Evaluation of genotoxicity and hematological effects in common carp (*Cyprinus carpio)* induced by disinfection by-products

Samina Perveen, Imran Hashmi and Romana Khan

ABSTRACT

Disinfection is intended to improve drinking water quality and human health. Although disinfectants may transform organic matter and form disinfection by-products (DBPs), many are branded as cyto- and genotoxic. Traditionally, research focuses on the effects of DBPs on human health, but cytogenic impacts on aquatic organisms still remain ill defined. The current study examines the potential toxic effect of chloroform and iodoform (DBPs) on *Cyprinus carpio*, selected as a model organism. Fish specimens were exposed to various concentrations of DBPs primarily based on LD_{50} values, where acute toxicity was monitored for 96 h. Headspace SPME extraction through gas chromatography was employed to assess the effects of spiked DBPs doses in fish blood. Cytotoxicity was monitored using Comet assay. Tail length, tail DNA, and olive tail moment values were quantified to be significant ($P < 0.05$) as compared to control. A statistically significant ($P < 0.05$) decrease in all blood parameters (hematology) was observed. Changes in biochemical indices (glucose, total protein, and alanine aminotransferase (ALT)) were also significant. ALT secretion was significantly increased (93 ± 0.05 and 82.8 ± 0.1 U/L) at higher concentration compared to control (56 ± 0.1 U/L), suggesting liver damage. Results demonstrated that iodoform was statistically more damaging as compared to chloroform.

Key words | biochemistry, chloroform, Comet assay, disinfection by-products, hematology, iodoform

Samina Perveen
Imran Hashmi (corresponding author)
Romana Khan
Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE),
National University of Sciences and Technology (NUST),
H-12 sector, Islamabad,
Pakistan
E-mail: *imranhashmi@iese.nust.edu.pk*

INTRODUCTION

The aquatic environment plays an important role in the stability and maintenance of the ecosystem. However, the system is being polluted in natural and anthropogenic ways, releasing toxic chemicals and xenobiotics. These toxic pollutants accumulate and contaminate various parts of water through water transposition processes. A polluted environment may lead to nutritional, behavioral, and reproductive complications within aquatic organisms (Çok *et al.* 2011). Provision of clean drinking water for human consumption became the prime health advancement of the 20th century. One of the major accomplishments is drinking water disinfection to generate safe and high quality potable water (Muellner *et al.* 2010). The process improves water quality via reduction in pathogenic load. However, disinfectants (chlorine, ozone, UV, and chloramines) added during disinfection have been shown to react with naturally occurring organic matter to produce a wide range of disinfection by-products (DBPs) which show cytotoxic and carcinogenic effects within aquatic organisms (Gustavino *et al.* 2005). Humic acid interacts with disinfectants and produces toxic by-products. Diverse halogenated DBPs include trihalomethanes (THMs), haloacetonitrile (HANs), and haloacetic acids (HAAs).

Chloroform is one of the most predominant DBPs detected in water (Monarca *et al.* 2004). Iodoform is

doi: 10.2166/wh.2019.261

formed as a result of disinfection when HOI (hypoiodous acid) reacts with naturally occurring iodide ion (I^-) (Bichsel 2000). Iodinated disinfection by-products (I-DBPs) have been classified as emerging and unregulated by-products (Pan *et al.* 2016). I-DBPs are more cytotoxic and genotoxic than chlorinated and brominated correspondents (Richardson *et al.* 2007). The maximum contamination limit for iodoform is 0.02–5 μg/L (Allard *et al.* 2012). The Environmental Protection Agency (EPA) established a maximum contaminant level (MCL) of 0.07 mg/L for chloroform. A MCL of 0.08 mg/L for trihalomethanes has also been established (USEPA 2006).

Abbas *et al.* (2015) monitored chlorination DBPs in the water distribution network of Islamabad and Rawalpindi. They reported average concentration of 575 and 595 μg/L of total trihalomethanes (TTHMs) and chloroform, which exceeded the permissible limits of the EU (100 μg/L) and USA (80 μg/L). Concentration of THMs in drinking water samples from different sectors of Islamabad was monitored. The value of THMs ranged from 116.4 to 774.97 μg/L among different sampling sites, which exceeds the US-EPA drinking water quality standard (80 μg/L) for different TTHMs (Qaiser *et al.* 2014).

Residual chlorine leads to the formation of DBPs in the presence of natural organic matter (NOM) in Rawal Lake. Farooq *et al.* (2008) monitored the total amount of residual chlorine in Rawal Lake treatment plant which ranged from 0.9 to 1.11 mg/L. Further, they also documented via a survey that the water treatment plant applied a chlorine dosage of 2 mg/L for the disinfection process after filtration.

In vivo treatments/*in situ* exposure of aquatic organisms are the most utilized approach to detect mutagenicity and carcinogenicity in water. Several epidemiological studies support the evidence of the carcinogenic and mutagenic nature of DBPs. Different invertebrates have been used as biomarkers to assess pollutants within water systems. Klobučar *et al.* (2010) reported fish as a suitable biomarker, having the ability to tolerate adverse environments and high sensitivity to changing conditions. Fish are widely distributed aquatic vertebrates and are frequently used in water pollution monitoring as they bio-accumulate toxic pollutants and respond very rapidly to ecological disturbance and mutations (Russo *et al.* 2004). Biomarkers may forecast change at species to ecosystem level and be considered as an indicator of short-term fluctuations (Whyte *et al.* 2000). Diverse aquatic biomarkers have been used to evaluate the genotoxic nature of DBPs. Genotoxicity measures the DNA damage which is associated with DNA single- or double-strand breakage indicating the mutagenic and carcinogenic character of the pollutants. Comet assay is considered as a powerful tool in toxicological studies to evaluate DNA damage and repair in aquatic organisms (Mitchelmore & Chipman 1998). Comet assay does not require that cells be present in a dividing state, which makes it a promising technique compared to other *in vivo* treatments. Therefore, it is considered as a useful tool for aquatic bio-monitoring (Dixon *et al.* 2002).

Common carp (*Cyprinus carpio*) is a cool to temperate water fish species and a most suitable model indicator for toxicological studies because of its economic importance and its being a main constituent of the food chain in many areas of the world. Several studies have been conducted to investigate oxidative stress induced by different DBPs in common carp. Hepatic antioxidant enzyme and total glutathione were used as oxidative stress indicators when exposed to three different disinfectants (Elia *et al.* 2006). Gustavino *et al.* (2005) detected cytogenetic damage in common carp erythrocytes after exposure to chlorinated DBPs (sodium hypochlorite, chlorine dioxide, and peracetic acid) with humic acid interaction. Mattice *et al.* (1981) identified toxicity of chloroform ($CHCl_3$) along with other THMs as being environmentally critical and susceptible for aquatic life. The stress response of common carp toward polluted water was evaluated both in natural and artificial environments using Comet assay and micronucleus testing by Klobučar *et al.* (2010). Cytotoxicity of iodoacid (IA) in *Salmonella typhimurium* and Chinese hamster ovary cells was 2.9 and 53.5 times higher than brominated and chlorinated acids (Plewa *et al.* 2011).

The aim of this study was to investigate the genotoxicity of DBPs by integrating *in vivo* toxicology with two prevalent DBPs, chloroform and iodoform using Comet assay. Hematological and biochemical malignancies were examined at different concentrations for both compounds. A comparative study was also done to observe which of the two was more noxious in nature.

MATERIAL AND METHODS

Study area

Rawal Lake was the study area of the present study. The lake is situated east of Islamabad and north-east of Rawalpindi, Pakistan (33°42′ N, 73°07′ E). It provides water to both cities and covers an area of 8.8 km^2. The storage capacity of Rawal dam is 47,500-acre feet and it generates 84,000-acre feet of water on average rainfall. The site is under pressure from human settlements in Bhara Kahu, Malpur, Bani Gala, Noorpur Shahan, etc. Untreated municipal, mainly domestic and agricultural waste is directly dumped into the reservoir which increases the demand for disinfection. Thereby, the resulting toxicant by-products showed cyto- and genotoxic effects within aquatic organisms. Figure 1 clearly describes the study area.

