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Original Article

Assessment of the embryotoxic potential of contaminated sediments using fish embryotoxicity tests for the river Buriganga, Dhaka, Bangladesh

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Abstract: Sediment samples from six different locations of Buriganga River following exposure to Zebrafish (*Danio rerio*) eggs and larvae displayed prominent effects on both whole sediments and sediment organic extracts. The acute and sublethal effects during 96 h exposure period included (i) a significant (P<0.05) increase in morality and abnormalities in zebrafish eggs and embryos; (ii) a significant (P<0.05) reduction in hatching success and heart rate; (iii) increased frequency of helical tail and lordosis after 96 h exposure to sediment extracts; (iv) developmental delay and yolk sac edema after exposed to whole sediments at 96h exposure period. Chemical analysis showed the increased concentrations of heavy metals (Zn, Cr, Cu, Pb, and Cd) in downstream (S1, S2, and S3) compared to upstream (S4, S5, and S6), where some ions such as Cd and Cr exceeded the Environmental Protection Agency's Threshold Effect Level (EPA TEL). The current study delineates the contamination of extremely toxic compounds in the sediment of Buriganga River, which may initiate toxic effects on the early life stages of fish. Therefore, integrating zebrafish embryo toxicity tests may be crucial for evaluating the sediment quality of polluted rivers.

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Introduction

Greater Dhaka, the capital city of Bangladesh, has eighteen million people, of which only 25% of households absorb the sewage treatment facilities (Islam et al., 2015a). Dhaka city is surrounded by Buriganga-Turag-Dhaleswari River systems (Fig. 1) which are being polluted by the untreated waste of more than 30,000 factories (Whitehead et al., 2018) situated on the bank of these rivers leading to extremely serious pollution scenario in Bangladesh where industrial pollution contributes 60% pollution of Dhaka watershed (Islam et al., 2015a). To acclimatize with the economic development of other Asian mega-cities, it is essential to improve the health of surrounding rivers, which support a number of communities, agriculture and industries of Dhaka city. On a worldwide scale, the toxic effluents from leather, textiles, bricks, steel, pharmaceuticals, paper, garments and battery factories cause severe metal and organic pollution leading to disruption of ecology and deterioration of water quality (Whitehead et al., 2018). Pollution takes away nine million lives (premature deaths) per year has been reported by the Lancet Commission on pollution (Landrigan et al., 2018). The UN sustainable development goal (SDG) 6.3 aims to achieve a good ecological and chemical status in surface waters (Schiwy et al., 2015) before 2030 (Whitehead et al., 2019). To achieve this goal, sediment ecotoxicology may play a crucial role because sediments can be a secondary source of pollution (Schiwy et al., 2015). On a worldwide scale, chemicals of anthropogenic origin may be absorbed in particulate matter and finally aggregate in the sediment (Ahlf, 1995l; Zeng

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et al., 2020). Therefore, a comprehensive assessment of contamination of an aquatic ecosystem depends not only on the toxicity of the water phase (Engwall et al., 1994) but also on the determination of the toxicity of sediment (Ahlf, 1995; Burton and MacPherson, 1995).

Sediment is the major reservoir of pollutants (Bartzke et al., 2010). The contaminants in the sediments can be remobilized by flooding or dredging and can affect organisms in the water column, thus representing a source of expansive pollution in the water compartment (Hollert et al., 2003). The aggregation level of toxic compounds in sediment could be higher than in the free water column (Hollert et al., 2002). As sediment pollutants are linked to aquatic organisms and human health via the food chain, it is imperative to give a comprehensive toxicity evaluation of river sediments and address strong environmental risks to support further management strategies for the restoration of the health of the whole river system. To see the effects of sediments on aquatic life, controlled toxicity and bioaccumulation tests are indispensable; however, we have succinct information on the concentration and bioavailability of water and sediment contaminants. In general, sediment contact assay using the whole sediment on zebrafish represents a real field-like exposure scenario. But it is necessary to extract sediment-bound chemicals to the dissolved phase to perform *in vitro* and *in vivo* tests using fish early life stage tests.

To isolate more polar chemicals in the hydrous phase, it is necessary to elute sediments with artificial water, which mimics remobilization during flood events. In contrast, sediment could be extracted with an organic solvent to rearrange the non-polar substances (Ho and Quinn, 1993). As the extraction procedure of eluates and extracts results in a small volume of eluates and extracts, therefore precautions should be maintained in selecting bioassays, which require a small sample volume. Under these preconditions, bioassays using primary or fish cells lines (Lee et al., 2020; Mennillo et al., 2020) and early life stages of fishes are appropriate (Dar et al., 2019; Cormier et al., 2021). As zebrafish, *Danio rerio* is comparatively easy to breed and eggs could be available throughout the year; the use of zebrafish provides some advantages over other models. Besides, the eggs and post-larvae of zebrafish are transparent to monitor continuously through a microscope, and embryonic development is relatively rapid and simultaneous (Von Nagel, 1986; Kimmel et al., 1995), which intrigues the suitability of this model in toxicity study of sediment.

The river Buriganga, one of the most polluted rivers, runs past Dhaka City, the capital of Bangladesh. Many tanneries in the Hazaribagh area discharged the wastes to the Buriganga until their shifting in 2017 (Harris et al., 2016) in the Savar area. Heavy metal in the water and sediments induces harmful effects on biota (Yi and Zhang, 2012), including the chances of cancer and cancer-related diseases (Kim et al., 2015). Heavy metal pollution in the Buriganag River is reported but there is no research available for sediment-bound contaminants with embryotoxicity or teratogenic potential on the early stages of fish from this river. Several studies have incorporated the early life stages of fish, especially zebrafish (Hollert et al., 2003, Strmac et al., 2002; Wu et al., 2010) and the cell line model (Mennillo et al., 2020) following exposure to sediments to extrapolate the chemical burden associated. With the benefit of hindsight, the purpose of the present study was (i) to estimate the concentration of some selected heavy metals from human-impacted regions of Buriganga, (ii) to examine the toxicity of whole sediment and sediment organic extracts on zebrafish early developmental stages and iii) to evaluate the suitability of zebrafish for the assessment of the toxicity of sediment.

