Journal of Hazardous Materials 461 (2024) 132532

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Bisphenol A contamination in Hilsa shad and assessment of potential health hazard: A pioneering investigation in the national river Ganga, India

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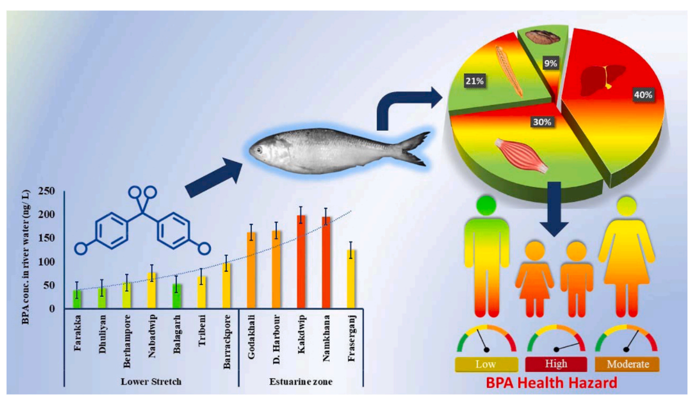
HIGHLIGHTS

- The first research on BPA in Hilsa Shad of River Ganga's lower and estuarine zone.
- The estuarine zone of River Ganga had higher BPA levels than the lower region.
- BPA concentration in fish tissues was found as liver>muscle>kidney>gonad.
- BPA content in fish tissues correlated strongly with various water quality indices.
- Human exposure to BPA from consuming Hilsa was assessed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Joao Pinto da Costa

Keywords:
Hilsa shad
Ganga-Hooghly River estuary
Migratory fish
Bisphenol A contamination
Health hazard

ABSTRACT

The anadromous Hilsa, often known as the "Queen of Fishes" (*Tenualosa ilisha*), is the most valuable fishery in the Ganga-Hooghly delta estuary. Although BPA exposure has been shown to be harmful to aquatic organisms, no research has looked at the effects of BPA on the commercially valuable Hilsa shad of river Ganga. To close this information vacuum, we examined BPA levels in Hilsa fish from the Ganga estuary. Liver, muscle, kidney, and gonads were all positive for BPA among the Hilsa fish of all ages. Liver BPA levels were highest in adult males (272.16 ± 0.38 ng/g-dw), and lowest in juveniles (5.46 ± 0.06 ng/g-dw). BPA concentrations in the Hilsa shad muscle were highest in reproductively mature females (196.23 ± 0.41 ng/g-dw). The study also discovered a correlation between fish development and BPA exposure, with higher levels of BPA being identified in adult Hilsa species. This is the first study to look at the impact of BPA pollution on aquatic ecosystems and fisheries, and it showed that Hilsa shad is contaminated with BPA and poses health hazards to human beings. The results, which demonstrate BPA contamination, are useful for protecting Hilsa in the river Ganga.

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https://doi.org/10.1016/j.jhazmat.2023.132532
Received 17 July 2023; Received in revised form 8 September 2023; Accepted 9 September 2023
Available online 12 September 2023

1. Introduction

River Ganga after originating from the upstream of the Himalayas traverses a long course of 2525 kilometers through uplands and plains and finally terminates into the Bay of Bengal. During its course of flow, it meets with several open water resources like rivers, streams, estuaries, etc. embracing huge amounts of organic and inorganic loadings. It is reported that annually over 0.12 million tonnes of plastic are discharged into marine habitats from the Gangetic network alone, making it one of 14 continental rivers into which more than a fifth of worldwide garbage is thrown. A study from 2019 showed that Polyethylene terephthalate and polyethylene were the prevailing plastic wreckages among the meso- and micro-plastic distribution in Ganga River sediments [97]. Owing to its versatility, polyethylene is the commonly used plastic found in sediments and water collected from the river Ganga [103]. Features like abandoned or discarded fishing gear, threads etc. are reported to the important sources of microplastic pollution in the Ganga River system and its related tributaries [82,84]. Increased levels of such microplastic pollution are recently been noticed by several workers from water and sediments of river Ganga sprawling from upper to lower stretches indicating a higher risk of potential health hazards [103,104,15,30,4,52,53,81,83]. Studies on the biological contamination of plastics especially in gastrointestinal tract, muscles, liver, and gills of few carnivore and herbivore wild caught Gangetic fishes has also shown a potential health risks [54]. Very recently, the Ganga River, especially its estuarine region has been reported with plasticizers and BPA with its estrogenic effect leading to increasing ecotoxicological risk [80]. The highest estimated ecotoxicological risk to the aquatic insects and fishes stemmed from the emerging concentration of BPA in river water studied over the entirety of the Ganga starting from Uttarakhand to Sunderban region [14].

Comprehensive studies on the alterations in fish physiology due to the effect of Bisphenol A (BPA) has been carried out by several researchers in vitro to identify the detrimental effect of BPA on freshwater fish health either alone or in combination with some other heavy metal/inorganic pollutant [113,12,122,19,25,27,42]. BPA is now an impending bio hazard creating immense vulnerabilities. To disrupt hormone function, BPA can act like the body's own hormones. BPA acts and behaves similarly to the hormone estrogen and diethylstilbestrol. Because of its similarity to estrogen, BPA can mimic its effects by binding to estrogen receptors and so altering processes as diverse as cell growth and repair, fetal development, and reproduction [124,26,40,43,72,75]. Although few references could be inferred from Northern Taiwan rivers [70], studies on the effect of the BPA in a wild-caught estuarine fish are meager. The Hooghly-Matlah estuary the largest estuary of India, is a positive mixohaline estuary that gets feed from the water of river Ganga. The river washes the entire city sewage of the Kolkata-Howrah metropolis and anthropogenic contaminants into the estuary bringing detrimental impact on the ecosystem. The species *Tenualosa ilisha* (Ham, 1822) belonging to the family Clupeidae (Clupeiformes) is an active migrant species possessing rich commercial significance in the eastern part of the country accounting for 15–20% of the total landings [7]. The species spends a crucial part of their life cycle in the brackish water and freshwater regions for its breeding cycle and offers extensive research subject due to its important behavioral patterns. A recent study on hilsa (*Tenualosa ilisha*) from Bangladesh documented for the first time the existence of microplastics in the gastrointestinal tract of fish [102]. Nonetheless, research into the BPA levels in river Ganga's Hilsa shad is lacking. This is the first study to measure BPA concentrations in Hilsa shad and establish a correlation with physicochemical parameters of water samples obtained from river Ganga. The primary goals of this study were to determine the average concentration of the plasticizer BPA in Hilsa shad (*Tenualosa ilisha*) sampled from the lower stretches and estuarine zones of the Ganga River in India and to evaluate the possibilities and potential health hazards associated with consuming BPA-exposed Hilsa.

2. Materials and methods

2.1. Fish and water sampling

For this study, a total of 184 numbers of Hilsa shad were collected during commercial fish catch for human consumption from the lower freshwater stretch of river Ganga [39 from Farakka (NFK), Murshidabad; 25 from Dhuliyan (DGLE); 27 from Berhampore (BPC), Murshidabad; 34 from Nabadwip (NDAE), Nadia; 47 from Balagarh (BGAE) and Tribeni (TBAE), Hooghly; 12 from Barrackpore (BP), North 24 Parganas] and a total of 163 individuals were collected from the estuarine zone of river Ganga [35 from Godakhali (GDK), 32 from Diamond Harbour (DH); 29 from Kakdwip (KWDP); 39 from Namkhana (NMKA); 28 from Fraserganj (FG)] comprising of male, female and juvenile individuals during the period of March 2022 to February 2023 (Fig. 1). River water samples were collected monthly for the analysis of physicochemical parameters following standard methods of APHA, 2017. The BPA level from each sampling site was also analysed simultaneously during the study period.

The details of no. of individuals collected, their total length, and body weight according to the maturity stages of the fish have been mentioned in Table 1. All of the sites chosen for sampling are significant nodes in the supply chain for the Hilsa fishery of river Ganga. The fish samples were collected during the early morning hours from fish landing centers. Fish samples, after collection were measured with a scale for total length (to the nearest 0.1 mm) and body weight (nearest to 0.1 g) using a weighing scale before freezing the samples (Fig. 2). Each fish was eviscerated immediately using pre-deionized treated instruments and the tissue samples were brought in ice-cold packets and were kept in a − 80 °C freezer for further analysis. Liver, muscle, kidney, and gonad samples were collected aseptically and stored in BPA-free vials in liquid nitrogen. The details of length-weight for male, female, and juvenile were shown in Fig. 3. River water samples were collected from all stations and stored at 4 °C until further use.

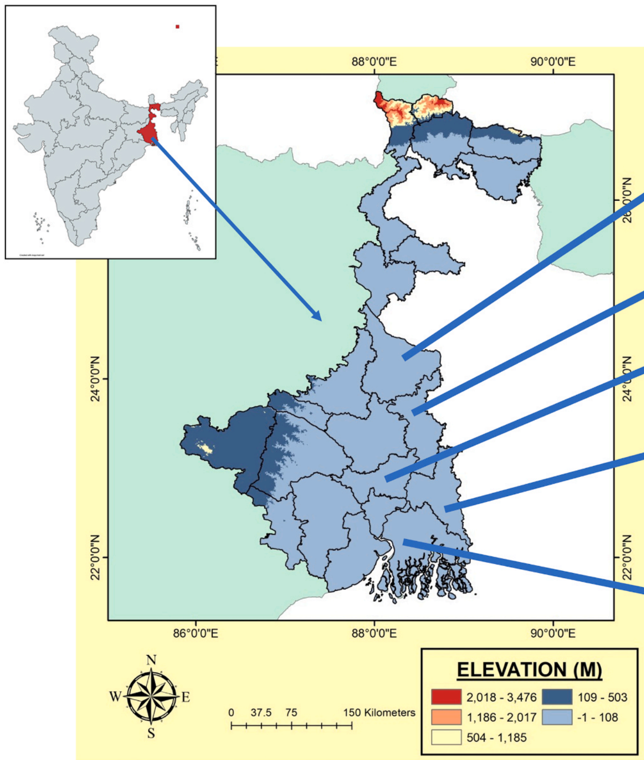
The stowaway containers are all made of glass or a material that does not leach BPA. All samples and pieces of equipment, including filters, were maintained under aluminum foil or in sealed glass containers to avoid contamination from the air. The sample handling process involved the use of cotton lab coats rather than polyester garments and nitrile gloves.

2.2. Chemicals and reagents

Dimethyl sulfoxide (DMSO), N, O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA), N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), chlorotrimethylsilane (TMCS), HPLC-grade methanol, acetonitrile, α-phosphoric acid (85% purity) and triethylamine were procured from Sigma-Aldrich (St. Louis, MO).

