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Zebrafish Early Stage Developmental Defects as Indicator of Site Specific Water Composition of River Yamuna

Chitra Bhasin[1]*, Padmshree Mudgal[2]*, Adita Joshi[4],[5]*, Anita Garg Mangla[2], Madhu[1], Varsha Singh[2], Sakshi Jain[2], Kritika Sharma[2], Kirti Saluja[2], Yogita Kapoor[2], Priyanka Kandola[1], Maniki Mathur[1], Nikita Khatri[1], Alisha Arora[1], Simran Motwani[2], Sakshi Jain[2], Arushi Taneja[2], Surbhi Chauhan[2], Kanika Arora[3], Surbhi Pandey[3], Ritika Chaudhary[3]

[1] Department of Zoology, [2] Department of Biochemistry,
[3] Department of Life Sciences,Daulat Ram College, University of Delhi,
[4] CSIR-IGIB South Campus, Delhi;[5] Sansriti Foundation, Delhi
*chitrabhasin@yahoo.co.in,padmshree.m@gmail.com,adita.joshi@igib.in

ABSTRACT

River Yamuna, the major water resource of Delhi has become heavily polluted due to anthropogenic interventions in the last few decades. Eco-toxicological assessment of Yamuna water on a live organism has been sparsely investigated so far. In the present study, we have used zebrafish, an organism amenable to toxicological evaluations, for examining the embryo-toxic effects of Yamuna water. We selected three different sites: Wazirabad barrage, Kashmiri gate mid stream and Okhla barrage in Delhi region for the present study. Zebrafish embryos at 1000 cell stage were exposed to water samples from the three sites and the effects were examined and described for a 96 hours post fertilization (hpf) exposure. Early life stage characteristics such as gastrulation, survival rates, hatching success, pigmentation, blood circulation, heartbeat, and regular morphology were evaluated during the exposure period. We observed significant differences for survival rate and hatching success of the exposed embryos among water samples from the three sites. An array of similar defects in early life stages were documented for zebrafish embryos exposed to all the three water samples. However, a few abnormalities were observed specifically in water sample from a single site. Baseline information pertaining to the water conditions that support aquatic life was obtained by quantifying standard physico-chemical parameters for the three water samples. Significant aberrations in the permissible values for major physico-chemical parameters such as dissolved oxygen and heavy metals were observed. The study represents the preliminary efforts for exploration of Yamuna water within the geographical boundaries of Delhi for assessing early stage embryo-toxicity in a living system

Keywords: Developmental defects; embryo-toxicity; pollutants; Yamuna river; Zebrafish.

INTRODUCTION

River Yamuna provides for about two third (70%) of Delhi's total water requirement (1).The 22 km stretch of Yamuna, which flows through Delhi entering at Wazirabad barrage and exiting at Okhla barrage, constitutes about 2% of the river's total length. However, as a catchment area the 22 km stretch accounts for almost 50-70% of pollution of the river as a whole (1). Several industrial, domestic and cultural practices (idol immersion) result in the entry of toxic contaminants such as organic effluents, untreated solid waste, wastewater and agricultural runoffs with pesticides into the river (2, 3). The Central Pollution Control Board (CPCB) has prescribed permissible range for physico-chemical and biological characteristics such as pH, Dissolved Oxygen (DO), Biological oxygen demand (BOD), Chemical oxygen demand (COD), conductivity, concentration of ammonia, phosphates and coli-forms to assess water quality for drinking, outdoor bathing, irrigation and aquaculture uses (3). However, the permissible ranges for these parameters have been considerably disturbed and have exceeded the threshold points due to augmentation of contaminants over time. A few of the additional unexplored contaminants include antibiotics in wastewater (4) and from the dairy or aquaculture farms (5, 6) or pharmaceutical industries. Further, e-waste disposals account for heavy metal pollutants (6). River water pollution has a direct hazardous impact on aquatic animals and plants and indirect consequences are manifested in humans via food chain (7, 8). Recently, lindane a new toxic contaminant commonly used in mosquito repellants was detected at an alarmingly high level in Yamuna water (9). It is speculated that lindane may enter the food chain via vegetables that are grown in the floodplain areas of the Yamuna river. Fish represent the highest tropic level of food chain in aquatic system and thus bioaccumulation of toxins in fish acts as a possible bio-transfer route to humans. Biological impacts of accretion of pollutants like DDT, endosulfan and heavy metals have been studied in fishes (7, 8) as well as on humans (10). The composition and levels of contaminants and physico- chemical parameters vary with respect to the anthropogenic activities associated with major sites and drains entering into the river (11). It would be interesting to delineate the contaminant specific affects on a living system and understand the long-term health outcomes for population in localities near major sites of pollution.

