

## Research Paper: Health Risk Assessment of *Liza Parsia* Fish Lipids: Heavy Metals and DDTs Contaminations



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## ABSTRACT

**Background:** Fish is a source of nutrition; however, toxic substances and heavy metals may enter human body due to consuming contaminated fish. The aim of this study was to investigate the levels of heavy metals and DDTs in *Liza parsia*, a fish species, and assess the health risk of the contaminants in the fish samples.

**Methods:** The extracted fish lipid was added with n-hexane and H<sub>2</sub>SO<sub>4</sub> and was kept for long time. The upper organic part was collected for the analysis of DDTs by gas chromatography equipped with electron capture detector. The fish samples were homogenized by wet digestion method, and the heavy metal contents were analyzed, using an atomic absorption spectrophotometer. The health risks of the fish samples were assessed based on target hazard quotient and target cancer risk.

**Results:** The pesticide contents of DDT, DDD, DDE and DDTs in the fish samples were 0.0414, 0.000253, 0.015 and 0.0567ng/g, respectively. Also, the mean concentrations of Mn, Fe, Cu and Zn were 35.42±10.05, 318.75±242.45, 41.67±4.78 and 281.25±5.42 mg/kg, respectively. Of note, the Cd and Pb contents were below the detection limits. The concentrations of other heavy metals were above the reference limits, which can cause various disease processes.

**Conclusion:** The contents of four heavy metals and DDTs in the samples were above the limits. The main sources of these substances are likely due to human anthropogenic activities. Fish and other edible aquatic animals should be monitored regularly and the heavy metal levels be determined and declared to the public.

**Keywords:** DDTs, Heavy metals, Health risks, Pesticides, Toxicity

## Introduction

**F**ish is an excellent food and a vital source of protein, minerals, vitamins, and fatty acids [1]. Generally, these essential ingredients are present in 442 marine species and 266 fish species caught locally in Bangladesh. Among these species, *Liza parsia* has gained much popularity among the local people due to its taste and nutritional value. This species lives in

slightly salty (brackish) water and is mainly found in the tropical and subtropical areas [2]. Beside the food ingredients, this fish may also contain toxic substances, such as pesticides (DDTs) and heavy metals. They are considered toxic pollutants, which can accumulate in both land and sea animals, and last long in nature. Although the use of DDTs has already been banned in many parts of the world, the developing countries are still using the pesticides for agricultural and various other purposes [3]. The concerned authorities in Bangladesh have restricted

the applications of DDTs but there is no systematic plan for monitoring its spread in the environment. Consequently, they are still detectible in aquatic as well as terrestrial ecosystems [4]. In aquatic environment, DDTs are transferred from water to fish and eventually enter the human body [3].

A study has shown that fish contributes only 10% to human diet whereas 90% of DDTs in the human body is due to consumption of animal fat including fish species [5]. The exposure of human body to DDTs can cause severe health risks, such as impaired immune system, disrupted thyroid function and other hormones, lower fertility, diabetes, and various cancers (pancreas, breast, and liver) [5]. Similar to DDTs, heavy metals, with specific densities more than 5g/cm<sup>3</sup>, are also toxic pollutants in human diet [6]. The heavy metals are originated in water from natural sources and human anthropogenic activities, such as mining, transportation, industrial and household wastes. In addition, application of wastewater and agrochemical compounds, such as fertilizers, pesticides, and insecticides from farmlands are the other popular sources of these pollutants [7]. When fish are cultivated in polluted waters, heavy metals accumulate in them and eventually enter the human food chain [8]. Heavy metals bind to nucleic acids, proteins, enzymes, and bio-membranes in human cells [9]. While some metal ions are biologically useful at low concentration, such as Mn, Fe, Cu and Zn but cadmium and lead have no useful biological benefit to human cells and tissues. These heavy metals are toxic at any concentration and their acute exposure has detrimental effects on both human and animal health [10]. For instance, the elevated level of iron can lead to high hemoglobin level, which may cause liver cancer and/or diabetes. Also, it retards the cognitive and intellectual development in children. Cadmium has fatal effects on human health, causing bone and kidney impairment, hepatic dysfunction, and certain physiological disorders. The excessive level of lead in foods reduces mental performance in children and cause hypertension in adults [11, 12].