Test materials and instruments

Standard analytes of chloroform and iodoform were purchased from Fluka (02487–5 mL) and Aldrich (109,452–5 g) with 99% purity. Standards were stored at −20 °C. Methanol with 99% purity for making dose suspensions was acquired from Merck (Germany). For Comet assay study, low melting point agarose (LMPA), normal melting point agarose (NMPA), and Tris–HCl for molecular biology grade was purchased from Scharlau (Spain). EDTA blood vacutainers were purchased from LABOVAC Italiano. For headspace analysis, SPME (75 μm Car-PDMS) fiber was obtained from Supelco (USA). Shimadzu GC-2010 (Japan) was used for gas chromatographic analysis equipped with electron capture detector (ECD). Hematology was analyzed using a fully automated Sysmex XP-100, whereas for biochemistry, an AMP Piccos II Chemistry Analyzer was used.

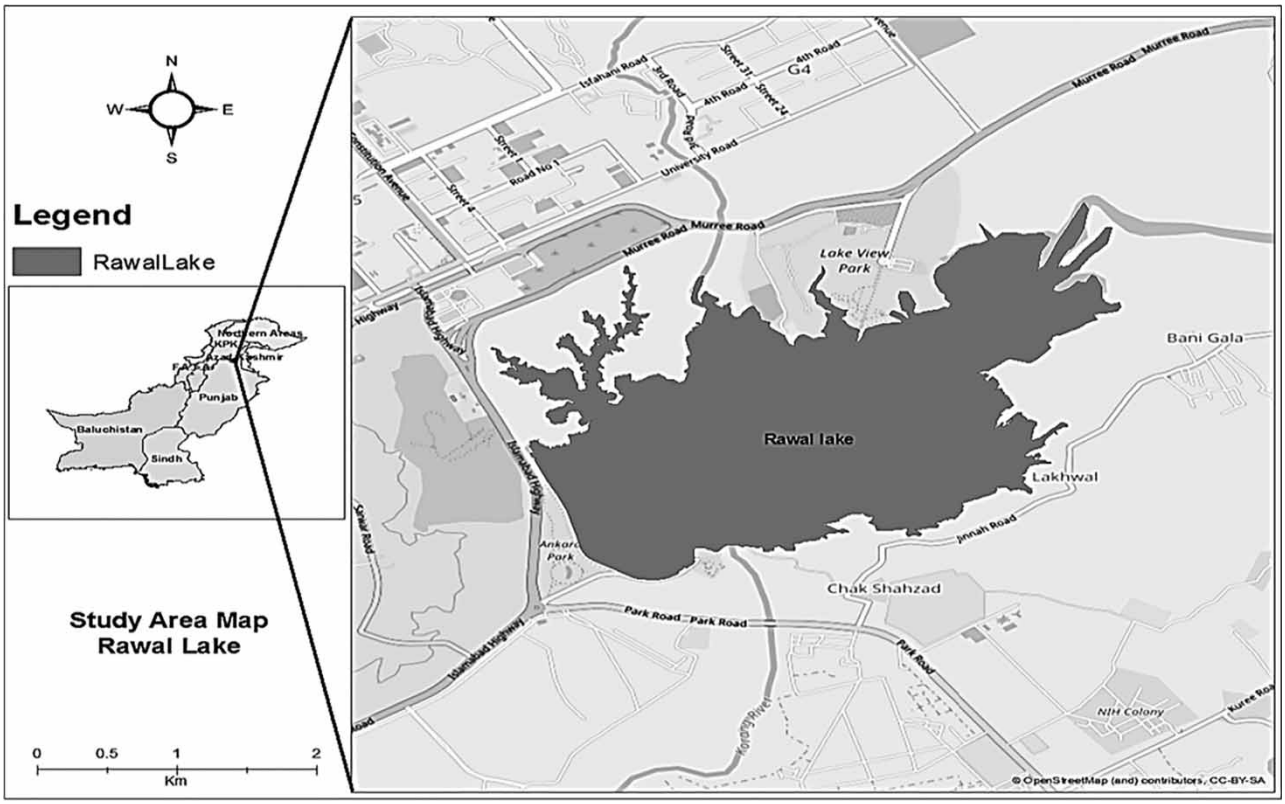

Figure 1 | Map of the study area.

Sample collection and fish care and handling

Healthy specimens (30–80 g, 14 cm, 60 days) were purchased from Punjab Hatchery Rawal Town (Aquaculture and Fisheries Program and Research Centre), Islamabad and transferred to oxygenated polyethylene bags. Special care was observed during transportation to the Environmental Toxicology Laboratory of IESE, National University of Sciences and Technology (NUST) and the fish were kept in glass tanks (dimension $3 \times 1.5 \times 1.5$ ft) containing 50 liters of tap water. Before commencement of the experiment, fish were acclimatized to laboratory conditions for a period of 2 weeks under control conditions (photoperiod of 12 days and night, proper aeration with 80% air saturation, room temperature 20–24 °C, etc.). They were fed with commercial food pellets containing soybean, rapeseed, rice, bran, corn, and wheat.

Water quality assessment

The physicochemical characteristic of experimental water was assessed using standard OECD (Organization for Economic Cooperation and Development) guideline method 203 (1992) (Table 1). Water quality was determined at the start of the experiment. The water was renewed every alternative day using tap water from the Environmental Toxicology Laboratory.

DBPs are strongly related to the concentration of total organic carbon (TOC) in water. Previous studies reported THMs' evolution as a function of different water quality parameters such as TOC, total dissolved solids (TDS), and residual chlorine concentration (Clark 1998; Chang *et al.* 2001; Jayana *et al.* 2009). Chowdhury *et al.* (2007) monitored water quality using different water parameters and found strong associations among TOC and formation of THMs.

Table 1 | Water quality parameters of experimental tanks

Measured parameters	Mean ± S.D.	Standard OECD guideline
Temperature (°C)	27.5 ± 0.2	20–24
pH	7.5 ± 0.1	6–8.5
Dissolved oxygen (mg/L)	4.5 ± 0.3	80% of air saturation
Hardness (mg/L)	222 ± 0.3	10–250 mg/L

Irfan & Khan (2009) studied the temporal and spatial variations in TOC in Rawal Lake, Islamabad, Pakistan, which is also the current study area. They reported temporal and spatial concentration of 24.08 and 16.6 mg/L of TOC in the studied lake.

Experiment design for determination of LD_{50}

A toxicity test was carried out to determine LD_{50} according to OECD guidelines 203 (1992). Based on a literature review, five varying concentrations were selected. For chloroform and iodoform, concentration range was defined as 30–90 and 1–3 mg/L, respectively. The fish were exposed to the defined concentrations for 96 h. The mortalities were observed after every 24 h and results recorded. On completion of the test, a single concentration was determined at which 50% of the population was killed.

Standard stock solutions

Standard stock solutions of chloroform and iodoform were prepared in methanol having a concentration of 200 and 100 ppm, respectively. The stock solutions were stored at −4 °C. Working solutions were prepared individually for each analyte from stock solutions with concentrations of 0.1, 1, 5, 10, 15, and 20 ppm to obtain linear calibration curves.

Chromatographic conditions

Calibration and optimization of chloroform and iodoform DBPs were conducted using a gas chromatography (Shimadzu GC-2010, Japan) system with fused silica capillary column (TRB-5 30 m × 0.32 mm × 1 μm with coating of 95% dimethylpolysiloxane) equipped with an electron capture detector (ECD). The optimized GC working conditions were column oven (200 °C), injector (220 °C), and detector temperature (280 °C), with flow of carrier gas (N_2) (3 mL/min).

