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Original article

Fish as bio indicators to determine the effects of pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay

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ABSTRACT

Objectives: The unpolluted freshwater is an essential ingredient for the healthy life of animals and humans. Aquatic pollution is the most important problem for living organisms. The main source of water pollution is industrial sewages. In the current study bioaccumulation and genotoxic effects of different metals e.g. Pb, Cd, Cr, Cu were determined in various tissues of *Puntius sophore* (*P. sophore*).**Methods:** The water sample and fish *P. sophore* were brought from different sites of the Gomati river (coordinates: 25°30'29"N 83°10'11"E), India. The heavy metals in water and fish tissues were determined by using ICP-MS. The mutagenic and genotoxic potential of the river water in *P. sophore* were evaluated using micronucleus and single-cell gel electrophoresis techniques.**Results:** We have observed that the concentration of heavy metals (Pb, Cd, Cr, Cu) in the river water and bioaccumulation of these metals in *P. sophore* are equal in order. Thus it was confirmed that fish act as a sentinel indicator of metal pollution of the surrounding environment. The formation of micronuclei, as well as fragmentation of DNA, were significantly increased ($p < 0.05, 0.01$) in river fish as compared to the control.**Conclusions:** Thus we might consider the fish for biomonitoring of aquatic ecosystems. Also, these findings confirmed that water of the Gomati river was contaminated with heavy metals. So, it is important to prevent the inflow of domestic and industrial sewage in the river.© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The water of rivers is contaminated due to anthropogenic activities, domestic waste, industrial effluents, and other pollutants which adversely impacted the aquatic biotas, human and animal healths. Nowadays the main source of pollution is wastewater form plastic, tanneries, mining, and cement industries (Lahiry, 2017). As a consequence, these industrial pollutants enter into the aquatic ecosystem (Environment Agency, 2013). Many types

of pollutants showed mutagenic and clastogenic properties (Waters et al., 1999; Tchounwou et al., 2012). The toxic heavy metals discharged in the environment are zinc, copper, nickel, mercury, cadmium, lead, and chromium (Fu and Wang, 2011). Heavy metals pollution is considered to be a serious world problem (Islam et al., 2014) due to bioaccumulation and bio magnifications in the food web and trophic level (Zhou et al., 2004; Sharma et al., 2007). Fish act as a biological indicator of water quality (Al-Ghanim et al., 2016) because of their affinity to accumulate the metals in their muscles (Zhao et al., 2012) that cause alterations in physiological, biochemical and genetic parameters in their body (Javed and Usmani, 2017). Andrade.

The river Gomati is a branch of the largest river Ganges of the Indian sub-continent (Welcomme, 1985). The Gomati rivers are the main source of water in Lucknow metro city. Recently some researchers reported bioaccumulation of metals in flora and fauna, contamination waters exerted dangerous effects on aquatic biota and human health (Gupta et al., 2009; Vaseem and Banerjee, 2013). Heavy metals (Cr, Cd, Pb, As) pollution was a many-fold

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increase in river Ganga water (Paul, 2017). The consumption of contaminated fish can pose harmful effects on human health (Puel et al., 1987). Andrade et al. (2004) documented that fish could be as a biological demonstrator for contamination of river water using the genotoxic test. Comet and micronucleus tests are simple and sensitive biomarkers to detect the genotoxic and mutagenic characteristics of pollutants (Ali et al., 2008). The present situation demands to investigate the current status of heavy metals accumulation in water and fish of the river Gomati. Therefore, freshwater fish *P. sophore* was used as a bioindicator/ biomarker for aquatic pollution to detect the genotoxic potential of river water.

2. Materials and methods

2.1. Collection of fish

We have collected the total 30 specimens of *Puntius sophore* (Hamilton, 1822) striped dwarf catfish using cast and drag nets with the help of fisherman from five locations of Gomati rivers at Lucknow region (26°52'N 80°07'E), during the period of May–June 2019. The samplings were done twice in a month. A total of 30 specimens were collected at a rate of 5 fish from each site. The details of the river are given in Fig. 1 (Tangri et al., 2018), and Table 1 (Gupta and Tripathi, 2017). The collected fish were