2.3. BPA extraction from water and fish tissue

To conduct chemical analysis, water samples are typically subjected to solid-phase extraction (SPE) to extract potentially harmful EDCs. Fisher C18 cartridges (SPE 24-port, Fisher Scientific, Pittsburgh, PA) were briefly installed on a vacuum manifold. The cartridges were cleaned by passing 40 ml of deionized water and 25 ml of methanol through them while the cartridges were placed in a low vacuum. This was done to remove any residual bonding agents that might have been present. The cartridges were then filled with one liter of river water from each region and forced through them under a mild vacuum (mean flow rate: 60 ml/min). Aqueous samples were loaded into SPE cartridges, where the target chemicals could be extracted. After loading, the cartridges were flushed with 20 ml of deionized water and then vacuum dried for 5 min to remove any residual moisture. The adsorbed analytes were extracted from the cartridges using 5 ml of methanol at a flow rate of 5 ml/min, which was then transferred to 10 ml vials. Derivatization of samples was performed in a rotary vacuum evaporator (Equitron Medica



District	Sampling Points	GPS Coordinates
Murshidabad	Farakka	24.813196, 87.916950
	Dhuliyan	24.689375, 87.965446
	Berhampore	24.092175, 88.244823
Nadia	Nabadwip	23.408018, 88.379639
Hooghly	Balagarh	23.126656, 88.469720
	Tribeni	22.989598, 88.403667
N 24 Parganas	Barrackpore	22.777282, 88.333585
S 24 Parganas	Godakhali	22.396144, 88.136690
	D. Harbour	22.179876, 88.192696
	Kakdwip	21.864800, 88.170776
	Namkhana	21.764763, 88.218801
	Fraserganj	21.568507, 88.244092

Fig. 1. Sampling locations of Hilsa shad from lower stretch and estuarine zone of river Ganga, India.

Table 1
Table showing the sampling year and month with no. of male, female and juveniles sampled during the study along with their total length (TL) in millimetre (mm), body weight (BW) in gram (g).

Year	Month	No. of Male	TL (mm)	BW (g)	No. of Female	TL (mm)	BW (g)	No. of Juveniles	TL (mm)	BW (g)
2022	Mar	12	289.33 ± 16.62	381.5 ± 49.42	26	326.09 ± 27.42	648 ± 34.81	6	89.75 ± 5.28	31.75 ± 4.94
2022	Apr	14	390.42 ± 25.94	639.17 ± 34.33	29	399.36 ± 52.67	794.17 ± 32.75	8	90.08 ± 10.04	24.25 ± 5.69
2022	May	17	502.17 ± 22.53	894.75 ± 47.0	24	435.55 ± 40.71	837.5 ± 51.58	9	108.42 ± 9.29	46.08 ± 12.37
2022	Jun	6	398.33 ± 14.35	863 ± 30.69	14	424.09 ± 41.24	755.17 ± 35.77	11	97.50 ± 3.62	30.25 ± 3.70
2022	Nov	13	433.67 ± 15.31	819.75 ± 40.29	19	420.64 ± 40.95	861.75 ± 36.82	5	114.42 ± 8.7	31.92 ± 8.38
2022	Dec	11	357.50 ± 21.46	717.75 ± 47.7	18	516.82 ± 96.18	965.42 ± 46.22	14	134.92 ± 21.7	35.92 ± 11.9
2023	Jan	5	250.75 ± 15.91	531.42 ± 50.77	16	373.36 ± 38.53	747.92 ± 39.84	19	114.25 ± 13.77	54.92 ± 15.45
2023	Feb	18	178.25 ± 9.32	234.17 ± 50.43	25	121.82 ± 9.76	475.25 ± 56.64	8	97.92 ± 7.87	33.50 ± 7.99

Private Limited, Mumbai, India) using methanol eluent from SPE to remove polar functional groups from BPA. We used BSTFA and 1% TMCS or MSTFA and 1% TMCS to derivatize the dry residues. Each reaction vial received 100 ml of the derivatization reagent. After that, the vials were sealed and heated at 65 degrees Celsius for 25 min. Test samples (100 g/l) were subjected to derivatization procedures for 10, 20, 25, 30, 35, and 40 min, each time in triplicate. Since no underivatized compounds were found after a 25-minute reaction time, this value was kept constant throughout the research. Following defrosting, aliquots of fish tissue were weighed and homogenized in a glass Dounce homogenizer. The homogenized samples were extracted with 8 ml of acetonitrile using a vortex for 2 min and an ultrasonicator for 10 min in a 15 ml BPA-free Falcon centrifuge tube. The samples were extracted once more with 2 ml of acetonitrile after being vibromixed for 1 min, centrifuged for 10 min at 2640g, and then extracted again. The combined supernatants were evaporated in a vacuum evaporator and was collected in 2 ml of acetonitrile before being applied to the SPE cartridge or stored at room temperature for further use. (Adapted from [11]). A detailed flowchart for BPA extraction method has been elucidated in Fig. 4.

2.4. Detection of BPA using sandwich-ELISA

In this study, we have used a sandwich-ELISA kit (Cat. No. MBS2602664) procured from My Biosource Inc. (San Diego, CA) which provides a quantitative assay detection range of < 0.05–300 ng/ ml. The kit contents were kept at room temperature for 30 min prior to the experiment. As per the manufacturer's protocol, all the reagents along

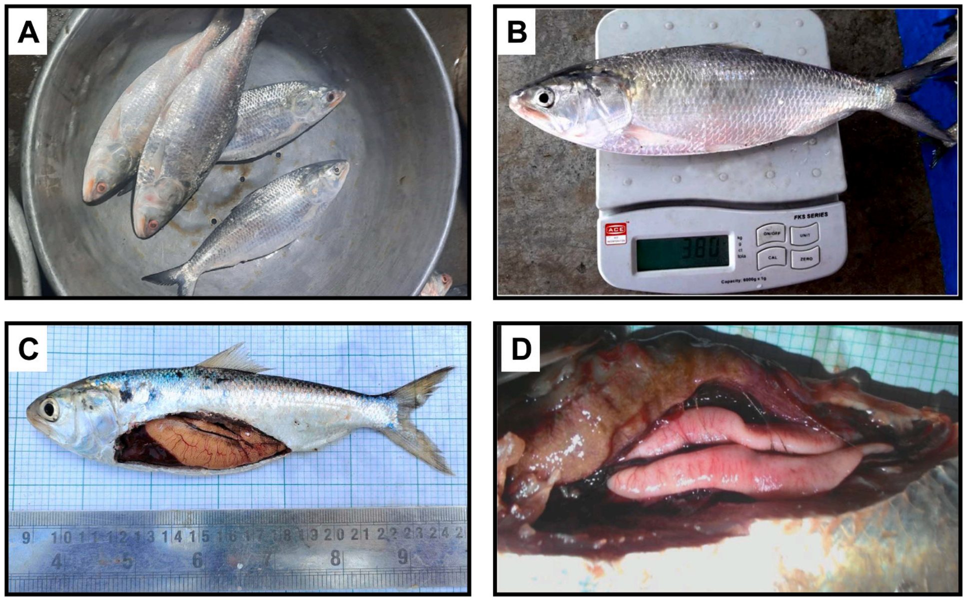


Fig. 2. Wild-caught Hilsa shad (A), a shad individual is being weighed (B), female individual with mature gonad (C) and mature male shad with gonad (D).

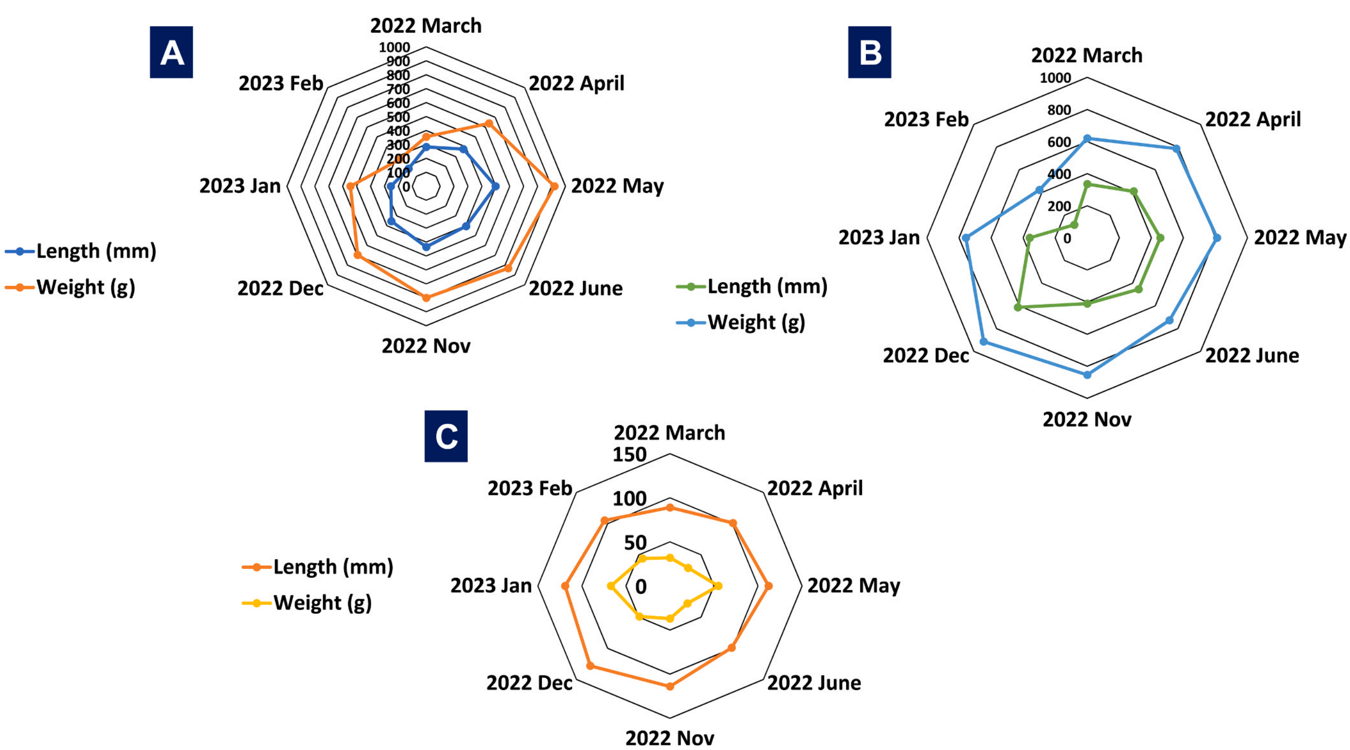


Fig. 3. Length (mm) and body weight (g) of male (A), female (B) and juvenile (C) hilsa shad collected during 2022–23.

with standards were reconstituted before the experiment. For making the standard curve, 1.0 ml of standard diluent was added to the lyophilized standard vial and was allowed to sit for 30 min after proper mixing. After the standard has completely dissolved, the concentrations used to achieve the standard curve are as followed: 200, 100, 50, 25, 12.5, 6.25, 3.12 ng/ ml. To state briefly, 100 µl of standards, as well as samples, were loaded to assigned ab-coated wells including blank. The plates (wells) were covered with the adhesive sealer provided with the kit and incubated at 37 ℃ for 90 min. After incubation, the wells were washed twice with the appropriate wash buffer and 100 µl of biotinylated Ab was added to each well. The wells were sealed again and incubated at 37 ℃ for 60 min. The wells were washed thrice after incubation and 100 µl of enzyme conjugate was added to each well. The wells were again incubated for 30 min at 37 ℃ after sealing. After the incubation period is over, wells were washed five times, and 100 µl of the prepared color reagent was added to individual wells (in dark) including the blank well, sealed, and incubated at 37 ℃ for 30 min. After the incubation, 100 µl of the color reagent C was added to individual wells, mixed, and OD was read at 450 nm using EPOCH 2 microplate spectrophotometer (Agilent, Santa Clara, CA).