This study investigated the pesticide contents of *Liza parsia* by a Gas Chromatography (GC) unit, equipped with Electron Capture Detector (ECD). Our findings revealed that DDTs were present in fish samples, likely because they were used as pesticides, despite being banned by the governmental policies in Bangladesh. This study further estimated the concentrations of various metals, such as Mn, Fe, Cu, Zn, Pb, and Cd by Atomic Absorption Spectroscopy (AAS). The concentrations of heavy metals exceeded the allowable limits in several cases. Based on our findings, we are concerned about the transfer and accumulation of heavy metals and DDTs in the human body through the consumption of contaminated fish, which may lead to carcinogenic and pathologic conditions in humans. Finally, this study as-

essed the various health risk indices for the heavy metals and DDTs in *Liza parsia* fish.

## Materials and Methods

**Geographic area:** Moheshshorkati is an area of Assasuni Upozilla under Satkhira district in the southern part of Bangladesh as shown in Figure 1. The area of Moheshshorkati is 402.36 Km<sup>2</sup> and is located between 21°36' and 22°54' north latitudes and between 88°54' and 89°20' east longitudes. It is surrounded by Tala, Satkhira sadar and Debhata Upazila on the north, Debhata and Kaligonj Upazila on the west, Shyamnagar Upazila on the south, and Khulna District on the east side of the country.

**Analysis of moisture:** A fixed amount of the fish samples were dried at 105°C in an oven for 10 hours and the moisture percentage was calculated by comparing the weight before and after drying [13].

**Analysis of ash:** A set portion of fish samples were burned in a muffle furnace at 550-600°C and the ash was quantified as the percentage of the fish [13].

**Analysis of lipid:** After evaporating the moisture, the samples were finely ground, and the lipid was extracted, using ethyl ether solvent. The solvent was then evaporated by heating and the percentage of the lipid content was determined [13].

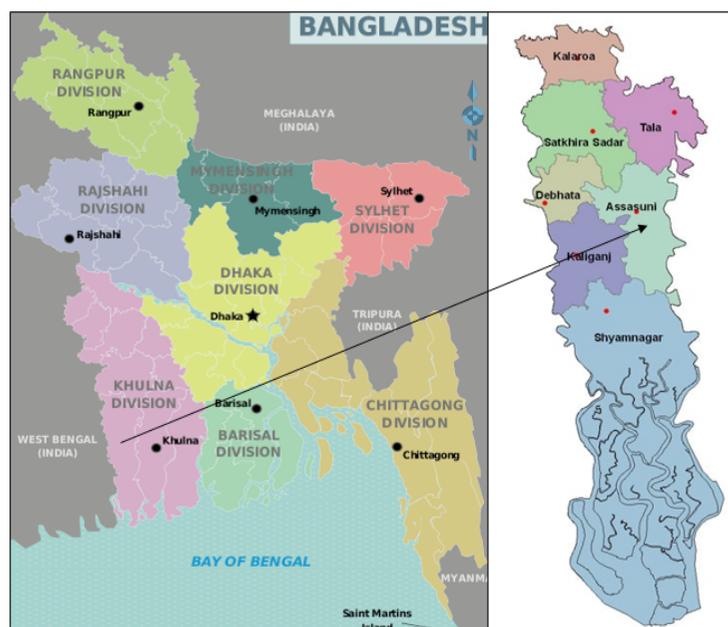
**Analysis of protein:** The percentage of protein contents in *Liza parsia* samples were estimated by micro-kjeldahl method [13].

**Analysis of DDTs:** The fish lipid was dissolved in n-hexane to which concentrated H<sub>2</sub>SO<sub>4</sub> was added. The upper layer was aspirated and analyzed in GC-ECD for quantification of DDTs [4].

**Analysis of heavy metals:** Tri-acid mixture (5:1:1; 70% HNO<sub>3</sub>, 70% H<sub>2</sub>SO<sub>4</sub>, and 65% HClO<sub>4</sub>) was added with a set portion of the fish samples. Then, the solution was digested at 80°C for 30 minutes and analyzed on an AAS unit (Shimadzu model AA-7000) for the estimation of heavy metal contents (Mn, Fe, Cu, Zn, Pb, and Cd) [14].