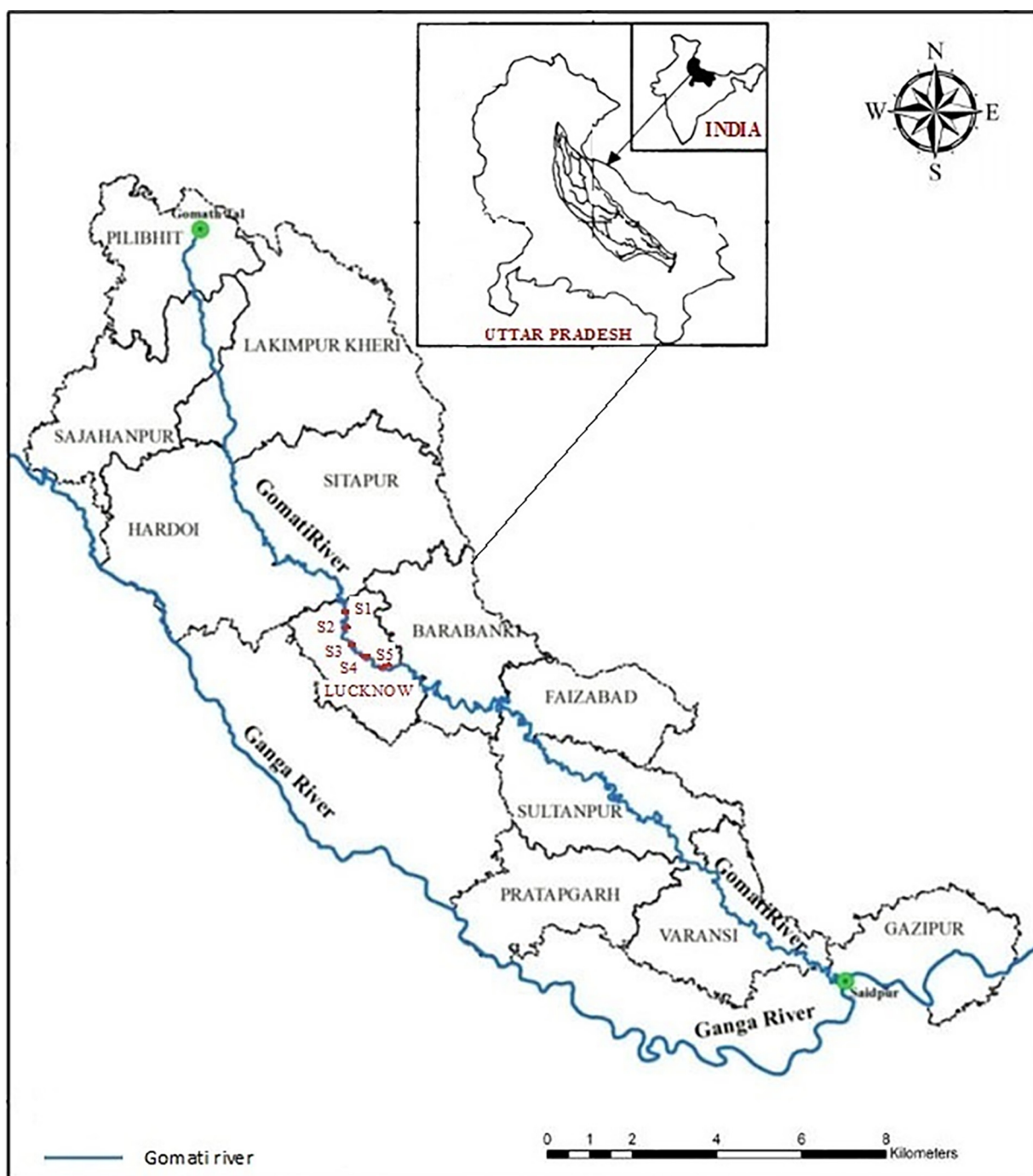


Fig. 1. Sampling sites of water, fish *P. sophore* in river Gomti in Lucknow metro city. The figure was taken and redrawn from Map of India.

Table 1
List of sampling sites.

Sampling sites	Sampling sites	Latitude and longitude of the sampling sites
S1	Pipa pul ghat	26.8871°N 80.9006°E
S2	Kuriyan Ghat	26.8741°N 80.9119°E
S3	Pakka Pul Ghat	26.8727°N 80.9162°E
S4	Saheed Smarak Ghat	26.8634°N 80.9286°E
S5	Gomati Barrage	26.8555°N 80.9694°E

washed with water; total length (cm) and body weight (g) of the fish were taken using scale and electronic balance respectively [Table 3](#). The fish were dissected out with the help of a sharp knife to take out the 3 g of dorsal muscle tissue from each individual of sample fish and placed in polythene bags which were properly marked with the specimen number and stored in the freezer at $-80\text{ }^{\circ}\text{C}$ for further analysis.