Headspace solid-phase microextraction using gas chromatography

An optimization experiment to evaluate the effect of DBPs in fish was determined through headspace solid phase

microextraction technique (HS-SPME) using gas chromatography. Fish blood samples were fortified with optimized concentrations of chloroform and iodoform in glass vials having PVC (polyvinyl chloride) covers and 1.5 mm thick PTFE (polytetrafluoroethylene) skiving silicone septum. The analytes were extracted with divinylbenzene carboxen-polydimethlysiloxane 75 μm (DVB-CAR-PDMS-75 μm) fiber using hot plate magnetic stirrer. The stirring was done for 30 min at 40 °C. After the adsorption of analytes, the fiber was retracted back and inserted into the GC injection port where they were thermally desorbed under the flux of carrier gas. Recovery efficiency (RE) was calculated using EPA method 555.1 (Munch & Hautman 1995):

$$R = 100(A-B)/C$$

where A = total measured concentration; B = background concentration; C = the fortifying concentration.

Acute toxicity test

Acute toxicity testing was performed according to OECD guidelines 203 (1992). Fish were divided into control and experimental groups containing five fish per batch. Applied concentrations were administered based on previous LD_{50} test values. The exposed sub-lethal doses determined after LD_{50} range from 2.1 to 2.9 and 77.5 to 87.5 mg/L for iodoform and chloroform, respectively. Significant health effects were noted at all observed concentrations for both compounds. Prominent changes such as erratic swimming, restlessness and loss of equilibrium, corrosion of fins, skin injuries and accelerated respiration, cyto- and genotoxicity were observed in experimental groups compared to controls. Our results were in accordance with the findings of Ejraei *et al.* (2015), Ramesh *et al.* (2015), Blahova *et al.* (2014), Mikulikova *et al.* (2013), Imanpoor & Kabir (2011), and Bolognesi *et al.* (2004), who reported similar changes in common carp exposed to different toxic compounds.

The desired doses were applied to each experimental group and toxicity was observed for 24, 48, 72, and 96 h of exposure for chloroform and iodoform. After every 24 h, blood samples were collected from the caudal vein using a heparinized syringe from each experimental group. Blood samples were immediately collected in EDTA vacutainers and processed for genotoxicity, hematology, and biochemical parameters.

The Comet assay

Comet assay was performed according to the method listed by Singh *et al.* (1988) with slight modifications. Prior to blood collection, microscopic slides were pre-coated with 1% normal melting point agarose (NMP) and dried for 15 min. Cell suspension containing 80 μL of 2% low melting point agarose (LMP) with 20 μL of blood sample were placed on pre-coated slides. The slides were solidified for 20 min on an ice box. Another layer of NMP agarose was deposited to fill any air cavities remaining during the second layering. Solidified slides were then placed in lysing solution containing high salts and detergents for 1–2 h (to dissolve the cellular membranes and liberate the DNA). The pH of the solution was adjusted to 10. After lysing, step slides were rinsed with distilled water and placed in alkaline buffer solution (pH > 13) for 20 min at room temperature to develop single-stranded DNA prior to electrophoresis. After alkaline unwinding, the slides were electrophoresed in the same buffer solution (pH > 13) for 30 min at 24 V and 300 mA to produce comets. Electrophoresis allows cell transfer indicating DNA damage. After electrophoresis, the slides were neutralized for 10–15 min with neutralization solution (pH 7.5). The process was repeated three to four times to produce high background for scoring of slides. At the end, slides were stained with 80 μL of fluorescent dye ethidium bromide (EtBr) three to four times and dried at room temperature. Slides were then visualized under a trinocular fluorescence microscope (Optika-B353FL) to determine genetic damage through comet tail, tail DNA length, and olive tail moment.

Hematological and biochemical analysis

Blood samples from each experimental group were collected in EDTA vacutainers through cardiac puncture using a 5 mL heparinized syringe. Over 96 h, test blood samples were collected every 24 h. Vials were slightly vortexed to avoid formation of clots. For blood serum production, the vials were centrifuged for 10 min at 400 rpm. Hematological indices including white blood cells (WBC), red blood cells

(RBC), platelets (PLT), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hgb) were examined. Biochemical profile was analyzed through glucose, total protein, and ALT indices.

Statistical analysis

CaspLab software was used to analyze comet images. The data were subjected to two-way analysis of variance (ANOVA) to determine significance of difference among control and experimental groups. P-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Gas chromatographic analysis

Linear calibration curves with regression coefficient (R^2) of 0.966 and 0.962 were obtained after gas chromatographic analysis for chloroform and iodoform, respectively, as prescribed in Figure 2(a) and 2(b).

Respective chromatographic peaks at concentration of 20 mg/L for chloroform and iodoform are reported in Figure 2(a) and 2(b). The well-resolved and identifiable chromatographic peaks at retention time 2.1 and 10.8 min were obtained for chloroform and iodoform, respectively. The analysis run time was 18 min.

HS-SPME analysis

HS-SPME analysis using GC/ECD was optimized through DVD-CAR-PMDS fiber with 30 min extraction time at 40 °C. The method validation was determined with relative recovery (R) efficiency using EPA method 555.1 (Delvaux *et al.* 2017). The results for recovery efficiency percentage for both DBPs at each concentration are presented in Table 2.

Recovery efficiency (R) within acceptable range (68–95% and 74–83%) was obtained for iodoform and chloroform, respectively. This verifies that HS-SPME is an accurate, fast, and reproducible technique for determining DBPs in fish blood samples. The results for recovery efficiency were in accordance with Delvaux *et al.* (2017), who reported percentage recovery efficiency of 76–120% for four different THMs (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) present in fish blood samples. Thus, it was confirmed that HS-SPME has adequate accuracy for the determination of different THMs in fish.

Khan *et al.* (2018) compared HS-SPME and LLE (liquid liquid extraction) techniques to analyze the response of THMs. Significant ($P < 0.1$) increase in peak areas for HS-SPME compared to LLE showed that HS-SPME was a more accurate, fast, and reproducible extraction technique for trihalomethanes. Iglesias & Medina (2008) determined volatile compounds from fish muscles through the HS-SPME extraction technique followed by polydimethlysiloxane fiber.

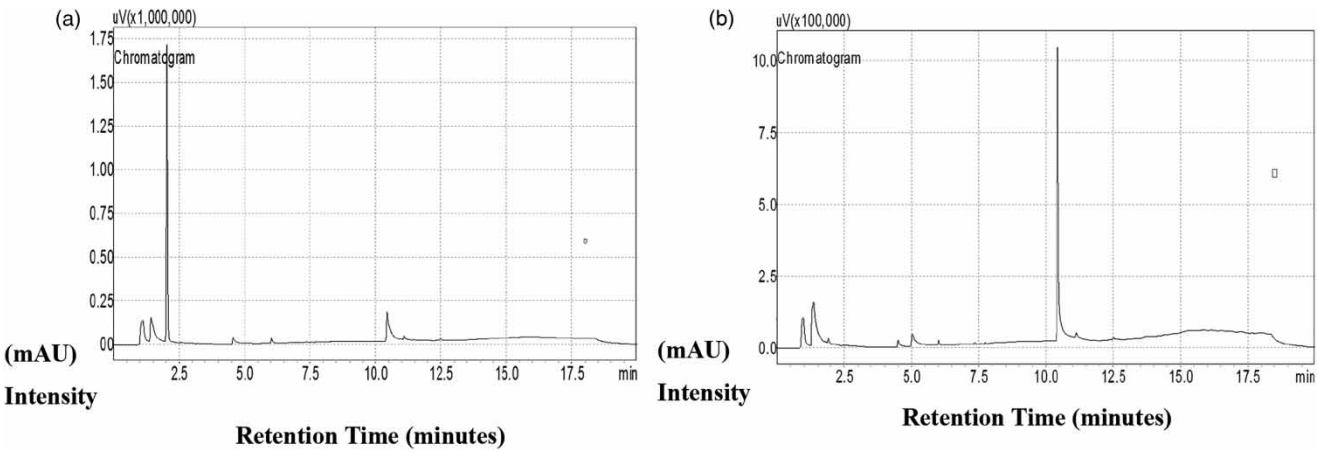

Figure 2 | Chromatographic peaks of chloroform (a) and iodoform (b) at concentration 20 ppm.