Materials and Methods

Sampling locations: Sediment samples (n=30) were drowned from six locations (within latitude 23°42'4.83"N and longitude 90°20'10.62"E of Dhaka city) of Buriganga River (Fig. 1) during the winter season of 2019 using stainless steel shovels with five

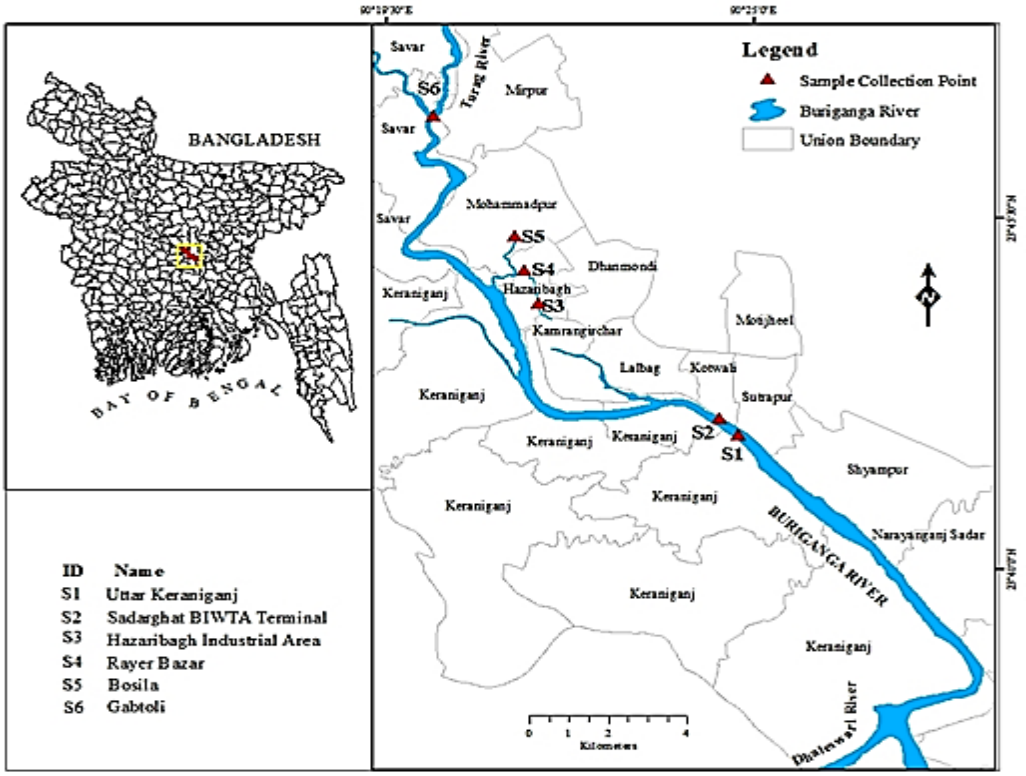

Figure 1. Location map of the river Buriganga indicating sampling spots.

replicates at each station. Among the sampling locations, Uttar Keraniganj (S1) has experienced massive industrial expansion from nearly 2000 SMEs in 2001 to 6766 in 2013 (Bangladesh Economic Review, 2017). Sadarghat BIWTA terminal (S2) is the largest terminal where millions of people aggregate daily to travel to different regions of Bangladesh leaving the port polluted. One of the most pollution sources of the Buriganga River is the Hazaribag industrial area (S3) which released untreated waste from a number of tanneries to the Buriganga River directly (Asaduzzaman et al., 2016). From 1960 to 2017, 90% of the tanneries were located in the Harzribagh area, downstream of the Buriganga River. But to reduce pollution and to achieve the national standard and SDG 6.3, the Government of Bangladesh has decided to shift the tanneries from Hazaribag to a new area in Savar named Harindhra on the bank of Dhaleswari River, connected to Buriganga (Harris et al., 2016). Therefore, to understand the effects of the relocation of tanneries on the ecosystem, it is urgent to study the toxicity of sediments associated with the Hazaribag area. In contrast, Rayer Bazar (S4) is a densely populated area with a huge density of slums. The tannery effluent from the Hazaribag area mobilizing to upstream of the Buriganga River makes this area highly polluted. Bosila (S5) is characterized by slums, brick fields and fertilizer industries. Nearly 9000 square meters of effluents from 104 fertilizer industries, located in Bosilla, Demra, Fatulla and Faridabad, are discharged into the Buriganga River per day (Rahman and Bakri, 2010). The station Gabtoli (S6) is the largest bus terminal for transportation to the different districts of Bangladesh. This area also contains dense population, numerous brick fields, landfills and many industries (Ahmed et al., 2016).

Chemical analysis of heavy metals*:* The collected sediment samples were transported to the laboratory by maintaining a cold chain and kept at -20°C until further processing. Heavy metals were determined by digesting the freeze-dried sediments with concentrated HNO_3 and $HCLO_4$ (Yang et al., 2020; Li et al., 2020) Briefly, the homogenized sediment samples (~1g) were taken into a separate beaker with

8 ml of concentrated HNO_3 (65%) and 4 ml of $HCLO_4$ (70%) and digested at 70°C. When the brown fumes disappeared, the digested mixture was filtered by the Whatman filter paper. Then, deionized water was added to make a final volume of 50 ml and subjected to Atomic Absorption Spectrophotometer (AAS) (AA-7000, Shmadzu, Japan) analysis.