2.2. Determination of physicochemical parameters

The fishes and water were collected all together at the time of sampling. The water was collected from five sites of the river such as S1, S2, S3, S4, and S5 ([Table 1](#)) (35 cm below the surface of the water) in a brown color dark bottle for DO analysis and transparent glass bottles for other parameters and the metal analysis. The physicochemical parameters such as conductivity ($\mu\text{mho/cm}$), pH, dissolved oxygen (DO) (mg/l), hardness (mg/l), total dissolved solids (TDS) (mg/l) were examined using standard methods as described by [APHA \(2005\)](#).

2.3. Tissue preparation for metal analysis

The tissue was homogenized, weighed, and dried at $80\text{ }^{\circ}\text{C}$ in Petri-plate for 48 hrs, and after cooling it was homogenized into a very fine powder. The tissue 500 mg were digested using HNO_3 (2.0 ml), H_2O_2 (1.0 ml), and H_2O (3.0 ml). The clear solution obtained was cooled and diluted with Milli-Q water.

2.4. Estimation of heavy metals by ICP-MS

These metals such as cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu) in water and tissue samples were determined by ICP-MS (Optima 8000, Perkin Elmer) in triplicates. The standards were prepared from stock solutions of considered elements at the rate of 1000 mg/l concentration procured from Perkin Elmer (USA). Simultaneously the blanks were used in each set of analyses for confirmation. The used absorption wavelengths (λ) for analysis of Pb, Cu, Cr, and Cd were 220.35 nm, 327.39 nm, 267.71, and 214.43 nm respectively and the minimum detection limit was 0.5 ppb for all the elements. The results obtained were quantified using calibration curves of different elements. The concentrations of the above metals were expressed as milligrams per liter (mg/l) and milligrams per kilogram (mg/kg) for water and tissue respectively.

2.5. Micronucleus (MN) assay

After sampling the specimens, the MN assay was carried out as described by [Ali et al. \(2009\)](#).

The formula for calculation of MN:

$$\text{MN}\% = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of counted}} \times 100$$

2.6. Geno toxicity assay

The genotoxicity of the effect of heavy metal in polluted water was evaluated by determining DNA damage in lymphocytes, gills, and muscle fish samples by comet technique as described by [Ali et al. \(2009\)](#).

2.7. Statistical analysis

The data was statistically calculated using ANOVA for the average concentration of metals in water and fish and fragmentation of DNA of different sites of river. The significant probability level was set as less than 0.05 and 0.01.

3. Results

3.1. Physico-chemical parameters and concentration of heavy metals

The physicochemical parameters such as pH, DO, hardness, conductivity, and TDS of experimental river water pf different sites were analyzed and details are listed in [Table 2](#).

The metals bioaccumulation in muscle tissue of *P. sophore* fish and water from different sites of river Gomati are listed in [Table 3](#). The Cd was present in less concentration in all sites of water samples in the present study ([Table 4](#)). The concentrations sequence of heavy metals fish muscles and in water were found as $\text{Pb} > \text{Cr} > \text{Cu} > \text{Cd}$ ([Table 4](#)). Metals concentrations in fish tissue and water sample procured from different sites were observed as significant ($p < 0.05$) among sites ([Table 4](#)). The bioaccumulation of Pb and Cd were also highest and lowest respectively from the samples of the fish collected from different sites and the same

Table 2
Physico-chemical parameters of the water samples collected from the Gomati River.