Table 2 | Percentage recovery efficiency (R) of chloroform and iodoform at each specified concentration

Compounds	Concentration (mg/L)	% Recovery efficiency (R)
Iodoform	2.1	95
	2.3	86
	2.5	80
	2.7	74
	2.9	68
Chloroform	77.5	83
	80	81
	82.5	78
	85	76
	87.5	74

They documented efficient and accurate extraction at 60 °C for 30 min. Moreover, they demonstrated that SPME is a simple, fast, and useful technique in monitoring targeted compounds within fish.

Acute toxicity test

LD_{50} for iodoform and chloroform was found to be 90 and 3 mg/L.

The results were in accordance with Mattice *et al.* (1981), who determined LD_{50} for chloroform, bromodichloromethane, dibromochloromethane, and bromoform (97.2, 67.4, 33.5, and 52.3 mg/L) exposed to common carp juveniles. LD_{50} of 2.92 mg/L was determined after 96 h for fathead minnow (*Pimephales promelas*) exposed to iodoform DBP (Brooke *et al.* 1984).

Fisher *et al.* (2014) determined acute and chronic toxicity of different DBPs such as dibromochloromethane, four haloacetic acids, and sodium bromate in fish. Acute toxicity of 96 h exposure LD_{50} of tribromoacetic acid was found in the range of 376.4 mg/L for *Cyprinodon variegatus* and 46.8 mg/L of both dibromochloromethane and sodium bromate for *Daphnia magna*. Teixidó *et al.* (2015) determined LD_{50} of 286.5 mg/L and EC_{50} of 100.3 mg/L of chloroform in zebra fish after 96 h of exposure. Toussaint *et al.* (2001) determined 96 h LD_{50} of 138 mg/L for chloroform in Japanese medaka fish.

Behavioral observations

The present study showed that the exposure of DBPs to relatively higher concentrations (2.5–2.9 and 82.5–87.5 mg/L for iodoform and chloroform, respectively) could negatively affect the health status of exposed specimens (50 (5 fishes/batch)) of common carp. Behavioral changes were noted in experimental tanks at all observed concentrations for both compounds in comparison to control groups. Prominent behavioral changes were observed at higher concentrations (2.9 and 87.5 mg/L of iodoform and chloroform) after 24 h exposure. Mild changes such as restlessness, abnormal swimming, and sluggish movement were observed after 48 h exposure at lower concentrations (2.1–2.3 and 77.5–80 mg/L for iodoform and chloroform, respectively). Whereas, corrosion of fins, skin injuries and signs of behavior associated with anxiety were observed at medium concentrations for both DBPs. On the other hand, sudden changes, such as erratic swimming, lethargy, skin injuries, increase in the frequency of opercular movements, and loss of equilibrium were noted at higher concentration (2.5–2.9 and 82.5–87.5 mg/L for iodoform and chloroform, respectively).

Our results are in line with the study conducted by Blahova *et al.* (2014), who reported similar behavioral changes in common carp (*Cyprinus carpio*). Similar trends of uncoordinated behavior were also reported by Imanpoor & Kabir (2011) in common carp after 96 h exposure to sublethal concentrations of chloramines-T in the range of 1–60 mg/L.

Table 3 | Comet parameter values for iodoform after 96 h acute exposure

		Concentrations (mg/L)				
Comet parameters	Control	2.1	2.3	2.5	2.7	2.9
Tail length (μm)	4.4 ± 0.04	11 ± 0.3	17 ± 0.2	25 ± 0.3	31 ± 0.3	41 ± 0.4
Tail DNA (%)	0.09 ± 0.03	5.1 ± 0.1	5.4 ± 0.2	7.8 ± 0.01	17.6 ± 0.3	23.1 ± 0.3
Olive tail moment	0.04 ± 0.01	1.2 ± 0.05	2.1 ± 0.3	3.9 ± 0.1	5.6 ± 0.3	11.3 ± 0.3

Table 4 | Comet parameter values for chloroform after 96 h acute exposure

		Concentrations (mg/L)				
Comet parameters	**Control**	**77.5**	**80**	**82.5**	**85**	**87.5**
Tail length (μm)	3.2 ± 0.1	6 ± 0.4	12 ± 0.4	15 ± 0.4	18 ± 0.2	22 ± 0.4
Tail DNA (%)	0.07 ± 0.01	1.1 ± 0.3	2.3 ± 0.2	3.7 ± 0.1	7.5 ± 0.1	7.9 ± 0.07
Olive tail moment	0.04 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.34 ± 0.02	0.67 ± 0.02	0.79 ± 0.01

Comet assay

Genotoxicity using Comet assay may present as a suitable biomarker for assessment of pollutants in aquatic organisms (González-Mille *et al.* 2010). The data obtained from Comet assay are presented as median of tail length, tail DNA, and olive tail moment in Tables 3 and 4. The comparison of data obtained from Comet assay as DNA migration at the beginning of exposure concentration (2.1 and 77.5 mg/L) and at the end of exposure concentration (2.9 and 87.5 mg/L) for both compounds (iodoform and chloroform) was statistically significant as compared to control groups.

The sensitivity of Comet assay was increased with increase in concentration and exposure time for both DBPs, where genotoxic signals increased significantly. Significantly higher DNA migration in zebra mussel cells was observed with increased disinfectants in surface drinking water (Bolognesi *et al.* 2004). Toxicity of chloroform and carbon tetrachloride in rainbow trout hepatocytes revealed that DNA single-strand breakage in treated cells was in accordance with higher toxicity (Råbergh & Lipsky 1997).

Statistical analysis shows that when comet parameters including tail length, tail DNA, and olive tail moment were compared for both compounds, a significant increase was observed in the case of iodoform as compared to chloroform. The significant increase in the tail length is determined through the migration of DNA toward the tail region which is quantified by fluorescence, measured as tail length (μm). DNA percentage present in the tail region also quantifies the amount of strands' breakage, which increases with increased dose concentration (Mitchelmore & Chipman 1998). Klobučar *et al.* (2010) detected significant increase in total percentage of tail DNA damage in crayfish juveniles at polluted sites (7.34 ± 1.25%, Zagreb 8.99 ± 0.88%, Sisak 14.17 ± 1.70%) compared to the reference site (4.46 ± 0.30%). The same trend (increased toxicity with higher concentration) was observed in olive tail moment (OMT) which shows a great advantage of Comet assay, as it presents DNA damage and migration of genetic material as a single unit (Theodorakis *et al.* 1994).

The relationship between % DNA damage and varying concentrations of iodoform and chloroform against tail length, tail DNA, and olive tail moment are presented in Figures 3 and 4, respectively. It is shown that comet parameters showed a direct relationship with respect to dose concentration and % DNA damage response which means that with the increase in dose concentration the DNA damage in terms of tail length, tail DNA, and OMT was also increased.

Tail length is considered to be one of the most important parameters to assess the DNA damage (Kumaravel & Jha 2006). It was observed that there is an increasing trend in dose-dependent manner for both DBPs for tail length. The mean tail length values for iodoform and chloroform (11, 17, 25, 33, 41 and 6, 12, 15, 18.3, 22 μm) were significantly increased as compared to control (4.4 μm). Notable damage in comet parameters (tail length, tail intensity, and tail moment) was observed in common carp exposed to pollutants in Lake Mogan (Çok *et al.* 2011).

Disinfection by-products indirectly affect human beings, as a bioaccumulation factor in fish. Exposure of trihalomethanes ($CHCl_3$, $CHCl_2Br$, $CHBr_3$, and CH_2Cl_2) to human cells showed a decrease in the cell viability, approximately 50% reduction in cell number for all treatments at higher concentrations. $CHCl_2Br$ and $CHCl_3$ were the most potent genotoxins among treated THMs and caused DNA damage through tail extent moment (Landi *et al.* 2003). The current study demonstrates the usefulness of Comet assay as a suitable tool for genotoxicity related to DBPs using common carp as model organism.