Organic matter extraction: Acetone extraction method was imposed to extract the sediment organic matter from the freeze-dried sediment sample (Hollert et al., 2002; Strmac et al., 2002; Wu et al., 2010). In short, dried sediment (20 g) was taken inside the thimble and extracted with acetone for 12-16 h using a Soxhlet apparatus. A vacuum rotary evaporator (Rotavapor, 400 mbar, 38°C) was used to reduce the volume of the acetone extracts. The extracts were concentrated by nitrogen dry followed by re-dissolved in 1 ml dimethylsulfoxide (DMSO) (i.e., 20,000 mg/ml as stock) and stored at 4°C for further sediment testing. DMSO (0.3% v/v ratio) was maintained in the solution.

Rearing and egg production of zebrafish: 3-month-old zebrafish with a ratio of 3:2 (males/females) were reared for egg production in fish breeding chambers according to the protocol of Braunbeck and Lammer (2006) with some modifications. Fishes were fed commercially available dry flake food (Tetra, Melle, Germany) and hard-boiled egg yolk two times daily. The surplus food particles and the faeces of zebrafish were removed twice a day while the aquaria screens were washed on each alternate day. The fertilized eggs were prudently transferred in Petri dishes (18 cm diameter) and dipped in artificial water (ISO 7346/3). The exposure tests were conducted using embryos within 2 h post-fertilization (hpf).

Testing of organic extracts: The methods described by Viganò et al. (2020) were implied to organic extract exposure. Artificial water (ISO) was used to dilute the organic extract according to ISO 7346/3. From the 20,000 mg/ml organic extract stock solution; 100 mg/ml working solutions were prepared. 2 ml working solution with 5 eggs was placed in every 20 wells while the other 4 wells of a 24-well plate were added 5 eggs with artificial water, used as the negative control. Hatching rate, abnormalities in development, swimming activity and mortality of embryos and larvae were recorded after 24, 48, 72 and 96 h toxicological endpoints using a stereomicroscope. Five parallel tests were performed for each sediment sample. A total of 350 eggs were used in this study: 5 rounds x 7 treatments (6 sites+ negative control) x 2 replicates x 5 eggs. Additionally, the deceased embryos and larvae were instantly removed from the Petri dishes.

Testing of whole sediments: The guidelines described by Hollert et al. (2003) were followed to carry out the whole sediment exposure with slight modification. Concisely, 3 g of freeze-dried whole sediment and 7.5 ml of 24 h ventilated artificial water (333 mg/mL H_2O) were mixed in a glass Petri dish. After 1 h, 5 fertilized eggs (2 h post fertilization) were transferred to each Petri dish and incubated at 26°C with 14 h light/10 h dark photoperiod. Five parallel tests were performed for each sediment sample. A total of 350 eggs were used in this study: 5 rounds x 7 treatments (6 sites+ negative control) x 2 replicates x 5 eggs. The developmental stages of fertilized eggs were recorded for lethal and sub-lethal endpoints during 24, 48, 72 and 96 h of post-fertilization. The observations were continued until the hatching of embryos and dead embryos were removed immediately.

Determination of cardiotoxicity: Zebrafish embryos were exposed to sediments for 48 h from different stations of Buriganga River to investigate the effects on heart rate (beat/min) in embryos (48-50 hpf). A microscope (Olympus, Japan) coupled with Olympus Cellsens entry software was used to determine heart rate as previously described by Babić et al. (2017).

Statistical inferences: Differences among the sampling locations were tested using one-way analysis of variance (ANOVA), with Duncan's Multiple Range Test. SPSS 19.0 (SPSS, USA) was used to perform all statistical analysis and the significance was tested at $P<0.05$ level of

Table 1. Heavy metal analysis (±SD) of sediments collected from six spots of Buriganga River during winter.

Heavy metal	Concentration in downstream (µg/g)			Concentration in upstream (µg/g)			EPA TEL
	S1	S2	S3	S4	S5	S6	
Zn	120.2±11.32	252.4±31.4	131.7±8.31	122.4±6.3	118±4.62	119±13.12	<90
Cr	68.8±4.17	72.91±5.47	45.76±6.12	35.72±2.61	41.74±5.14	43.10±6.0	37.3
Cu	23.9±2.74	41.91±3.26	19.70±1.13	21.±2.42	14.51±1.5	16.16±4.1	35.7
Pb	13.2±2.14	27.6±1.18	24.2±2.94	18.5±1.10	11.3±1.02	13.0±3.2	35
Cd	0.95±0.013	1.64±0.03	1.12±0.43	0.74±0.018	0.59±0.03	0.62±0.06	0.596

*SD, standard deviation, EPA TEL= Environmental protection agency's threshold effect level.

significance.

Results

Chemical Analysis of Sediments: The heavy metal concentrations in the sediment of different sampling stations of Buriganga River were found in the decreasing order of Zn>Cr>Cu>Pb>Cd (Table 1). Of all the five metals investigated, the concentrations of Zn in different stations are higher than that of other metals. The highest concentration of Zn was found in S2 (252.4±31.4 µg/g) whereas the lowest was detected in S5 (118±4.62 µg/g). The concentrations of Cr from the sediments of the present study of Buriganga River were ranged from 35.72±2.61 (S4) to 72.91±5.47 µg/g (S2). Except for S4, all stations exceeded the limit of EPA TEL.

Concentrations of Cu were in decreasing order of S2>S1>S4>S3>S5. The highest (41.91±3.26 µg/g) and the lowest (14.51±1.5 µg/g) Cu concentrations were found in station 2 and station 5, respectively. Cu concentrations of all the stations were within the acceptable limit of EPA TEL except for the S2. The highest concentration of Pb was found in S2 (27.6±1.18 µg/g) followed by S3 (24.2±2.94 µg/g), S4 (18.5±1.10 µg/g), S1 (13.2±2.14 µg/g) and S5 (11.3±1.02 µg/g), respectively. The concentration of Pb from all the stations was below the acceptable limit of EPA TEL. Similarly, Cd was found maximum in S2 (1.64±0.03 µg/g) followed by S3 (1.12±0.43 µg/g), and S5 (0.59±0.03 µg/g). Sediment samples from all the stations exceeded the EPA TEL limit except S5.