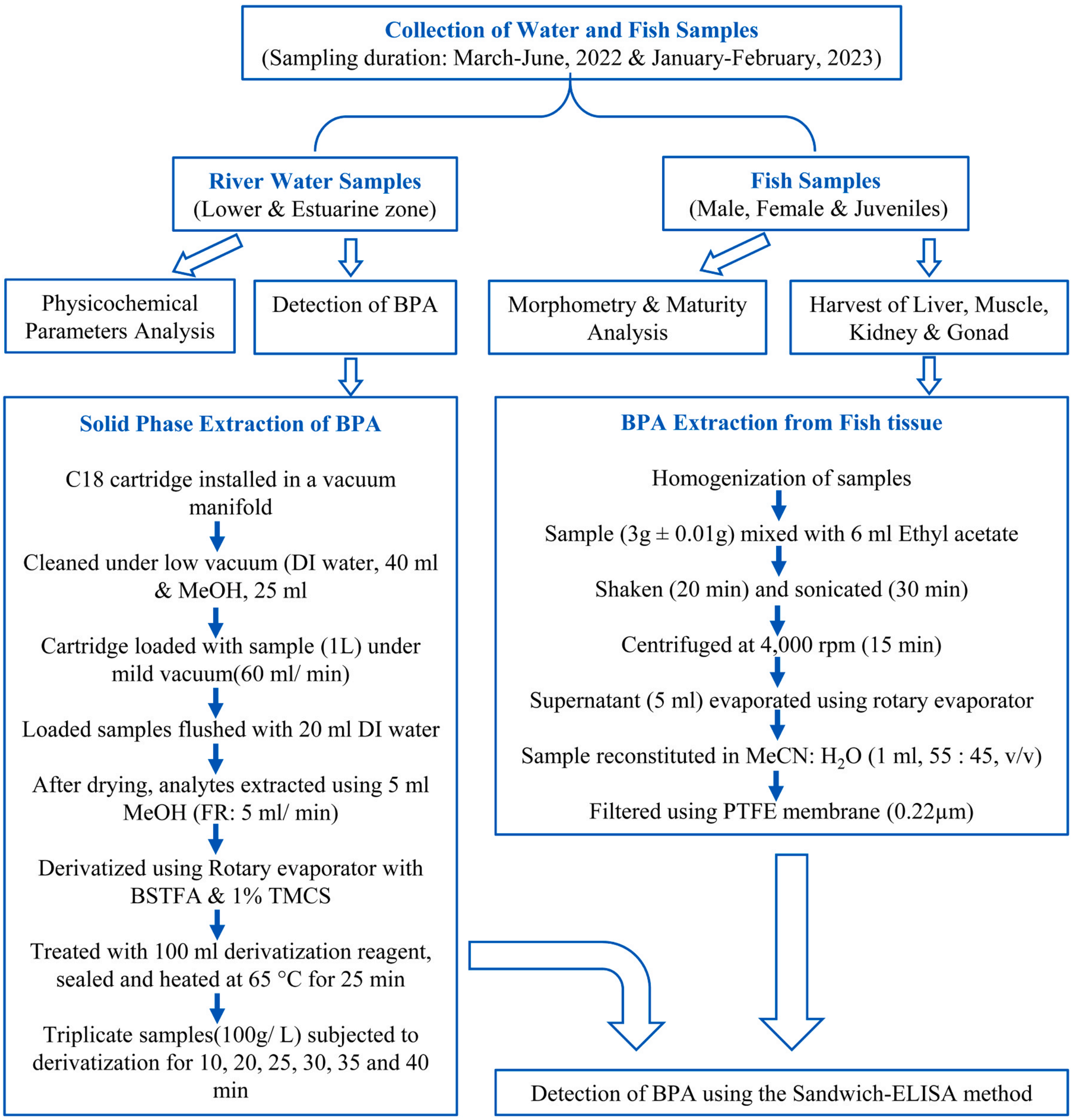


Fig. 4. A detailed flowchart of BPA extraction method from water and fish tissue samples.

2.4.1. *ELISA cross-reactivity testing*

The cross-reactivity of the BPA ELISA was assessed by investigating the potential of the polyclonal antibody to exhibit cross-reactivity with endocrine disruptors and compounds that share a structural resemblance to BPA (100% reactivity). The most significant cross-reactivity was observed for bisphenol S (11.7%); bisphenol F (10.44%); bisphenol E (10.2%); bisphenol E (9.6%); bisphenol B (7.4%) and bisphenol AF (6.2%). Cross-reactivity for phenol and nonylphenol was 0.17% and 0.12% respectively. For all other structures similar to BPA like Bis (2-ethylhexyl) phthalate, 17β-estradiol, and estrone, the cross-reactivity was found to be < 0.02%. Each compound was tested following the ELISA test procedure as described above.

2.4.2. *Experimental reproducibility of the ELISA method*

To confirm the reproducibility of the method, 50 μl of BPA standard solution (3.12, 6.25, and 12.5 ng/ ml) was added to 50 μl of blank sample with three replications. The reproducibility (%) of the test was found to be 93.8 ± 0.79, 92.66 ± 0.64, and 98.78 ± 0.29 for 3.12, 6.25, and 12.5 ng/ ml respectively for the BPA standards added. The values represent the average of five replicas, and the data is presented as the mean value ± SEM. The observed variances were found to be less than 1%, indicating a high level of precision and consistency in the ELISA approach, as evidenced by the exceptional dependability and repeatability of the obtained data.

2.5. *BPA exposure and hazard*

Central tendency exposures can be estimated using the mean values of the concentration of BPA in fish, while a conservative estimate of the mean concentration can be obtained using the 95% upper confidence limit of the mean concentration as suggested by U.S. Environmental Protection Agency (USEPA). Therefore, the mean and 95th percentile was used to indicate moderate and high exposure levels in the exposure evaluation.

2.5.1. *Estimated daily intake*

Based on the concentration in muscle samples of *T. ilisha* (n = 347), the recommended dose of fish intake for adults (100–200 g of muscle/

week) and children (25–40 g of muscle/week) recommended by the National Institute of Nutrition, ICMR [73], and a body weight of 60 kg for adult men, 55 kg for adult women, and 25.1 kg for children ages 7–9 years, the EDI of individual bisphenols was calculated. Most estimates of dietary exposure have been provided as intervals between a lower (LB) and an upper bound (UB). Toxin concentrations below the LOD or limit of quantification (LOQ) are often not included in LB calculations, while values over the LOD or LOQ are assigned a value of one. Average toxin concentrations are used to estimate UB, and values below the LOD or LOQ are given a value equal to the LOD or LOQ (WHO, 2023) unless otherwise specified. The EDI was calculated by the following equation [5]:

$$EDI_i = \frac{C_{iX} \quad B_f}{BW_i} \tag{1}$$

Where, C_i is the daily recommended intake of fish by an individual (g/day); B_f is the BPA concentration in the fish muscle (ng/kg); BW_i is the individual's body weight (kg); and EDI_i is the individual's total daily exposure (ng/kg bw/ day).

2.5.2. Target hazard quotient

The Target Hazard Quotient (THQ) is evaluated by using the potential health risks for each chemical and then summing them to obtain the hazard index. The value is obtained by dividing the median exposure to Bisphenol A (BPA) in nanograms per kilogram per day by the corresponding Reference Dose (RfD) in the same units to calculate the hazard quotient for BPA. The hazard index was then calculated for the median exposure level by summing up the individual hazard quotients obtained in the previous step (up to 95th percentile exposure levels). If the Total Hazard Quotient (THQ) is less than 1, there is limited or no clear evidence of significant health risks to the exposed population. However, if the THQ exceeds 1, it indicates the potential existence of negative health consequences. It is important to note that without the specific values and calculations mentioned in the study, it is not possible to provide precise hazard quotients or hazard indices for the Indian population in this context. The THQ was calculated by the following equation [5]:

$$\text{THQ} = \frac{EDI_i}{\text{TDI}} \tag{2}$$

where 'EDI_i' is the estimated daily intake obtained from Eq. 1; TDI is the available oral toxicity benchmarks (i.e., 0.2 ng/kg bw/ day).

2.6. Statistical analysis

For the statistical analysis software tool SPSS v. 29.0.1 (IBM Corporation, USA) was used. For descriptive statistics like mean, standard deviation etc. Microsoft Excel (Microsoft Office 2019) was utilized. To compare the concentration of BPA in surface water among the sampling sites, a one-way analysis of variance (ANOVA) was employed, followed by the Bonferroni correction. When $p < 0.05$, differences were deemed significant. The values are shown as the mean ± SEM (n = 387).

3. Results

3.1. Detection of BPA in river water

The concentrations of BPA found in surface river water are shown in Fig. 5. Data shows that the concentration of BPA ranged between 39.22 and 96.6 ng/ L in the water samples collected from the middle stretch of river Ganga with the highest value recorded at Barrackpore (96.6 ng/ L) and the lowest was found at Farakka (39.22 ng/ L). As compared to the middle stretch, we found a much higher concentration of BPA in the lower stretch of the river (estuarine zone) ranging between 124.35 and 198.67 ng/ L.

3.2. Detection of BPA in Hilsa shad

Our study, for the first time, measured the concentration of BPA in several tissues of Hilsa shad sampled from multiple landing sites of river Ganga. Fig. 6 showed the concentration of BPA (ng/ g-dw) in the liver, muscle, kidney, and gonad of male Hilsa shad sampled during Mar-Jun, Nov-Dec, 2022 and Jan-Feb, 2023.

Fish liver is considered as a detoxifying organ which may accrue several pollutants or their metabolites and typically considered for the study of toxicity. The present study detected the mean concentration of BPA in the liver sampled from male, female, and juvenile shad. Fig. 6 A showed that BPA was found in all liver samples ranging 219–288 ng/ g-dw in males, 172–222 ng/ g-dw in females, and 20.8–27.5 ng/ g-dw in juveniles.

