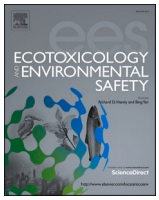
Ecotoxicology and Environmental Safety 233 (2022) 113320

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Evaluation of cytogenotoxic potential and embryotoxicity of KRS-Cauvery River water in zebrafish (*Danio rerio*)

Abass Toba Anifowoshe [a,b,*], Debasish Roy [a,c], Somit Dutta [a], Upendra Nongthomba [a]

[a] Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India
[b] Cell Biology and Genetics Unit, Department of Zoology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria
[c] Journal of Visualized Experiments (JoVE), 1 Alewife Center Suite 200, Cambridge, MA 02140, USA

ARTICLE INFO

Edited by Dr Yong Liang

Keywords:
Confocal microscopy
Developmental defects
Microplastics
Oxidative stress
ROS (Reactive oxygen species)
Zebrafish

ABSTRACT

In the Cauvery River (CR), indiscriminate discharge of waste causes unexplained skeletal deformity in some fish species present in the water. To investigate this phenomenon, we analyzed the biological, physical, and chemical parameters present in the water and then evaluated the toxicity effects on the zebrafish (*Danio rerio*) model. The zebrafish were treated with KRS-CR water samples collected from three stations (fast-flowing water [X], slow-flowing [Y], and stagnant [Z] water), before and after filtration. Firstly, we detected microscopic organisms (MO) such as *Cyclops, Daphnia, Spirogyra, Spirochaeta,* and total coliform *(Escherichia coli),* which are bio-indicators of water pollution present in the samples. All physicochemical parameters analyzed, including heavy metals before and after filtration of the water with Millipore filter paper (0.45 μm), were within the acceptable limits set by standard organizations, except for decreased dissolved oxygen (DO), and increased biochemical oxygen demand (BOD), and chemical oxygen demand (COD), which are indicators of hypoxic water conditions, as well as the presence of microplastics (polybutene (< 15 μm), polyisobutene (≤ 20 μm), and poly-methylpentene (≤3 mm)) and cyclohexyl in CR water samples. Zebrafish embryos treated with the water samples, both before and after filtration exerts the same cytogenotoxic effects by inducing increased reactive oxygen species (ROS) production, which triggers subcellular organelle dysfunctions, DNA damage, apoptosis, pericardial edema, skeletal deformities, and increased mortality. As a result, we observed that both water samples and zebrafish larvae had significantly less oxygen using SEM and EDS. Our findings show that KRS-CR water can induce cytogenotoxic and embryotoxic defects in zebrafish due to hypoxic water conditions triggered by the microplastics influx. The present study would provide valuable insights for health hazards evaluation and future river water treatment strategies.

1. Introduction

Water pollution and its effects on human health are of significant concern today. Indiscriminate discharge of a considerable amount of chemicals and waste materials, most of which are unknown, is responsible for pollution of the majority of the water bodies, including the rivers (Wang et al., 2020; Escher et al., 2020; Tian et al., 2021). Two-thirds of human long-term sickness risk cannot be explained exclusively by genetics; it may be caused by environmental or gene-environment interactions (Rappaport and Smith, 2010). Contaminated water serves as a vehicle for the transfer of pathogens, which could lead to massive or sporadic outbreaks of disease (La Rosa et al., 2020) and is responsible for harmful effects, including developmental abnormalities, neurological disorders, respiratory infections, liver and kidney dysfunction, endocrine disorders, reduced fertility, depressed immune functions, DNA damage, higher risk of cancer and increased mortality (Chandra et al., 2006; Speizer et al., 2006; Gaziano et al., 2006; Abdel-moneim et al., 2012; Chennaiah et al., 2014; Yu et al., 2014; Bhasin et al., 2016; WHO, 2017; Shuliakevich et al., 2022; Xie et al., 2022).

Abbreviations: KRS-CRW, Krishnaraja Sagara Cauvery River Water; DO, Dissolve Oxygen; BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand; SEM-EDS, Scanning Electron Microscopic & Energy dispersed X-ray; WHO, World Health Organization; BIS, Bureau of India Standard; C, Chorion; TR, Tail Region; HR, Head Region; YS, Yolk Sac; PC, Pericardial; YSE, Yolk Sac Edema; HW, Head Wane; PE, Pericardial Edema; DE, Deformed Embryo; DL, Deformed Larvae; SC, Scoliosis; BT, Bent Tail; ST, Straight Tail; UHE, Unhatched Embryo.

* Corresponding author at: Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India.
E-mail address: anifowoshea@iisc.ac.in (A.T. Anifowoshe).

https://doi.org/10.1016/j.ecoenv.2022.113320
Received 3 November 2021; Received in revised form 25 January 2022; Accepted 14 February 2022
Available online 17 February 2022

The Cauvery River (CR), which is the eighth largest among the 14 major river basins in India, is an inter-state river that flows eastward from the state of Karnataka through Tamil Nadu and drains into the Bay of Bengal. The CR provides potable water for over 150 million humans and animals and has long sustained fishing and irrigation. One of the largest dams erected on the CR, the Krishna Raja Sagara (KRS) Dam, acts as a critical reservoir for drinking water to the Karnataka cities of Mysore, Mandya, and Bangalore. The Central Pollution Control Board (CPCB) in the country has reported that the Cauvery water parameters fall within the permissible limits for drinking, in terms of pH, nitrate levels, heavy metals, among others, except for biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total coliform in Karnataka state (CPCB, 2015; Sakala and Sampath, 2019). Despite the anthropogenic activities and multiple contradictory reports on the complex admixture of biological, physical, and chemical components in the Cauvery River (EMPRI, 2017), there has been no research on other water pollutants, like microplastics; which has attracted global attention.

Microplastics (MPs) are plastic particles with diameters less than 5 mm. They have been shown to induce increased ROS production, leading to hypoxia, inflammation, cell death, metabolome dysfunction, skeletal deformities due to oxidative stress caused by MPs in targeted tissues (Yin et al., 2018; Yu et al., 2020; Shengchen et al., 2021). In recent years, some fish species (*Labio* sp.) caught from the KRS-CR water have shown skeletal deformities [Supplementary information (Fig. S1)]. To our knowledge, no study has explained the origin of this phenotype, and it has not been described in any environmental literature or databases despite continuous monitoring of the river. We hypothesized that a potential biomarker of environmental contaminants' toxicity could produce oxidative stress by promoting the generation of reactive oxygen species (ROS), leading to skeletal deformities in the fish (Zhao et al., 2015; Wang et al., 2019a, 2019b; Shengchen et al., 2021). To investigate this, we used zebrafish (*Danio rerio*) as an animal model to evaluate the ecotoxicological and developmental consequences of KRS-CR water (CRW).

The zebrafish (*D. rerio*) is an effective vertebrate model organism for toxicity testing (Dai et al., 2014). It has emerged as a primary vertebrate model for investigating the developmental defects, genotoxic, mutagenic, and carcinogenic potential of contaminants found in water samples. It has been employed in various studies as an indicator of aquatic environmental pollution because it can digest, concentrate, and retain water-borne contaminants (Moșneang et al., 2015). According to Howe et al. (2013), 70% of human genes have one zebrafish orthologue. Therefore, in this study, we have used zebrafish as an animal model to understand the toxicological and developmental implications of KRS-CR water pollution. The current study could potentially aid in assessing and identifying the ecotoxicological risk posed by pollutants like MPs in KRS-CR water.

2. Material and method

2.1. Sampling

Collection of water was done using the method described by Loos et al. (2017). Water was collected in three replicates from the KRS Dam during the pre-monsoon seasons (15th March–14th May), between 2019 and 2021 to avoid any surge in contaminants during heavy water flow due to the monsoon season. With the help of fishermen, cast nets were used to collect fish samples for morphological identification. Three points along the entire course of the KRS-CR water were selected concerning its speed and overall pollution. From its spring, where the water is fast flowing, at KRS I (moderately polluted site), the middle of the river, where the water becomes slow-flowing, at KRS II (semi-polluted site) and to its downstream, where it became stagnant, at KRS III (polluted site) [Supplementary information (Fig. S2)]. About 1 L of water samples were collected in cleaned brown glass bottles, placed in coolers with icepacks, and transported to the laboratory within 4 h of collection. The map of the three sites was constructed using Tableau 2019.1.3 software. The ranking of these three stations was done based on the appearance and physicochemical reports of the water carried out at Ramaiah Advanced Testing Laboratory in Bangalore, India. In the further experiments, these three stations were represented by X, Y, and Z for KRS I, KRS II, and KRS III, respectively. Before the water samples collection, we had obtained ethical approval from Cauvery Neeravari Nigama Limited (A Govt. of Karnataka Enterprise).

2.1.1. Microscopic and bacteriological analyses of the water samples

For microscopic examination of the water samples, we used a modified method of Plutzer and Törökné (2012). The water samples collected from the three sites on the river were gently stirred to ensure uniform distribution of organisms in the water. Briefly, about 1 mL of each water sample was dropped using a dropper on separate clean, sterile microscopic glass slides and spread by placing a coverslip on top at 45 °C. Blotting paper was used to absorb excess water from the slides. Each slide was then observed using a stereomicroscope (Olympus SZ51 stereomicroscope) to identify viable and non-viable organisms present in the water. The steps were repeated twenty times by taking 1 mL of the water samples before and after filtration using a 0.45 μm Millipore filter paper. Bacteriological analysis was performed using the most probable number (MPN) method as described by Malathy et al. (2017) and Phyo et al. (2019).

2.2. Evaluation of water quality before and after filtration

2.2.1. Physicochemical parameters

Water quality parameters such as pH, temperature, and total dissolved solids (TDS) were monitored using Hanna multiparameter (H198121 & H198301) directly from the field. DO was calculated using Winkler's method. Other parameters such as BOD, COD, chloride (Cl), sulfate (SO_4), fluoride (F), nitrate (NO_3), and cyanide were analyzed by titrimetric method-UV-Vis-spectrophotometer. Heavy metals such as Pb, Cu, Fe, Cr, Ni, and Cd were analyzed in water samples using inductively coupled plasma-mass spectrometry (ICP-MS). Color (Hazen units), phenol, insecticide, oil and grease, and anion detergent were analyzed using Gas chromatography-mass spectrometry (GC-MS). The manual of standard methods for evaluating water and wastewater was used to sample and analyze the river water (APHA, AWWA, 2007).

2.2.2. Scanning electron microscopic and energy dispersed X-ray (SEM/EDS) for nano and micro characterizations of water samples

KRS-CR water samples collected from the three stations were used for these experiments. The standard method of Yao et al. (2017) was employed with modifications. Water samples from the three sites were dropped separately on a metal plate and desiccated overnight before analysis. SEM/EDS (ULTRA 55) was used to characterize the dry samples at the Centre for Nano Science Engineering (CeNSE), Indian Institute of Science. The ULTRA 55 combines ultra-high resolution scanning electron imaging with material spectroscopic techniques to represent the most recent advancement in GEMINI technology. This ULTRA 55 includes a fully integrated Energy and angle selective Backscattered electron (EsB) detector based on the SUPRA 55. The new EsB detector has a built-in filtering grid that improves image quality without additional changes. Because the EsB detector is less susceptible to edge contrast and charge effects, it can image and measure boundaries, particles, and features with more precision.