**Metal Pollution Index (MPI):** Metal pollution index was used to assess the contamination of heavy metals for each of the fish samples as Formula 1:

$$1. MPI = (CM_1 \times CM_2 \times CM_3 \times \dots \times CM_n)^{1/n}$$



**Figure 1.** The geographic map of the study area (Moheshshorkati, Assasuni, Satkhira)

Where,  $CM_1$  is the concentration of first heavy metal;  $CM_n$  is the concentration of “nth” heavy metal (mg/kg of dry weight) in the fish samples. If MPI was greater than one, it indicated the existence of contamination. The  $MPI < 1$  indicated no contamination [15].

**Health risk assessment:** The carcinogenic and non-carcinogenic conditions due to heavy metals were assessed by the following health risk model.

**Estimated Daily Intake (EDI):** The estimated daily intake value was calculated, using the Formula 2:

$$2. EDI = \frac{C_m \times I_g \times C_f}{W_b}$$

Where,  $C_m$  is the concentration of heavy metals in the fish samples (mg/kg of dry weight);  $I_g$  is the consumption rate of fish for Bangladeshi people (62.58 g/day);  $W_b$  is the average body weight of Bangladeshi people (49.5 kg), and  $C_f$  is the conversion factor (0.3227) [16, 17].

**Target hazard quotient for heavy metals:** The Target Hazard Quotient (THQ) was evaluated, using the Formula 3:

$$3. THQ = \frac{C_m \times I_g \times E_f \times D_e \times C_f}{D_f \times W_b \times T_{avncar}}$$

Where,  $E_f$  is the exposure frequency and is equal to 365 days/year.  $D_e$  is the exposure duration (71.8 years).  $D_f$  is the reference dose of Mn, Fe, Cu, Zn, Cd and Pb that has been considered as 0.14, 0.7, 0.037, 0.30, 0.0005 and 0.0036 mg/kg/day, respectively (Table 2).  $T_{avncar}$  indicates the average time for non-carcinogens (365 days/year  $\times D_e$ ) [17].

**Hazardous Index (HI):** The hazard index was calculated by the Formula 4 [17]:

$$4. HI = \sum THQ = THQ_{Mn} + THQ_{Fe} + THQ_{Cu} + THQ_{Zn} + THQ_{Pb} + THQ_{Cd}$$

**Target cancer risk:** The target cancer risk was calculated by the Formula 5:

$$5. TCR = \frac{C_m \times I_g \times E_f \times D_e \times S_{cps} \times C_f}{D_f \times W_b \times T_{avncar}}$$

Where,  $S_{cps}$  is the carcinogenic potency slope. The  $S_{cps}$  values of Cd and Pb are 6.1 and 0.0085 mg/kg body weight/day, respectively [17]. Some heavy metals like Mn, Fe, Cu and Zn do not have carcinogenic effects on the human body. So, the  $S_{cps}$  values for these heavy metals have not been established yet [18].

**Health risk assessment:** Exposure to pesticides, such as DDTs causes various fatal diseases in humans. The probability of causing cancer and other diseases can be evaluated by estimating the daily intake and target quotients for DDTs, leading to the determination of target cancer risk as described below.

**Estimated Daily Intake (EDI):** The estimated daily intake of DDTs was calculated as Formula 6.

$$6. EDI = \frac{C_D \times I_g}{W_b}$$

Where,  $C_D$  is the concentration of DDTs (ng/g ww),  $I_g$  is the consumption rate of fish (62.58 g/day) and  $W_b$  is

**Table 1.** Concentrations of heavy metals and residual amounts of DDTs (ng/g) in *Liza parsia* samples

Sample Name	Mn	Fe	Cu	Zn	Cd	Pb
<i>Liza parsia</i>	31.25	584.38	37.50	275.00	ND	ND
	28.13	262.50	46.88	284.38	ND	ND
	46.88	109.38	40.63	284.38	ND	ND
Mean±SD	35.42±10.05	318.75±242.45	41.67±4.78	281.25±5.42	ND	ND
Range	28.13-46.88	109.38-584.38	37.50-46.88	275.00-284.38	ND	ND
Reference value	5.40 <sup>a</sup>	10-56 <sup>a</sup>	40.00 <sup>a</sup>	50.00 <sup>b</sup>	0.50 <sup>a</sup>	2.00 <sup>a</sup>
<i>Liza parsia</i>	DDT (2,4'-DDT + 4,4'-DDT)	DDD	DDE	Total DDTs	Ratio of DDT/DDTs	
	0.0414	0.000253	0.0150	0.0567	0.7302	
<i>Liza parsia</i>	Ash	Moisture	Protein	Lipid		
	2.12	67.69	17.80	12.39		
	2.01	68.23	17.20	12.56		
	2.08	67.27	17.74	12.91		
Mean±SD	2.07±0.06	67.73±0.48	17.58±0.33	12.62±0.27		
Range	2.01-2.12	67.27-68.23	17.20-17.80	12.39-12.91		