It is highly recommended that fish muscle tissues must be examined for chemical contaminants as muscle tissue is being consumed by human being directly as fish fillets. The value of BPA in muscle was found to be highest in May 2022 (178 ng/ g-dw) whereas the lowest was recorded in February 2023 (98 ng/ g-dw) in male shad. A similar trend was observed

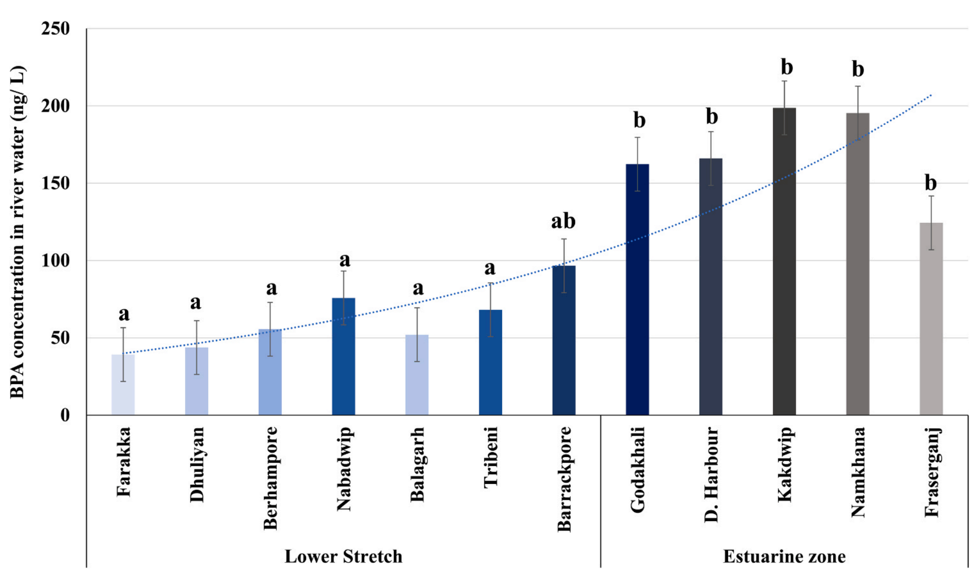


Fig. 5. Concentration of BPA (ng/ L) in surface water collected from 7 sampling sites of lower stretch and 5 sampling sites from the estuarine zone of river Ganga. Different letters differ significantly (p < 0.05) among different sampling stations.

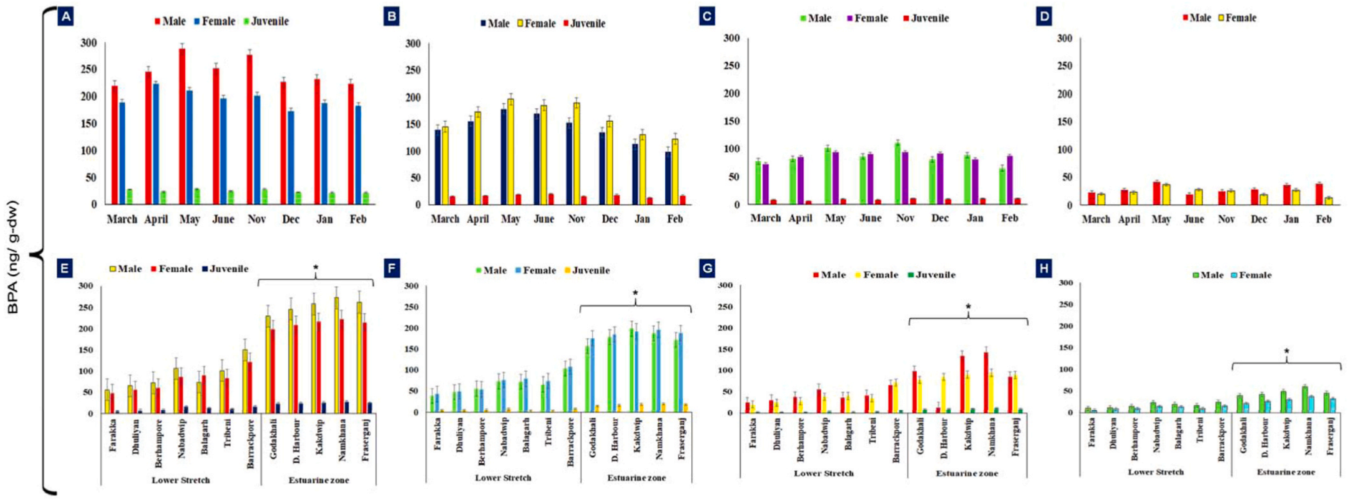


Fig. 6. Concentration of BPA (ng/ g-dw) in the liver (A), muscle (B), kidney (C), and gonad (D) samples collected from male, female, and juvenile Hilsa shad from Mar-June and Nov-Dec, 2022; and Jan-Feb, 2023. The concentration of BPA (ng/ g-dw) in the liver (E), muscle (F), kidney (G), and gonad (H) samples collected from male, female, and juvenile Hilsa shad from 7 sampling sites of lower stretch and 5 sampling sites from the estuarine zone of river Ganga. The estuarine region differs significantly (*$p < 0.05$) from the lower stretch of river Ganga.

in the muscle samples from female hilsa shad (Fig. 6 B) where the highest recorded value was 196 ng/ g-dw in May 2022 and the lowest was found to be 122 ng/ g-dw in February 2023. In juvenile muscle samples, BPA concentration ranged from 12.4 to 19.15 ng/ g-dw throughout the sampling (Fig. 6 B).

The kidney plays a crucial role in the life of a fish, serving as a vital organ responsible for maintaining the overall balance of fluids and electrolytes within its body. By efficiently filtering and excreting waste materials, the kidney contributes to the fish's overall health and survival in its aquatic habitat. When fish are exposed to BPA, either through direct contact with contaminated water or by ingesting food containing BPA, it can lead to various negative impacts on their kidney function. We, therefore, measured the BPA content in the kidney of hilsa shad male, female, and individuals sampled from both stretches. The kidney content of BPA was found to be highest in male shad (111 ng/ g-dw) followed by females (94 ng/ g-dw) and juveniles (10.6 ng/ g-dw). The lowest in males (65 ng/ g-dw) was detected in February 2023, in females (72 ng/ g-dw) in March 2022, and in juveniles (6.2 ng/ g-dw) in April 2022 (Fig. 6 C).

One significant effect of BPA on fish gonads is endocrine disruption. BPA can mimic or interfere with the natural hormones in fish, particularly estrogen. This disruption can lead to alterations in the hormonal balance and regulation of the reproductive system, affecting the development and functioning of the gonads, changes in reproductive behaviors, gamete production, and overall reproductive success. Prior to these, the detection of BPA in fish gonads is necessary. Therefore, we aimed to detect the concentration of BPA in male and female hilsa shad. Male gonad BPA concentrations peaked in May 2022 (41 ng/ g-dw) and dropped gradually to their lowest levels (18 ng/ g-dw, Fig. 6 D). The ovarian value of female shad ranged from a peak of 36 ng/ g-dw in May 2022 to a low of 12 ng/ g-dw in February 2023 (Fig. 6 D). Male shad gonads had a significantly greater BPA content than female individuals in all the samples examined.

3.3. Comparison between lower stretch and estuarine zone BPA levels in Hilsa shad

The possible harmful consequences of BPA contamination on aquatic ecosystems and human health have made BPA pollution in the Ganga river a topic of discussion. Researchers have looked into the presence and concentrations of BPA in the Ganga and its tributaries on multiple occasions. There have been no scientific investigations about the Hilsa shad population in the Ganga's estuary zone or lower reaches. So, we analyzed the BPA levels in the liver, muscle, kidney, and gonads of Hilsa shad from 7 different locations along the lower stretch of the Ganga river and 5 different locations along the estuarine zone, and the results are shown in Fig. 6 (lower panel). Liver samples from males, females, and juveniles living along the lower reaches of the Ganga river were analyzed for BPA levels (Fig. 6 E). Barrackpore had the highest BPA content among males (149.57 ng/ g-dw), while Farakka had the lowest (55.43 ng/ g-dw); BPA contamination was also highest among the females (121.63 ng/ g-dw) and lowest (47.84 ng/ g-dw). Values in juveniles varied from 16.12 to 5.44 ng/ g-dw, which is also a surprise. The results were very different when samples were taken from the river's estuary. The male liver BPA level increased significantly across all 5 sampling locations, with the greatest value reported at Namkhana (272.09 ng/ g-dw) and the lowest value recorded at Godakhali (229.11 ng/ g-dw). Similarly, female liver samples (222–198 ng/ g-dw) and juvenile liver samples (27.5–22.54 ng/ g-dw) found the values. Fig. 6 E shows unambiguously that the BPA concentration in the livers of fish caught in the estuary zone is higher than that of fish caught in the lower river section.

No previous study has compared BPA levels in the muscle of Hilsa shad in river Ganga, as far as we are aware of. We examined the level of BPA in the muscle sample collected from all individuals sampled from both stretches and found that BPA content was significantly higher ($p < 0.05$) in estuarine zone muscle samples when compared to the lower stretch of river Ganga (Fig. 6 F). BPA ranged from 156.67 to 198.34 ng/ g-dw in males; 176–196 ng/ g-dw in females and 14.1–19.15 ng/ g-dw in juvenile samples respectively. In all individuals, muscles samples were shown to be highly contaminated in the samples collected from Barrackpore, and the value was recorded lowest at Farakka (37.69 ng/ g-dw in Males, 42.53 ng/ g-dw in females and 3.92 ng/ g-dw in juveniles) among all the sampling points of the lower stretch of the river (Fig. 6 F). Fig. 6 G & H showed the values of BPA contamination obtained from kidney and gonad samples from Hilsa shad from both stretches. We found a similar trend in liver and muscle BPA content as the result depicts a significant increase ($p < 0.05$) in the BPA content in the kidney and gonad samples from the estuarine zone than the samples from lower stretch in all individuals. Our study showed that BPA content in Hilsa shad tissues followed the order of Liver> Muscle> Kidney> Gonad.

3.4. Correlation matrix

The correlation coefficient, ranging from − 1.0 to + 1.0, was employed to assess the strength and direction of the relationships. Values falling between ± 0.8 and ± 1.0 were considered indicative of a robust positive or negative connection, while values between ± 0.5 and ± 0.8 denoted a moderate correlation.

3.4.1. Correlation between BPA concentration of river surface water and water quality parameters

Fig. 7 shows a heatmap of the results of an investigation of the correlation coefficient matrix, which shows a substantial association between the content of BPA in surface water and several physicochemical properties of water. Surface water BPA concentrations varied from 39.22 ± 0.6–198.72 ± 1.57 ng/ L, with the greatest levels found in the KWDP (estuarine area) and the lowest levels found in Farakka (lower stretch). Similarly, there was a robust positive connection between water temperature and the content of BPA in surface water measured in NDAE, BP, DH, and NMKA. The BPA concentration in surface water was shown to have a high positive association with temperature in DGLE, NDAE, and BP, and a moderate positive correlation with BOD in GDK and NMKA. These results suggest that higher amounts of BPA in surface water in these regions are associated with higher concentrations of organic matter, as measured by BOD.