2.2.3. Raman spectroscopy

Following the Takahashi et al. (2020) method, KRS-CR water samples were analyzed for the presence of microplastics (MPs) and other chemical functional groups using Raman spectroscopy-LabRam (HR) UV system (spectral range 0–3000 cm^{-1}, 585 nm laser) at the Centre for Nano Science and Engineering (CeNSE), Indian Institute of Science,

Bangalore. Lab RAM HR can acquire wide area Raman images in seconds/minutes using the X, Y, and Z mapping characteristics. With a 325 nm and 514 nm LASER and a CCD detector, UV and Visible Raman may be detected. The gathered Raman spectra were compared to those found in the SLOPP Microplastics Library and the KnowItAll software's spectral library (20.1.210.0). (Bio-Rad Laboratories, Inc.). Hit Quality Index (HQI) similarities of more than 80 were deemed satisfactory.

2.3. Maintenance, breeding, embryo collection, and exposure of zebrafish

2.3.1. Routine maintenance

Adult zebrafish (*D. rerio*) were kept in a glass aquarium with a capacity of 50 L at the Zebrafish Facility of the Developmental and Biomedical Genetics Laboratory (DBGL), Indian Institute of Science, Bangalore, India, in continuously well-aerated water containing 2 mg/L instant ocean salt at approximately 28 °C under a 14:10 h light-dark photoperiod. Two times a day, in the morning and evening, they were fed with commercial fish food (Tetra, Melle, Germany) and live *Artemia nauplii* (INVE Aquaculture, Thailand). In aquarium water, standard processes were utilized to test and maintain water quality parameters such as pH, DO, and total hardness (APHA, AWWA, 2007).

2.3.2. Breeding and embryo collection

Adult zebrafish in the ratio of two (2) males to two (2) females were placed in the aquaria housing breeding chamber for pair inter-crosses. Separators were placed in the breeding chamber to prevent adult zebrafish from eating the eggs. The entire system was kept at a 28 °C ambient temperature and a 14:10 h light-dark photoperiod. Breeding took place over time and began at the first sign of light the following day. With a Pasteur pipette and siphoning, eggs were retrieved from the bottom of the breeding chamber after 30 min. At 28 °C, the eggs were incubated in E3 medium (distilled deionized water containing 60 mg/mL instant sea salt), also known as egg water. Embryos were checked every 30 min to remove the dead ones. Healthy developed fishes were selected using a stereomicroscope at the blastula stage (6 h post-fertilization) following the previously reported embryonic development stages (Kimmel et al., 1995; Hosen et al., 2013). Unfertilized eggs that entered inadvertently during separation can be easily identified at the blastula stage. For additional investigation, the selected embryos were treated with water samples collected at three distinct KRS-CR water locations.

2.3.3. Exposure

Embryos (n = 5) at the blastula stage (6 h post-fertilization) were separated into the wells of a six well-plate and exposed to KRS-CR water for 96 h post-fertilization (hpf). The eggs were incubated each with 10 mL of 1X E3 medium (control), X (KRS I), Y (KRS II), Z (KRSIII) before and after filtration, and W (Tap water) for further experimentation. The experiment was performed in three replicates (N = 15 individuals/groups). All the experiments were carried out after receiving the required ethical approval from the Institutional Animal Ethics committee (IAEC) (Project No.: CAF/Ethics/684/2019).

2.3.4. Developmental defects

The exposed zebrafish embryos were checked every 24 h to screen for mortality, hatching, heartbeat, and morphological abnormalities for 96 hpf using a stereomicroscope (Olympus SZ51 stereomicroscope). Every observation was recorded at 24 hpf, 48 hpf, 72 hpf, and 96 hpf, according to Bachmann (2002) and Nagel and Dar (2002), Yumnamcha et al. (2015, 2020).

2.4. Reactive oxygen species (ROS) measurement

This was done using two different methods described by Mugoni et al. (2014) with slight modification.

2.4.1. Single-cell ROS detection method

Single-cell ROS detection was done using a standard protocol with slight modifications (Zhu et al., 2015). Embryos were cultured for 96 h in 10 mL of each sample. Ten larvae were taken from each group and rinsed three times with cold phosphate-buffered saline (PBS) (pH 7.4). The larvae were homogenized in cold buffer (0.32 mM sucrose, 20 mM HEPES, 1 mM $MgCl_2$, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), after being rinsed. The pH of the solution was raised to 7.4 using a 1 N solution of NaOH. The homogenates were then centrifuged for 20 min at 15,000 × *g* at 4 °C. 20 μL of the homogenate supernatant were added to each well of a black 96-well plate and incubated for 5 min at room temperature before adding 100 μL of PBS (pH 7.4) and 8.3 μL of dichlorodihydrofluorescein diacetate (H_2DCFDA) (D6883, Sigma Aldrich, USA) stock solution (dissolved in DMSO, 10 mg/mL). The final concentration of DCFH-DA for each well was ~ 6.5 mg/mL. The plates were incubated for 30 min at 37 °C, and the fluorescence intensity was measured with a spectrophotometer (Tecan-infinitepro.ink/MODEL INFINITE 200 PRO) and a flow cytometer (BD Accuri C6 Flow Cytometer equipped with Accuri C6 Software, manufactured by BD Biosciences).

2.4.2. Whole-mount ROS detection method

The detection of ROS in entire zebrafish larvae was carried out using the modified methods of the Wu et al. (2011) and Mugoni et al. (2014) protocols. After 96 h of exposure to the water samples, ten (10) larvae from each sample were randomly selected and washed three times with egg water for five minutes. The larvae were treated for 2 h in the dark at room temperature with 1 mg/mL cell-permeable CM-H_2DCFDA. Larvae were anesthetized in melting ice for 1 min before being mounted on a clean glass slide using propyl gallate mounting solution to avoid photobleaching. The larvae were imaged using a confocal microscope (Carl Zeiss LSM 510 META), and all images were processed using ImageJ-win64 software (version 1.52p).

2.5. Cell viability

Cell viability was carried out using the modified method of Zhu et al. (2015) and Piao et al. (2018). Briefly, zebrafish embryos were incubated in 10 mL of each water sample until 96 hpf. Ten larvae from each group were collected and rinsed thoroughly with cold PBS (pH 7.4) thrice. After rinsing, the larvae were homogenized in a cold buffer, and the pH of the solution was adjusted to 7.4. The homogenates were then centrifuged at 15,000 *g* at 4 °C for 20 min. Then, 20 μL of the homogenate supernatant was added to a 96-well plate and incubated at room temperature for 5 min. After 5 min, 8.5 μL of trypan blue (0.2%) was added to the homogenate and mixed using a micropipette. From the mixture, 10 μL was pipetted and released gently on a clean glass slide. Before imaging, a glass coverslip was then placed gently on each slide and sealed with nail polish. Each slide was imaged using a fluorescence microscope (Olympus BX51) at 10x magnification. Viable cells (%) was calculated using the formula below;

$$\text{Viable cell}(\%) = \frac{\text{total number of viable cells}}{\text{total number of cells}} \times 100$$

2.6. Measurement of mitochondrial membrane potential (Δψm (JC-1 staining))

Mitochondrial Δψ was measured using confocal microscopy after staining with lipophilic cationic fluorescence JC-1 dye (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) (ENZ-52304, India) as described previously in Giordo et al. (2020) with minor modifications. Briefly, the manufacturer's instructions were followed to prepare a 1.5 mM stock JC-1 solution in DMSO. 96 hpf zebrafish embryos were treated in 5.0 μM JC-1 solution in PBS for 30 min in the dark. Following the incubation period, the embryos were rinsed three times with 1X E3 medium and then treated for 30 min with 10 μL of our water

samples. Using a 96-multiwell dark plate with 5 embryos per well, fluorescence intensity was quantified using a microplate reader with both red (535–590 nm) and green (485–535 nm) channels. A confocal microscope with red and green channels was used to obtain the images. The ratio of average green/red fluorescence signals, representing the measurement of the degree of Δψm level, was used to express our findings (Posadino et al., 2015; Giordo et al., 2020).

2.7. Cell death analysis using specific dye

Cell death analysis was assessed using acridine orange (AO) dye (A6014, Sigma-Aldrich, USA). The standard method was followed with minor modifications (Slaats et al., 2014). Briefly, after 96-h exposure to the water samples, ten (10) zebrafish larvae from the well plate were selected and rinsed three times with 1X E3 medium for 5 min before incubating with 2 mg/mL AO solution at room temperature for 20 min. Before imaging, larvae were anesthetized with melting ice for 1 min, mounted using propyl gallate mounting medium, and imaged using a fluorescence microscope (Olympus BX51). All figures were processed using ImageJ-win64 software (version 1.52p).

2.8. Caspase 3 assay

Polyclonal antibody to Caspase 3 (Casp 3) (PAA626Hu01) was purchased from Cloud Clone Corp. Using Sorrells et al. (2013) method; whole embryos were fixed in 4% PFA overnight at 4 °C and incubated with Casp 3 (1:150) primary antibody overnight at 4 °C. The embryos were stained with secondary antibody (Alexa 488 goat-anti-rabbit (Invitrogen A11034)) 1:200 and visualized on an SP8 confocal microscope.

2.9. DNA damage analysis

2.9.1. Blood collection

Adult zebrafish were euthanized in an ice water bath at 4 °C for 1 min to collect peripheral blood (Babaei et al., 2013). The zebrafish were taken out of the cold water and placed in 30 °C warm water for 5 s. The zebrafish were then removed, dried with tissue paper, and placed in a modified microcentrifuge tube with the tail facing downward (cut at the tip). The modified microcentrifuge tube was immediately assembled with a collecting tube prepared with a 2% EDTA solution after making an incision at the end of each zebrafish's anal fin. After that, centrifuged for 5 min at 50 g and 11 °C. Another cut behind the previous cut was made for each fish to remove blood clots, and centrifugation was repeated at the same speed, temperature, and time. A micropipette was used to transfer blood from the collecting tube treated with EDTA solution into a pre-chilled microcentrifuge tube. The pooled blood was frozen at − 20°C for further investigation.

2.9.2. Micronucleus assay

After 96 h of exposure to water samples, the frequency of micronuclei and other nuclear anomalies in the erythrocytes of adult zebrafish was determined. About 2 μL of blood were pipetted and put onto precleaned glass slides, which were then left to dry overnight. The micronucleus test was carried out with minor modifications following protocols described previously (Ueda et al., 1992; Cavas et al., 2005; Anifowoshe et al., 2020; Yumnamcha et al., 2020).

2.9.3. Comet assay

The alkaline comet assay was performed as described previously by Olive and Banath (2006), Yumnamcha et al. (2020) with slight modification. Briefly, cell-coated slides were soaked in lysis buffer for 1 h at 4 °C, electrophoresed, stained with ethidium bromide (EtBr), and examined under confocal microscopy.

2.9.4. Genomic DNA Analysis

Genomic DNA was extracted from zebrafish larvae (n = 10) from control, X, Y, Z, and W using a genomic DNA extraction kit (Qiagen). Following the manufacturer's instructions, about 180 μL buffer ATL was added to the larvae in a 1.5 mL microcentrifuge tube followed by 20 μL proteinase K. The mixture was vortexed and incubated at 56 °C until tissues were wholly lysed. After completing the steps, isolated genomic DNA bands were visualized in agarose gel stained with EtBr (0.5 μg/mL) using an ultraviolet trans-illuminator system. We also used a 1 kb DNA ladder. This experiment was repeated three times to document the best images using the gel documentation system, Alpha DigiDoc RT2 (JH Bio).