Sources: <sup>a</sup> [20], <sup>b</sup> [8]; ND: Not Detected; Concentrations: mg/kg of dry weight; Amounts of DDTs: ng/g

the average body weight of Bangladeshi people (49.5 kg) [16, 19].

**Target hazard quotient for DDTs:** The non-carcinogenic risk due to the consumption of fish contaminated with DDTs was calculated by the Formula 7 Target Hazard Quotient (THQ):

$$7. THQ = \frac{R_f D \times W_b}{C_d}$$

Where,  $R_f D$  is the reference dose for DDTs (500ng/kg/day);  $W_b$  is the average body weight of Bangladeshi people (49.5kg) [5, 17].

**Target cancer risk:** The Target Carcinogenic Risk (TCR) for DDTs due to the consumption of the contaminated fish was calculated by the Formula 8:

$$8. TCR = \frac{R_L \times W_b}{C_d \times OSF}$$

Where,  $R_L$  is the cancer risk level ( $1 \times 10^{-6}$ ); OSF is the cancer oral slope factor ( $3.4 \times 10^{-7}$  ng/kg/day) for DDTs [5, 17].

**Statistical analyses:** The means, standard deviations, one way ANOVA and Pearson's correlations of the data were calculated, using SPSS software, version 16.

## Results

Food safety has become a great challenge in developing countries due to frequent application of agricultural chemicals and dumping of waste materials in the ecosystem. To ascertain the food safety and assess both the carcinogenic and non-carcinogenic risks to human health, the concentrations of the following heavy metals, Mn, Fe, Cu, Zn, Cd and Pb, and DDTs in *Liza parsia* were determined. The levels of Mn, Fe, Cu, and Zn were found to be 35.42±10.05, 318.75±242.45, 41.67±4.78 and 281.25±5.42 mg/kg, respectively. Whereas, the concentrations of Pb and Cd were below the detection limits. In this study, the most abundant metal in the fish was Fe, with the concentration being 318.75±242.45 mg/kg. The concentrations of Mn, Fe, Cu and Zn were above the safe limits. In decreasing order, the concentrations of these heavy metals were found as follows: Fe > Zn > Cu > Mn.

As shown in Figure 2, the concentration of DDT varieties (2,4'-DDT + 4,4'-DDT), DDD, DDE and DDTs were