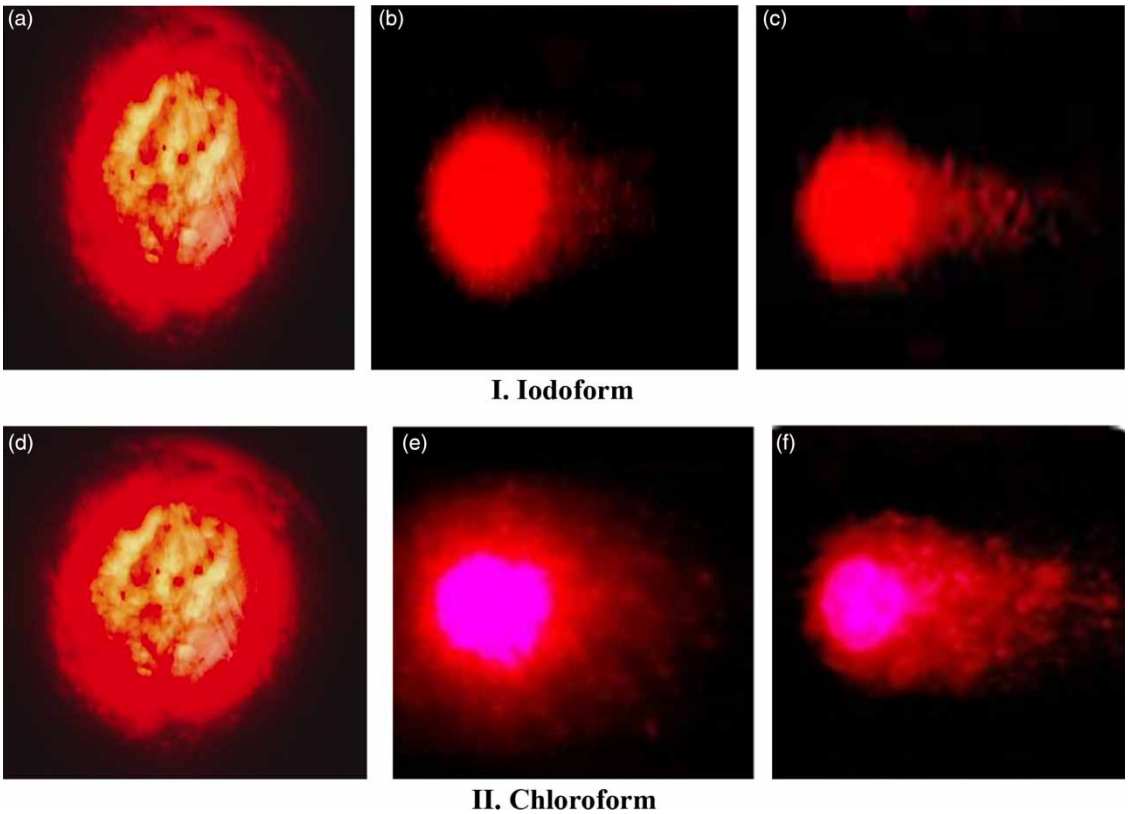

Figure 3 | Comet assay applied in common carp specimens: (a and d) without DNA damage, (b and e) low level DNA damage, (c and f) DNA damage with long tail for iodoform and chloroform. I. Iodoform. II. Chloroform.

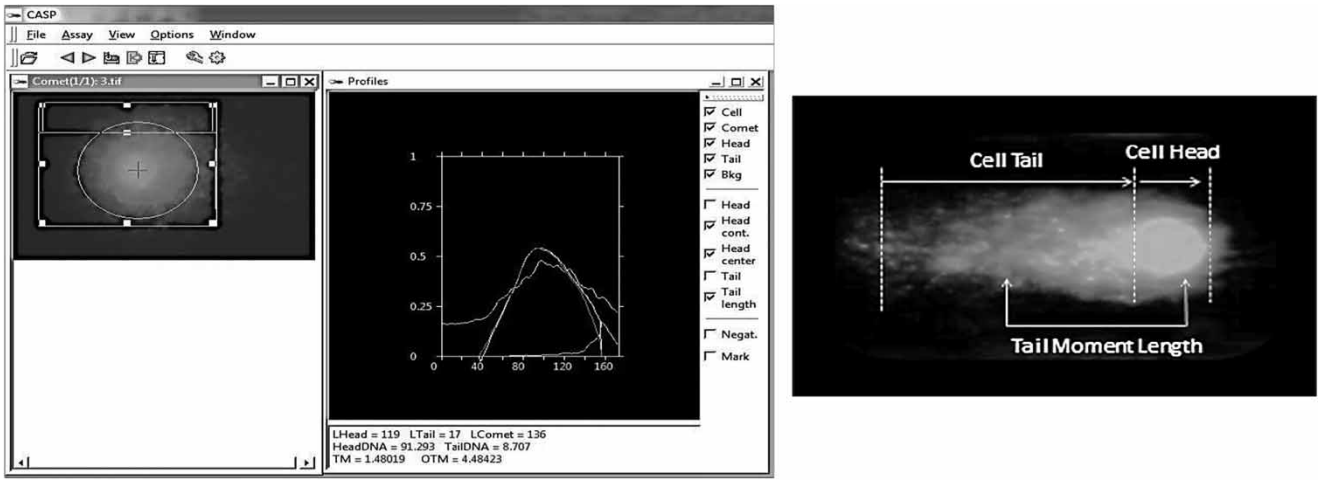

Figure 4 | Calculation of comet parameters (tail length, tail DNA%, and OMT) using CASPLAB software.

Hematological analysis

The stress induced in carp juveniles reflected the hematology of experimental groups. The change in water quality under the effect of toxicants alters the hematology of aquatic life through physiological changes (Van Vuren 1986). Therefore, water quality becomes one of the important factors for discrepancies in fish hematology, being closely related to

Table 5 | Hematological indices values for iodoform after 96 h acute exposure

		Concentrations (mg/L)				
Hematological indices	**Control**	**2.1**	**2.3**	**2.5**	**2.7**	**2.9**
WBC ($\times10^3/\mu L$)	119.5 ± 0.3	74.2 ± 0.05	67.4 ± 0.25	58.2 ± 0.05	43.1 ± 0.10	32.7 ± 0.05
RBC ($\times10^6/\mu L$)	0.38 ± 0.01	0.29 ± 0.01	0.25 ± 0.01	0.21 ± 0.03	0.17 ± 0.01	0.06 ± 0.01
HGB (g/dL)	5.3 ± 0.04	2.18 ± 0.01	2.15 ± 0.01	2.11 ± 0.03	1.8 ± 0.05	1.1 ± 0.05
HCT (%)	7.7 ± 0.05	3.6 ± 0.1	3.1 ± 0.2	2.7 ± 0.11	1.9 ± 0.15	1.3 ± 0.20
PLTS ($\times10^6/\mu L$)	98 ± 0.1	45 ± 0.2	42 ± 0.2	37 ± 0.2	31 ± 1	24 ± 0.1
MCV (fL)	192.5 ± 0.5	110.8 ± 0.05	105.6 ± 0.05	101.3 ± 0.05	93.2 ± 0.05	88.4 ± 0.04
MCH (pg)	67.1 ± 0.05	32.5 ± 0.04	31.3 ± 0.05	27.4 ± 0.05	22.8 ± 0.15	19.6 ± 0.20
MCHC (g/dL)	67.5 ± 0.05	53.1 ± 0.05	50.2 ± 0.05	46.1 ± 0.5	43.9 ± 0.2	37.3 ± 0.3

WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; PLTS, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 6 | Hematological indices values for chloroform after 96 h acute exposure

		Concentrations (mg/L)				
Hematological indices	**Control**	**77.5**	**80**	**82.5**	**85**	**87.5**
WBC ($\times10^3/\mu L$)	119.5 ± 0.3	87.3 ± 0.3	81.1 ± 0.1	78.5 ± 0.2	72.4 ± 0.4	67.8 ± 0.5
RBC ($\times10^6/\mu L$)	0.38 ± 0.02	0.26 ± 0.05	0.21 ± 0.04	0.17 ± 0.03	0.14 ± 0.03	0.11 ± 0.04
HGB (g/dL)	5.3 ± 0.07	4.4 ± 0.2	4.1 ± 0.12	3.8 ± 0.07	3.1 ± 0.12	2.1 ± 0.1
HCT (%)	7.7 ± 0.1	6.1 ± 0.4	5.5 ± 0.20	5.1 ± 0.41	4.6 ± 0.25	4.1 ± 0.3
PLTS ($\times10^6/\mu L$)	98 ± 0.4	80 ± 1.5	76 ± 0.01	65 ± 0.01	53 ± 1.5	41 ± 1.5
MCV (fL)	192.5 ± 0.6	180.1 ± 0.3	177.6 ± 0.3	173.2 ± 0.3	166.4 ± 0.2	154.3 ± 0.2
MCH (pg)	67.1 ± 0.3	58.5 ± 0.05	51.4 ± 0.05	48.7 ± 0.05	43.1 ± 0.2	39.8 ± 0.05
MCHC (g/dL)	67.5 ± 0.15	51.3 ± 0.2	46.4 ± 0.2	42.7 ± 0.1	38.9 ± 1.4	33.1 ± 0.2

environment and sensitive to fluctuations occurring within habitats.