2.10. Zebrafish larvae fixation and elemental analysis using SEM and EDS

Previously established methodologies were used to characterize and determine accumulated tiny chemical components and oxygen levels in zebrafish larvae (Kalishwaralal et al., 2017), with minimal modifications. The larvae were treated with KRS-CR water samples and then incubated with 1% paraformaldehyde before being washed three times with 1X PBS (pH 7.0). The zebrafish larvae were mounted on a copper tape, desiccated overnight, placed in gold sputtering, and images and elemental composition were analyzed using SEM and mapped using EDS.

2.11. Statistical analysis

All graphs were created using GraphPad Prism (version 8.0) and Origin 2021 software. The data were statistically analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test to measure a significant difference between the control and treated groups. Statistical significance was at the level of $^*p < 0.05$. The mean and standard error were used to present all data.

3. Results

3.1. Microscopic analysis of the KRS-CR water shows the presence of microscopic organisms

According to Jakhar (2013), microscopic organisms can be utilized as a water quality indicator. Microscopic analysis of the samples [Supplementary information (Fig. S3)] showed the presence of *Cyclops* and *Daphnia* in all the three samples tested. Whereas, *Spirogyra* and *Spirochaeta* were present in water samples from the Y and Z sites, respectively. To our knowledge, this is the first instance reporting the presence of these microbes in the KRS-CR water. These organisms are signs of contamination in the water by increasing turbidity and enhancing oxygen depletions. The total coliform count (MPN/100 mL) is shown in Table 1. The presence of total coliform (*E. coli*) in drinking water indicates sewage contamination from animals, humans, or both. However, no microorganisms were found after filtration of the water samples with a 0.45 μm Millipore filter [Supplementary information (Fig. S4)].

3.2. Most of the physico-chemical parameters of the collected water fall within the permissible limit for potable water

The Physico-chemical properties of KRS-CR water samples collected and monitored at three sampling sites are shown in Table 1. Most of the parameters assessed were within the permitted limit and acceptable for drinking water set by the standard organizations (SO) (BIS: Bureau India Standards & WHO: World Health Organisation). However, in Y and Z stations, analysis of the samples revealed low DO and high levels of BOD and COD, indicating a drop in oxygen levels at both locations (Table 1). All the heavy metals detected were within the limits allowed by SO for potable water (Table 1). All other measured parameters were below or

Table 1
Amounts of bacteria present, physicochemical and heavy parameters of KRS-CR water before and after filtration.

Parameter	X x̄±SD	Y x̄±SD	Z x̄±SD	Limits as per BIS 10500:2012	WHO limit guideline
*Total Coliform (MPN/100 mL)	1.95±0.02	2±1.000	4±1.001	0	0
pH	8.2±0.153	7.3±0.265	7.7±0.153	6.5-8.5	6.5-8.5
Temperature (°C)	25±1.000	23±3.000	24±2.000	25	25
DO (mg/L)	9.4±0.176	7.5±0.153	8.1±0.153	[a]	[a]
BOD (5 days at 20 °C), (mg/L)	4.0±1.000	20.2±0.060	6.0±1.732	[b]	[b]
COD (mg/L)	18±1.528	51±1.700	28±1.858	-	10
BOD/COD ratio	0.22±0.660	0.39±0.034	0.21±0.930	[c]	[c]
TDS (mg/L)	267±2.000	172±2.517	241±3.215	500-2000	1000
Fluorides (mg/L)	0.3±0.060	0.7±0.200	0.8±0.153	1-1.5	1.5
Chlorides (mg/L)	19±1.000	87±3.000	121±2.517	250-1000	250
Cyanides (mg/L)	ND	ND	ND	0.05	0.1
Nitrates (mg/L)	0.2±0.153	5.1±0.300	21±4.000	45	10
Sulphates (mg/L)	5±2.000	14±3.000	24±2.179	200-600	400
Colour (Hazen units)	4.95±0.045	25±2.000	50±1.000	5-15	15
Phenolic compounds	ND	ND	ND	0.001-0.002	-
Insecticides	ND	ND	ND	-	0.001
Anionic detergents	ND	ND	ND	0.2-1.0	1.0
Oil and Grease	0.05±0.02	ND	0.05±0.02	0.1	-
Cd (mg/L)	BDL	BDL	BDL	0.01	0.003
Cr (mg/L)	0.001±0.000	BDL	0.003±0.001	0.05	0.05
Se (mg/L)	BDL	BDL	BDL	0.01	0.01
Pb (mg/L)	0.001±0.000	BDL	BDL	0.05	0.05
Cu (mg/L)	0.02±0.000	0.008±0.002	0.008±0.002	0.05-1.5	2.0
As (mg/L)	BDL	BDL	0.001±0.000	0.05	0.05
Fe (mg/L)	0.03±0.010	0.02±0.000	0.06±0.02	0.3	<0.3

*After filtration, the data for physicochemical and heavy parameters results approximately remain the same except for bacteriological analysis (total coliform [MPN/100mL]) where X=Y=Z=0.
x̄±SD= Mean and Standard Deviation; BIS: Bureau Indian Standards; WHO: World Health Organization; ND: Not detected; BDL: Below Detection Limit.

[a]Dissolved oxygen (DO) mg/L
0-4.0 – No fish will survive
>4.0-6.5 - Very few fish will survive
>6.5-9.5 - Only big fish will survive
>9.5 - All fish can survive

[b]BOD Level (mg/L)	Interpretation	Site
1-2	Clean water	-
3-5	Moderately clean	X
6-10	Polluted water	Y
>10	Poor water quality	Z

[c]BOD/COD = 0.5. Value <0.5 indicates that the sample includes a high concentration of organic chemicals (Gerba and Pepper, 2015).

substantially within acceptable limits in all sampling sites, and they met India's drinkable water standards.

3.3. SEM-EDS and Raman spectroscopy analyses of the water samples revealed depleted oxygen levels and the presence of microplastics

Since atomic absorption spectroscopy, which is quite sensitive, but is not adequate to examine the accumulation of tiny chemical materials in water and zebrafish larvae, due to the large volume of samples required, we utilized SEM and EDS which allow the detection of trace elements and multi-element determination of nanoparticles. We noticed a significant decline in oxygen levels in all the water samples before and after filtration [Supplementary information (Fig. S5 a and b)]). The presence of other trace elements was minimal. Furthermore, with the help of a reliable, more robust, sensitive, and faster spectroscopy (Raman spectroscopy), we found for the first time, the presence of novel microplastics (polymethyl pentene, polybutene, and polyisobutene), toxic functional groups such as cyclohexyl, and many unknown particles in the KRS-CR water samples. These particles may contribute to the low level of oxygen observed in this study (Table 2; [Supplementary information (Fig. S6)]). Similar results (low oxygen levels) were also observed in zebrafish larvae exposed to these water samples [Supplementary information (Fig. S7)].

3.4. KRS-CR water samples induced developmental defects and reduced heartbeat rate in zebrafish embryos

In recent years, fishes caught from the KRS-CR water have been reported to show abnormalities [Supplementary information (Fig. S1)]). Thus, we wanted to evaluate whether KRS-CR water is capable of causing developmental toxicity. To do this, zebrafish embryos were incubated in the water samples collected from the rivers until 96 hpf, and the hazardous endpoints were assessed every 24 h (Fig. 1). Different developmental abnormalities in zebrafish exposed to water samples are shown in Supplementary information (Table S1). Pericardial edema (PE), skeletal deformities such as spinal curvature (SC), kyphosis (K), lordosis (LO), bent tail (BT) and deformed embryo/larvae (DE/DL), yolk sac edema (YSE), and late larvae hatching, were among the developmental defects observed. Prolong exposure of the hatched larvae in control water developed properly, whereas larvae exposed to the KRS-CR water samples resulted in additional/increased abnormalities and a delay in hatching (Fig. 1 B and C). At 96 hpf, the heartbeats of zebrafish larvae per minute decreased dramatically, in the order $Z > Y > X$ (Fig. 1D). The presence of MPs and altered BOD and COD resulting in depletion of oxygen reported at Y and Z sites may be responsible for a decrease in the heartbeat rate of the treated zebrafish larvae. Compared to the control group, the treated group's survival and mortality rates were lower and significant (Fig. 1 E and F). The developmental abnormalities in zebrafish larvae were not regulated or reduced by filtration of the water samples [Supplementary information (Fig. S8)]. It was observed that the developmental and toxicity effects, including mortality, delayed hatching, reduced heartbeat rate, and morphological abnormalities persist even after the filtration. Thus, microscopic organisms were not responsible for developmental and physiological defects. In addition, all of the treated fish had significantly lower hatching rates and heartbeats per minute [Supplementary information (Fig. S8 B–F)].

3.5. The KRS-CR water samples can induce oxidative stress, cellular damage, and DNA strand breaks

All diseases or anomalies are characterized mainly by generating reactive oxygen species (ROS) in a stress condition. To determine the potential role of oxidative stress induced by the water samples in zebrafish under hypoxic conditions, we measured the intracellular ROS using two approaches. In the first approach (whole-mount ROS-detection method), treatment with the water samples from Y and Z led to increased production of ROS in zebrafish larvae in head and tail regions as evidenced by H_2DCFDA staining using a confocal microscope [Supplementary information (Fig. S9)]. In the second approach (single-cell ROS-detection method), an elevated level of ROS was also observed in all the samples compared to the control, indicating oxidative stress as evidenced by H_2DCFDA staining using a spectrophotometer and flow cytometer [Supplementary information (Fig. S9 C and D)].

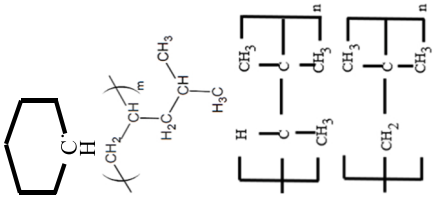
Next, we determined whether KRS-CR water can induce cellular damage (cytotoxicity) via ROS generation. Hence, we evaluated the apoptosis and viability of the cells. Apoptotic cells were observed using AO stain, which appeared mainly in the head and tail regions of the fishes exposed to water samples [Supplementary information (Fig. S10 A)]. We also observed a significant increase in mean cell death in the fish exposed to the KRS-CR water samples [Supplementary information (Fig. S10 B)]. Apoptosis is connected with increased mitochondrial membrane permeability, cytochrome c synthesis, and caspase activation (Chaudhary et al., 2016). Confocal microscopy images revealed that mitochondria in control cell showed red fluorescence intensely, indicating $\Delta\psi m$ polarization, whereas the green fluorescence, indicating $\Delta\psi m$ depolarization, was increased in KRS-CR water treated cells (Fig. 2). The Casp 3 assay gives a reliable result in a fixed cell for clear evidence of apoptosis. Supplementary information (Fig. S11) clearly shows the induction of apoptotic cells in zebrafish larvae treated with KRS-CR water (X + Y + Z) mixture, both before and after filtrations. All the samples were used as a mixture since all the treated groups showed induction of apoptotic cells, as evidenced by the AO stain. Surprisingly, we found approximately PE, YSE, and structural abnormalities in the tail region of zebrafish treated with X + Y + Z filtrates [Supplementary information (Fig. S12)]. In addition, the heartbeat per minute was three times lower when compared to the control (Supplementary Video I). The fact that the treated groups had more AO staining than the control larvae and Casp 3 would suggest that the water samples increased programmed cell death during development. Furthermore, as evidenced by trypan blue exclusion assay, KRS-CR water from Y and Z induced cytotoxicity (Fig. 3).