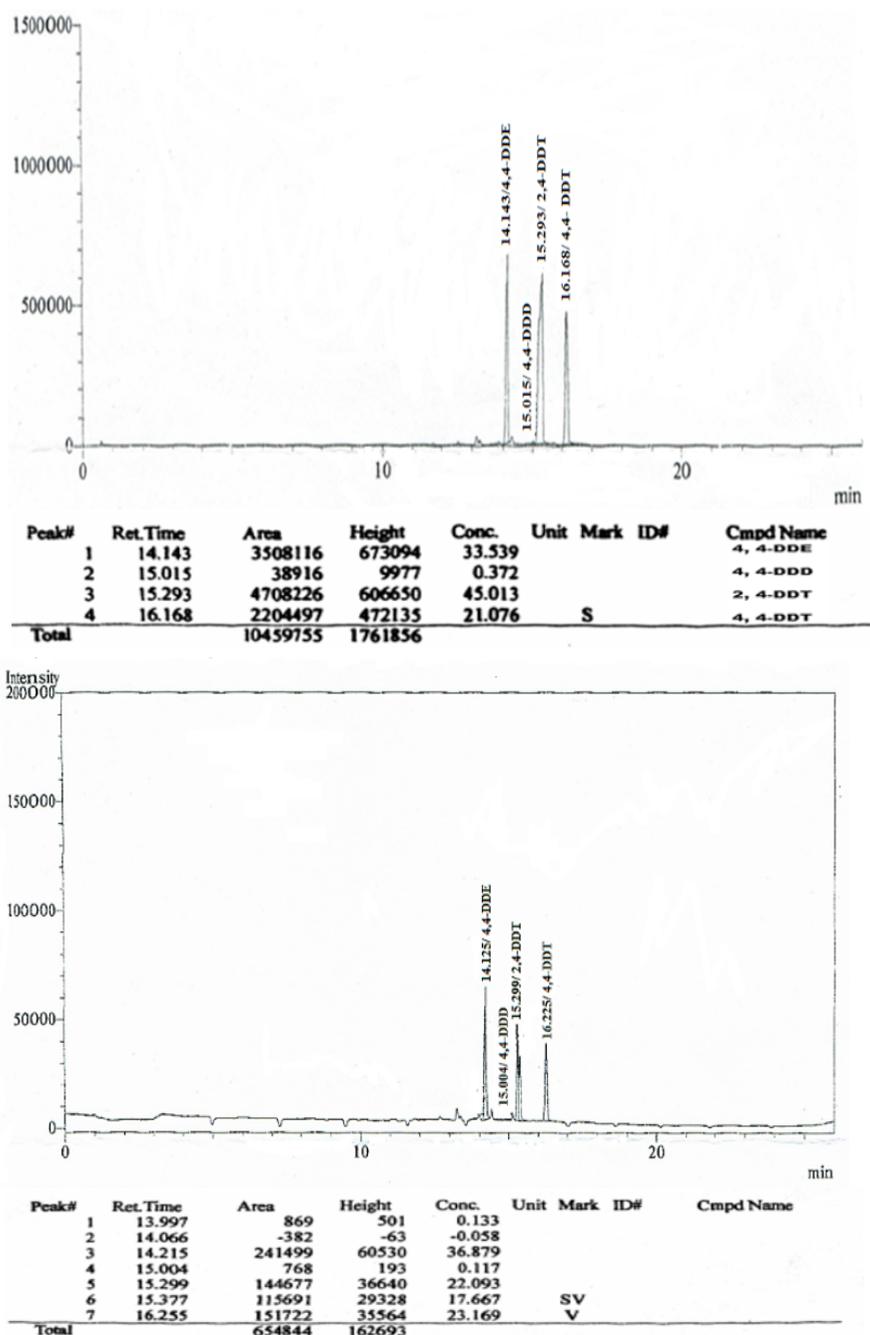


Figure 2. Gas chromatograms of (A) standard and (B) DDTs in *Liza parsia* samples

found to be 0.0414, 0.000253, 0.015 and 0.0567 ng/g, respectively. In this study, the approximate composition of *Liza parsia* was determined to support the level of DDTs in the fish lipid. The moisture, ash, lipid and protein contents were found to be  $67.73 \pm 0.48\%$ ,  $2.07 \pm 0.06\%$ ,  $17.58 \pm 0.33\%$  and  $12.62 \pm 0.27\%$ , respectively. Table 1 presents the heavy metal concentrations, DDTs and approximate composition of *Liza parsia*.

## Discussion

Moheshshorkati area is located in the southern part of Bangladesh. Due to various anthropogenic activities, heavy metals enter waters, hence into the aquatic animals in this area. These toxic metals are transferred to human body through the consumption of foods and affect the normal functions of the various organs. Therefore, regular monitoring of heavy metals and pesticide in fish products is essential to ensure the safety and quality