Results obtained after 96 h acute toxicity testing are presented in Tables 5 and 6. Acute exposure of iodoform and chloroform results in significant changes in all parameters of hematological indices. Significant changes were observed in all experimental groups exposed to the highest concentrations, whereas a non-significant effect was observed in the lowest applied concentrations. A significant decrease ($P < 0.05$) as compared to the control was observed at higher concentrations (87.5 and 2.9 mg/L) for iodoform and chloroform, respectively. Statistical analysis interprets that when hematological parameters were compared for both compounds, significantly higher values were observed in the case of iodoform as compared to chloroform.

Blood parameters including hemoglobin, hematocrit, and red blood cells are considered as an indication of the health status and oxygen-carrying capacity of fish (Tavares-Dias 2006). The significant decrease ($P < 0.05$) in the level of said parameters (red blood cells, hemoglobin, and hematocrit) compared to control, suggests that the common carp was highly adapted to anemic conditions prevailing in Rawal Lake (natural habitat) as a result of increased chlorination demand.

The mean decrease in MCV (88.4 ± 0.04 and 154.3 ± 0.25 fL) compared to control (192.5 ± 0.6 fL) presents the

number and size of RBC and abnormality in the cell division process, thus supporting the significant decrease of red blood cell count ($P < 0.05$). Decreases in MCV count under the effect of toxicants determine physiological changes in one or more hematological parameters of fish (Nussey 2000). White blood cells are the defensive cells of the body which protect it against infections (Tavares-Dias 2006). The responses of WBC and platelets confirm that under the effect of the applied dose, the values of both parameters significantly decreased ($P < 0.05$) at all concentrations after 96 h exposure. Decrease in all hematological indices was observed in Indian catfish exposed to vanadium metal (Chakraborty *et al.* 1998). Ejraei *et al.* (2015) reported marked variability in hematological and blood plasma indices of grass carp under the effect of age and hormonal treatments.

Statistical analysis through two-way ANOVA of blood indices revealed significant variance ($P < 0.05$) in all parameters. The decrease in the level of MCH and MCHC as compared to control is an indication of less synthesis of hemoglobin under stress, prompted by acute exposure of iodoform and chloroform. A decrease in the level of MCH in Chinese grass carp under the exposure of mercuric chloride suggested physiological stress (Shakoori *et al.* 1994). The results suggested that the observed change in hematological indices was attributed to the aggressive mechanism of carp juvenile against acute exposure. Further, it is due to disruption occurring in their metabolic and physiological processes.

Biochemical analysis

Biochemistry of fish blood was evaluated to examine toxicant level and physiological impacts. Biochemical indices such as glucose, total protein, and ALT were used to monitor stress caused by environmental toxicants. Mean values ± standard deviation of statistical data recorded in all experimental groups are presented in Tables 7 and 8.

Our investigation of blood biochemical indices reveals predominant alterations in all treatments after acute exposure of chloroform and iodoform. Glucose and total protein level in all treatments as compared to control were decreased significantly. The change in glucose level has been related to the feeding habits and normal activities of fish; therefore, its level is high in active fish (56.7 ± 0.14 mg/dL) that were not exposed. Significant decrease in glucose at all treatments (40.6 ± 0.05–15.3 ± 0.15 and 39.1 ± 0.2–19.4 ± 0.1 mg/dL) was observed for both iodoform and chloroform, respectively. The difference was significantly higher in the case of iodoform as compared to chloroform experimental groups. Further, it reflects the stress response of exposed juveniles as a consequence of intense metabolic activity. These changes in metabolic processes are

Table 7 | Biochemical indices values for iodoform after 96 h acute exposure

		Concentrations (mg/L)				
Biochemical indices	**Control**	**2.1**	**2.3**	**2.5**	**2.7**	**2.9**
GLU (mg/dL)	56.7 ± 0.1	40.6 ± 0.05	37.3 ± 0.1	30.9 ± 0.05	25.1 ± 0.2	15.3 ± 0.1
TP (g/dL)	35 ± 0.3	26.3 ± 0.05	21.5 ± 0.1	18.3 ± 0.1	11.4 ± 0.1	9.3 ± 0.1
ALT (U/L)	56 ± 0.2	67.3 ± 0.1	70.8 ± 0.05	77.3 ± 0.1	80.9 ± 0.2	93.6 ± 0.05

GLU, glucose; TP, total protein; ALT, alanine aminotransferase.

Table 8 | Biochemical indices values for chloroform after 96 h acute exposure

		Concentrations (mg/L)				
Biochemical indices	**Control**	**77.5**	**80**	**82.5**	**85**	**87.5**
GLU (mg/dL)	56.7 ± 01	39.1 ± 0.2	33.4 ± 0.05	28.9 ± 0.1	21.7 ± 0.1	19.4 ± 0.1
TP (g/dL)	35 ± 0.1	26.1 ± 0.1	21.3 ± 0.1	19.6 ± 0.1	13.2 ± 0.1	10.3 ± 0.1
ALT (U/L)	56 ± 0.1	65.1 ± 0.1	69.3 ± 0.1	74.1 ± 0.1	78.9 ± 0.1	82.8 ± 0.1

characterized through a decrease in liver glycogen and serum cortisol levels (Cicik & Engin 2005).

The significant decrease in the level of total proteins (9.3 ± 0.1 and 10.3 ± 0.1 g/dL for iodoform and chloroform, respectively) at 96 h as compared to the control (35 ± 0.1 g/dL) was due to increased demand and lessened synthesis of protein. Under stressed conditions, fish mobilized synthesized protein to all parts of the body to encounter energy demands and to maintain physiological activities, which decrease its level. The results were in accordance with Ramesh *et al.* (2015), who determined the toxic impacts of furadan pesticide on hematological and biochemical parameters in common carp. They reported a significant decrease in the level of plasma glucose and protein under higher concentrations of toxic chemical. Blood chemistry in many fish species was assessed previously. Salmonids, including golden and rainbow trout, showed lower total protein, potassium, and calcium levels (Hille 1982). The decrease in the level of total protein and glucose was observed in cyprinid fish species (Groff & Zinkl 1999). Physiological (total protein) and immunological (hepatocytes) count was investigated in caged cray fish as a biomarker of undergoing stress (Klobučar *et al.* 2010).

Liver is considered as the main targeted organ, which quickly responds and bio-accumulates toxicants. The significant increase {67.3 ± 0.1–93.6 ± 0.05 and 65.1 ± 0.1–82.8 ± 0.1 (U/L) for iodoform and chloroform, respectively} in ALT secretion ($P < 0.05$) at all concentrations after 96 h exposure was observed. Statistical analysis showed that ALT secretion was increased with increased dose concentration for both compounds. Secretion of ALT in higher amounts indicates liver function disorder in exposed common carp. This was attributed to modification in the metabolism process which causes changes in liver histology and specifies liver damage.