The present study aims to use Zebrafish (Danio rerio) as an animal model to assess the impact of Yamuna water on early zebrafish embryo and larval development. Zebrafish is a small tropical fresh water fish that can be procured from pet shops and can be easily maintained in mini aquariums or tanks in laboratory. Though simpler than humans, zebrafish are complex vertebrates and share 70% genetic homology with humans (12) and approximately 84% of disease-associated genes in humans, are conserved in zebrafish.

Early embryonic developmental stages are finely described in zebrafish (13). Zebrafish develop all the vital organ systems as in humans and offer the advantage of rapid organ development within 3-4 days post fertilization (dpf) (14). Thus, zebrafish is an ideal model for toxicological evaluation of developmental, physiological and biochemical processes in vertebrates. Many of these evaluations can be accomplished without invasive procedures due to transparent and externally fertilized nature of early zebrafish embryos and larvae. These advantages allow zebrafish to serve as a live biological assessment tool that provides a connect between *in vitro* and *in vivo* animal worlds.

Early embryonic development in zebrafish is very sensitive to environmental insult due to dynamic changes in processes of cellular differentiation, proliferation and migration that form multi-cellular cell types, tissues and organs. The majority of organs, including nervous system, cardiovascular system, liver and kidneys can be studied by 5 dpf, when larvae are still only 3-4 mm in length, providing feasibility for performing multi-well plate assays (15, 16, 17). Amidst increasing regulations in use of animal testing, zebrafish offers the alternative of an inexpensive animal model for screening and toxicological evaluation of river water systems. As the polluted Yamuna water poses crucial environmental and health concerns, it is very pertinent that a comprehensive study be undertaken to investigate the impact of contaminants present in Yamuna.

MATERIAL AND METHODS

Zebrafish Maintenance, Breeding and Egg Collection

Wild type ASWT (18) zebrafish (*Danio rerio*) were obtained from CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi. Adult fish were maintained at 28 ± 2^0C, in light controlled room (14 hours light and 10 hours dark cycle). Male and female fish were kept separately in manually maintained tanks in water supplemented with sea salt (0.06 g ocean sea salt/litre of RO water). The water was changed daily, and the adult fishes were fed dry flake fish feed (Tetra, Germany) twice a day. While handling the fish, utmost care was taken to minimize animal suffering.

Breeding tanks were set up in the evening, a day before, for egg collection. A plastic separator was placed in the middle of the breeding tank to separate male and female fish (Figure I A). An hour after feeding, male and female fish was transferred into the breeding tanks on either side of the separator (Figure I B). The breeding tanks were left undisturbed overnight. Next morning, the separator was removed as soon as the lights were turned on. The fish were allowed to mate and spawn and fertilized eggs were collected. The fertilization rate was found to be > 90%. The viable, fertilized eggs were then transferred into petriplates containing embryo water (0.06 g ocean sea salt/litre of RO water) and were grown in the incubator at 28 ^{0}C.

A **B**

Figure-I: Zebrafish laboratory breeding set up.

(A) An illustration depicting key parts of a zebrafish laboratory breeding tank. (B) A cartoon depicting a breeding set up.

Water Sample Collection

Yamuna river water samples were collected from three sites (Figure II, III), namely site 1:Wazirabad upstream (WUP), site 2:Kashmere Gate mid-stream (KGMS) (5 Km downstream of Wazirabad), and site 3:Okhla upstream (OKDS) (14 Km downstream of Wazirabad) in the months of December, 2015 and January, 2016. Samples were collected in 500 ml polyvinyl carbonate (PVC) bottles from just below the water surface near the river bank.

Some of the physical and chemical parameters such as total dissolved solids (TDS), conductivity, pH, temperature, transparency and salinity were recorded at the site using multi-parameter water analyzer (PCStestr™ 35, EUTECH Instruments, OAKTON, Made in Singapore), and turbidity was determined using Secchi disc. The sample bottles were transferred to the laboratory in iceboxes. After reaching the laboratory, the samples collected from each site were filtered using 0.45 micron filter and were stored at 4 ^{0}C till further use.