Site name	Physicochemical parameters	Values
S1	Temperature	$24 \pm 2\text{ }^{\circ}\text{C}$
	pH	7.90 ± 1.0
	DO	$6.8 \pm 1.80\text{ mg/l}$
	TDS	$232.5 \pm 3.20\text{ mg/l}$
	Total Hardness	$252.0 \pm 3.0\text{ mg/l}$
	Total Conductivity	$441 \pm 4.0\text{ }\mu\text{mho/cm}$
S2	Temperature	$23.5 \pm 1.5\text{ }^{\circ}\text{C}$
	pH	7.80 ± 1.20
	DO	$5.60 \pm 2.40\text{ mg/l}$
	TDS	$238.42 \pm 4.20\text{ mg/l}$
	Total Hardness	$255.0 \pm 5.0\text{ mg/l}$
	Total Conductivity	$440 \pm 2.0\text{ }\mu\text{mho/cm}$
S3	Temperature	$24 \pm 1\text{ }^{\circ}\text{C}$
	pH	7.87 ± 1.6
	DO	$4.80 \pm 1.60\text{ mg/l}$
	TDS	$242.7 \pm 5.40\text{ mg/l}$
	Total Hardness	$265.0 \pm 6.0\text{ mg/l}$
	Total Conductivity	$438 \pm 5.0\text{ }\mu\text{mho/cm}$
S4	Temperature	$23.8 \pm 2.4\text{ }^{\circ}\text{C}$
	pH	7.88 ± 1.2
	DO	$5.10 \pm 2.20\text{ mg/l}$
	TDS	$241.4 \pm 3.90\text{ mg/l}$
	Total Hardness	$255.0 \pm 2.0\text{ mg/l}$
	Total Conductivity	$440 \pm 3.0\text{ }\mu\text{mho/cm}$
S5	Temperature	$24.2 \pm 1\text{ }^{\circ}\text{C}$
	pH	7.90 ± 1.0
	DO	$4.55 \pm 1.40\text{ mg/l}$
	TDS	$244.5 \pm 7.10\text{ mg/l}$
	Total Hardness	$261.0 \pm 4.0\text{ mg/l}$
	Total Conductivity	$437 \pm 4.0\text{ }\mu\text{mho/cm}$

Table 3

Parameters of length, weight and their regression equations of *Puntius sophore* collected from the river Gomati.

Sites	Sample quantity	L (cm)	W (g)	Regression equation
Gomati river	50	5–17	10–50	$-1.471 \pm 2.378 \text{ Log L}$

Symbol: L = length, W = weight.

sequence (Pb > Cr > Cu > Cd) of metal accumulation was detected out as found in the water samples Table 4.

3.2. Mutagenicity

MN production in erythrocytes was observed and induction of MN was found more in fish of highly metal-contaminated sites (Fig. 2 a). The effect of contamination of metals on MN production was according to sampling sites. The frequency of MNi obtained in this study is shown in Fig. 2 a. The correlation of frequency of MNi induction and DNA damage in collected fish is shown in Fig. 2 b.

3.3. Genotoxicity

After collecting fish from different sites of Gomati rivers the fragmentation of DNA was determined in selected tissues of *P.sophore* using comet assay and DNA fragmentation was found in fish according to metal bioaccumulated fish tissues

Table 4

Mean concentrations of heavy metals ± SE in the water samples and in the muscle tissues of freshwater fish *P. sophore* collected from different sites of the Gomati river.

Sampling sites	Heavy metals	Concentration of metals in water sample (mg/L)	Concentration metals in muscle tissue (mg/kg)
S1	Pb	0.34 ± 0.10	0.28 ± 0.21
	Cd	0.018 ± 0.05	0.014 ± 0.07
	Cr	0.22 ± 0.78	0.20 ± 0.40
	Cu	0.201 ± 0.75	0.18 ± 0.12
S2	Pb	0.40 ± 0.19	0.35 ± 0.20
	Cd	0.027 ± 0.20	0.016 ± 0.22
	Cr	0.34 ± 0.26	0.35 ± 0.80
	Cu	0.111 ± 0.38	0.098 ± 0.26
S3	Pb	0.36 ± 0.72	0.31 ± 0.40
	Cd	0.020 ± 0.13	0.011 ± 0.40
	Cr	0.25 ± 0.11	0.23 ± 0.36
	Cu	0.107 ± 0.17	0.41 ± 0.30
S4	Pb	0.38 ± 0.12	0.34 ± 0.10
	Cd	0.023 ± 0.17	0.014 ± 0.22
	Cr	0.29 ± 0.09	0.30 ± 0.28
	Cu	0.110 ± 0.62	0.46 ± 0.24
S5	Pb	0.49 ± 0.29	0.44 ± 0.39
	Cd	0.030 ± 0.52	0.022 ± 0.31
	Cr	0.35 ± 0.08	0.33 ± 0.29
	Cu	0.113 ± 0.18	0.52 ± 0.20

(Fig. 3 a,b,c,d). We have observed the higher fragmentation of DNA was occurred in gill cells (Fig. 3a, d). A significant effect on genotoxicity was observed in metal bioaccumulated in fish (Fig. 3a-d).

