.97	38.25 ± 2.08
November	76.22 ± 1.66	297.50 ± 54	558.92 ± 33.85	38.92 ± 0.9
December	78.12 ± 10.86	394.50 ± 52.7	631.775 ± 97.43	40.02 ± 2.39
Total Mean ± S.E	**78.21 ± 3.13**	**367.12 ± 26.43**	**665.7 ± 36.94**	**40.64 ± 0.82**

The mean (± S.E.) weight of plankton masses was ($0.597 \pm 0.094 \times 10^{-3}$ gr m^{-3} dry wt per cubic meter volume of water). The highest mean was seen in December ($1.138 \pm 0.782 \times 10^{-3}$ gr m^{-3} dry wt per cubic meter volume of water), while the lowest mean was recorded in September ($0.268 \pm 0.052 \times 10^{-3}$ gr m^{-3} dry wt per cubic meter volume of water), (Table 2).

The transmission amounts of T-Hg from plankton's masses to muscle tissue of silver carp during the months of July to December 2010 in the SGR were calculated [25] as shown in Table 2. According to the mentioned method:

Eqn. 1:

Amount of T-Hg transmission by plankton to the next trophic level (ng m^{-3} dry wt per cubic meter volume of water) = plankton's T-Hg concentration (ng g^{-1} dry wt) × plankton's masses amount (gr m^{-3} dry wt per cubic meter volume of water)

BMF_{Hg} for different months are demonstrated in Table 3. It was calculated by dividing mean accumulated T-Hg concentration in the muscle tissues of silver carps as the consumer of planktons (predator) into mean accumulated T-Hg concentration in planktons as silver carp's staple food source (prey) [26]. During the studying months, all calculated BMF_{Hg} were higher than one (Table 3).

Table 2. Mean T-Hg transmissions and 95% confidence interval (in parenthesis) for planktons during July to December 2010 from Sanandaj Gheshlagh Reservoir.

Months	T-Hg in plankton mass (Mean± S.E.), (ng g^{-1} dry wt) (A)	Plankton mass (Mean ± S.E. × 10^{-3}) (gr m^{-3} dry wt) (B)	T-Hg transmission × 10^{-3} m^{-3} (ng dry wt) (A × B)
July	86.60±7.32	0.585 ± 0.091	50.66 (27.8, 41.14)
August	86.81 ±10.9	0.662 ± 0.158	57.46 (0, 96.14)
September	68.00 ± 2.52	0.268 ± 0.052	18.22 (0, 78.95)
October	73.51 ± 7.90	0.401± 0.057	29.47 (0, 107.88)
November	76.22 ± 1.66	0.528 ±0.142	40.24 (10.7, 67.73)
December	78.12 ± 10.86	1.138 ± 0.782	88.9 (0, 187.77)
Mean of total	**78.21 ± 3.13**	**0.597 ± 0.094**	**46.69 (37.07, 57.73)**

Table 3. Biomagnification factor (±S.E.) of $Hg_{(plankton-fish)}$ for different months (July to December, 2010) in Sanandaj Gheshlagh Reservoir.

July	August	September	October	November	December	Mean total
4.28 ± 0.59	5.78 ± 0.87	4.50 ± 0.79	4.52 ± 0.46	3.90 ± 0.61	5.05 ± 0.68	4.69 ± 0.35

DISCUSSION

In aquatic ecosystems, planktons assumed as the primary trophic level in a grazing food chain. They have short bio-cycle in aquatic ecosystems and respons quickly to the environmental changes and pollutions. Consequently, these organisms are appropriate bio-indicators for measuring accumulation of heavy metals such as Hg in these ecosystems particularly in lotic water sources [8, 23]. In addition, silver carp is an economic species, cultivated more than any other species of fish in different parts of the world after grass carp. Therefore, propagation of this species has to be accompanied with a great care and high quality assurance. Accordingly, assessing the amount of any kind of contamination including the concentration of heavy metals in edible tissues of silver carp is vital in terms of human health and safety. SGR is a naturally mercury polluted aquatic ecosystem [21, 22, 27]. Nevertheless, the qualities of SGR fisheries products have to be assessed and monitored to ensure health considerations.

The comparison of T-Hg in muscle tissue of silver carp in the present study with other species of fish in other parts of the world showed that the level of T-Hg concentration in SGR's silver carp was high and this indicated that Hg bioaccumulation occurred in muscle tissue of our silver carp (Tables 1 and 4). Mean concentration of T-Hg in plankton biomass was also remarkable (Table 5). This was expected as it was reported that the level of Hg in SGR water was higher than Iranian standard and WHO limits (1 µg l^{-1}) [28].