**Table 2.** Comparison of heavy metal concentration (mg/kg) and DDTs (ng/g) with other studies

Country	Mn	Fe	Cu	Zn	Pb	Cd	DDTs	Ref.
Iran	--	4.65	--	--	--	--	--	[1]
Ethiopia	--	--	0.54	3.68	0.004	--	--	[3]
	--	--	0.58	3.67	0.003	--	10.83	
Bangladesh	--	--	0.65	5.30	0.003	--	81.37	[4]
	--	--	--	--	--	--	22.40	
Italy	--	--	--	--	--	--	3.10	[5]
Turkey	--	31.08	4.04	25.45	49.93	3.44	--	[8]
	--	18.45	3.66	24.53	32.80	2.68	--	
Janadeleh	--	19.97	27.13	46.88	39.94	7.22	--	[10]
	--	--	0.003	0.01	0.0004	0.0002	--	
Ethiopia	--	--	0.004	0.02	0.0007	0.0004	2.33	[19]
	--	--	--	--	--	--	4.38	
Saudi Arabia	--	--	--	--	--	--	4.55	[20]
	0.96	55.70	3.52	--	1.40	0.16	--	
Bangladesh	--	--	5.14	--	3.79	0.12	--	[21]
Iran	--	--	--	--	--	--	33.00	[29]
	--	--	--	--	--	--	31.00	
South Africa	--	--	--	--	--	--	645-2399	[30]
Bangladesh	--	--	12.10	--	13.88	0.39	--	[31]
China	--	--	--	--	--	--	3.40-7.80	[32]
India	--	185.00-215.00	--	1.40-272.20	29.70-44.8	0.11-0.15	--	[33]
Bangladesh Moheshshorkati	35.42	318.75	41.67	281.25	ND	ND	0.0567	Present study
	28.13-46.88	109.38-584.38	37.50-46.88	275.00-284.38	ND	ND	--	

of human foods. In this study, six heavy metals were investigated and analyzed in the fish species. Among the heavy metals, Mn, Fe, Cu, and Zn are biologically important whereas Cd and Pb are hazardous to human organs. As shown in Table 1, the levels of Mn, Fe, Cu, and Zn, except for Pb and Cd, were found to be above the safe limits. These elements might have originated from various sources. For instance, the sources of Mn in the

environment are cement materials, petroleum products and metallurgy processes. Iron, which is an essential element for the physiological functions in humans, may come from disintegrating metal parts, fertilizers, plant and animal wastes. Further, excess Cu and Zn in the environment may originate from transportation, liquid petroleum stations, battery, oil wastes, and oil leakage from boats and ships [14].

**Metal Pollution Index (MPI):** The metal pollution indices of heavy metals in *Liza parsia* were found as  $101.49 \pm 14.80$ . In this study, MPI was greater than one ( $MPI > 1$ ), indicating that the fish samples were contaminated with heavy metals [7]. This value also exceeded the maximum permissible limit of 3.83 mg/kg. Shehawy, et al. analyzed the MPI in some aquatic species and reported that the maximum value traced in *Sardinella longiceps* was 1.82 mg/kg whereas the minimum value detected in *Sparus aurata* was 0.44 mg/kg [20]. Ahmed, et al. studied *Liza parsia*, in which the MPI was 3.65 mg/kg [21]. This value was much lower than that detected in the current study. Based on the results from this study, we determined that the levels of heavy metals were higher than the acceptable international limits.

**Estimated Daily Intake (EDI):** This study revealed that the estimated daily intake values for Mn, Fe, Cu and Zn were less than the corresponding reference doses ( $D_r$ ) for these metals. The New York State Department of Health (NYSDOH) has suggested that if the ratio of  $EDI/D_r$  is  $\leq 1$ , its health risk is minimal. If the  $EDI/D_r$  ratio is 1-5 times above the  $D_r$ , it is associated with low health risks. When  $EDI/D_r$  ratio is greater than 5-10 above the  $D_r$ , it is associated with moderate health risks. Finally, if the  $EDI/D_r$  ratio is 10 times above the  $D_r$ , it is associated with high health risks [22]. Based on our results, Zn, Mn and Fe posed minimum to low health risks whereas Cu posed high carcinogenic risk to humans.

**Target Hazard Quotient (THQ):** The target hazard quotient is the measure of non-carcinogenic health risks and the acceptable value is considered equal or less than one [23]. In this regard, Ambedkar, et al. have concluded that if the THQ value of individual metal exceeds its limit, it may cause serious health risks [24]. Based on our THQ measures, the Mn, Fe, Cu and Zn contents were at safe limits.

Furthermore, the combined impact of Hazard Index (HI) of all heavy metals was 1.13 that was higher than the acceptable limit of 1.0 in *Liza parsia* fish samples. Therefore, we can assume that the consumption of this fish might not be associated with carcinogenic health risk [25]. In general, the combined adverse effects may occur if more than one toxic substance are consumed by humans [26]. Moreover, the long-term exposure of humans to carcinogenic foods may lead to cancerous conditions.