Our results were in accordance with Råbergh & Lipsky (1997), who determined hepatocyte toxicity in fish by measuring the released concentrations of LDH, GSH, and protein after acute exposure of chloroform and carbon tetrachloride. At higher concentration of $CHCl_3$, total lysis of cells and release of LDH, GSH was noted and completely depleted. Ozmen *et al.* (2008) monitored xenobiotic and organochlorine (OC) in Sariyar Dam Lake, Turkey and reported increased enzymatic activity (ALT, AST, LDH) in exposed fish liver (*Cyprinus carpio* and *Capoeta tinca*) during the spring and autumn seasons.

Mikulikova *et al.* (2013) recorded an increase in the activities of all enzymes (ALT, AST, LDH, GSH) which were important indicators of liver damage. Elia *et al.* (2008) studied alterations in biochemical parameters (hepatic antioxidant enzymes and total glutathione) in carp liver under different disinfectants. They reported significant increase in enzymatic activity after exposure to chlorine compound as compared to peracetic acid disinfectants. Based on findings, it was observed that fish hepatocytes were sensitive toward chloroform and iodoform DBPs.

CONCLUSIONS

The conclusions are as follows:

- LD_{50} was determined to be 3 and 90 mg/L for iodoform and chloroform, respectively.
- Recovery efficiency was within acceptable range (74–83% and 68–95%) to quantify that HS-SPME is an accurate and efficient technique for determining DBPs within fish blood samples.
- Comet assay demonstrates significant ($P < 0.05$) DNA damage, depicted via tail length, tail DNA, and olive tail moment.
- Acute toxicity manifested that both iodoform and chloroform (DBPs) are toxic to common carp and cause significant changes in hematological and biochemical parameters. Significant alterations were observed in hepatocyte and cell counts.
- Statistical data represent that iodoform is more toxic and causes significant changes in common carp as compared to chloroform, confirming the hepatotoxic nature of iodoform.

Recommendations

Common carp continuously exposed to DBPs are dangerous and hazardous to human consumption if these DBPs exceed WHO standard limits (<80 μg/L). Therefore, histopathological studies are recommended to find morphological alterations in fish tissues due to emerging and unregulated

DBPs. Similar studies related to defense mechanisms of fish against emerging DBPs are also recommended.

ACKNOWLEDGEMENTS

The authors are grateful to the Institute of Environmental Sciences and Engineering and Environmental Toxicology Laboratory of National University of Sciences and Technology (NUST) for providing assistance throughout the research work. The authors also acknowledge support from Higher Education Commission (HEC), National Research Program for Universities (NRPU) (5995/Federal/NRPU/R&D/HEC/2016) and postgraduate research fund for providing financial assistance. The authors acknowledge the useful contribution provided by diagnostic laboratory of Pakistan Institute of Medical Sciences (PIMS) throughout the work.

REFERENCES

Abbas, S., Hashmi, I., Rehman, M. S. U., Qazi, I. A., Awan, M. A. & Nasir, H. 2015 Monitoring of chlorination disinfection by-products and their associated health risks in drinking water of Pakistan. *Journal of Water and Health* **13** (1), 270–284.

Allard, S., Charrois, J. W., Joll, C. A. & Heitz, A. 2012 Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry. *Journal of Chromatography A* **1238**, 15–21.

Bichsel, Y. 2000 *Behavior of Iodine Species in Oxidative Processes During Drinking Water Treatment*. Doctoral dissertation, ETH Zurich, Switzerland.

Blahova, J., Modra, H., Sevcikova, M., Marsalek, P., Zelnickova, L., Skoric, M. & Svobodova, Z. 2014 Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp (*Cyprinus carpio* L.) after acute exposure to atrazine herbicide. *BioMed Research International* **2014** (4), 980948.

Bolognesi, C., Buschini, A., Branchi, E., Carboni, P., Furlini, M., Martino, A. & Rossi, C. 2004 Comet and micronucleus assays in zebra mussel cells for genotoxicity assessment of surface drinking water treated with three different disinfectants. *Science of the Total Environment* **333** (1–3), 127–136.

Brooke, L. l., Call, D. J., Geiger, D. L. & Northcott, C. E. (eds) 1984 *Acute Toxicities of Organic Pollutants to Fathead Minnow (Pimephales promelas)*, Vol. 1. Center for Lake Superior Environmental Studies, Superior, WI, USA.

Chakraborty, A., Oinam, S., Karmakar, R. & Chatterjee, M. 1998 Vanadium toxicology – an assessment of general health, haematological aspects and energy response in an Indian catfish *Clarias batrachus* (Linn). *Biometals* **11** (2), 95–100.

Chang, E. E., Chiang, P. C., Ko, Y. W. & Lan, W. H. 2001 Characteristics of organic precursors and their relationship with disinfection by-products. *Chemosphere* **44** (5), 1231–1236.

Chowdhury, S., Champagne, P. & Husain, T. 2007 Fuzzy risk-based decision-making approach for selection of drinking water disinfectants. *Journal of Water Supply: Research and Technology-AQUA* **56** (2), 75–93.

Cicik, B. & Engin, K. 2005 The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). *Turkish Journal of Veterinary and Animal Sciences* **29** (1), 113–117.

Clark, R. M. 1998 Chlorine demand and TTHM formation kinetics: a second-order model. *Journal of Environmental Engineering* **124** (1), 16–24.

Çok, İ., Ulutas, O. K., Okusluk, Ö., Durmaz, E. & Demir, N. 2011 Evaluation of DNA damage in common carp (*Cyprinus carpio* L.) by comet assay for determination of possible pollution in Lake Mogan (Ankara). *The Scientific World Journal* **11**, 1455–1461.

Delvaux Jr., N. A., de Queiroz, M. E., Neves, A. A., Oliveira, A. F., da Silva, M. R., Faroni, L. R. & Heleno, F. F. 2017 Headspace solid phase microextraction-gas chromatography for the determination of trihalomethanes in fish. *Microchemical Journal* **133**, 539–544.

Dixon, D. R., Pruski, A. M., Dixon, L. R. & Jha, A. N. 2002 Marine invertebrate eco-genotoxicology: a methodological overview. *Mutagenesis* **17** (6), 495–507.

Ejraei, F., Ghiasi, M. & Khara, H. 2015 Evaluation of hematological and plasma indices in grass carp, *Ctenopharyngodon idella*, with reference to age, sex, and hormonal treatment. *Archives of Polish Fisheries* **23** (3), 163–170.

Elia, A. C., Anastasi, V. & Dörr, A. J. M. 2006 Hepatic antioxidant enzymes and total glutathione of *Cyprinus carpio* exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. *Chemosphere* **64** (10), 1633–1641.

Elia, A. C., Fanetti, A., Dörr, A. J. M. & Taticchi, M. I. 2008 Effects of concentrated drinking water injection on glutathione and glutathione-dependent enzymes in liver of *Cyprinus carpio* L. *Chemosphere* **72** (5), 791–796.

Farooq, S., Hashmi, I., Qazi, I. A., Qaiser, S. & Rasheed, S. 2008 Monitoring of coliforms and chlorine residual in water distribution network of Rawalpindi, Pakistan. *Environmental Monitoring and Assessment* **140** (1–3), 339–347.

Fisher, D., Yonkos, L., Ziegler, G., Friedel, E. & Burton, D. 2014 Acute and chronic toxicity of selected disinfection byproducts to *Daphnia magna*, *Cyprinodon variegatus*, and *Isochrysis galbana*. *Water Research* **55**, 233–244.

González-Mille, D. J., Ilizaliturri-Hernández, C. A., Espinosa-Reyes, G., Costilla-Salazar, R., Díaz-Barriga, F., Ize-Lema, I. & Mejía-Saavedra, J. 2010 Exposure to persistent organic pollutants (POPs) and DNA damage as an indicator of environmental stress in fish of different feeding habits of Coatzacoalcos, Veracruz, Mexico. *Ecotoxicology* **19** (7), 1238–1248.

Groff, J. M. & Zinkl, J. G. 1999 Hematology and clinical chemistry of cyprinid fish: common carp and goldfish. *Veterinary Clinics of North America: Exotic Animal Practice* **2** (3), 741–776.