Figure-II: Map showing the location of the three sites along the 22 Km stretch of River Yamuna in Delhi.
Yellow circles mark the three sites namely: WUP/ Site 1 (Wazirabad upstream); KGMS/Site 2 (Kashmere gate mid stream); and OKDS/Site3 (Okhla downstream).(Courtesy: Google maps)

Figure-III: Sampling sites along river Yamuna in Delhi.
Students and staff collecting water samples at the three sites: A, B: Wazirabad upstream; C: Kashmere gate midstream; D: Okhla Downstream.

Chemical analysis Water samples were analyzed for multiple chemical parameters using the standard methods (19). The water samples were processed for DO analysis at the site immediately after collection. DO levels were measured using modified Winkler's method.

Nitrate and nitrite levels were quantified using Greiss method and ammonia levels were estimated with Nessler's method. The levels of sulphates and phosphates were calculated using Turbidimetric method and Ascorbic acid reduction method respectively. Atomic Absorption Spectroscopy (AAS) was employed for analysis of heavy metal contaminants. For AAS analysis, water samples were filtered and transferred into 60 ml bottles and fixed by adding 1 ml concentrated nitric acid (HNO_3) in each bottle.

Samples were then sent for heavy metal analysis at Atomic Absorption Spectrophotometer facility, Department of Environmental studies, University of Delhi. A summary of parameters analyzed and the respective methods used is given in Table-I.

Table-I: Analytical methods used for testing of standard physico-chemical parameters to assess water quality.

PARAMETER	ANALYSIS
Temperature	Multiparameter
Transparency	Secchi Disc method
Conductivity	Multiparameter
pH	Multiparameter
Salinity	Multiparameter
Dissolved oxygen (DO)	Modified Winkler's
Total dissolved solids	Multiparameter
Content of Ammonia	Nesslerization method
Content of Nitrate	Greiss method
Content of Nitrite	Greiss method
Content of Sulfate	Turbidimetric method
Content of Phosphate	Ascorbic acid
Content of heavy metals (Pb, Cu, Cr, Zn, Cd)	Atomic absorption spectrophotometry

Experimental Design

Fertilized eggs were collected and maintained at 28 ^{0}C as described previously. Embryos that had progressed till 1000 cell stage (3.5 hpf) were transferred into 6-well experimental plates with a density of 25 embryos/8ml/well. Four replicates each for test samples and control were set up for the study (n=100 embryos per treatment). For all experiments, embryo water was used as control. Yamuna river water collected from the three designated sites, Wazirabad upstream (WUP), Kashmere Gate mid-stream (KGMS) and Okhla downstream (OKDS) were the test samples. All the treatment studies with zebrafish embryos were done within fifteen days of water sampling. For all the three test samples, neat; 1:2; and 1:4 dilutions were investigated (Figure IV). Dilution of the test water samples was done using embryo water. Separate 6-well plates were maintained for control & test water samples. Equal number of embryos were then transferred in 6-well plates (25 embryos / 8ml / well) containing embryo and test water respectively. Each group had four replicate wells. After every 24 hours, respective water was changed (water samples were brought to 28^0 C before embryo transfers) and dead embryos, if any were removed. The embryos were visualized and photographed using digital camera (MIPS series, Magnus) mounted on Olympus

stereomicroscope (Magnus). The embryos were monitored up to 96 hpf and various parameters like mortality rate, hatching rate, heart beat rate and other morphological and developmental anomalies were scored. All images were processed using Adobe Photoshop.

RESULTS

A. Physico- chemical analysis of the water samples.

The appearance of the three water samples was evaluated manually. On visual evaluation of the three water samples, the WUP water sample appeared comparatively transparent (Figure V A) than KGMS and OKDS water samples, which appeared dark colored with negligible transparency (Figure V B, C) Additionally, KGMS and OKDS water samples carried a foul smell that was fairly absent in WUP sample. We evaluated the three water samples for various standard physico-chemical water quality parameters. The pH values were estimated at the site of collection. The pH value for WUP site was recorded to be 8.9, whereas KGMS and OKDS recorded a near neutral pH of 7.7-7.8. Conductivity, salinity, TDS, and turbidity values were recorded at the site of water collection (Table-II). Dissolved Oxygen (DO) content at WUP was measured to be around 5 ppm whereas DO content for KGMS and OKDS sites was determined to be negligible or zero (Table-II). Ammonia levels were quantified to be above the permissible limit of 0.4 ppm at all the three sites and measured 1.8 ppm, 23.6 ppm, 26.6 ppm for WUP, KGMS and OKDS respectively (Table-II). The content of sulfates, phosphates, and nitrates were within permissible limits (Table-II). Additionally, the water samples were analyzed for the presence of heavy metals like, cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) using atomic absorption spectrophotometry.