Embryo toxicity tests

The survival rate in the whole sediment: In DMSO control solution, zebrafish embryos developed normally and the survival rate was 99.5% in the whole sediment and sediment organic extract exposure. The whole sediments from six different stations (S1, S2, S3, S4, S5 and S6) revealed survival rates from 60±5.6 to 86±8.3 % after 96 h exposure period (Fig. 2). Among the six different stations, samples from both the downstream and upstream exhibited the significant (P<0.05) reduction in survival rate compared to the negative control, with the highest (86%) and the lowest (60%) survival rate in S5 and S3, respectively. The survival rates from six different stations were of decreasing order of S5>S6>S1>S2>S4>S3 (Fig. 2).

The survival rate in sediment organic extracts: The zebrafish embryos exposed to sediment organic extracts from six stations showed the highest survival rate ranging from 70 to 94%, which was 8% higher than the whole sediment exposure. The increased survival rate reflected the much better condition of zebrafish embryos exposed to sediment extracts. The highest survival rate (94%) was observed in S4 while the lowest (70%) in S3 (Fig. 2). Among the stations, S1, S2 and S6 exhibited moderate survival rates of 77, 78 and 88%, respectively (Fig. 2). In comparison with the DMSO control, organic extracts from both upward and downward stations displayed significant (P<0.05) reduction in survival rate (Fig. 2).

Developmental abnormalities in the whole sediment: No abnormal embryo was observed in the control solution. But, some developmental abnormalities in the zebrafish embryos were observed when exposed to sediment samples from

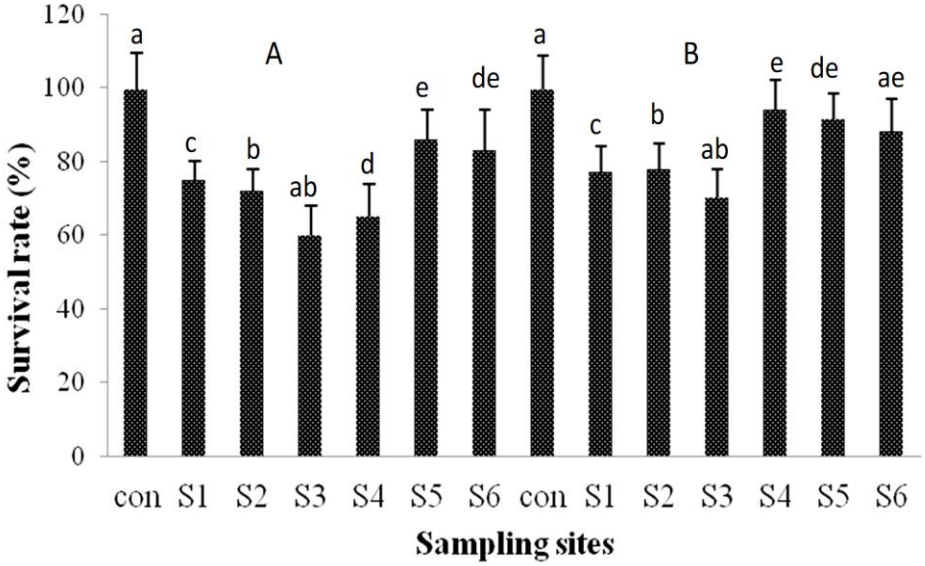

Figure 2. Survival rate of zebrafish embryos after the exposure to the (A) whole sediment and (B) organic sediment extracts for 96h exposure in the Buriganga River. Bar (mean±SD) with a different letter is significantly different (ANOVA, $P<0.05$).

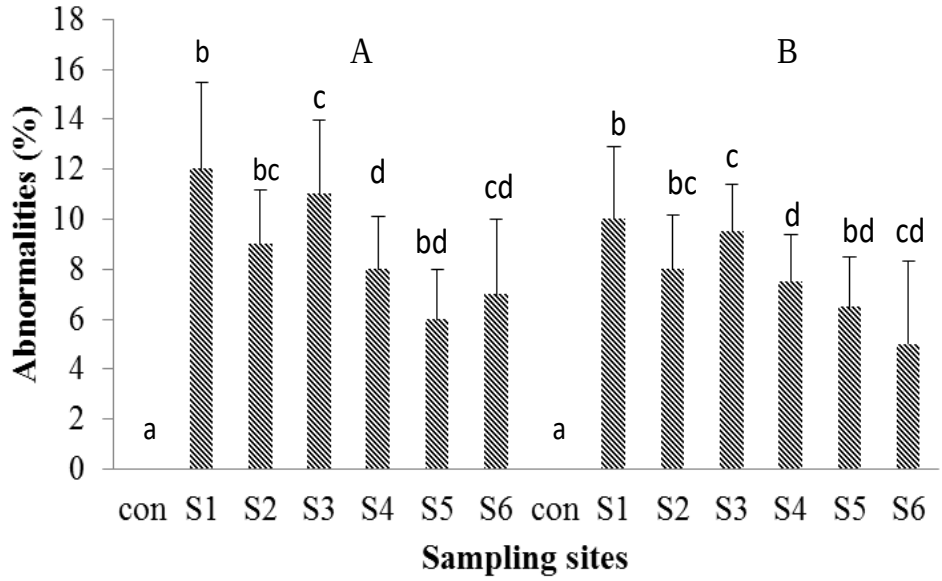

Figure 3. Percentage of abnormalities observed among zebra fish embryos exposed to the (A) whole sediments and (B) organic extracts for 96h from six sites in Buriganga River. Bar (mean±SD) with a different letter is significantly different (ANOVA, $P<0.05$).