The next step was to see if KRS-CR water samples could cause DNA strand breaks via generating ROS. We used micronucleus and comet assays to evaluate the fidelity of the genome. Micronuclei (MN) and other nuclear abnormalities (NA) like blebbed, reniform, deformed, nucleoplasmic bridge, binucleated, and multinucleated were detected in treatment groups (X, Y, and Z). These abnormalities were increased in KRS-CR water samples compared to the control group. The comet assay, which measures DNA breaks, revealed that water samples from Y and Z had more cellular DNA tails than control samples. This was confirmed by genomic DNA extracted from zebrafish tissues, which exhibited genomic DNA damage in the treatment group compared to the control group in terms of the quality of bands (Fig. 4).

4. Discussion

Treatment of zebrafish embryos with the KRS-CR water samples, both before and after filtration of the MO, exerts the same cytogenotoxic effects by generating ROS, which triggers subcellular organelle dysfunctions, DNA damage, apoptosis, pericardial edema, skeletal deformities, and increased mortality. Previous studies have shown that industrial effluents, agricultural runoff, municipal and domestic sewage, plastic wastes, as well as pedogenic background contributions, are the principal causes of pollution in the Cauvery River, without examining the pollution impacts (Mahadev and Gholami, 2010; Susheela et al., 2014; EMPRI, 2017; Sakala and Sampath, 2019; Lalitha et al., 2021). These wastes and many barriers, including check dams, small hydel, and large dams, have contributed to a decline in fish population over time (Prakash et al., 2015; Sharma et al., 2020). In the present study, we have shown that water samples collected from KRS-CR are hypoxic, contain microplastics, increase mortalities and cause toxicological and

Table 2
Functional groups/microplastics (MPs) found in the KRS-CR water samples before and after filtration.

Control	X	Y	Z	W	Size	Structure
–	Cyclohexyl	Cyclohexyl	Cyclohexyl	–	–	
–	–	Polymethylpentene	Polymethylpentene	–	≤3 mm	
–	–	Polybutene	Polybutene	–	< 15 μm	
–	–	Polyisobutene	Polyisobutene	–	≤ 20 μm	
				Unknown		

MPs: Polymethylpentene, Polybutene and Polyisobutene.

developmental defects in the developing embryo of zebrafish.

The biological, physical, and chemical substances in water influence the amount of dissolved oxygen (DO). Low DO levels can inhibit the bacterial metabolism of some organic compounds, putting aquatic organisms at risk of oxidative stress. In most cases, the ratio BOD/COD is approximately 0.5 in value. When this ratio drops below this value, as shown in Table 1, it indicates that the sample includes a high concentration of organic chemicals that are difficult to break down by bacteria (Gerba and Pepper, 2015).

All three water samples (X, Y, and Z) contained *Cyclops* and *Daphnia*. However, *Spirogyra* and *Spirochaeta* were only found in water samples from the Y and Z sites, respectively. *Cyclops* and *Daphnia* belong to an aquatic arthropod, and *Spirogyra* belongs to aquatic plants. All three KRS-CR water samples showed the presence of total coliform. Total coliform in drinking water could signal a treatment system failure, infiltration in the distribution system, regrowth, sewage contamination, or animal wastes, all of which could have significant health consequences. As a result, coliforms are still considered a reasonable indicator of treatment and disinfection performance (Tallon et al., 2005). However, we were able to eliminate all of these microscopic organisms using 0.45 μm Millipore filter paper [Supplementary information (Fig. S4)].

The physical and chemical parameters of the KRS-CR water showed concentrations that were within the limit acceptable by standard organizations (BIS and WHO), except for DO, BOD, and COD in the three sites before and after filtration (Table 1). Parameters from our study are in agreement with previous reports (Krishna, 2012; Ramya and Ananthu, 2014; Susheela et al., 2014; CPCB, 2015; Sakala and Sampath, 2019). One of the most extensively used metrics for determining water quality is BOD. It reveals how much of the organic load in water is quickly biodegradable (Jouanneau et al., 2014). In samples Y and Z, we found lower levels of DO and higher levels of BOD and COD, indicating that oxygen levels are low at both locations. Higher COD levels indicate that the sample contains more oxidizable organic material lowering the DO levels. Anaerobic conditions can result from a decrease in DO, which is harmful to higher aquatic life forms. DO in the aquatic environment is an essential requirement for survival. Characterization of the KRS-CR water samples using SEM-EDS also confirmed a significant decrease in oxygen in the water samples and zebrafish larvae.

The major sources of pollution of KRS-CR water are industrial effluents, agricultural runoff, municipal and domestic wastes, return flow, paper and plastic wastes (Susheela et al., 2014; EMPRI, 2017). According to Ravit et al. (2019), organic compounds may be associated with microplastic pollution. Because of various physicochemical features that make microplastics multifunctional stressors, understanding their influence is quite difficult. On the one hand, microplastics transport dangerous chemicals across ecosystems. Still, they are also a cocktail of hazardous compounds introduced willingly as additives to boost polymer characteristics and extend their life (Campanale et al., 2020). In this study, we found the presence of novel microplastics (MPs) – such as polymethylpentene (≤3 mm), polybutene (< 15 μm), and polyisobutene (≤ 20 μm) and cyclohexyl for the first-time using Raman spectroscopy.

Variations in DO, BOD, and COD parameters leading to declining oxygen in the water and zebrafish samples may be attributed to the presence of these MPs (polymethylpentene, polybutene, and polyisobutene) and cyclohexyl (Skyberg et al., 1990). To the best of our knowledge, studies on the toxicity of these plastic polymers are very sparse. There are numerous applications for these MPs. For instance, polybutene is used in agricultural films and crop protection, while polyisobutene is used in tire inner liners and tubes, sealants, adhesives, condenser caps, pharmaceutical stoppers, and chewing gums, formulation of lipsticks, eye, and facial makeup (Barczikai et al., 2021). Polymethylpentene (thermoplastic polymer of 4-methyl-1-pentene) is used to manufacture cosmetic caps/tubes, and synthetic leather, while cyclohexyl isocyanate is used to make agricultural chemicals and pharmaceutical materials. There is evidence that hypoxia, as well as the

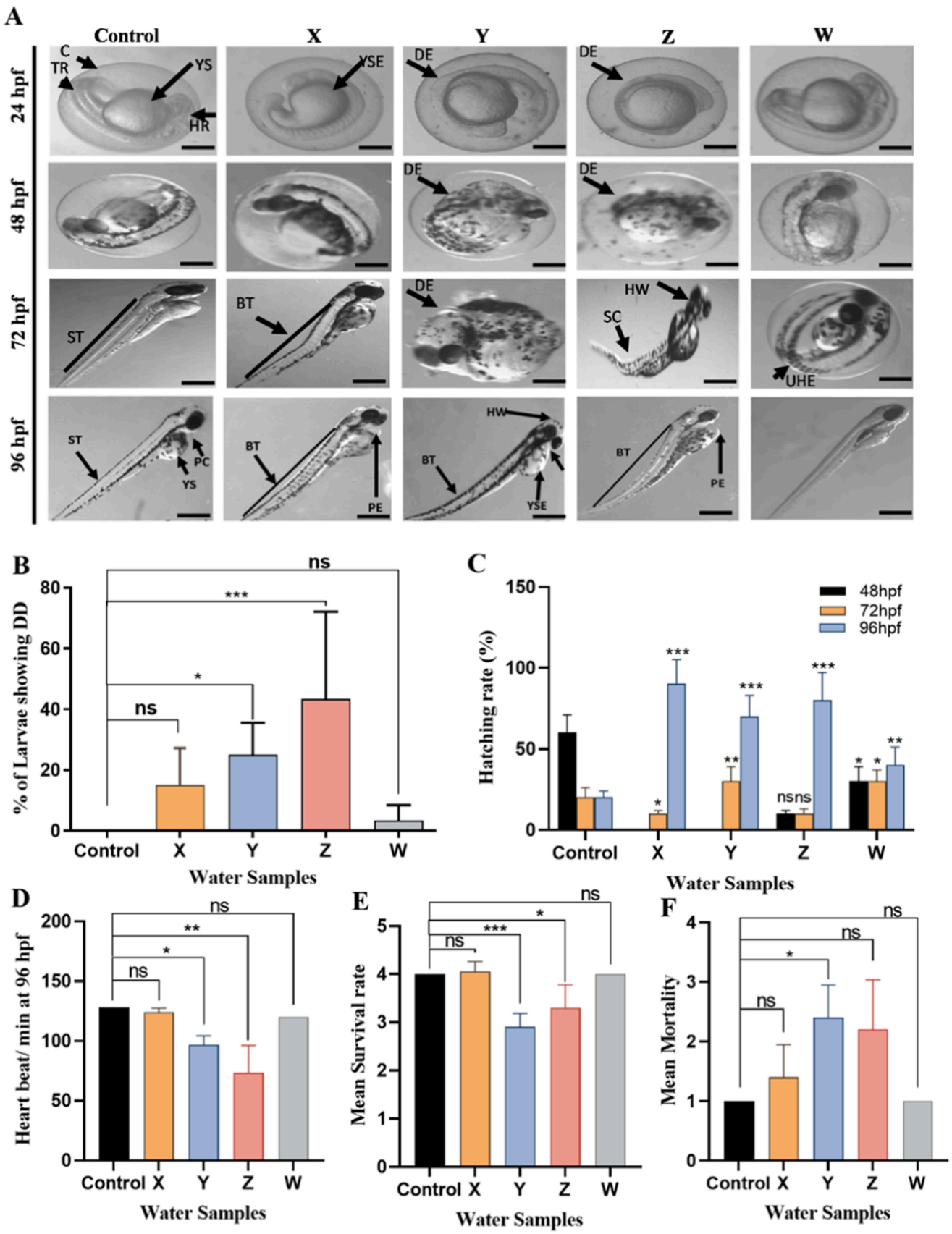


Fig. 1. Developmental toxicity test shows that KRS-CR water can induce heartbeat, hatching rate, developmental defects and mortality in zebrafish. A. The Control, and test samples X, Y, Z and W show various phenotypic alterations in zebrafish (24–96 hpf). **B.** Quantification of various anomalies observed in the zebrafish. **C.** Rate of heartbeat per minute in zebrafish larvae at 96 hpf in both control and test samples. **D.** Hatching rate (%) at 48–96 hpf. E. and F. Mean survival and mortality rates observed in both control and test samples. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. ns: not significant. DD: Developmental Defects.