The analysis revealed significantly high concentration of Zn, and Pb in water samples from all the three sites. WUP water samples showed highest concentration of Zn (5.8 ppm), followed by KGMS (3.9 ppm) and then OKDS (1.9 ppm) (Table-II). Pb was also highest in KGMS (2.25 ppm), followed by WUP (1.27 ppm) (Table-II). Pb was found to be under permissible limits in OKDS. Cu and Cd levels were detected to be higher than permissible limits in all the river samples. Cr was the only metal, the concentration of which was measured to be under the permissible limits in all water samples.

A) Wazirabad upstream (WUP) B) Kashmere gate mid stream (KGMS) and C) Okhla downstream (OKDS). WUP sample is fairly transparent whereas the KGMS and OKDS samples exhibit a dirty and turbid appearance.

B. Yamuna water affects Survival rate in early Zebrafish embryos.

B) Zebrafish embryos at the 1000 cell stage were exposed to and grown in the water samples obtained from the three sites. Survival rate of zebrafish embryos in different water samples was studied up to 96 hpf and number of dead embryos were counted (Figure VI).

Table-II: Basic Physical and Chemical conditions at three sampling sites along the Yamuna River in January' 2016

Physicochemical Parameters	Wazirabad Upstream	Kashmere Gate Mid stream	Okhla Downstream	WHO Permissible range
Latitude	28.7257802^0 N	28.6675^0 N	28.5315291^0 N	
Longitude	77.2277069^0 E	77.2281^0 E	77.2718407^0 E	
Water temperature	14.6-14.8	19.0-19.3	17.8	
pH	8.95-9.07	7.65-7.8	7.68-7.81	6.5-9.2
Conductivity (uS)	1441-1485	1692-1714	1837-1846	1000
Salinity (ppm)	726-730	839-844	925-926	
TDS (ppt)	1.02-1.05	1.20-1.21	1.30-1.38	0.5
Transparency %	2.5	1	1	90
Turbidity %	97.5	99	99	10
Ammonia (ppm)	1.8	23.6	26.6	0.4
Nitrate (ppm)	37.0	17.0	8.0	45
Nitrite (ppm)	40	9.0	8.0	0.1-0.2
Sulfate (ppm)	95.2	102.4	106.4	250
Phosphate (ppm)	13.5	48	35	45
DO (ppm)	5	0	0	≥ 6
Zn (ppm)	5.8089	3.3893	1.9268	1.000
Cu (ppm)	0.0513	0.0396	0.0117	0.020
Cr (ppm)	0.0014	0.0434	0.0366	0.050
Pb (ppm)	1.272	2.252	0.0136	0.050
Cd (ppm)	0.0263	0.0266	0.0289	0.005

In neat WUP water, the survival rate of the embryos was found to be comparable to EW nearing to about 80% by 72 hpf, whereas 100% mortality was observed in neat KGMS and OKDS water by 72 hpf (Figure VI A). A similar trend was observed for the three water samples in the 1:2 (Figure VI B) and 1:4 dilutions and maximum mortality was documented in the OKDS water sample for all the three dilutions tested (Figure VI C).

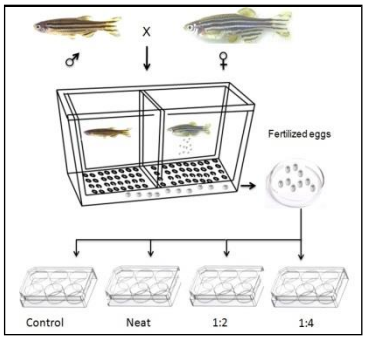

Figure-IV: Experimental design illustrating exposure of early stage zebrafish embryos to varied dilutions of water samples from Yamuna river.

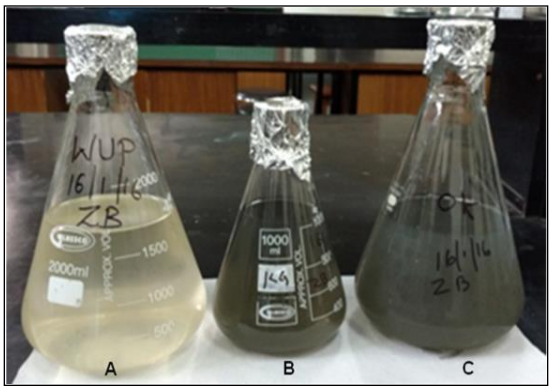

Figure-V: Water samples from the three sampling sites.