different stations of the Buriganga River. Developmental abnormalities of zebrafish after exposure to whole sediments varied from 5(±0.91) to 12(±2.2)%. Station 1 (S1) showed the highest (12%) while site 5 displayed the lowest rate (5%) of abnormalities (Fig. 4). Station 3 exhibited the abnormalities in between the highest and the lowest rates. Significantly ($P<0.05$) higher abnormalities were recorded in zebrafish embryos after 96 h exposure to sediments from both upstream and downstream stations compared to the negative control. The sublethal effects in the whole sediments were lack of desorption of the yolk sac after 24 h exposure (Fig. 4C), changes in the yolk sac severe pericardial edema after 48 h (Fig. 4F), and enlargement of the yolk sac, pericardial edema and yolk sac edema after 72 h (Fig. 4I), developmental delay, yolk sac edema, bent tail and haemorrahage in the gut region after 9 h expose (Fig. 4L).

Developmental abnormalities in sediment organic extracts: Embryos developed normally in the DMSO control solution and no abnormalities were

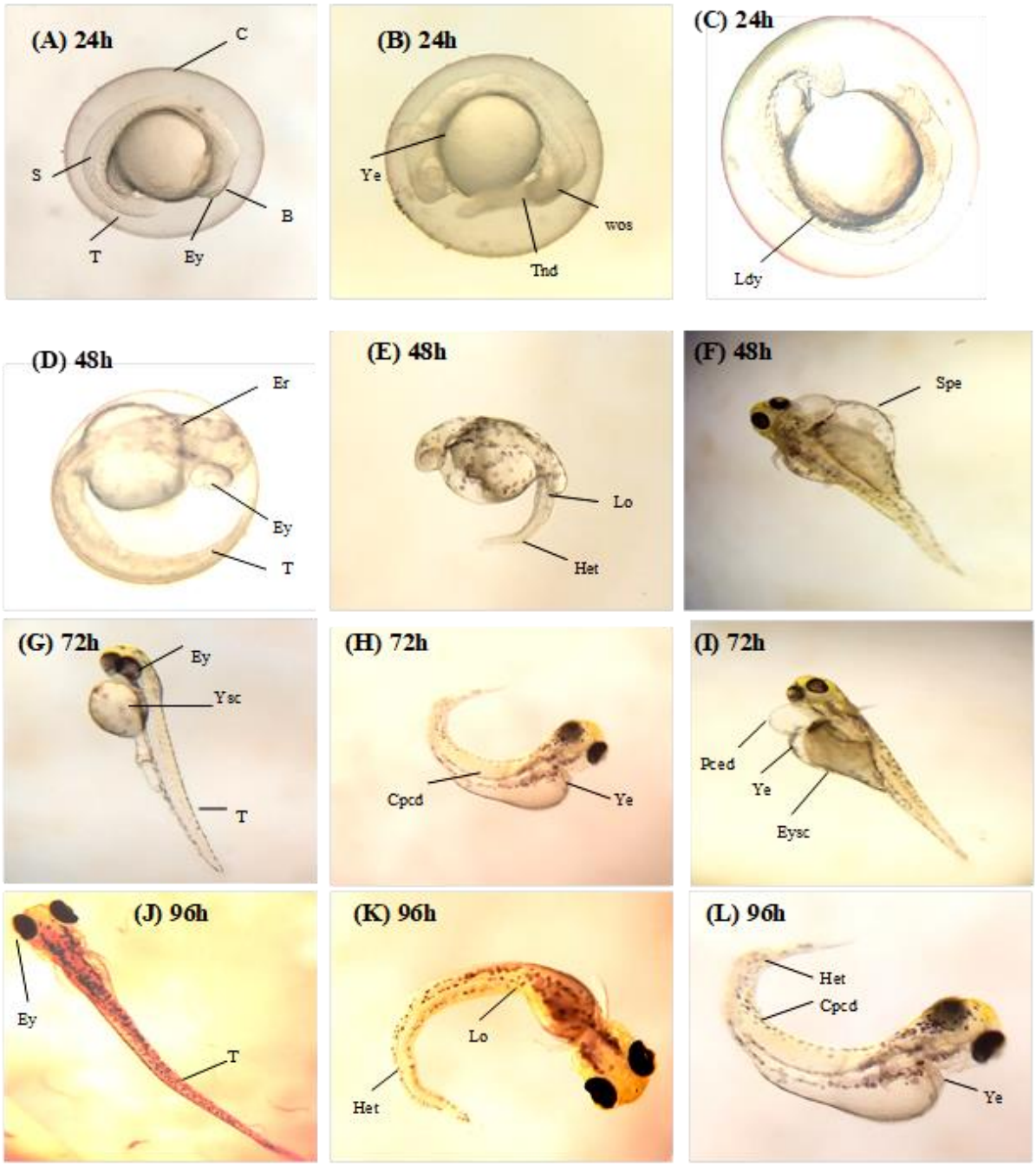

Figure 4. Changes in zebrafish embryos after exposure of sediment at specific time periods: (A) Represented normal embryo after 24h; (B) exhibited developmental delay, lack of somite formation and failure of tail detachment after 24h exposure to sediment extracts; (C) showed lack of desorption of yolk sac after 24h exposure to whole sediments; (D) exhibited normal embryo after 48h; (E) demonstrated changes in yolk sac and larva with bent tail and lordosis after 48h exposure to organic extracts; (F) represented changes in yolk sac and severe pericardial edema after 48h exposure to whole sediments; (G) represented normal embryo after 72h; (H) highlighted the complete curvature of spinal cord, yolk sac edema and complete changes in the pigmentation after 72h exposure to sediment extracts; (I) demonstrated the enlargement of yolk sac, severe pericardial edema, and yolk sac edema after 72h exposure to whole sediment; (J) represented the normal embryo after 96h; (K) exhibited helical tail and lordosis after 96h exposure to sediment extracts; (L) showed developmental delay, yolk sac edema and helical tail after exposed to whole sediments. B, brain; S, somites; T, tail; Ey, eyes; Ye, yolk sac edema; Tnd, tail not detached; Wos, without somites; Ldy, lack of desorption of yolk sac; Er, ear; Lo, lordosis; Het, helical tail; Spe, severe pericardial edema; Ysc, yolk sac; Cpcd, curvature of spinal cord; Pced, pericardial edema; Eysc, enlargement of yolk sac.