3.4.2. Correlation between BPA concentration of river surface water and hilsa shad organs

A heatmap analysis was conducted to examine the correlation between the concentrations of Bisphenol A (BPA) in the lower stretch and estuarine zone of the river Ganga and the BPA levels in targeted organs of hilsa shad (Fig. 8). The results depicted in Fig. 8 revealed a significant association between tissue BPA content and BPA concentrations in surface water, although these relationships exhibited considerable variations based on the sexes and age groups of the shad. Notably, a positive correlation was observed between the levels of BPA in the livers of shad collected from the lower stretch (NFK and BP) and the corresponding BPA concentrations in surface water ($p < 0.05$), except in the DH region. Furthermore, a strong positive correlation was found between BPA levels in surface water and the BPA concentrations in liver tissue from males at DGLE and NDAE. Similar significant positive associations ($p < 0.05$) were observed for liver samples from males in NFK and DH, as well as from juveniles in BP. Surprisingly, a moderately negative correlation was identified between female liver samples from DGLE and NDAE, as well as NMKA. Moreover, female muscle samples from BPC exhibited a robust relationship with BPA concentrations of surface water. Conversely, muscle samples from DGLE juveniles and DH females displayed a strong inverse correlation with surface water BPA levels. Additionally, female kidney samples from TBAE exhibited a substantial inverse relationship with BPA concentrations in surface water ($p < 0.05$).

3.5. Health risk assessment: estimated daily intake, target hazard quotient

Bisphenol A (BPA) has been the subject of numerous studies and health risk assessments due to concerns about its potential effects on human health. We in the present study, calculated the estimated daily intake (EDI) and target hazard quotient (THQ) for the shad individuals collected from the lower stretch and estuarine zone of river Ganga. Unlike other studies, where HI is calculated by adding the THQ values of the individual target hazard quotients of the elements (e.g., different heavy metals, derivatives of bisphenol other than BPA) assessed for food/ fish type [2,28,5,51], we were unable to measure the HI as the hazard element detected in this study, is only BPA.

3.5.1. Estimated daily intake

The daily intake of BPA was estimated and compared with the recommended daily intake (RDI) and tolerable daily intake (TDI) levels, which are considered good monitoring parameters for human exposure to BPA. The manual of "Dietary Guidelines for Indians" published by the National Institute of Nutrition, Indian Council of Medical Research (2011) suggested consuming fish frequently with a weekly recommendation of a minimum of 100–200 g/ week which renders a value of 28.57 g/ day fish intake. We calculated the EDI value based on this and according to Table 2, the EDI of Hilsa shad muscles in the current study is ranging from 13.357 to 64.91 ng/ kg body weight/ day in the case of men, 14.571–70.811 ng/ kg body weight/ day in case of women and 31.93–155.164 ng/ kg body weight/ day in case of children.

3.5.2. Target hazard quotient (THQ)

The target hazard quotient (THQ) is a measure used to assess the potential health risk associated with exposure to BPA. It is calculated by dividing the estimated daily intake of the substance by its reference dose (RfD) or acceptable daily intake (ADI). As per EFSA [23], the RfD or ADI is 0.2 ng/kg bw/ day, the current acceptable amount of BPA exposure. Table 2 shows that the highest THQ was found in BP (174.06) among the other lower stretch regions and in KWDP (324.55) among the estuarine

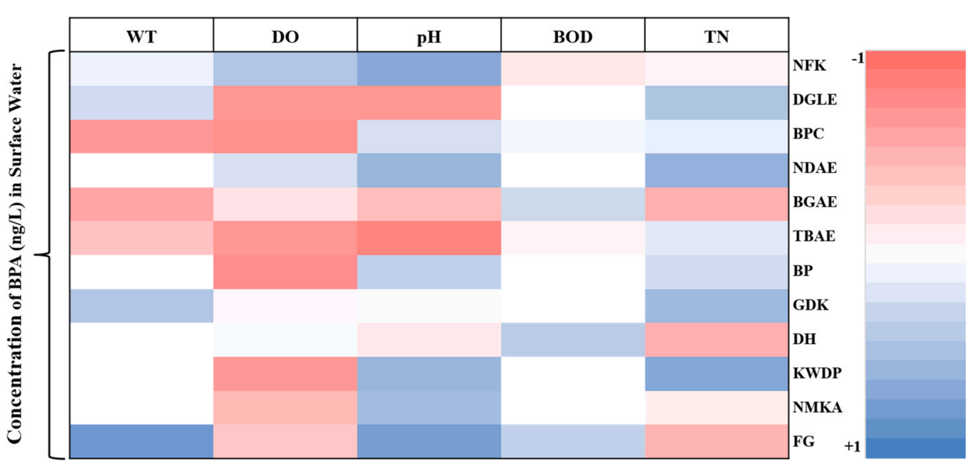


Fig. 7. Heat map of correlation coefficient matrix showing the BPA concentration in surface water was correlated with the Physico-Chemical Parameters of water samples collected from different sampling sites. The red shades denote lower values, rose quartz and baby blue medium, and blue higher, with dark red representing the negative and dark blue the positive values. White boxes denote a significant ($p < 0.05$) correlation. WT: water temperature (°C), DO: Dissolved oxygen, BOD: Biological Oxygen Demand TN- Total Nitrogen; NFK: Farakka, DGLE: Dhuliyan, BPC: Berhampore, NDAE: Nabadwip, BGAE: Balagarh, TBAE: Tribeni, BP: Barrackpore, GDK: Godakhali, DH: Diamond Harbour, KWDP: Kakdwip, NMKA: Namkhana, FG: Fraserganj.

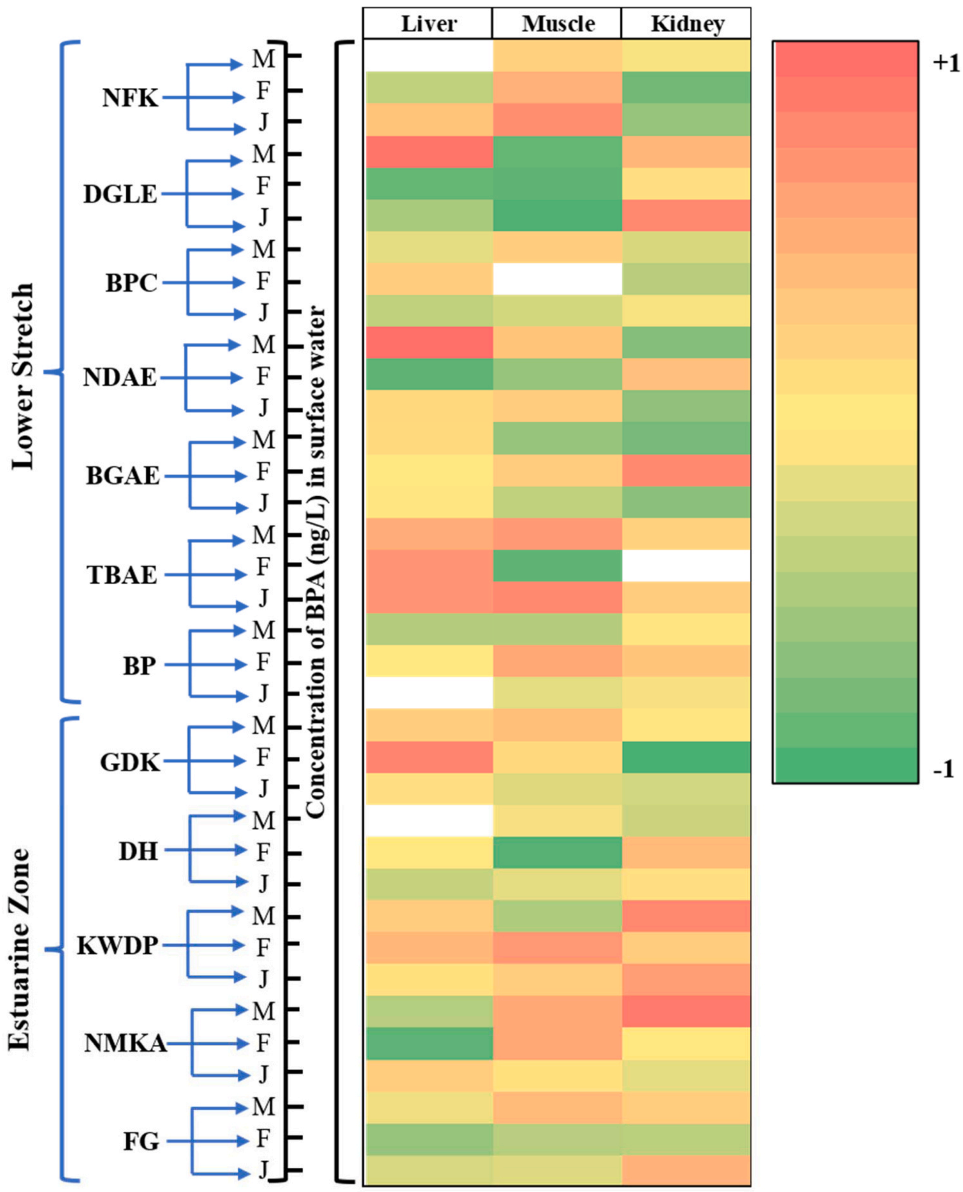


Fig. 8. Heat map of correlation coefficient matrix showing the BPA concentration in surface water was correlated with the BPA levels in the hilsa shad's liver, kidney, and muscle tissue. The green shades denote lower values, amber medium, and red higher, with dark green representing the negative and dark red the positive values. White boxes denote a significant ($p < 0.05$) correlation. M: Male, F: Female, J: Juvenile; NFK: Farakka, DGLE: Dhuliyan, BPC: Berhampore, NDAE: Nabadwip, BGAE: Balagarh, TBAE: Tribeni, BP: Barrackpore, GDK: Godakhali, DH: Diamond Harbour, KWDP: Kakdwip, NMKA: Namkhana, FG: Fraserganj.

regions in men. In women, the THQ ranged between 72.85 and 354.05 with a mean value of 189.88 at BP and 354.05 at KWDP. In children, the story is completely different showing the highest THQ among all the individuals with a mean value of 775.82 at KWDP of the estuarine zone. The lowest value of THQ in children was observed at NFK (159.65). THQ values for BPA were above 1 for all the individuals and considered a higher scenario of exposure.