The calculated $BMF_{Hg\ (plankton-fish)}$ demonstrated that Hg biomagnification was occurred from planktonic food level to silver carp's level (Table 3). Although, the higher level of T-Hg could be due to the vary sources (i.e, direct absorption from Hg polluted water and or different food sources), it seems that SGR's plankton biomass is the main sources of accumulated T-Hg in the muscle tissue of silver carp as a planktivorous fish [20]. Furthermore, this means that consumption of the SGR's silver carp must be accompanied by serious health considerations because silver carp is the most commonly consumed fish in the region. The concentration of T-Hg in the muscle tissue of all samples weighted more than 850 gr was higher than the limits allowed by US Environmental Protection Agency (300 ng g^{-1}) and WHO (500 ng g^{-1}) [24, 29] during all study months during present research. The highest Provisional Tolerable Weekly Intake (PTWI) of Hg for every kg of human body is 5 ppb (5 ng g^{-1} or 5 µg/kg), [27]. Hence, a person weighing 70 kg is allowed a safe intake of up to 350 ng g^{-1} Hg per week. Accordingly, for the silver carp from SGR, the maximum permitted consumption would be 953 gr of fish per week, with this assumption that there is no other source of Hg in the diet.The highest

BMF_{Hg} was observed in August and since weight and length are among the most influential factors for increasing Hg concentration in the muscle tissue of fishes [2, 15, 30, 31], it seems that the higher level of measured T-Hg in the muscle tissue of SGR's silver carp in this month was due to the increasing the length and weight of fish samples during this time (Table 1 and Figure 3). Similar results were reported in previous studies [30, 31]. Also, our results showed that sex has no significant influence on the Hg accumulation rate in silver carp from SGR and Hg content is only affected by silver carp's weight and size [29].

In Tables 4 and 5, we compared T-Hg in the muscle tissue of silver carp and plankton's biomass from SGR with similar studies in other aquatic ecosystems in different parts of the world. These comparisons showed that the level of T-Hg in fish muscle depends on species type, season (time), level and sources of pollution [2]. We suggest a lab experiment to evaluate the effect of species type and different exposure time as well as different type of Hg speciation on the rate of Hg absorption in edible tissues of fish and upper trophic level members (e.g., Human) for future studies.

Table 4. Comparison of mean T-Hg in muscle tissue of different fish species from different regions in the world.

Species	T- Hg (µg g^{-1} dry wt) Muscle	Site	Refrences
Chondrostoma toxostoma	3.25	Cecina River, Italy	[32]
Tilapia mossambica	0.14	Mexicali valley, Mexico	[33]
Myleus rubripinnis	0.013	Maroni River (French Guiana)	[34]
Epinephelus coides	3.923		
Tinca tinca	0.32	Aquatic Zahlinice Ecosystem	[18]
Ctenopharyngodon idella	0.05	(Czech Republic)	
Hypophthalmichthys molitrix	**0.367**	**SGR, Iran**	**This study**
	T- Hg (µg g^{-1} wet wt)		
Ciclha monoculus	0.35	Amazonian region, Brazil	[35]
Hoplias malabaricus	0.41		
Colossoma macropomum	0.04		
Piaractus mesopotamicus	0.09		
Cyprinus carpio	0.79	Ya-Er Lake, China	[36]
Hypophthalmichthys molitrtix	0.429		
Carassius carassius	0.423		
Ophiocephalus argus cantor	0.827		
Ctenochromis horei	0.15	Lake Tanganyika, Tanzania	[37]
Neolamprologus boulengeri	0.2		
Mastacembelus cunningtoni	0.22		
Clarias theodorae	0.22		

Table 5. Comparison of mean T-Hg (ng g^{-1} dry wt) of planktons in different regions of the world and the result of this study.

Plankton	Location	Mean T-Hg (ng g^{-1} dry wt)	References
Phytoplankton	Terra Nova bay, south pole	39	[17]
Zooplankton		65	
Plankton	Natural lakes of Quebec, Canada	85 – 432	[38]
	Littoral zones of reservoirs	360 – 671	
	Pelagic zones of reservoirs	70 – 538	
Zooplankton	15 study lakes, Wisconsin, USA	33 – 206	[39]
Zooplankton	Superior lake, Canada	44.5-101	[40]
Plankton	Talawaan watershed, Indonesia	400 –118000	[8]
Phytoplankton	Grande marsh, Colombia	520	[11]
Zooplankton		940	
Plankton	**SGR**	**78.21**	**This study**

CONCLUSION

The planktons and silver carps from the SGR are contaminated by mercury. Furthermore, during the studying period, all calculated $BMF_{Hg\ (plankton\text{-}fish)}$ were higher than one, which indicated the transmission of Hg from plankton to silver carp was significant. Due to the toxic effects of Hg on human health, it is necessary to implement sever health consideration in related to consumption of silver carp from SGR. Since this reservoir is the most important fishery and drinking water source in the region, further surveys are necessary to verify the results of this study and assess the negative health effects of Hg pollution on local population as well as tracking total Hg in other living parts of SGR ecosystem.

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