Gustavino, B., Buschini, A., Monfrinotti, M., Rizzoni, M., Tancioni, L., Poli, P. & Rossi, C. 2005 Modulating effects of humic acids on genotoxicity induced by water disinfectants in *Cyprinus carpio*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **587** (1), 103–113.

Hille, S. 1982 A literature review of the blood chemistry of rainbow trout, Salmo gairdneri Rich. *Journal of Fish Biology* **20** (5), 535–569.

Iglesias, J. & Medina, I. 2008 Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography A* **1192** (1), 9–16.

Imanpoor, M. R. & Kabir, M. 2011 Effects of sublethal concentration of chloramine T on growth, survival, haematocrit and some blood biochemical parameters in common carp fry (*Cyprinus carpio*). *Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux)* **4** (3), 280–291.

Irfan, M. & Khan, U. Z. 2009 *Analysis of Temporal and Spatial Variations in Rawal Lake Water Quality Parameters*. Pakistan Navy Engineering College and Institute of Environmental Science and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology, Islamabad, Pakistan.

Jayana, B. L., Prasai, T., Singh, A. & Yami, K. D. 2009 Assessment of drinking water quality of madhyapur-Thimi and study of antibiotic sensitivity against bacterial isolates. *Nepal Journal of Science and Technology* **10**, 167–172.

Khan, R., Hashmi, I., Nasir, H., Rasheed, S. & Campos, L. C. 2018 Incidence of chlorination by-products in an institutional drinking water distribution network, Islamabad, Pakistan, using response surface methodology. *Journal of Water, Sanitation and Hygiene for Development* **8** (4), 740–751.

Klobučar, G. I., Štambuk, A., Pavlica, M., Perić, M. S., Hackenberger, B. K. & Hylland, K. 2010 Genotoxicity monitoring of freshwater environments using caged carp (*Cyprinus carpio*). *Ecotoxicology* **19** (1), 77–84.

Kumaravel, T. S. & Jha, A. N. 2006 Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. *Mutation Research* **605** (1), 7–16.

Landi, S., Naccarati, A., Ross, M. K., Hanley, N. M., Dailey, L., Devlin, R. B. & DeMarini, D. M. 2003 Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **538** (1), 41–50.

Mattice, J. S., Tsai, S. C., Burch, M. B. & Beauchamp, J. J. 1981 Toxicity of trihalomethanes to common carp embryos. *Transactions of the American Fisheries Society* **110** (2), 261–269.

Mikulikova, I., Modra, H., Blahova, J., Kruziková, K., Marsalek, P., Bedanova, I. & Svobodova, Z. 2013 Recovery ability of common carp (*Cyprinus carpio*) after a short-term exposure to terbuthylazine. *Polish Journal of Veterinary Sciences* **16** (1), 17–23.

Mitchelmore, C. L. & Chipman, J. K. 1998 DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis* **399** (2), 135–147.

Monarca, S., Zani, C., Richardson, S. D., Thruston Jr., A. D., Moretti, M., Feretti, D. & Villarini, M. 2004 A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. *Water Research* **38** (17), 3809–3819.

Muellner, M. G., Attene-Ramos, M. S., Hudson, M. E., Wagner, E. D. & Plewa, M. J. 2010 Human cell toxicogenomic analysis of bromoacetic acid: a regulated drinking water disinfection by-product. *Environmental and Molecular Mutagenesis* **51** (3), 205–214.

Munch, D. J. & Hautman, D. P. 1995 Method 551.1: Determination of chlorination disinfection byproducts, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extraction and gas chromatography with electron-capture detection. In: *Methods for the Determination of Organic Compounds in Drinking Water*, Hodgeson, J. W. & Cohen, A. L. (eds). USEPA, Cincinnati, OH, USA.

Nussey, H. H. 2000 Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae), from Witbank Dam, Mpumalanga. *Water SA* **26** (2), 269–284.

OECD 1992 *Guidelines for Testing of Chemicals, Guideline 203 Fish Acute Toxicity Test.* Organisation for Economic Co-operation and Development, Paris, France.

Ozmen, M., Ayas, Z., Güngördü, A., Ekmekci, G. F. & Yerli, S. 2008 Ecotoxicological assessment of water pollution in Sariyar Dam Lake, Turkey. *Ecotoxicology and Environmental Safety* **70** (1), 163–173.

Pan, Y., Li, W., An, H., Cui, H. & Wang, Y. 2016 Formation and occurrence of new polar iodinated disinfection byproducts in drinking water. *Chemosphere* **144**, 2312–2320.

Plewa, M. J., Wagner, E. D. & Mitch, W. A. 2011 Comparative mammalian cell cytotoxicity of water concentrates from disinfected recreational pools. *Environmental Science and Technology* **45** (9), 4159–4165.

Qaiser, S., Hashmi, I. & Nasir, H. 2014 Chlorination at treatment plant and drinking water quality: a case study of different sectors of Islamabad, Pakistan. *Arabian Journal for Science and Engineering* **39** (7), 5665–5675.

Råbergh, C. M. & Lipsky, M. M. 1997 Toxicity of chloroform and carbon tetrachloride in primary cultures of rainbow trout hepatocytes. *Aquatic Toxicology* **37** (2–3), 169–182.

Ramesh, M., Narmadha, S. & Poopal, R. K. 2015 Toxicity of furadan (carbofuran 3% g) in *Cyprinus carpio*: haematological, biochemical and enzymological alterations and recovery response. *Beni-Suef University Journal of Basic and Applied Sciences* **4** (4), 314–326.

Richardson, S., Plewa, M., Wagner, E., Schoeny, R. & DeMarini, D. 2007 Occurrence, genotoxicity and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutation Research* **636** (3), 178–242.

Russo, C., Rocco, L., Morescalchi, M. A. & Stingo, V. 2004 Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. *Ecotoxicology and Environmental Safety* **57** (2), 168–174.

Shakoori, A. R., Iqbal, M. J., Mughal, A. L. & Ali, S. S. 1994 Biochemical changes induced by inorganic mercury on the blood, liver and muscles of freshwater Chinese grass carp (*Ctenopharyngodon idella*). *Journal of Ecotoxicology & Environmental Monitoring* **4** (2), 81–92.

Singh, N. P., McCoy, M. T., Tice, R. R. & Schneider, E. L. 1988 A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research* **175** (1), 184–191.

Tavares-Dias, M. 2006 A morphological and cytochemical study of erythrocytes, thrombocytes and leukocytes in four freshwater teleosts. *Journal of Fish Biology* **68** (6), 1822–1833.

Teixidó, E., Piqué, E., Gonzalez-Linares, J., Llobet, J. M. & Gómez-Catalán, J. 2015 Developmental effects and genotoxicity of 10 water disinfection by-products in zebrafish. *Journal of Water and Health* **13** (1), 54–66.

Theodorakis, C. W., D'Surney, S. J. & Shugart, L. R. 1994 Detection of genotoxic insult as DNA strand breaks in fish blood cells by agarose gel electrophoresis. *Environmental Toxicology and Chemistry: An International Journal* **13** (7), 1023–1031.

Toussaint, M. W., Rosencrance, A. B., Brennan, L. M., Beaman, J. R., Wolfe, M. J., Hoffmann, F. J. & Gardner Jr., H. S. 2001 Chronic toxicity of chloroform to Japanese medaka fish. *Environmental Health Perspectives* **109** (1), 35–40.

USEPA (US Environmental Protection Agency) 2006 National primary drinking water regulations: stage 2 disinfectants and disinfection byproducts rule: final rule. *Fed. Reg.* **71** (2), 388.

Van Vuren, J. H. 1986 The effects of toxicants on the haematology of Labeo umbratus (Teleostei: *Cyprinidae*). *Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology* **83** (1), 155.

Whyte, J. J., Jung, R. E., Schmitt, C. J. & Tillitt, D. E. 2000 Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Critical Reviews in Toxicology* **30** (4), 347–570.

First received 28 September 2018; accepted in revised form 28 May 2019. Available online 23 July 2019