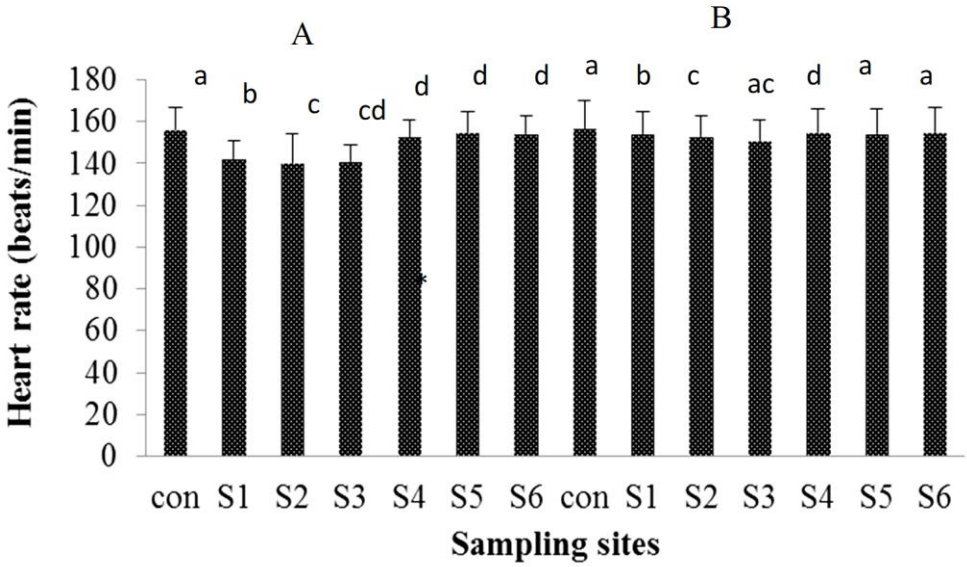

Figure 5. Percentage of heart rate observed among zebrafish embryos exposed to the (A) whole sediments and (B) organic extracts for 48h from six spots in Buriganga River. Bar (mean ± SD) with a different letter is significantly different (ANOVA, P<0.05).

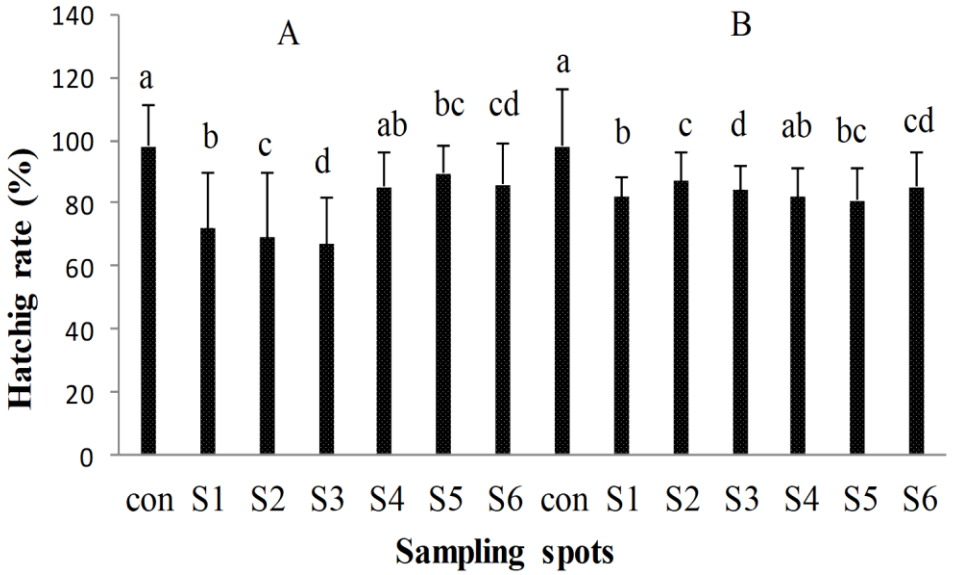

Figure 6. Hatching rates of zebrafish embryos exposed to A) whole sediments and B) organic extracts for 96h from six sites of Buriganga River. Bar (mean ± SD) with a different letter is significantly different (ANOVA, *P*<0.05).

noted. The abnormalities of zebrafish embryos ranged from 5(±1.16) to 10(±2.31) % after exposure to the sediment organic extracts. The highest abnormalities were observed S1 while the lowest in S6. The abnormality rates of zebrafish embryos from six different stations were of decreasing level of S1>S3>S2>S4>S5>S6 (Fig. 3). Significant differences in abnormalities were also observed in sediments from the control and the sampling stations. Sublethal effects in zebrafish embryos were also observed after exposure to the sediment organic extracts (Fig. 4B, E, H and K).

Heart rate in the whole sediment: The heart rate of zebrafish embryos in response to the whole sediment was recorded after 48 h of the exposure period. The unexposed embryos exhibited heat rate of 155±17.2 beat/min. Among the sampling stations, downstream sampling stations (S1, S2 and S3) exhibited a significant (*P*<0.05) reduction in heartbeats compared to the control, while the sediments from

the upstream (S4, S5 and S6) showed similar indices to the negative control (Fig. 5A). These results confirmed that the sediments from the downstream stations (S1, S2 and S3) were effective to induce the cardiotoxic effects.