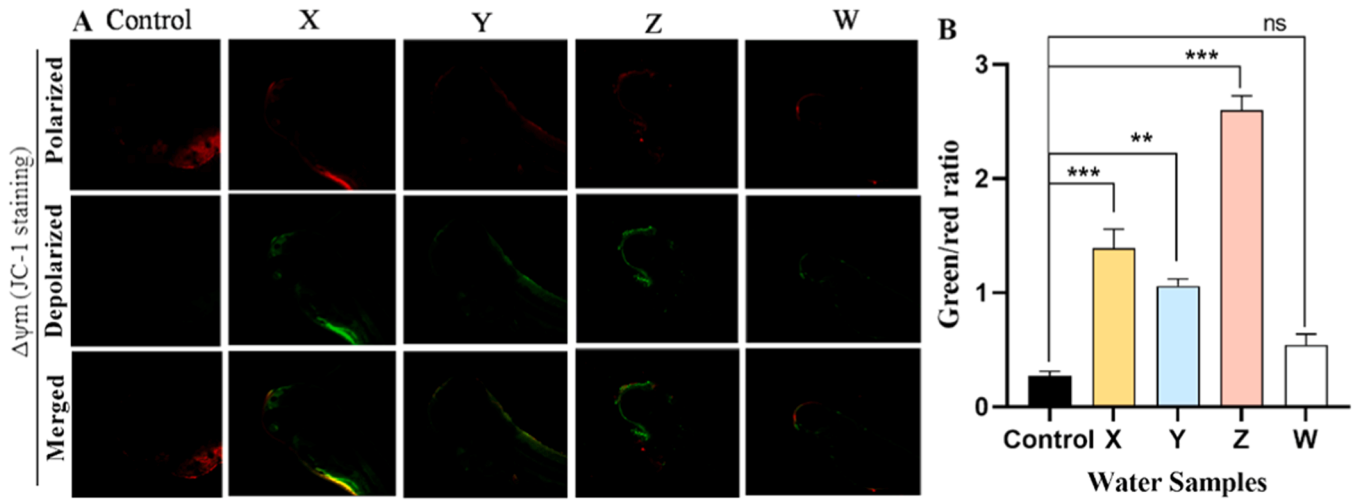


Fig. 2. Measurement of Mitochondrial membrane potential using JC-1 dye in 96-hpf zebrafish embryos shows mitochondrial depolarization in the test samples compared to control. A. Confocal microscopy images show mitochondrial membrane potential (ΔΨm) in control and treated (X, Y, Z and W) embryos (Mag. × 10). **B.** Quantitative analysis of the zebrafish embryos stained with JC-1 dye at 96 hpf. Mitochondrial depolarization (damaged state) or polarization (healthy state) are revealed by a decrease or increase in the green/red fluorescence intensity ratio respectively. Values are shown as mean ± SD, $n = 15$. **p < 0.01; ***p < 0.001; ns: not significant.

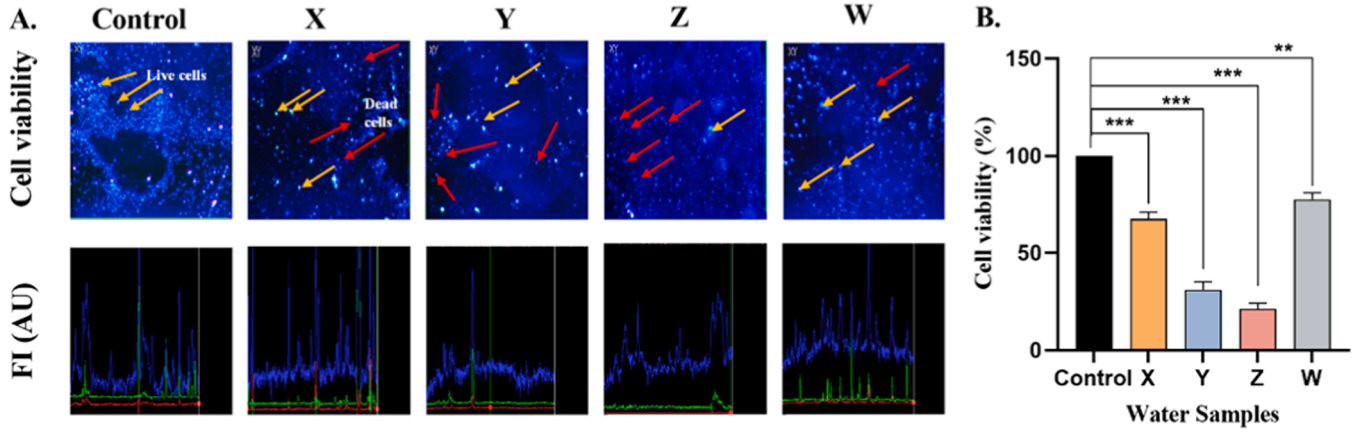


Fig. 3. KRS-CR water causes reduce cell viability. A. Cell viability analyzed by Trypan blue exclusion measured using fluorescence microscope (Mag. × 10). The intensity of the viable and non-viable cells is shown below each group. Orange arrows indicate live cells (viable) and red arrows indicate dead cells (non-viable). **B.** The intensity of the live cells reduced drastically in Y and Z. **p < 0.01; ***p < 0.001. FI: Fluorescent Intensity (AU: Arbitrary unit).

presence of MPs in water, increases ROS production and inhibits the intracellular antioxidant system in aquatic organisms, leading to physiological changes, such as increased gestation, damaged nerve fibers, inhibit acetylcholinase (AchE) activity, reduced feeding and growth rates, and increased mortality (Camargo et al., 2005; Tallon et al., 2005; Pollock et al., 2007; Jeong et al., 2016; Rist et al., 2017; Yu et al., 2019, 2021). In mammals, MPs damage skeletal muscle regeneration due to oxidative stress (Shengchen et al., 2021). Thus, to determine the toxicological and developmental effects of hypoxia and MPs in KRS-CR water, we used the zebrafish model organism. The chorion in the zebrafish embryo operates as a sieve rather than a shield (Ali et al., 2017) and has pores with an average diameter of 0.5–0.7 μm which can be found at distances of 1.5–3 μm. This demonstrated that chorion enabled both solute and particles (in nano and micro sizes) to pass through its pore channels (Lee et al., 2012; Kais et al., 2013). Our data indicate that water samples from Y and Z sites promoted ROS generation which triggers subcellular organelle dysfunctions, DNA damage, apoptosis, pericardial edema, skeletal deformities, and increased mortality in the zebrafish embryo. As evidenced in *Labio* sp. caught from the KRS-CR water, which showed skeletal deformities [Supplementary information (Fig. S1)], several body malformations such as skeletal deformities (spinal curvature, kyphosis, and lordosis), PE, YSE, deformed body, among others, were observed in the zebrafish larvae treated with KRS-CR water. The heart and skeletal system were mainly affected in fishes treated with KRS- CR water. This was confirmed by the reduced heartbeat rate observed in fishes from treated groups (Fig. 1D, [Supplementary information (Fig. S8 D and Video I)]). As a result, cardiac abnormalities in the early life stages of fishes are symptomatic of stressor reactions, and an increase in oxidative stress is related to a decreased heartbeat, as seen in this study (Shang and Wu, 2004). If the quantity of dissolved oxygen in the water is constantly low, the growth of aquatic animals will be hindered, and they will be more vulnerable to infectious diseases (Nimesh et al., 2012). Low oxygen levels cause a wide range of issues, from mass mortality of aquatic species (fish and marine mammals) to local extinction of fauna, as well as ecological disruption and fishery productivity decreases (Diaz, 2001; Pollock et al., 2007; Domenici et al., 2017; Roman et al., 2019).

We found that sites Y and Z had a 50% mortality rate compared to the control. In all treated groups, the hatching rate was likewise delayed (X, Y, and Z). This finding is consistent with Kaur and Toor (2011), who

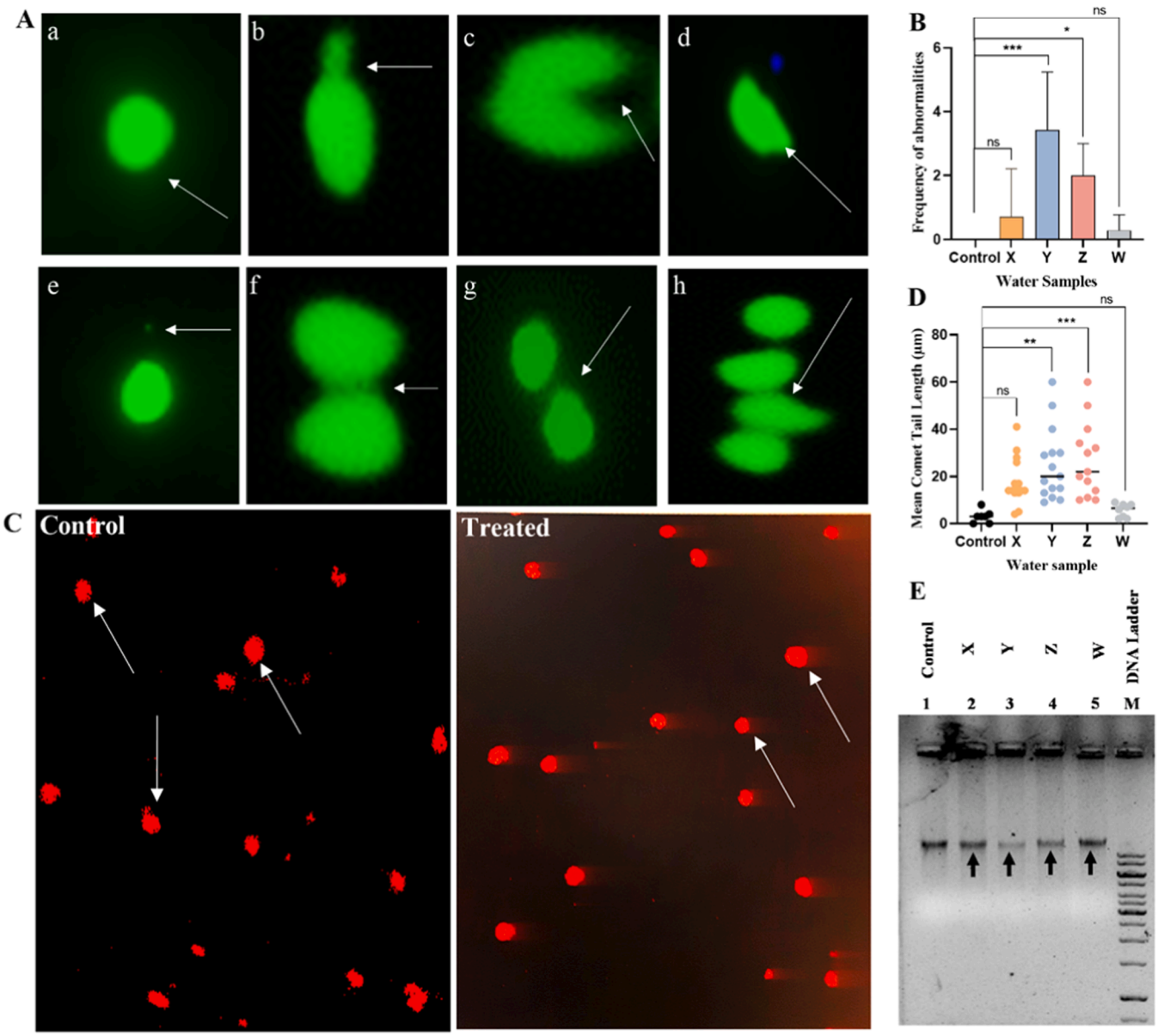


Fig. 4. Genotoxic test shows induction of micronuclei, nuclear anomalies and DNA damage by the KRS-CR Water. A. Micronuclei (MN) and other nuclear abnormalities (NA) (blebbed, reniform, deformed, nucleoplasmic bridge, binucleated and multinucleated observed in treated groups (X, Y and W) zebrafish embryos stained with AO dye at 96 hpf. (Mag. × 60). **B.** Quantitative analysis of MN and NA observed in zebrafish embryos at 96 hpf. *$p < 0.05$; $p < 0.001$. **C.** DNA damage evaluated by single cell gel electrophoresis (comet assay) (Mag. × 10) in both control and treated groups. **D.** Quantification of the water samples from Y and Z sites shows significant induction of DNA strand breaks as indicated by comet tail. **$p < 0.01$; ***$p < 0.001$; ns: not significant. **E.** Agarose gel electrophoresis of genomic DNA exposed to the water samples. The genomic DNA of the Y and Z are becoming fragmented, thus fading when compared to the control (Black arrows). a – Normal Nucleus; b – Blebbed Nucleus; c– Reniform Nucleus; d – Deformed Nucleus; e – Micronucleus; f – Nucleoplasmic Bridge; g – Binucleated; h – Multinucleated.