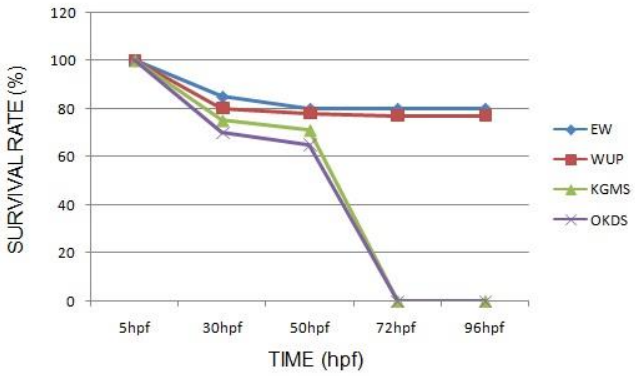

Figure-VI A: Survival rate (%) of zebrafish embryos exposed to neat WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100).

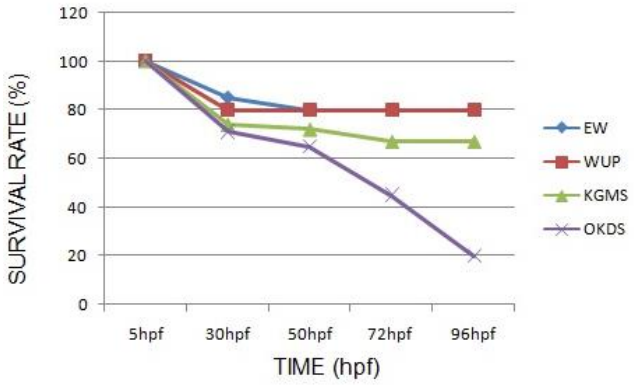

Figure-VI B: Survival rate (%) of zebrafish embryos exposed to 1:2 diluted WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100)

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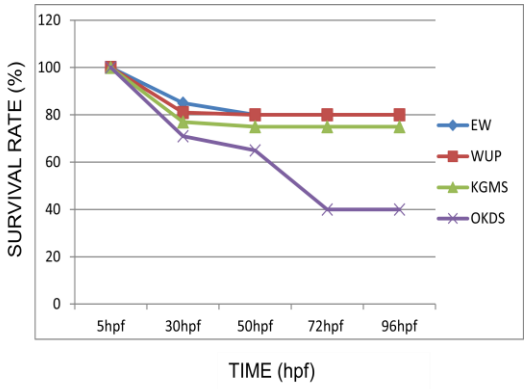

Figure -VI C: Survival rate (%) of zebrafish embryos exposed to 1:4 diluted WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100).

C. Zebrafish embryos exposed to Yamuna water display delayed hatching

Hatching delay, an index of developmental toxicity, was observed in embryos treated with all the three river water samples. Maximum hatching delay was observed in neat KGMS and OKDS water (Figure VIIA) samples, and by 72hpf, 10% and 20% of the treated embryos hatched respectively. Neat WUP water sample did not show a significant delay in hatching with 80% of the embryos hatched when compared to EW which showed 100% hatching success by 72 hpf.
We observed a similar trend for 1:2 (Figure VII B) and 1:4 dilutions (Figure VII C) of the water samples. At 1:4 dilutions, embryos in OKDS water showed considerable hatching delay, with only 45% embryos hatching by 72 hpf, whereas 90% and 100% embryos had hatched in 1:4 diluted KGMS, WUP water and EW respectively. By 96 hpf 100% hatching had occurred in all water samples at 1:4 dilution. In, embryos treated with 1:2 dilution of WUP, KGMS, and OKDS water samples, the hatching rate was similar to that shown in 1:4 dilution of the respective water samples.

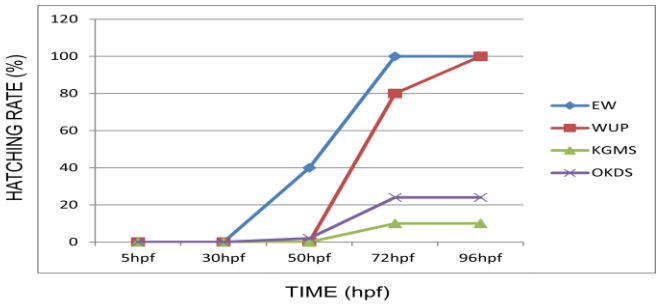

Figure-VIIA: Hatching rate (% of alive) of zebrafish embryos exposed to neat WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100).