4. Discussion

The high concentration of Bisphenol A (BPA) in the estuarine region of a river can be attributed to several factors. Estuaries serve as a receiving zone for pollutants transported from upstream sources, including industrial and urban areas. BPA, being widely used in various industrial processes and consumer products, can enter the river system through runoff, industrial discharges, and wastewater treatment plant effluents. Nearly half of Kolkata's population discharges untreated residential sewage directly to Hooghly River through many stormwater outlets, accounting for 47% of West Bengal's total contribution [13]. This continuous influx of BPA from upstream areas contributes to its accumulation in the estuarine region. Recently, Tiwari et al. [110] identified the estuarine region as "Unhealthy" as compared to the middle stretch of River Ganga by assessing water quality indices. In another study using river water sampled from riverine and estuarine regions, it has been documented that the average concentration of BPA in surface water was 732.7 ± 19.87 ng/L (riverine region) whereas, the value was 137.4 ± 28.34 ng/L near the rural estuarine region and 98.11 ± 34.96 ng/ L at the port region [80]. Our data in the present study showed a greater value of BPA in the lower stretch of river Ganga as compared to the freshwater region. This might happen due to the unique characteristics of estuaries, such as slow water circulation and sedimentation, which can lead to the retention and accumulation of contaminants like BPA. The sediments in estuaries act as sinks, trapping and retaining BPA particles transported by the water. Over time, BPA

Table 2
Estimated daily intake (EDI) and target hazard quotient (THQ) of Bisphenol A (BPA) estimated for adult men, adult women and children.

Sampling Stations	Estimated Daily Intake of BPA (EDI) (ng/ kg body weight/ day)*						Target hazard quotient (THQ)					
	Adult Men		Adult Women		Children (7–9 yrs)		Adult Men		Adult Women		Children (7–9 yrs)	
	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile
NFK	13.357	4.697	14.571	5.124	31.93	11.22	66.785	23.485	72.855	25.62	159.65	56.1
DGLE	15.863	5.63	17.306	6.141	37.921	13.458	79.315	28.15	86.53	30.705	189.605	67.29
BPC	18.119	6.393	19.766	6.974	43.313	15.282	90.595	31.965	98.83	34.87	216.565	76.41
NDAE	24.699	8.72	26.944	9.513	59.041	20.845	123.495	43.6	134.72	47.565	295.205	104.225
BGAE	24.232	9.432	26.435	10.289	57.926	22.546	121.16	47.16	132.175	51.445	289.63	112.73
TBAE	22.455	8.776	24.496	9.574	53.677	20.979	112.275	43.88	122.48	47.87	268.385	104.895
BP	34.812	12.725	37.976	13.881	83.216	30.418	174.06	63.625	189.88	69.405	416.08	152.09
GDK	50.125	19.744	60.136	21.539	131.773	47.198	250.625	98.72	30.68	107.695	658.865	235.99
DH	60.202	21.411	65.675	23.358	143.91	51.183	301.01	107.055	328.375	116.79	719.55	255.915
KWDP	64.91	22.821	70.811	24.895	155.164	54.552	324.55	114.105	354.055	124.475	775.82	272.76
NMKA	63.909	22.232	69.719	24.253	152.772	53.145	319.545	111.16	348.595	121.265	763.86	265.725
FG	59.977	21.01	65.43	22.92	143.373	50.223	299.885	105.05	327.15	114.6	716.865	251.115

* Unit recommended by EFSA (2023)

can accumulate in these sediments, resulting in higher concentrations in the estuarine region compared to other parts of the river. Table 3 shows a compilation of the results obtained in previous studies on BPA concentration in waterbodies in various parts of the world in comparison with our study.

According to our findings, the average concentration of BPA was significantly greater in the liver of the shad compared to the concentration in the muscle of all the individuals accounted for. A recent study on the Yangtze River in China, it has been shown that bisphenols had the greatest accumulative potential in muscle, followed by brain and liver tissues, with BPA being the dominating BPs in brain tissues. Researchers found a positive correlation between the levels of bisphenols in the traditionally dissolved phase and the colloidal phase and the levels of bisphenols in the muscle of wild fish with different feeding habits [66]. The highest levels of bisphenols were found in the muscle of filter-feeding fish, followed by omnivorous fish, and then herbivorous fish. BPA concentrations in fish caught off the coast of Portugal are within the range of values previously reported for fish caught in the Northeast Atlantic Ocean. Previously published research has indicated lower amounts of BPA in wild fish from different places. In fish from the Netherlands' estuaries and coastlines, concentrations ranged from 5 to 11 ng/g in muscle and 2–75 ng/g in liver [6]; in fish from Spain's Basque Coast, concentrations ranged from 20 to 28 ng/g in muscle and 47–97 ng/g in liver [93]; in fish from Greenland, the LOQ was up to 59. Recent reports of BPA levels in shark muscle samples from NE Greenland (up to 105.3 ng/g) were lower than those found in the present study for both male and female shad. Similar results were found in other fish species from Dutch estuaries and coastal areas [6] and the Tyrrhenian Sea, Italy [76], with the mean BPA concentrations being highest in the liver and lowest in the muscle for all of the individuals. An in vitro investigation confirmed our findings by showing that after 12 days of exposure to BPA, rainbow trout stored more of the BPA in their liver than in their muscles [63].

Histopathological and biochemical analysis of the freshwater mosquito fish, *Gambusia affinis* and guppy-fish, *Poecilia reticulata* [24]; cyprinid, *Ctenopharyngodon idella* [25]; freshwater catfish, *Heteropneustes fossilis* [86]; freshwater bighead carp, *Aristicthys nobilis* [1] showed that BPA exposure led to degenerative alterations in the liver and kidneys, as well as oxidative damage in both organs, even at sublethal concentrations. To date, no study except the present research work has detected the level of BPA in fish tissues especially in the kidney from wild-caught freshwater/ estuarine zone fish.

Over the last 15 years, researchers documented the role of BPA administered externally either alone or in combination with other estrogenic compounds to fish and examined their abnormalities in the gonadal morphology, including alterations in testicular and ovarian development. These alterations can lead to reduced fertility, impaired reproductive capacity, and even reproductive failure in fish populations. Vacuolization of the follicular cytoplasm, degeneration of cell components, and a marked rise in the percentage of atretic follicles were all seen in a female zebrafish study utilizing BPA in vitro [78]. When exposed to BPA, adult female rare minnow (*Gobiocypris rarus*) showed disruption in gonadal development and altered expression of steroidogenic genes [131]. Wang et al. [113] showed that BPA triggered a drop in 11-KT levels, which impaired spermatogenesis and led to immature testis formation in male goldfish (*Carassius auratus*). In male zebrafish, BPA exposure led to higher damage in fish gonad development when compared with estrogen exposure [108]. Sublethal dosages of BPA caused substantial gonadal damage in *Heteropnuestes fossilis*, likely as a result of excessive free radical generation and the disruption of defense mechanism in the gonadal tissue [106]. Exposure to BPA in one generation of fish can lead to epigenetic modifications that can be inherited by subsequent generations. These epigenetic changes can alter gene expression patterns in the gonads, potentially perpetuating the negative effects of BPA on reproductive health across multiple generations. In 2016, researchers showed that BPA interferes with zebrafish reproduction, most likely through estrogenic pathways and that exposure to ambient quantities of BPA is linked to changes in the transcription of critical enzymes involved in DNA maintenance methylation [55]. The transcriptional alterations of DNA methylation/demethylation-asso ciated genes in adult zebrafish ovaries and testes were also studied and found to be regulated by BPA [65]. Very recently, using mutant zebrafish as a model, the role of BPA on sexual differentiation has been investigated. Researchers showed that BPA negatively impacted multiple stages of reproduction, including puberty, sexual development, gametogenesis, and spawning [105]. So, it has been well established that BPA exposure can have detrimental effects on fish reproductive health, including altered gonadal development, reduced fertility, and impaired reproductive success. Therefore, detection of BPA in Hilsa shad is a priority job because the presence and abundance of Hilsa in rivers and estuaries serve as indicators of healthy and well-functioning aquatic ecosystems. Hilsa requires clean and well-oxygenated waters for spawning and growth. Therefore, conservation and restoration efforts focused on protecting Hilsa habitats often involve measures to improve water quality, maintain ecological balance, and protect biodiversity.

Our data shows unambiguously that the BPA concentration in the livers of fish caught in the estuarine zone is higher than that of fish caught in the lower river section (Fig. 6 E). This might be due to the water flow, which is relatively faster in the lower stretch of the river Ganga compared to the estuarine zone. The river may exhibit higher

Table 3
Comparison of BPA concentrations in river water across studies.

Year	Country	Waterbody	Quantity of BPA	References
1998	Spain	Loja River, Granada	52.0–219.5 ng/L	[34]
2001	Germany	Elbe River	8.9–776 ng/L	[38]
	Germany	South-west river	< 50–272 ng/L	[8]
2003	Germany	Elbe River	4–92 ng/L	[107]
2004	USA	Bayou River	9–44 ng/L	[9]
	USA	Canals River	1.9–158 ng/L	[59]
	Germany	Elbe River	16–100 ng/L	[118]
	China	Hangzhou-Qiantang River	0.33–25.0 ng/L	[127]
	UK	Sussex	< 5.3–24	[68]
	Japan	Tama River, Aki River, Asa River,	2–230 (8.8 × 10^{-12} to 1.0 × 10^{-9} M)	[109]
	China	Tianjin-Haihe River	19.1–106 ng/L (8300 in 1 sample)	[50]
	China	Haihe River	106.0 ng/L	[49]
2005	China	Wuhan-Changjiang River	ND - 198.7 ng/L	[120]
2006	China	Qingdao-Jiaozhou Bay	< 3.8–161 ng/L	[58]
	China	Shanghai-Huangpu River	170–3520 ng/L	[71]
	Japan	Tokyo Bay	< 500–900 ng/L	[136]
	Japan	Mukogawa River	46.5 ± 0.85 pg/ml	[95]
2008	China	Dalian River	23.1–714 ng/L	[128]
	China	Guangzhou-Pearl River	97.8–540 ng/L	[32]
	China	Harbin-Songhua River	29–64 ng/L	[99]
2009	China	Guangzhou-Pearl River/ Dong River	43.5–639 ng/L	[33]
2010	Italy	River Po	10–100 ng/L	[3]
	Brazil	Santa Maria do Leme	2.0 × 10^{-7} mol/L	[10]
	Taiwan	Kao-Pin River	0.037–4.23 μg/L	[16]
2011	China	Zhujiang River	0–9.934 μg/ L	[134]
	China	Qinghe River	0–224 ng/L	[123]
2012	Malaysia	Langat River Basin	1.3–215.0 ng/L	[96]
2013	China	Shenzhen River	5.51 ± 0.21 nM/L	[47]
	China	Maozhou River	7.82 ± 0.37 nM/L	
	China	Guanlan River	6.14 ± 0.33 nM/L	
	China	Longgang River	3.39 ± 0.16 nM/L	
	China	Pinshan River	9.24 ± 0.41 nM/L	
	Japan	Naruo-shin river	68 ng/L	[39]
	Portugal	Cavado River	30.5–41 ng/L	[92]
	Portugal	Douro River	43 – 50.5 ng/L	
	Portugal	Ferro River	28.7 ng/L	
	Portugal	Sousa River	40.9 ng/L	
	Portugal	Vizela River	46.2 ng/L	
	Portugal	Ave River	29.8 – 98.4 ng/L	
2014	China	Yangtze River	0.98 – 43.8 ng/L	[101]
2015	Japan	Edogawa River, Arakawa River, Tamagawa River	30.7 ng/L	[121]
	Korea	Han River, Nakdong River, Yeongsan River	105.7 ng/L	
	China	Pearl River, West River	58 ng/L	
	India	Cooum River, Adyar River, Korttalaiyar River	441 ng/L	
	China	Dongfeng River	3–300 ng/ml	[130]
	France	River-La Chaudanne	10^{-11} M	[126]
2016	China	Tongyu River	0.82–3.14 μM	[65]
	China	Liaohe River	47 ng/L	[48]
	China	Hunhe River	40 ng/L	
	USA	Tennessee River	0.53 fM (DS) 0.31 fM (US)	[17]
2017	Poland	Warta River	5–95 ng/L	[20]
	China	Hun river	2–20 nM	[37]
2018	Brazil	Sinos River basin, Rio Grande do Sul	ND-517 ng/L	[88]
	Czech Republic	Nisa River	2.28 μg/ L	[36]
	China	Local River	182.43 ng/L	[100]
	China	Jialing river	ND-54.35 pM	[125]
	China	Yangtze river	ND-53.74 pM	
2019	Japan	Tama River, Tokyo	117 ng/L	[89]
2020	Italy	Aniene Valley River	1–20 μM	[46]
	China	Yellow River	42.6 ng/L (DRS) 33.0 ng/L (RNS)	[133]
2021	India	Ganga River	0.04–4.46 μg/L	[14]
	India	Hooghly river (Riverine Region, Urban)	64.65 – 8800 μg/L	[80]
	India	Hooghly river (Riverine Region, Suburban)	43–7037 μg/L	
	India	Hooghly river (Estuarine Region, Urban)	97.12 – 157.4 μg/L	
	India	Hooghly river (Estuarine Region, Suburban)	57.14 – 154.6 μg/L	
	China	Minjiang River	0.067 ng/ml	[69]
	Romania	Danube River, Jiu River	< 2.3–135 ng/L	[18]
2022	South China	Pearl River	34.9 ng/L	[112]
	Nigeria	Local river of Ondo state	0.41–5.19 μg/L	[44]
	Indonesia	Bengawan Solo and Brantas	219–222 ng/L	[45]
	Bosnia and Herzegovina	Vrbas River	33–354 ng/L	[98]
2023	China	Pearl River Delta	550 kg/y	[60]
	India	Ganga River	39.2–198.67 ng/L	Present Study