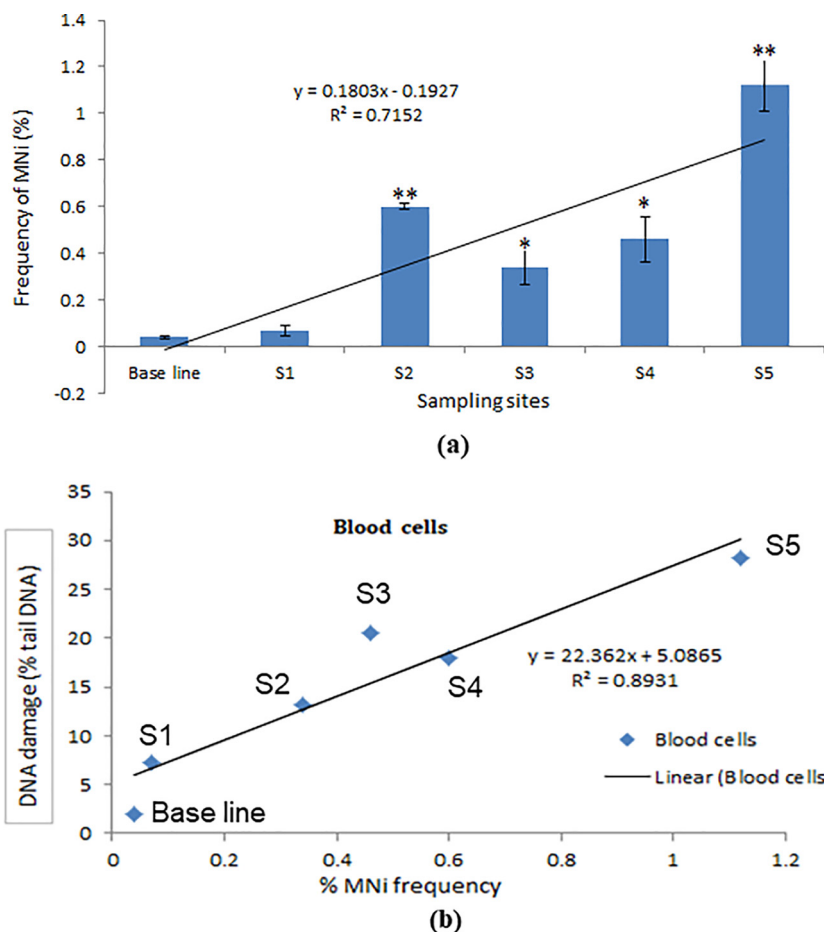


Fig. 2. (a) Induction of MNi (%) in erythrocyte cells of freshwater *P. sophore* fish at different sampling sites. (b) Correlation of induction of DNA damage and MN for different sampling sites. Each value represents the mean ±SE of three experiments. *p < 0.05, **p < 0.01 vs. control.