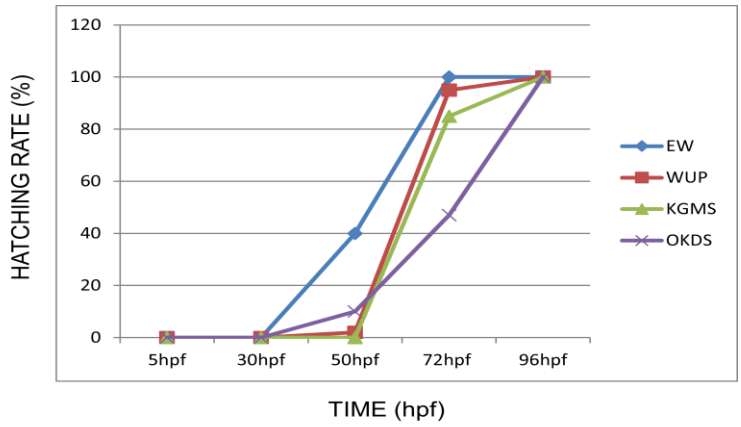

Figure-VII B: Hatching rate (% of alive) of zebrafish embryos exposed to 1:2 diluted WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100).

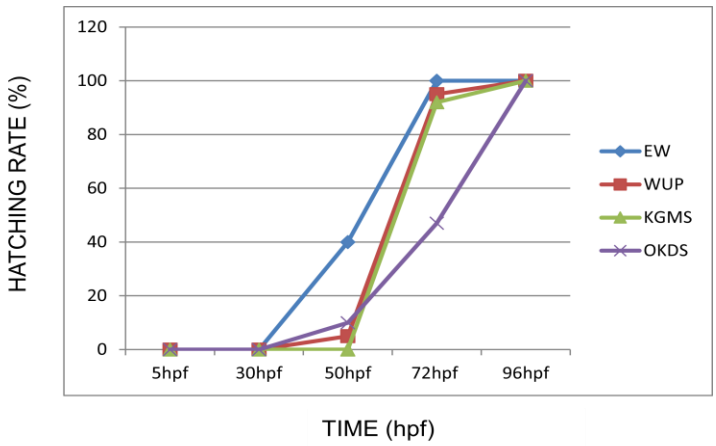

Figure-VII C: Hatching rate (%) of zebrafish embryos exposed to 1:4 diluted WUP; KGMS and OKDS water samples. EW: embryo water (control); (n=100).

D. Early stage Developmental defects

All the sets of embryos that were treated with Yamuna water samples were observed for gross morphological abnormalities from 1 days post fertilization (dpf) till 5 dpf. At 24 hpf, embryos in all water samples, at all dilutions appeared normal with no evident phenotypic abnormality. At 50 hpf, about 50% of embryos treated in WUP water sample at all dilutions developed, pericardial edema and head edema was documented for 10% of the WUP treated embryos (Figure VIII).
Embryos treated with KGMS and OKDS water samples at all dilutions displayed yolk sac edema, pericardial edema (Figure VIII). A small number of embryos treated with the WUP and OKDS samples displayed blood accumulation in the pericardial cavity. However, no instances of head edema were observed in the KGMS and OKDS treated group. Curved body axis defects and bent tail morphology were primarily documented for the OKDS and KGMS treated groups respectively (Figure VIII). Pigmentation was observed to be affected and degree of pigmentation was decreased in KGMS water.

Embryos growing in sample waters from the three different sites exhibited both axial and dorsal curvature defects, and bent tail. Embryos developing in neat KGMS and OKDS water samples displayed significant mortality by 96 hpf. The embryos exhibiting the above described defects are represented in Figure IX, X. The control embryos displayed normal development in embryo water.