(continued on next page)

Abbreviations: ng/L- nanogram/ Liter, ng/ml- nanogram/ mililiter, M- molar, pg/ml- picogram/ milliliter, μg/ L- microgram/ Liter, nM/L- nanomole/ Liter, μM- micromole, pM- picomole, fM- femtomole, kg/y- kilogram/ year, DS-downstream, US- upstream, DRS- dry season, RNS- rainy season, ND- not detected.

turbidity and sediment load due to the upstream flow and potential runoff from agricultural areas. In the estuarine zone, the water flow slows down as the river meets the tidal influence of the Bay of Bengal. The water in this zone may have lower turbidity and sediment load due to the settling of suspended particles in the river. Toxins are primarily stored, bio-transformed, and eliminated from the body through the liver, which also has essential roles in basic metabolism [41]. It is evident from a comparison of the values in Fig. 6 F-H that the BPA content in fish sampled in the estuarine zone is significantly higher than that observed in the fish caught in the lower stretch. The results are in line with those found in research done by Mukhopadhyay and Chakraborty in the year 2021. Using Hilsa shad collected from Bangladesh, it has been shown for the first time, that all the individuals were contaminated with microplastic [102]. Another recent study from a coastal area in Bangladesh showed that liver, muscle, and gill tissues from Hilsa shad were contaminated with seven toxic trace elements and the study suggested continuous monitoring regularly to ensure the safety of Hilsa shad for human consumption [91]. Hilsa shad collected from Rupsa River, Bangladesh also showed carcinogenic molecule accumulation in their muscle [87]. A moderate amount of metal contamination (Cd, Pb, Cu, Co, Ni) was also detected in the muscle, liver, and gill of Hisa shad caught from the coastal waters of Khuzestan, located in the northwest of the Persian Gulf [85]. No research has been done yet, outside of the aforementioned studies, to assess the effect of any detrimental water pollutant on Hilsa shad collected wild from a riverine system and its effects on human health. Our study, for the first time, detected the level of BPA, a potent endocrine-disrupting chemical, in surface water and in fish tissue of Hilsa shad wild-caught from river Ganga. The fishing and commercial fishing sectors around the coast rely heavily on Hilsa shad. It's important because it helps fishermen, processors, traders, and exporters make a living. Both domestic consumption and export markets for Hilsa shad contribute to the country's economy. Therefore, the conservation of Hilsa shad (*Tenualosa ilisha*) is essential to safeguard its population and the ecological balance of the aquatic ecosystems it inhabits. In Table 4, we compared our findings with those of other research that have measured BPA levels in fish tissues from throughout the world.

In the present study, the quantity of BPA was shown to be positively correlated with water temperature over a wide range of sampling sites. [31] research confirmed that temperature has a significant role in determining how much BPA is leached into the water. As was the case in BP, a substantial ($p < 0.05$) positive association was observed between the BPA concentration in surface water and both water temperature and biochemical oxygen demand (BOD) at KWDP, while a moderately significant ($p < 0.05$) negative correlation was observed between BPA and dissolved oxygen (DO). The results of this investigation corroborate those of a previous study [132] showing an inverse relationship between BPA concentrations in rivers and DO concentrations. This indicates that BPA may be resistant to degradation in low-pH and low-DO river water. Faster biodegradation of BPA and thus lower BPA concentrations in river water is facilitated by higher DO and the presence of BPA-degrading bacteria in aquatic habitats [56]. Intriguingly, we discovered that greater concentrations of BPA were linked to lower pH levels. According to research by Wang et al. [114], BPA degrades quickly under alkaline pH circumstances. There are probably fewer detectable quantities of BPA in the river water since the alkaline climate aids the breakdown process. As mentioned by Mohapatra et al. [77], lower oxygen levels impede the breakdown of BPA, hence the combination of greater temperature and lower DO may be responsible for the highest BPA concentration in surface water of KWDP.

A higher concentration of BPA in surface water suggests that BPA is continuously released into the river basin from nearby factories and cities. The existence of two sewage water treatment facilities (STP) (Lat Bagan and Anandapuri) in BP, as well as the vicinity of several additional STPs and wastewater treatment plants (WWTPs), likely explains why BP had the highest BPA concentration in the lower stretch. As indicated by [29], only 18% of phthalate esters (PAEs) can be removed with standard settings in WWTPs, and these sources contribute to excessive levels of plasticizers.

Our data showed that there is a strong correlation between water temperature and BPA levels in surface water. More BPA was found than was expected, and the results suggest that BPA flow from industrial and urban regions along the river basin is to blame. These findings highlight the significance of effective management techniques to prevent the negative environmental implications of BPA contamination in surface water and provide useful insights for understanding the dynamics of this contamination. Our findings (Fig. 7) provide scientific evidence of the interplay between BPA concentrations in the river Ganga's lower stretch and estuarine zone and the BPA levels in the targeted organs of hilsa shad, emphasizing the significance of considering various factors such as sex, age, and organ specificity when evaluating the associations.

A recent study from the North-East Atlantic Ocean showed that EDI for BPA was 5.917 and 5.966 μg/ kg bw/ day in children and adults respectively which was almost 2–7 folds higher based on their percentiles [5]. The European Food Safety Authority (EFSA) issued a temporary tolerated daily intake (t-TDI) for BPA of 4 μg/kg bw/ day in 2015. In 2015, the TDI was made temporary when experts at the European Food Safety Authority (EFSA) found several data gaps and uncertainties, which they pledged to re-evaluate as more information became available, most notably information from 2-year research conducted by the United States National Toxicology Program. 0.2 ng/ kg bw/ day is the new TDI as on 2023 set by EFSA. The prior t-TDI was 4 μg/ kg bw/ day, therefore the new one is 20,000 times lower than the 2015 value. The current acceptable amount of BPA exposure is 0.2 ng/ kg bw/ day, although the estimated dietary exposure from the EFSA opinion in 2015 is two to three orders of magnitude greater. For people of all ages, exposure to BPA levels that are far greater than the new TDI is cause for concern. EU legislators will use EFSA's scientific opinion on BPA to guide future talks on how to safeguard consumers. (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Lambré , C et al., 2023).

Our THQ data corroborates with the earlier study with the THQ values above 1 as calculated in the muscles of *Dicentrarchus labrax*, *Trachurus trachurus,* and *Scomber colias* sampled from the North East Atlantic Ocean [5]. It is pretty clear from our study as well as the study by Barboza and team (2020), that wild-caught fishes are more exposed to BPA present in their environment rather than canned seafood where BPA exposure is insignificant in terms of THQ values which was below 1 [61,62].

5. Conclusion and future direction

The level of ambient BPA must be determined because it has an impact on aquatic creatures' development, growth, and reproduction. Fish seems to be the species that are most sensitive among freshwater organisms. This is the first study to show BPA in Ganga river surface water and wild-caught Hilsa shad. BPA contamination of Ganga river water has been reported before, but this study assessed its content stretch-wise and found that it grew progressively from the lower stretch to the estuarine zone. BPA content in fish tissues is liver > muscle > kidney > gonad, while estuarine zone BPA is substantially greater than lower river stretch. Our findings also demonstrated that surface water BPA concentration was positively correlated with water temperature, biochemical oxygen demand, and male liver tissue BPA content. We calculated the estimated daily intake of this fish and discovered that children have a higher EDI than adult men and women. As an endocrine disruptor, BPA prevents the body from effectively creating, secreting, transporting, acting upon, and eliminating its natural hormones. It has been suggested that infants and young children are particularly vulnerable to BPA's side effects. Therefore, we calculated the target hazard quotient to estimate the possible health risks associated with BPA exposure, and our results showed that children are at a high health risk, as contrasted to adult women, who are under moderate risk, and adult men, who were determined to be in the low-risk zone. The THQ describes the hazardous element's non-carcinogenic health risk. If the THQ is < 1, non-carcinogenic health impacts are unlikely. If the THQ is > 1, health problems may occur. Recycling, safe BPA disposal, and waste management are needed to reduce BPA contamination. Local governments should treat urban runoff to prevent BPA and other pollutants from entering metropolitan waterways. Indian Council of Agricultural Research (ICAR)-Central Inland Fisheries Research Institute (CIFRI), Barrackpore, Kolkata, under the flagship program of the National Mission for Clean Ganga (NMCG), is using environmental education and awareness programs to reduce pollution and preserve and revitalize India's most important river.