**Target carcinogenic risks:** Some analyzed heavy metals, such as Mn, Fe, Cu, and Zn are not carcinogenic and the level of Pb and Cd were below the detection limits. Thus, *Liza parsia* fish species in this area may not contribute to the carcinogenic effects in local people [18].

The TCR value for DDTs was found to be  $2.6E^3$  that is above the safe limit ( $10^4$ ). Therefore, the fact that the fish samples contained the detected level of DDTs contamination is a cause for human health concern.

**Analysis of DDTs contents:** In this study, the concentration of DDTs was higher than DDD and DDE. The DDTs might originate in the fish feeds. DDTs gradually convert to DDD and DDE over time. Moreover, the ratios of  $DDT/\Sigma DDTs$  in *Liza parsia* was found to be 0.7302 ng/g. From these results, it might be predicted that the fish had been exposed to pesticides recently. Given that the ratio of  $DDT/\Sigma DDTs$  was greater than 0.5, it suggests that recent biotransformation of DDT to DDD and DDE had occurred [4]. The accumulation of DDTs depends on the lipid content in the fish. The lower the content of water (90%) in the fish, the higher are the contents of lipid and protein [27, 28]. In this species, the water content was found to be  $67.73 \pm 0.48\%$ , suggesting higher percentages of protein and lipids in the fish (Table 1). DDTs are lipid-soluble organic compounds; therefore, they accumulate in species that have high lipid content [29]. Further, the lipid content in the fish may vary in different seasons, species, geographical location, age and the maturity of the species [28]. With changes in these parameters, the total lipid content also varies.

With the  $THQ \leq 0.2 \text{ ng/g}$ , DDTs may cause negligible adverse effects on human health while if the THQ values exceed 0.2 ng/g, the adverse effect on human health also rises [30]. Considering that the THQ values for DDT, DDD, DDE and DDTs in this study were  $6.0E^5$ , 9.8E, 1.7E and 4.4E ng/g, respectively, the adverse effects in humans may reach fatal conditions.

Lastly, both the developed and developing countries are cautious about their foods and their environment becoming polluted with toxic substances. At present, various studies are underway to monitor the sources and levels of environmental pollutants, and establish specific data bases to combat the problem. In this context, the current study has compared its findings with those of other studies conducted at various parts of the world to provide useful information on variations in the pollution status versus the safety of human food products.

## Conclusions

In this study, the levels of Fe, Cu, and Zn were above the safe limits for humans in the *Liza parsia* fish samples, but the contents of Cd and Pb were acceptable. Anthropogenic activities, such as the use of pesticides, fertilizers, wastewaters in agricultural fields might be the potential

sources of contaminations of heavy metals and DDTs. The indices of various health risks, such as EDI and THQ were assessed for heavy metals and DDTs. The TCR was also assessed for the carcinogenic effects of DDTs in the human body. These indices indicated the potential health risks in humans. The consumption of contaminated fish products is the most significant concern for carcinogenic and non-carcinogenic risks to human health. Moreover, the findings of this study may serve as effective references for future investigations on the subject. In this regard, we believe that necessary steps should be taken to raise the public awareness about heavy metals and DDTs contaminations in the aquatic species and the food products that originate from them in Bangladesh.

**Limitations of the study:** This study had limited laboratory resources to comprehensively evaluate the levels of all heavy metals and DDTs.

**Recommendations for future research:** Regular monitoring of the levels of heavy metals and pesticides is warranted in this geographic area of Bangladesh. Thus, the findings of this study may be useful as a data base for the further research.

## Ethical Considerations

### Compliance with ethical guidelines

The study measured the toxic substances (heavy metals and DDTs) in a fish species, and did not involve other animals or human subjects. Therefore, the ethical guidelines were not applicable to this study.

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### Author's contributions

Conceptualization and supervision: Mosummath Hosna Ara and Muhammad Shamim Al Mamun; Methodology: Md. Abu Rayhan Khan; Investigation, writing review and editing: Mosummath Hosna Ara and Muhammad Shamim Al Mamun; Writing -original draft: Muhammad Shamim Al Mamun and Md. Abu Rayhan Khan; Data collection: Md. Abu Rayhan Khan; Data analysis: Muhammad Shamim Al Mamun and Md. Abu Rayhan Khan; Funding acquisition and resources: Mosummath Hosna Ara and Muhammad Shamim Al Mamun.

## Conflict of interest

The authors declared no conflict of interest.

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