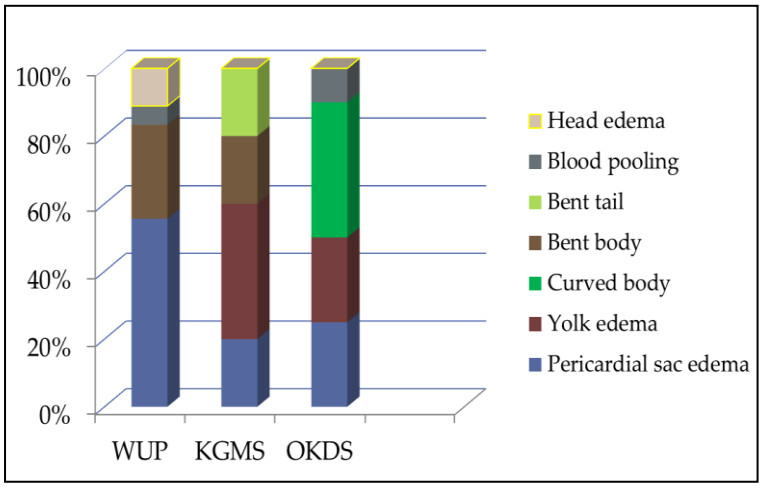

Figure-VIII: Developmental abnormalities observed in Zebrafish embryos, exposed to Yamuna river water samples from three different sites.
Site1: Wazirabad upstream (WUP); Site2: Kashmiri Gate midstream (KGMS); Site3: Okhla downstream (OKDS), (n = 100).

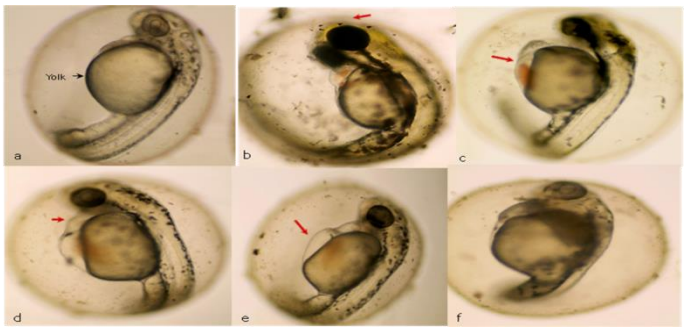

Figure-IX: Zebrafish embryos displaying developmental defects as documented at 50 hpf

(a) Control embryo in EW at 50 hpf. (b) An embryo showing head edema. (c) An embryo exhibiting blood accumulation in the pericardial cavity. (d) An embryo displaying pericardial edema. (e) An embryo displaying yolk sac edema. (f) A deformed embryo showing bent body (red arrow heads mark the above described abnormalities). Images taken at10X.

(b)

(c)

Figure-X: Zebrafish embryos displaying developmental defects when exposed to Yamuna river water as documented at 96 hpf.

(a) Control 96 hpf embryo in EW. (b) Embryos with yolk edema; the yolk is left unutilized as compared to the control 96 hpf embryo. (c) An embryo with cardiac edema; the pericardial cavity exhibiting fluid accumulation is easily seen. (d) Embryos displaying body curvature defects and yolk edema. (e) Embryo showing blood accumulation in the pericardial cavity. (f) Embryo exhibiting body curvature defects. Images taken at 2.5 X.

DISCUSSION

Our study aims to assess the effects of Yamuna river water in the Delhi region on the survival, successful hatching, and embryonic development of juvenile zebrafish larvae. The early life stages of zebrafish are transparent and undergo rapid development, thus the embryo-toxic responses that may manifest in gross morphological features such as eye, otolith, pigmentation, body curvature can be documented effortlessly (20). Often the changes in morphology or tissues of the living juvenile larvae are an outcome of water quality that is determined by several physico-chemical parameters. The measurements of physico-chemical parameters for all the three sampling sites, WUP, KGMS and OKDS, revealed values that were either reasonably lower or substantially higher than the threshold values or permissible limits as per WHO norms. These include parameters such as conductivity, salinity, turbidity,TDS, DO, and ammonia levels. The

data obtained from the physico-chemical analysis of the three water samples suggests a steady decrease in the water quality in terms of unique pollutant compositions as the river takes it course from WUP to KGMS and subsequently to OKDS, the exit point of Yamuna in Delhi.