found that DO levels below 6.0 mg/L were insufficient for the proper survival of scale carp eggs (*Cyprinus carpio* communis). About two decades ago, over 1000 seabirds in the North Sea were reported to be demobilized and killed by polyisobutylene (PIB) (Camphuysen et al., 1999). Therefore, the decline in fish species in CR as reported by EMPRI (2017), and increased mortality as observed in this present study may also be attributed to the presence of PIB.

Waterborne genotoxic pollutants impact aquatic organisms and humans through contaminated drinking water and fish consumption through the food chain (Ali et al., 2008; Rocha et al., 2009; Xian et al., 2021). Physiological alterations in aquatic organisms living in contaminated rivers have been observed in recent investigations. Hariri et al. (2018) found DNA damage in sampling fish erythrocytes, liver, and gill during an in situ examination of the Karaj River's genotoxic impact. In our study, developmental defects such as skeletal deformities, PE, YSE, and UHE were linked to oxidative stress caused by low oxygen levels at the Y and Z stations, which activated cytotoxic and genotoxic processes such as increased apoptosis, decreased cell viability, mitochondrial damage, and increased DNA damage. Mitochondrial dysfunctions are the primary source of intracellular ROS, and they play a critical role in the development and progression of many diseases (Piao et al., 2018). Our findings suggest that embryos treated with KRS-CR water samples experienced mitochondrial damage triggered by the presence of MPs. To our knowledge, this is the first time that these types of MPs have been found in the Cauvery river. Therefore, all these could contribute to the developmental defects observed in zebrafish (Fig. 5), which correlate with abnormalities of the fish body caught and dwindling numbers of fish species in KRS-CR water.

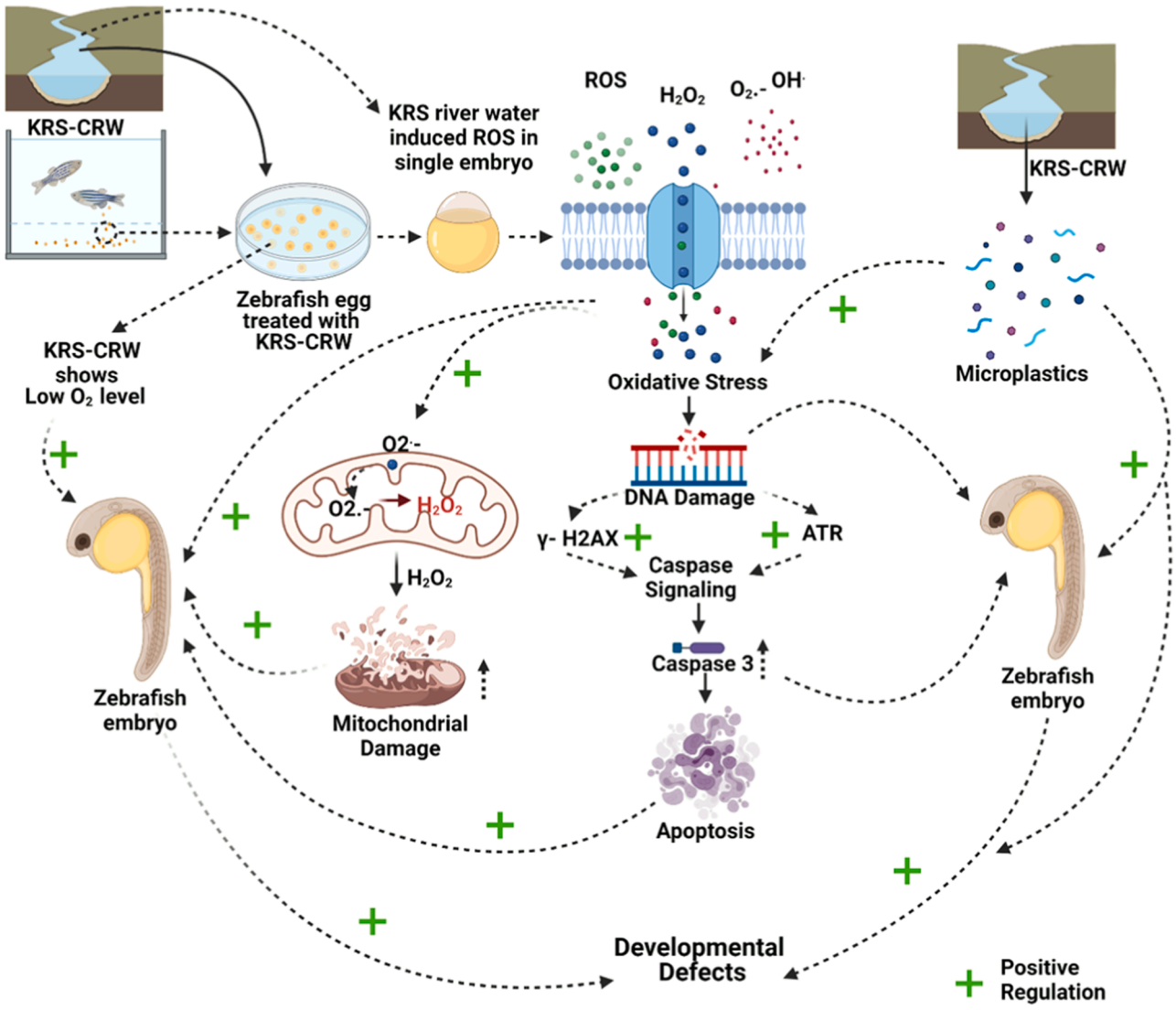


Fig. 5. Summary of the experiments. KRS-CR water induces developmental defects in zebrafish by increasing apoptosis through oxidative stress and destruction of cellular organelles. It enhances the production of reactive oxygen species (ROS), which causes oxidative stress, mitochondrial dysfunction, and DNA damage, leading to apoptotic cell death and developmental defects, primarily skeletal deformities and pericardial edema in zebrafish.

5. Conclusion

In conclusion, for the first time, our study demonstrates the pollution effects of CR water, at KRS-CR water sampling sites. Y and Z sites, where the water flows slowly or stagnant, both samples induced cytotoxicity and genotoxicity in zebrafish due to reduced DO and excess amounts of BOD/COD. The alterations in these parameters leading to a hypoxic condition in the water and fish samples may be attributed to other contaminants like microplastics (polymethylpentene, polybutene, and polyisobutene) and cyclohexyl, which require further investigations. Our study also highlights that the KRS-CR water can generate developmental defects in the zebrafish, which correlates with phenotypic abnormalities observed in fishes caught from KRS-CR water in recent years. The findings from the present study may prove helpful in providing valuable insights for future treatment of the water and the potential health hazards of using KRS-CR water for drinking, fishing, and irrigation.

Ethics approval and consent to participate

All the experiments were carried out after receiving the required ethical approval from the Institutional Animal Ethics committee (IAEC) (Project no.: CAF/Ethics/684/2019). Prior to the water and fish samples collections, we had obtained ethical approval from Cauvery Neeravari Nigama Limited (A Govt. of Karnataka Enterprise), India.

Funding

This study was funded by the Department of Biotechnology, Government of India (DBT) and The World Academic of Science (TWAS), Italy postgraduate fellowship (FR number 3240300004) granted to author ATA tenable at the Indian Institute of Science (IISc), and supported by, The Department of Science and Technology (DST), Govt. of India, (DST FIST, Ref. no. SR/FST/LSII-036/2014), the University Grant Commission (UGC-SAP to MRDG: Ref. no. F.4-13/2018/DRS-III (SAP-II)), and the Department of Biotechnology (DBT), Govt. of India, (DBT).

CRediT authorship contribution statement

Abash Toba Anifowoshe (ATA): Formal analysis, Investigation, Data curation, Funding acquisition, Writing – original draft, Visualization. **Debasish Roy (DR):** Methodology, Validation, Investigation, Data curation. **Somit Dutta (SD):** Writing – review & editing. **Upendra Nongthomba (UN):** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Consent for publication

All the authors have gone through the manuscript and the data reported and consented to publication.

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

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

We thanked Dr. T. V. Ramachandra, Centre for Ecological Science, Indian Institute of Science (IISc), and his students, particularly Mr. Ravi, for their technical assistance during this work. We are grateful to the Central Imaging Facility staff, Bioscience building, Indian Institute of Science, for their help and support during image acquisition and processing, and we thank Mr. Bilal for giving technical assistance while utilizing the flow cytometer. For sample processing, analysis, and data interpretation, we thank the Centre for Nano Science Engineering (CeNSE) at IISc, particularly Amit Kumar and Archana Venkateswarlu. Our gratitude also goes to Mr. Abhilash and Mr. Ae Kishore of KRS Dam for their help during water and fish samples collection and Cauvery Neeravari Nigama Limited (A Govt of Karnataka Enterprise) for providing ethical consent to collect water samples. Thanks to Amartya Mukherjee for revising the text prior to its final submission.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113320.

References

Abdel-moneim, A.M., Al-kahtani, M.A., Elmenshawy, O.M., 2012. Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. Chemosphere 88, 1028–1035. https://doi.org/10.1016/j.chemosphere.2012.04.001.

Ali, F.K., El-Shewawi, A.M., Seehy, M.A., 2008. Micronucleus test in fish genome: a sensitive monitor for aquatic pollution. Afr. J. Biotech. 7, 606–612. https://doi.org/10.5897/AJB2008.000-5020.

Ali, M.K., Saber, S.P., Taite, D.R., Emadi, S., Irving, R., 2017. The protective layer of zebrafish embryo changes continuously with advancing ages of embryo development (AGED). J. Toxicol. Pharmacol. 1, 009.