Heart rate in sediment organic extracts: Significant (P<0.05) differences in the average heartbeat of zebrafish embryos exposed to upstream and downstream (S1, S2, S3 and S4) sediment extracts were recorded compared to DMSO control solution (Fig. 5A).

Hatching rate in whole sediments: In the whole sediment and sediment extracts, in DMSO control solution, the zebrafish embryos survived and hatched normally. The highest (89.5±14.4%) and the lowest (72±9.47%) hatching rates were found in sediments from S5 and S2 after 96h exposure, respectively (Fig. 6). Significant differences (P<0.05) in hatching rate for the exposure period of 96 h, were observed in sediments from all the sampling stations compare to the negative control.

Hatching rate in sediment organic extracts: In the control, the zebrafish embryos hatched normally. Like the effects of whole sediments, S2 and S5 showed the highest (87±18.5%) and the lowest (81±15.52%) hatching rate, respectively after 96 h exposure period (Fig. 6). Significant differences (P<0.05) in the hatching rate for the exposure period of 96 h, were observed in sediments from all the sampling spots compare to the negative control.

Discussion

In human-impacted regions, hundreds of thousands of chemicals from industry, domestic and household sources (Viganò et al., 2015a) funnel into the aquatic environment leading to potential ecotoxicity on sediment inhabitant biota (Dekker et al., 2006). The most sensitive stages in fish development are the early stages as demonstrated by 173 toxicity tests (Suter et al., 1987). Fry survival is usually critical for fish development (Woltering, 1984). The early life stages are the most sensitive stages as exposure at these stages may result in serious alterations and permanent malformations (Viganò et al., 2015b). Thus, assessment of sediment toxicity using zebrafish embryos is of great importance because the fish community might be directly or indirectly exposed to sediment chemicals. This study showed that zebrafish embryos exposed to sediment extracts and whole sediment from Buriganga River demonstrate different responses. The cytotoxic effects following exposure to the whole sediment were prominent and stronger than the effects obtained by the exposure to sediment organic extracts, which is similar to that observed by Hollert et al. (2003) and Wu et al. (2010). The effects of sediments from upstream (S4, S5 and S6) and downstream (S1, S2 and S3) sampling stations collaborate with the results of chemical analysis in this study.

Exposure of zebrafish embryos to whole sediment and sediment organic extracts for 96 h resulted in 40 and 30% mortality, respectively. These findings highlighted the early life stages test with brown trout exposed to sediment elutes and water samples of Krahenbach and Korsch for 60 days where mortality was reported 30% (Luckenbach et al., 1999). In another study by Wu et al. (2010), the whole sediment from the Yangtze River induced 39% mortality similar to this study (40% mortality in S3). Another study by Hallare et al. (2005) on Laguna Lake demonstrated the highest mortality rate of embryos in sediment extracts compared to the whole sediments, which was opposite to the present finding. In the present study, the survival rate of whole sediment and sediment organic extracts was 60±5.6 to 86±8.3% and 70 to 94%, respectively, leading to high mortality in whole sediments. As whole sediment testing using zebrafish embryos represents a field-like exposure condition, the highest 40% mortality of zebrafish embryos exposed to whole sediment (S3) documents the severity of pollution of Buriganga River, leading to the scenario of the complete decline of the whole fish population in the short-term exposure period. As sediment induces low oxygen concentration or oxygen depletion during whole sediment exposure (Kuster and Altenburger, 2008), the changes in the mortality

of embryos in this study, maybe not only to the effects of toxicants in sediments but also to the low oxygen as well (Wu et al., 2010). The chorion may protect the embryos not by completely preventing but by slowing down the uptake of toxicants (Van Leeuwen et al., 1985). In contrast, lipothilic compounds (PAHs and PCBs) in sediment extracts easily penetrate the embryos, but the heavy metals mostly remain on the surface of the chorion (Mitchibata, 1981). Moreover, due to the underdeveloped metabolic systems of embryos, the pollutants cannot interact with the enzymatic system leading to mortality (Ensenbach and Nagel, 1995).

Developmental abnormality rates of zebrafish embryos after exposure to whole sediments and sediment organic extracts varied from 5±0.91 to 12±2.2% and 5±1.16 to 10±2.3%, respectively. Wu et al. (2010) found the abnormality rates in the six sites of the Yangtze River varied from 1.33 to 9.33% in whole sediment and 4.71 to 11.81% in organic extracts that were nearly similar to the current study. Besides acute cytotoxicity, the present study reported the sublethal effects on zebrafish embryos exposed to sediment extracts and whole sediments highlighting the changes in developmental delay, lack of somite formation, failure of tail detachment, lordosis, yolk sac and severe pericardial edema which were not only similar to *Crassostrea gigas* embryos and larvae exposed to sediment-associated PAHs and heavy metals (Geffard et al., 2001) but also similar to zebrafish embryos exposed to contaminated sediments from Nidda River, Germany (Schweizer et al., 2018). Thus, the abnormal changes i.e. curvature of the spinal cord, enlargement of the yolk sac and helical tail may be due to unspecific reactions of zebrafish embryos to toxicants and can be better represented by the pronounced differences in the reaction of zebrafish embryos to sediment extracts (Strmac et al., 2002). Several studies have been carried out by different authors on different rivers for the assessment of sediment quality and contamination level using zebrafish embryos and they found induced developmental abnormalities in both whole sediments and sediment organic extracts (Hollert et al., 2003; Strmac et al., 2002; Wu et al., 2010; Li et al., 2016). During the preparation of the sediment organic extracts, the soxhlet extraction process desorbed the lipothilic substances, such as persistent organic pollutants (PAHs, PCBs etc), which could easily penetrate the chorion and harm the embryos. On the other hand, whole sediment exposure contained hydrophilic substances such as heavy metals, which might be intercepted by the chorion on the surface (Michibata, 1981), leading to the toxicity of the embryos (Van Leeuwen et al., 1995).