WUP is the site of entry of river Yamuna in Delhi. We observed that the DO levels reduce drastically as the Yamuna flows into the city at KGMS site and finally exits at OKDS site. We speculate that the deterioration in water quality at KGMS site, which is located 5 Kms downstream of Wazirabad, could be attributed to the industrial, chemical and untreated sewage waste discharges by the Najafgarh drain in to the river (21). Further deterioration is observed when Yamuna flows down from KGMS to OKDS as additional drains including, Hindon cut canal of Hindon river and Power houses drain join the river (22). We consider that decreased DO levels could be due to increased organic matter contributed by sewage drained into the river after it enters the city at Wazirabad. Low DO levels, less than 4 ppm are detrimental to aquatic life. Our data from the physico-chemical analysis reveals, KGMS and OKDS to be devoid of DO with a value of 0 ppm. The negligible levels of DO strongly correlate with the low survival rate observed for the embryos treated with KGMS and OKDS water samples. Another factor that leads to significant embryo-toxicity is the amount of ammonia present in a water sample. The WUP sample recorded approximately five times higher ammonia levels when compared with the acceptable limits. A similar trend was reported for WUP water in a recent report in local news daily (23). Alarmingly high levels of ammonia with about fifty (50) fold increase above acceptable limits was recorded in KGMS and OKDS samples which could be due to the industrial and chemical discharge along these sites.

Although, physico-chemical analysis provides a near complete assessment of water quality, it cannot impart information on effects of contaminated/polluted water on the physiology and developmental in an organism. The use of zebrafish embryos for understanding developmental toxicity and physiological alterations fills in for this gap. Physiological and developmental defects due to adverse water quality can be studied *in vivo* using zebrafish. In the present study, morphological defects were visible by 50 hpf in embryos exposed to all the three river water samples. These defects included yolk sac edema, pericardial sac edema, body curvature, tail deformation, blood accumulation, and changes in body curvature. The defects were observed independent of the survival success of the embryos in the water samples. Embryos treated with WUP water sample exhibited maximum survival rate as compared to KGMS and OKDS, yet exhibited key abnormalities (Figure VIII). Change in body curvature and altered tail formation may be due to compromised skeletal and muscle development. A recent study reported high incidence of bone deformities of limbs in communities living on the banks of Hindon river, especially in the Baghpat district (24). Independent studies have shown that enormous content of heavy metals such as mercury, lead, zinc, phosphate, sulphide, cadmium, iron, nickel, and manganese to be present in the Hindon river water.

The physico- chemical analysis of the three water samples done in this study uncovers the presence of high amounts of heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) (Table-II). All water samples showed significantly high concentration of Zn, and Pb. WUP water samples exhibited the highest concentration of Zn followed by KGMS and then OKDS. Pb concentration was highest in KGMS, closely followed by WUP, however it was observed to be under permissible limits in OKDS. Cu and Cd were also found to be higher

than permissible limits in all the river samples. Morphological defects may be attributed to high levels of heavy metals found in river water samples. This speculation seems feasible with the example of WUP sample that exhibited similar survival and hatching rates as the control yet displayed developmental defects.

While some of the defects observed were common to embryos exposed to all the three water samples, we noticed that a few defects were recorded only for a specific water sample. This could be attributed to singular specific contaminants or a mixture of contaminants unique to the water composition at that particular site. Thus, this preliminary study presents prospects of advancing the experimental design by choosing unique components and understanding their effects on the early development and organ function in zebrafish embryos.

CONCLUSIONS

The study has a few caveats. The data presented describes the embryo-toxic effects of multiple components and contaminants present in the water samples from river Yamuna. However, as a preliminary study, we discovered interesting leads with respect to differences in response of the embryos to the three water samples, in terms of specific defects that were unique to water sample from a single site. WUP water sample showed manifestation of head edema, not seen prominently in the other two water samples. Similarly, KGMS water sample specifically displayed a significant proportion of embryos with a bent-tail defect. For future studies, we intend to improvise our experimental design by selecting single components and delineating their effects on organ function and developing tissues in juvenile zebrafish larvae.

Bacteriological analysis of fecal and total coliforms is another important parameter for examining the water quality (3). However, for this study, we chose to focus on the physico-chemical parameters and bacteria related studies were out of the scope of our expertise. Further embryo-toxic outcomes due to polluted river water exposure may change with temporal and spatial variations (20). For the present study, we collected the water samples during the winter months of December and January. The scope of evaluating the time and spatial variations is testable.

The study underscores the importance and potential of usage of zebrafish as a model to understand the impact of polluted water bodies on community health. Use of a live model for assessing water standards is a step ahead, as it has the potential to provide key links to some of the subtle yet important changes in the health outcomes of the community. A more detailed study needs to be undertaken to assess developmental toxicity, teratogenicity and tissue specific effects of individual components and their association with potential health risks.

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