Anifowoshe, A.T., Oyebanji, J.B., Oladipo, O.S., Oyeyemi, F.B., Abdulrahim, M.Y., et al., 2020. Histological changes, micronuclei induction and nuclear abnormalities in the peripheral erythrocytes of *Clarias gariepinus* (Burchell 1822) exposed to water sample from Apodu reservoir. JLBSR 1, 1–7. https://doi.org/10.38094/jlbsr113.

APHA, AWWA, 2007. WEF, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, and Water Environment Federation.

Babaei, F., Ramalingam, R., Tavendale, A., Liang, Y., Yan, L.S.K., et al., 2013. Novel blood collection method allows plasma proteome analysis from single zebrafish. J. Proteome Res. 12, 1580–1590. https://doi.org/10.1021/pr3009226.

Bachmann, J., 2002. Development and Validation of a Teratogenicity Screening Test with Embryos of the Zebrafish (*Danio rerio*) (Dissertation). Technical University of Dresden.

Barczikai, D., Domokos, J., Szabó, D., Molnar, K., Juriga, D., et al., 2021. Polyisobutylene—new opportunities for medical applications. Molecules 26, 5207. https://doi.org/10.3390/molecules26175207.

Bhasin, C., Mudgal, P., Joshi, A., Mangla, A.G., Madhu, et al., 2016. Zebrafish early stage developmental defects as indicator of site-specific water composition of river Yamuna. DU J. Undergrad. Res. Innov. 2, 40–55.

Camargo, J.A., Alonso, A., Salamanca, A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58, 1255–1267. https://doi.org/10.1016/j.chemosphere.2004.10.044.

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., Uricchio, V.F., 2020. A detailed review study on potential effects of microplastics and additives of concern on human health. Int. J. Environ. Res. Public Health 17, 1212. https://doi.org/10.3390/ijerph17041212.

Camphuysen, K.C., Barreveld, H., Dahlmann, G., Van Franeker, J.A., 1999. Seabirds in the North Sea demobilized and killed by polyisobutylene (C_4H_8)n (PIB). Mar. Pollut. Bull. 38, 1171–1176. https://doi.org/10.1016/s0025-326x(99)00152-6.

Cavas, T., Garanko, N.N., Arkhipchuk, V.V., 2005. Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. Food Chem. Toxicol. 43, 569–574. https://doi.org/10.1016/j.fct.2004.12.014.

Chandra, V., Pandav, R., Laxminarayan, R., Tanner, C., Manyam, B., et al., 2006. Neurological disorders. In: Jamison, D.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.), Disease Control Priorities in Developing Countries, 2nd edition. Oxford University Press and The World Bank, pp. 627–645.

Chaudhary, A.K., Yadav, N., Bhat, T.A., O'Malley, J., Kumar, S., et al., 2016. A potential role of X-linked inhibitor of apoptosis protein in mitochondrial membrane permeabilization and its implication in cancer therapy. Drug Discov. Today 21, 38–47. https://doi.org/10.1016/j.drudis.2015.07.014.

Chennaiah, J.B., Rasheed, M.A., Patil, D.J., 2014. Concentration of heavy metals in drinking water with emphasis on human health. Int. J. Plant Anim. Environ. Sci. 4, 205–214.

CPCB, 2015. ⟨https://cpcb.nic.in/⟩, (Accessed 26th October 2020).

Dai, Y.J., Jia, Y.F., Chen, N., Bian, W.P., Li, Q.K., et al., 2014. Zebrafish as a model system to study toxicology. Environ. Toxicol. Chem. 33, 11–17. https://doi.org/10.1002/etc.2406.

Diaz, R.J., 2001. Overview of hypoxia around the world. J. Environ. Qual. 30, 275–281. https://doi.org/10.2134/jeq2001.302275x.

Domenici, P., Steffensen, J.F., Marras, S., 2017. The effect of hypoxia on fish schooling. Philos. Trans. R. Soc. B 372, 20160236. https://doi.org/10.1098/rstb.2016.0236.

EMPRI, 2017. Assessment of current status of cauvery river in Karnataka and study of 300 m buffer zone on both sides of the river. ⟨https://empri.karnataka.gov.in/storage/pdf-files/Reports/Cauvery-Final%20Report.pdf⟩, (Accessed 31st December 2021).

Escher, B.I., Stapleton, H.M., Schymanski, E.L., 2020. Tracking complex mixtures of chemicals in our changing environment. Science 367, 388–392. https://doi.org/10.1126/science.aay6636.

Gaziano, T., Reddy, K.S., Paccaud, F., Horton, S., Chaturvedi, V., 2006. Cardiovascular disease. In: Jamison, D.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.), Disease Control Priorities in Developing Countries, 2nd edition. Oxford University Press and The World Bank, pp. 645–663.

Gerba, C.P., Pepper, I.L., 2015. Gentry: Environmental Microbiology, 3rd edn. Academic press, pp. 583–606. https://doi.org/10.1016/B978-0-12-394626-3.00025-9.

Giordo, R., Nasrallah, G.K., Al-Jamal, O., Paliogiannis, P., Pintus, G., 2020. Resveratrol inhibits oxidative stress and prevents mitochondrial damage induced by zinc oxide nanoparticles in zebrafish (*Danio rerio*). Int. J. Mol. Sci. 21, 3838. https://doi.org/10.3390/ijms21113838.

Hariri, M., Mirvaghefi, A., Farahmand, H., Taghavi, L., Shahabinia, A., 2018. *In situ* assessment of Karaj River genotoxic impact with the alkaline comet assay and micronucleus test, on feral brown trout (*Salmo trutta* fario). Environ. Toxicol. Pharmacol. 58, 59–69. https://doi.org/10.1016/j.etap.2017.12.024.

Hosen, M.J., Vanakker, O.M., Willaert, A., Huysseune, A., Coucke, P., et al., 2013. Zebrafish models for ectopic mineralization disorders: practical issues from morpholino design to post-injection observations. Front. Genet. 4, 1–17. https://doi.org/10.3389/fgene.2013.00074.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., et al., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503. https://doi.org/10.1038/nature12111.

Jakhar, P., 2013. Role of phytoplankton and zooplankton as health indicators of aquatic ecosystem: a review. Int. J. Innov. Stud. 2, 489–500.

Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., et al., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441.

Jouanneau, S., Recoules, L., Durand, M.J., Boukabache, A., Picot, V., et al., 2014. Methods for assessing biochemical oxygen demand (BOD): a review. Water Res. 49, 62–82. https://doi.org/10.1016/j.watres.2013.10.066.

Kais, B., Schneider, K.E., Keiter, S., Henn, K., Ackermann, C., 2013. DMSO modifies the permeability of the zebrafish (*Danio rerio*) chorion-Implications for the fish embryo test (FET). Aquat. Toxicol. 140–141, 229–238. https://doi.org/10.1016/j.aquatox.2013.05.022.

Kalishwaralal, K., Jeyabharathi, S., Sundar, K., Muthukumaran, A., 2017. Mapping of accumulation of SeNPs in developing zebrafish embryos and larvae: a new method using SEM with energy dispersive X-ray spectrometer. Micro Nano Lett. 12, 497–499. https://doi.org/10.1049/mnl.2016.0692.

Kaur, K., Toor, H.S., 2011. Effect of dissolved oxygen on the survival and hatching of eggs of scale Carp. Prog. Fish. C 40, 35–37. https://doi.org/10.1577/1548-8659(1978)40[35:EODOOT]2.0.CO;2.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dyn. 203, 253–310. https://doi.org/10.1002/aja.1002030302.

Krishna, J.M., 2012. Physicochemical and bacteriological study of Kaveri-river at Kudige, Kodagu district, Karnataka. Intern. J. Environ. Sci. 2, 2040–2049.

La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S., Suffredini, E., 2020. Coronavirus in water environments: occurrence, persistence and concentration methods – a scoping review. Water Res. 179, 115899 https://doi.org/10.1016/j.watres.2020.115899.

Lalitha, M., Dharumarajan, S., Kalaiselri, B., Shivanand, K., Koyal, A., et al., 2021. Hydrochemical characterization and groundwater quality in Cauvery deltaic fluvial plains of Southern India. ESPR 28, 44861–44876. https://doi.org/10.1007/s11356-021-13467-8.

Lee, K.J., Browning, L.M., Nallathamby, P.D., Desai, T., Cherukui, P.K., et al., 2012. *In vivo* quantitative study of sized-dependent transport and toxicity of single silver nanoparticles using zebrafish embryo. Chem. Res. Toxicol. 25, 1029–1046. https://doi.org/10.1021/tx300021u.

Loos, R., Tavazzi, S., Mariani, G., Suukuusk, G., Paracchini, B., et al., 2017. Analysis of emerging organic contaminants in water, fish and suspended particulate matter (SPM) in the Joint Danube Survey using solid-phase extraction followed by UHPLC-MS-MS and GC–MS analysis. Sci. Total Environ. 607–608, 1201–1212. https://doi.org/10.1016/j.scitotenv.2017.07.039.

Mahadev, J., Gholami, S., 2010. Heavy metal analysis of Cauvery river water around KRS Dam, Karnataka, India. J. Adv. Lab. Res. Biol. 1, 1–5.

Malathy, B.R., Sajeev, S.K., Thampy, S., Guruvayurappan, K., Ajitha, S.P., 2017. Bacteriological analysis of drinking water by MPN method from Chennai, India. IOSR-JESTFT 11, 57–64.

Mugoni, V., Camporeale, A., Santoro, M.M., 2014. Analysis of oxidative stress in zebrafish embryos. J. Vis. Exp. 89, e51328 https://doi.org/10.3791/51328.

Moșneang, C.L., Dumitrescu, E., Muselin, F., Ciulan, V., Grozea, A., et al., 2015. Use of zebrafish eggs as early indicators of aquatic environmental pollution. Pol. J. Environ. Stud. 24, 2079–2085. ⟨https://doi.10.15244/pjoes/58649⟩.

Nagel, R., Dar, T., 2002. The embryo test with the zebrafish *Danio rerio* – a general model in ecotoxicology and toxicology. ALTEX 19 (Suppl. 1), S38–S48.

Nimesh, N., Virdi, G.S., Jain, S., 2012. Evaluation of Oxygen Tolerance in Fresh Water Fishes, III. Voyager (ISSN: 0976-7436, (e): 2455-054X).

Olive, P.L., Banath, J.P., 2006. The comet assay: a method to measure DNA damage in individual cells. Nat. Protoc. 1, 23–29. https://doi.org/10.1038/nprot.2006.5.

Prakash, N., Menon, G., Basavaiah, A., 2015. Building Stake to Conserve River Related Biodiversity Using Otters as Flagship Species in Cauvery river basin in Karnataka. Final Techincal Report submitted by Paadhai Trust and Nityata Foundation to CEPF-ATREE Western Ghats Program.

Phyo, S.S.M., Yu, S.S., Saing, K.M., 2019. Bacteriological examination of bottled drinking water by MPN method. Haya: Saudi J. Life Sci. 4, 227–232. https://doi.org/10.21276/haya.2019.4.7.2.

Piao, M.J., Ahn, M.J., Kang, K.A., Ryu, Y.S., Hyun, Y.J., et al., 2018. Particulate matter 2.5 damages skin cells by inducing oxidative stress, subcellular organelle dysfunction, and apoptosis. Arch. Toxicol. 92, 2077–2091. https://doi.org/10.1007/s00204-018-2197-9.