Regarding the hatching rate, distinct differences were observed in zebrafish embryos after exposure to sediment extracts and whole sediments from the Buriganga River. In this study, zebrafish embryos exhibited significantly decreased hatching in response to whole sediments after 96 h exposure. In contrast, a significant decrease in the hatching rate was also found in sediment extracts after 96 h exposure. Li et al. (2016) investigated the embryotoxicity of sediments from the Yangtze River estuary using zebrafish embryos and showed reduced hatching rates varied from 87.5 to 95.83% after 96 h exposure; similarly, a decrease in hatching rate was also recorded in zebrafish embryos after 96 h exposure to surface water and wastewater (Wu et al., 2010; Thellmann et al., 2015; Ribeiro et al., 2020). The increased hatching rate indicates exposure to low toxicants concentration, which can be compared with the symptoms of hysteresis (Strmac et al., 2002). The decreased hatching rate can be explained by the remarkable differences in the reaction of embryos and larvae to sediment extracts and whole sediment, which might be due to the diverse composition of sediment extracts.

Zebrafish embryo chorion is responsible for the oxygen transport, nutrient in and excretory substances (Rawson et al., 2001) out of embryos but the minute pores (500-700 nm) on chorion help to enter the toxic chemicals within embryos leading to changes in hatching rate (Wang et al., 2017) by inhibition of hatching enzyme chorionase (Strmac et al., 2002). It was reported that heavy metals in

effluents can delay hatching by changing the expression of hatching-related genes (Jezierska et al., 2008). Zebrafish embryos were exposed to Zn, Ni and Cr for 96 h (Ansari and Ansari, 2015) and to Cu, Cd and Pb (Jezierska et al., 2008) might delay hatching by inhibiting the expression of hatching-related genes. Generally, zebrafish develop glands in their head region before hatching, which secrets chorionase, subsequently disrupting the chorion membrane to facilitate the hatching process. But heavy metal interferes with the development of this gland, thus involved in the decreased secretion of chorionase, resulting in delayed hatching (Samson and Shenker, 2000; Williams and Holdway, 2000).

After 96h exposure, the sediments from downstream sampling sites (S1, S2 and S3) exhibited a significant reduction in heartbeats compared to the control while the sediments from the upstream (S4, S5 and S6) showed similar indices to the control. In contrast, the sediment extracts from all the sampling sites (S5 and S6) did not exhibit any cardiotoxic effects on zebrafish embryos. A similar result was postulated by Strmc et al. (2002), where lower heartbeat frequency was observed in sediments exposed to zebrafish embryos for 96 h, similar to Wu et al. (2010), where estuary sediments from the upstream have more cardiotoxic effects on zebrafish embryos than that from downstream. Likewise, zebrafish embryo toxicity test showed that wastewater from upstream increased heartbeat and cardiotoxic effects after 96 h exposure (Ribeiro et al., 2020). The present study confirms that heartbeat is a suitable biomarker (Ribeiro et al., 2020) to access the toxicity of sediments on zebrafish embryos (Babic et al., 2017). The changes in heartbeat frequencies may be due to toxicants associated with sediments that invoke lethal alterations (Strmc et al., 2002) on embryos.

Chemical analysis data showed the increased concentrations of some heavy metal ions in downstream (S1, S2 and S3) stations compared to upstream (S4, S5 and S6) where some ions such as Cd and Cr exceeded the Environmental protection agency's threshold effect level (EPA TEL). High metal concentrations in the study area might be due to the obsessive industrial discharge from different industries and dismissal of domestic sewage combined with natural and anthropogenic processes (Ahmad et al., 2010; Islam et al., 2015b). The variation in metal concentration from diverse locations might be due to the river streaming and water disposal system of industries (Alam et al., 2003). The high concentration of Zn from this study may be due to the extensive discharge of industrial wastage as well as the mixing of domestic wastewater into the river (Dong et al., 2012), while the concentration of Pb and Cu was much lower than the previous study (Ahmed et al., 2010).

Gouva et al. (2020) found that exposure to various concentrations of heavy metals in zebrafish larvae decreased the hatching and survival rate. Zebrafish embryos exposed 1 to 1000 μM $CdCl_2$ or above result in reduced survival, delayed in hatching, lack of somite formation, detachment of tail, and pericardial edema (Cheng et al., 2000) similar to that observed in zebrafish embryos exposed to sediment extracts from Buriganga River. In contrast, exposures to $CdCl_2$ in zebrafish early life stages includes delay in hatching and altered swimming ability (Capriello et al., 2019) in zebrafish embryos.

The lethal and sublethal effects of zebrafish embryos following exposure to sediments demonstrated a clear difference in downstream samples compared to upstream and collaborated conclusions drawn from chemical analysis. However, from the chemical analysis data, a clear gradient of contamination was not found among the different sediment sites in the Buriganga River, suggesting the complex nature of the chemical mixture of sediments. Therefore, the non-defined effects induced by sediments might be due to the presence of stressors, such as phytotoxins and bacterial toxins, responsible for the additive or synergistic toxic effects on the early life stages of zebrafish.

Conclusion

The present study assessed the toxic effects of

polluted sediments on zebrafish early life stages. The reduced survival rate, hatching rate, and induced developmental abnormalities were observed after exposure to the whole sediment and sediment organic extracts. Chemical analysis showed that Cd and Cr exceeded the Environmental protection agency's threshold effect level (EPA TEL). From the acute and sublethal toxicity, it was clear that the sediment effects on zebrafish embryos agreed with the chemical analysis results. Therefore, the identification of pollution sources, especially heavy metals in the river Buriganga is essential for the routine monitoring of river health. New policy measures on sediment toxicity should be adopted to protect the deteriorating sediment quality and public health especially in the river Buriganga. Fish early life stages especially with zebrafish embryos might be a sensitive indicator for the comprehensive evaluation of sediment quality of sediments from Bangladesh Rivers.

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