Environmental implication

The chronic exposure of Hilsa shad to BPA in surface water may pose health and environmental risks. Thus, BPA's potential influence was

Table 4
Comparison of BPA concentrations in fish around the world.

Country	Study Area	Fish Habitat	Fish/ Species	Concentration of BPA (ng/g)			References
				Muscle	Liver	Kidney	
Italy	Gulf of Naples	Mw	*Sarpa salpa*	5	6		[76]
			Mugil cephalus	3.8	3.6		
			Diplodus sargus	0.7	1.8		
			Umbrina cirrhosa	0.6	1.8		
			Morone labrax	0.6	1.4		
	Latium coast	Mw	*Sarpa salpa*	2	1.9		
			Mugil cephalus	0.5	1.2		
			Diplodus sargus	0.6	1.4		
			Umbrina cirrhosa	0.4	1		
			Morone labrax	0.3	0.8		
Taiwan	Dansheui river	Bw, Fw	*Oreochromis niloticus*	410 (ww)			[57]
	Keelung river	Bw, Fw	*Oreochromis niloticus*	8300 (ww)			
	Laojie river	Bw, Fw	*Oreochromis niloticus*	1280 (ww)			
	Koya river	Bw, Fw	*Oreochromis niloticus*	1120 (ww)			
	Jhuoshuei river	Bw, Fw	*Oreochromis niloticus*	540 (ww)			
		Fw	*Spinibarbus hollandi*	2330 (ww)			
	Dahan river	Mw	*Mugil cephalus*	530 (ww)			
	Sinhuwei	Bw, Fw	*Oreochromis niloticus*	710 (ww)			
		Fw, Bw	*Cyprinus carpio carpio*	1480 (ww)			
	Erren	Mw, Bw, Fw	*Chanos chanos*	680 (ww)			
		Mw, Bw, Fw	*Arius maculatus*	25200 (ww)			
		Mw, Bw	*Elops machnata*	1390 (ww)			
	Love	Fw, Bw	*Channa striata*	1950 (ww)			
		Mw, Fw, Bw	*Megalops cyprinoides*	1700 (ww)			
China	Pearl river delta	Fw	*Aristichthys nobilis*	0.19 (ww)			[117]
			Clarias fuscus	0.2 (ww)			
			Ctenopharyngodon idella	0.013 (ww)			
			Mugil cephalus	0.06 (ww)			
			Siniperca knerii	0.19 (ww)			
			Cirrhinus molitorella	0.2 (ww)			
			Monopterus albus	0.05 (ww)			
			Channa asiatica	0.06 (ww)			
			Channa maculata	0.13 (ww)			
			Oreochromis mossambicus	0.14 (ww)			
		Mw	*Platycephalus indicus*	0.006 (ww)			
			Priacanthus aracanthus	0.07 (ww)			
			Siganus punctatus	0.007 (ww)			
			Nemipterus virgatus	0.08 (ww)			
			Epinephelus coioides	0.07 (ww)			
			Trachinotus blochii	0.1 (ww)			
			Cynoglosus robustus	0.11 (ww)			
			Pseudosciaena crocea	0.09 (ww)			
			Acanthopagrus latus	0.05 (ww)			
Portugal	North-East Atlantic Ocean	Mw, Fw, Bw	*Dicentrarchus labrax*	21.8 (dw)	25.6 (dw)		[5]
		Mw	*Trachurus trachurus*	3.4 (dw)	29.7 (dw)		
		Mw, Bw	*Scomber colias*	1.6 (dw)	40.7 (dw)		
China	East china sea (Yangtze River delta)	Mw, Bw	*Platycephalus indicus*	2.58 (ww)			[35]
China	Xiangjiang river		*Parabramis pekinensis, Cyprinus carpio, and Siniperca chuatsi*	3.51	61.9		[135]
China	Pearl river estuary	Fw, Bw	*Parabramis pekinensis*	0.76 (HM), 4.51 (JM), 0.43 (MDM), 1.23 (YM) (dw)			[22]
		Mw, Bw	*Pampus chinensis*	1.18 (HM), 3.19 (JM), 0.16 (MDM), 4.36 (YM) (dw)			
		Mw, Fw, Bw	*Mugil cephalus*	0.68 (HM), 1.27 (JM), 0.19 (MDM), 0.697 (YM) (dw)			
China	Luoma lake	Fw, Bw	*Ctenopharyngodon idella*	8.2 (dw)			[64]
		Mw, Fw, Bw	*Lateolabrax japonicus*	7 (dw)			
Italy	Northern coast of Sicily	Mw	*Mullus surmuletus*	46.7–58.9	35–77.6		[135]
China	Panlong river (Yunnan-Guizhou Plateau)	Fw, bw	*Carassius auratus*	5–25	30–65		[111]
		Fw, bw	*Cyprinus carpio*	6–38	63–175		
Spain	Urdaibai estuary (Bay of Biscay)	Mw, Fw, Bw	*Chelon labrosus*	20–28	47–97		[93]
China	Taihu lake		*Fish*	37.3–475 (dw)			[115]
China	Yundang lagoon	Mw, Bw	*Acanthopagrus schlegelii*	177.35 (lw)			[129]
		Mw, Fw, Bw	*Sparus latus*	93.02 (lw)			

(*continued on next page*)

Table 4 (*continued*)

Country	Study Area	Fish Habitat	Fish/ Species	Concentration of BPA (ng/g)			References
				Muscle	Liver	Kidney	
		Bw, Fw	*Oreochromis* sp.	54.15 (lw)			
France	Rhone river	Fw	*Barbus barbus*	3.2 (dw)			[74]
		Fw, Bw	*Abramis brama*	19.8 (dw)			
		Fw, Bw	*Blicca bjoerkna*	9.6 (dw)			
			Squalius cephalus	18.6 (dw)			
Iran	Anzali wetland	Fw, bw	*Cyprinus carpio*	1580 ± 260 (dw)	2150 ± 190 (dw)		[79]
China	Dianchi lake	Fw, bw	*Carassius auratus*	10–38.7 (dw)	25.7		[67]
		Fw, bw	*Cyprinus carpio*	20–58 (dw)	106.7		
		Fw	*Anabarilius alburnops*	18–83.5 ng/g (dw)			
China	Hong Kong fish market	Mw	*Platycephalus indicus*	6.2 ± 2.44 (ww)			[119]
			Trachinotus blochii	3.54 2.45 (ww)			
			Big eye	3.03 ± 0.78 (ww)			
			Tongue sole	2.82 ± 0.57 (ww)			
			Nemipterus virgatus	1.79 ± 0.64 (ww)			
			Larimichthys crocea	2.20 ± 0.49 (ww)			
			Epinephelus coioides	8.97 ± 5.88 (ww)			
		Fw	*Carassius carassius*	5.54 ± 1.99 (ww)			
			Rice field eel	4.90 ± 3.27 (ww)			
			Ctenopharyngodon idella	2.96 ± 1.59 (ww)			
			Snakehead	4.48 ± 3.54 (ww)			
			Mugil cephalus	7.02 ± 5.63 (ww)			
			Channa punctata	2.92 ± 1.30 (ww)			
			Siniperca chuatsi	3.93 ± 1.44 (ww)			
			Oreochromis sp.	5.48 ± 2.84 (ww)			
			Cirrhinus molitorella	5.97 ± 2.50 (ww)			
China	Nanjing fish market	Fw, Bw	Bream	4			[116]
Italy	Italian Border Health Authorities	Mw	Swordfish	0.6–3.2			[21]
		Mw, Bw	Tuna	0.8–7.1			
		Mw, Bw	Cod	0.6–1.1			
		Mw	Surmullet	0.9 ± 0.2			
Taiwan	Lao-Jie river		Fish	1.87 ± 2.75 (dw)			[69]
	Nankan river		Fish	1.85 ± 3.13 (dw)			
	Touqian river		Fish	1.63 ± 2.50 (dw)			
	Tamsui river		Fish	1.24 ± 0.971 (dw)			
	Mediterranean Sea	Mw	*Lepidopus caudatus*	1–11	2–75		[94]
Spain	Guadalquivir river basin		Fish	ND–59.09 (dw)			[90]
	Llobregat		Fish	223.91 (dw)			
India	Ganga river	Mw, Fw, Bw	*Tenualosa ilisha*	1.98–198.34 (dw)	6.92–272.09 (dw)	1.91–142.32 (dw)	Present study

Abbreviations: ng/g- nanogram/ gram, dw- dry weight, ww- wet weight, lw- lipid weight, Mw- Marine water, Fw- Fresh water, Bw- Brackish water, HM- Humen outlet, JM- Jiaomen outlet, MDM- Modaomen outlet, YM-Yamen outlet, ND- not detected.

assessed using toxicity data for adult men, women, and children. Human ecotoxicity of BPA was assessed using Target Hazard Quotient (THQ). THQ > 1.0 was used to rank risk. BPA's THQ values were > 1.0, indicating moderate to high risk for men, women, and children.

Funding

This important research work is funded by the National Mission for Clean Ganga, Ministry of Jal Shakti, Government of India, grant ID: T-17/2014 15/526/NMCG-Fish and Fisheries, Ministry of Jal Shakti, Govt. of India.

CRediT authorship contribution statement

Author 1: **Sourav Kundu**: Sample collection, Experimental design, Performing experiments, Writing the original draft of the MS, Editing, Reviewing. Author 2: **Ayan Biswas**: Experimental data curation, Performing experiments, Editing, Reviewing. Author 3: **Archisman Ray**: Data interpretation, Methodology upgradation, Validation, Editing, Reviewing. Author 4: **Shreya Roy**: Statistical analysis, Editing. Author 5: **Subhadeep Das Gupta**: Sample collection, Experimental data procurement, Editing, Reviewing. Author 6: **Mitesh Hiradas Ramteke**: MS preparation. Author 7: **Vikas Kumar**: MS preparation. Author 8: **Basanta Kumar Das**: Conceptualization, Overall Composition, Supervision, Validation, Editing, Reviewing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The National Mission for Clean Ganga (NMCG), Ministry of Jal Shakti, Government of India, is gratefully acknowledged by the authors for providing the funding necessary to carry out the study. The authors are appreciative of the local Ganga River fisherman for their kind assistance with sampling during the study period. The authors are thankful to Dr. Santhana Kumar V, Scientist, ICAR-CIFRI for his suggestions and continuous guidance for the improvement of the quality of the manuscript.

Author's contribution

Fish samples have been collected by SK, SDG, and AR. SK has conducted tests, performed analyses, and organized the MS. AB analyzed the results and prepared MS. Fish biology, data interpretation, and statistical analysis were handled by AR and AB. SDG and SR helped with the lab analysis. MHR and VK helped in MS preparation. The work was conceptualized by BKD and SK, and BKD also looked into the overall composition. The final manuscript was read and approved by all authors.

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