Plutzer, J., Törökné, A., 2012. Free-living microscopic organisms as indicators of changes in drinking-water quality. Water Pract. Technol. 7, 050. https://doi.org/10.2166/wpt.2012.050.

Pollock, M.S., Clarke, L.M.J., Dubé, M.G., 2007. The effects of hypoxia on fishes: from ecological relevance to physiological effects. Environ. Rev. 15, 1–14. https://doi.org/10.1139/a06-006.

Posadino, A.M., Cossu, A., Giordo, R., Zinellu, A., Sotgia, S., et al., 2015. Resveratrol alters human endothelial cells redox state and causes mitochondrial-dependent cell death. Food Chem. Toxicol. 78, 10–16. https://doi.org/10.1016/j.fct.2015.01.017.

Ramya, R., Ananthu, K.M., 2014. Appraising water quality aspects for an expanse of river Cauvery alongside Srirangapatna. Intern. J. Emerg. Technol. Comput. Appl. Sci. 9, 71–75.

Rappaport, S.M., Smith, M.T., 2010. Environmental and disease risks. Science 330, 460–461. https://doi.org/10.1126/science.1192603.

Ravit, B., Cooper, K., Buckley, I., Yang, I., Deshpande, A., 2019. Organic compounds associated with microplastic pollutants in New Jersey, USA surface waters. AIMS Environ. Sci. 6, 445–459. https://doi.org/10.3934/environsci.2019.6.445.

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in *Daphnia magna* – quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407. https://doi.org/10.1016/j.envpol.2017.05.048.

Rocha, P.S., Luvizotto, G.L., Kosmehl, T., Boettcher, M., Storch, V., et al., 2009. Sediment genotoxicity in the Tietê River (São Paulo, Brazil): in vitro comet assay versus in situ micronucleus assay studies. Ecotoxicol. Environ. Saf. 72, 1842–1848. https://doi.org/10.1016/j.ecoenv.2009.04.013.

Roman, M.R., Brandt, S.B., Houde, E.D., Pierson, J.J., 2019. Interactive effects of hypoxia and temperature on coastal pelagic zooplankton and fish. Front. Mar. Sci. 6, 1–18. https://doi.org/10.3389/fmars.2019.00139.

Sakala, B.T., Sampath, K.M.C., 2019. Assessment of physico-chemical characteristics of selected study area of Cauvery Basin. Int. J. Latest Trends Eng. 15, 051–054. https://doi.org/10.21172/1.152.09.

Shang, E.H.H., Wu, R.S.S., 2004. Aquatic hypoxia is a teratogen and affects fish embryonic development. Environ. Sci. Technol. 38, 4763–4767. https://doi.org/10.1021/es0496423.

Sharma, A., Hipel, K.W., Schweizer, V., 2020. Strategic insights into the Cauvery river dispute in India. Sustainability 12, 1286. https://doi.org/10.3390/su12041286.

Shengchen, W., Jing, L., Yujie, Y., Yue, W., Shiwen, X., 2021. Polystyrene microplastics-induced ROS overproduction disrupts the skeletal muscle regeneration by converting myoblasts into adipocytes. J. Hazard. Mater. 417, 125962 https://doi.org/10.1016/j.jhazmat.2021.125962 (ISSN, 0304-3894).

Shuliakevich, A., Muz, M., Oehlmann, J., Nagengast, L., Schröder, K., 2022. Assessing the genotoxic potential of freshwater sediments after extensive rain events – lessons learned from a case study in an effluent-dominated river in Germany. Water Res. 209, 117921 https://doi.org/10.1016/j.watres.2021.117921.

Skyberg, K., Skaug, V., Gylseth, B., Pederse, J.R., Iversen, O.H., 1990. Subacute inhalation toxicity of mineral oils, C_{15}–C_{20} alkylbenzenes, and polybutene in male rats. Environ. Res. 53, 48–61. https://doi.org/10.1016/s0013-9351(05)80129-x.

Slaats, G.G., Ghosh, A.K., Falke, L.L., Le Corre, S., Shaltiel, I.A., et al., 2014. Nephronophthisis-associated CEP164 regulates cell cycle progression, apoptosis and epithelial-to-mesenchymal transition. PLoS Genet. 10, e1004594 https://doi.org/10.1371/journal.pgen.1004594.

Sorrells, S., Toruno, C., Stewart, R.A., Jette, C., 2013. Analysis of apoptosis in zebrafish embryos by whole-mount immunofluorescence to detect activated caspase 3. J. Vis. Exp. 82, e51060 https://doi.org/10.3791/51060.

Speizer, F.E., Horton, S., Batt, J., Slutsky, A.S., 2006. Respiratory diseases of adults. In: Jamison, D.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.), Disease Control Priorities in Developing Countries, 2nd edition. Oxford University Press and The World Bank, pp. 681–695.

Susheela, S., Srikantaswamy, S., Kumar, D.D., Gowda, A., Jagadish, K., 2014. Study of Cauvery river water pollution and its impact on socio-economic status around KRS Dam, Karnataka, India. J. Earth Sci. Geotechn. Eng. 4, 91–109.

Takahashi, T., Liu, Z., Thevar, T., Burns, N., Mahajan, S., et al., 2020. Identification of microplastics in a large water volume by integrated holography and Raman spectroscopy. Appl. Opt. 59, 5073–5078. https://doi.org/10.1364/AO.393643.

Tallon, P., Magajna, B., Lofranco, C., Leung, K.T., 2005. Microbial indicators of faecal contamination in water: a current perspective. Water Air Soil Pollut. 166, 39–166. https://doi.org/10.1007/s11270-005-7905-4.

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science 371 (6525), 185–189. https://doi.org/10.1126/science.abd6951.

Ueda, T., Hayashi, M., Ohtsuka, Y., Nakamura, T., Kobayashi, J., et al., 1992. A preliminary study of the micronucleus test by acridine orange fluorescent staining compared with chromosomal aberration test using fish erythropoietic and embryonic cells. Water Sci. Technol. 25, 235–240. https://doi.org/10.2166/wst.1992.0297.

Wang, S., Chi, Q., Hu, X., Cong, Y., Li, S., 2019a. Hydrogen sulfide-induced oxidative stress leads to excessive mitochondrial fission to activate apoptosis in broiler myocardia. Ecotoxicol. Environ. Saf. 183, 109578 https://doi.org/10.1016/j.ecoenv.2019.109578.

Wang, S., Li, X., Wang, W., Zhang, H., Xu, S., 2019b. Application of transcriptome analysis: oxidative stress, inflammation and microtubule activity disorder caused by ammonia exposure may be the primary factors of intestinal microvilli deficiency in chicken. Sci. Total Environ. 696, 134035 https://doi.org/10.1016/j.scitotenv.2019.134035.

Wang, Z., Walker, G.W., Muir, D.C.G., Nagatani-Yoshida, K., 2020. Toward a global understanding of chemical pollution: a first comprehensive analysis of national and regional chemical inventories. Environ. Sci. Technol. 54, 2575–2584. https://doi.org/10.1021/acs.est.9b06379.

WHO, 2017. Guidelines for Drinking-water Quality, fourth ed. incorporating the 1st addendum. ⟨https://www.who.int/publications/i/item/9789241549950⟩, (Accessed 30 March 2019).

Wu, Y.T., Lin, C.Y., Tsai, M.Y., Chen, Y.H., Lu, Y.F., et al., 2011. β-Lapachone induces heart morphogenetic and functional defects by promoting the death of erythrocytes and endocardium in zebrafish embryos. J. Biomed. Sci. 18, 70. https://doi.org/10.1186/1423-0127-18-70.

Xian, H., Tang, M., Chen, Y., Lian, Z., Li, Y., et al., 2021. Indigenous fish–based assessment of genotoxic potentials of the Helong reservoir in Guangzhou, China. Environ. Toxicol. Chem. 1–9. https://doi.org/10.1002/etc.5043.

Xie, Z., Wang, M., Deng, Y., Li, J., Li, J., 2022. Acute toxicity of eucalyptus leachate tannins to zebrafish and the mitigation effect of Fe^{3+} on tannin toxicity. Ecotoxicol. Environ. Saf. 229, 113077 https://doi.org/10.1016/j.ecoenv.2021.113077.

Yao, J., Arey, B.W., Yang, L., Zhang, F., Komorek, R., et al., 2017. *In situ* characterization of Boehmite particles in water using liquid SEM. J. Vis. Exp. (127), e56058 https://doi.org/10.3791/56058 (10.3791/56058).

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). J. Hazard. Mater. 360, 97–105. https://doi.org/10.1016/j.jhazmat.2018.07.110.

Yu, H., Peng, J., Cao, X., Wang, Y., Zhang, Z., et al., 2021. Effects of microplastics and glyphosate on growth rate, morphological plasticity, photosynthesis, and oxidative stress in the aquatic species Salvinia cucullate. Environ. Pollut. 279, 116900 https://doi.org/10.1016/j.envpol.2021.116900.

Yu, L.M., Zhang, W.H., Han, X.X., Li, Y.Y., Lu, Y., et al., 2019. Hypoxia-induced ROS contribute to myoblast pyroptosis during obstructive sleep apnea via the NF-κB/HIF-1α signaling pathway. Oxid. Med. Cell. Longev. 4596368, 1–19. ⟨https://doi.10.1155/2019/4596368⟩.

Yu, X., Zuo, J., Li, R., Gan, L., Li, Z., et al., 2014. A combined evaluation of the characteristics and acute toxicity of antibiotic wastewater. Ecotoxicol. Environ. Saf. 106, 40–45. https://doi.org/10.1016/j.ecoenv.2014.04.035.

Yu, Y., Chen, H., Hua, X., Dang, Y., Han, Y., et al., 2020. Polystyrene microplastics (PS-MPs) toxicity induced stress and intestinal injury in nematode *Caenorhabditis elegans*. Sci. Total Environ. 726, 138679 https://doi.org/10.1016/j.scitotenv.2020.138679.

Yumnamcha, T., Devi, M.D., Roy, D., Nongthomba, U., 2020. Evaluation of developmental toxicity and genotoxicity of aqueous seed extract of *Croton tiglium* L. using zebrafish. Drug Chem. Toxicol. 6, 1–9. https://doi.org/10.1080/01480545.2019.1708094.

Yumnamcha, T., Roy, D., Devi, M.D., Nongthomba, U., 2015. Evaluation of developmental toxicity and apoptotic induction of the aqueous extract of *Millettia pachycarpa* using zebrafish as model organism. Toxicol. Environ. Chem. 97, 1363–1381. https://doi.org/10.1080/02772248.2015.1093750.

Zhao, X.J., Li, Z.P., Wang, J.H., Xing, X.M., Wang, Z.Y., et al., 2015. Effects of chelated Zn/Cu/Mn on redox status, immune responses and hoof health in lactating Holstein cows. J. Vet. Sci. 16, 439–446. https://doi.org/10.4142/jvs.2015.16.4.439.

Zhu, L., Mu, X., Wang, K., Chai, T., Yang, Y., et al., 2015. Cyhalofop-butyl has the potential to induce developmental toxicity, oxidative stress and apoptosis in early life stage of zebrafish (*Danio rerio*). Environ. Pollut. 203, 40–49. https://doi.org/10.1016/j.envpol.2015.03.044.