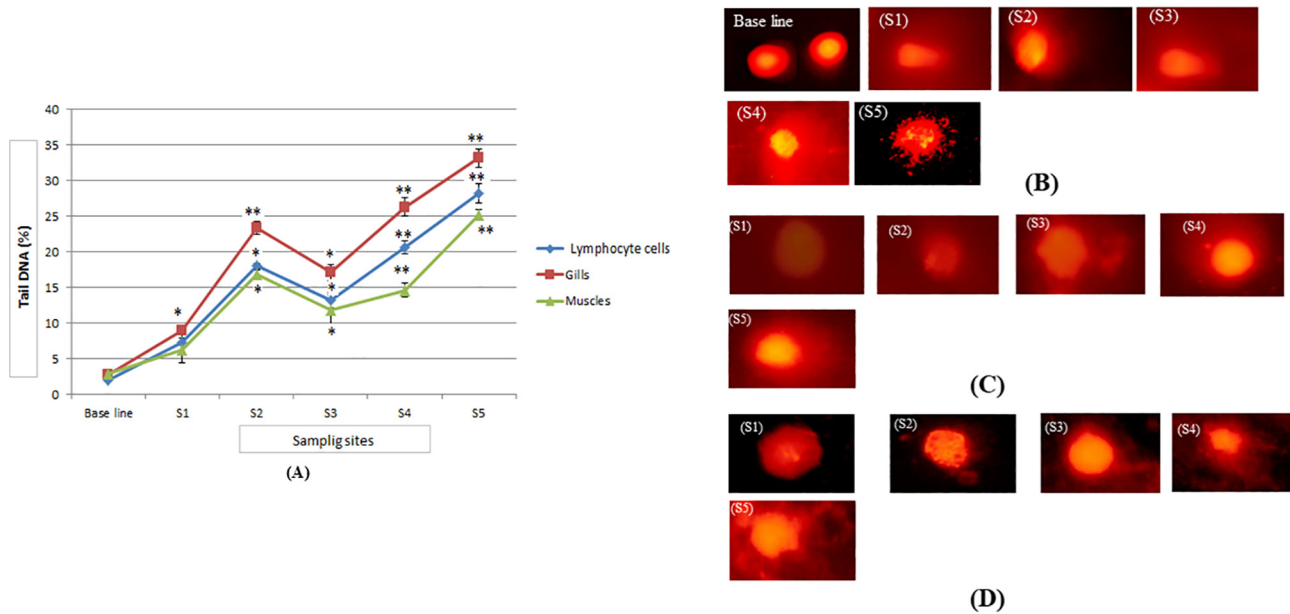


Fig. 3. (A) DNA fragmentation in different tissues of freshwater fish *P. sophore* (B) Images of DNA fragmentation in lymphocyte cells (C) Images of DNA fragmentation in muscle cells (D) Images of DNA fragmentation in gills cells of *P. sophore* of different sampling sites. Each value represents the mean \pm SE of three experiments. * $p < 0.05$, ** $p < 0.01$ vs. control.

4. Discussion

Nowadays the development of rivers water pollution is increased by runoff of sewage and agricultural production into the river system (Javier et al., 2017). We have determined the concentration of metals (Pb, Cr, Cu, Cd) in inhabiting fish *P. sophore* and water samples of the Gomati river. The metal concentrations in muscle were lower than the consumption limit (FAO, 1983). The sequence of accumulated metals was established as Pb > Cr > Cu > Cd and it was accordance in both water samples and fish muscles collected from the same sites of river. Hashim et al. (2014) reported that metals accumulation was depended upon fish species. Also, the accumulation of metals is governed by topography, hydrology, geology, local climate, anthropogenic influence, pH, alkalinity, water hardness, season, sex, and locality in the aquatic organism (Monikh et al., 2013). There was no remarkable variation in pH of water samples but it was slightly alkaline while conductivity, DO, TDS showed variations in the water samples along with the various sites in the present study. The concentration of Pb, Cr, Cu, Cd in organism tissue, and water were found more at site S5 in comparison to other sites. Out of four metals (Pb, Cr, Cu, Cd) in water samples, Pb metals were higher to other metals at different sampling sites. Cu was highly accumulated in muscles of *P. sophore* and its concentration was more at site S1, S5. Cd was least accumulated heavy metal but its concentration was higher in different sites of the river. Some researchers reported that heavy metals are accumulated in river fish (Gupta et al., 2009). Generally, the mean concentration of all metals present in fish of all sites was below the permissible limit (FAO, 1983, Table 5). The mean concentration of heavy metals obtained from fish and water samples indicated that serious attention should be taken because the con-

centration of these metals was approaching the permissible limits. Pb is a non-essential element and can be toxic to humans when ingested or inhaled in high concentrations. Pb level in the present findings ranged from 0.28 ± 0.21 to 0.44 ± 0.39 mg/kg which is within the recommended permissible food consumption limits of hazardous substances for aquatic products. Fish being sensitive to Pb (Lagerwerff, 1972), its concentration increased in the fish muscle with the increase in concentration in the surrounding water as recorded at different sites in the river in the present study. According to Pb can cause an extreme nuisance in fish because it is responsible for the formation of mucus and cause low metabolism resulting in decreased growth and survival rate (Burger et al. (2002).

Cd is a serious contaminant, highly toxic element, and the permissible limit for human consumption is 0.1 mg/kg. However, in the present study, mean concentrations of Cd ranged from 0.014 ± 0.07 to 0.022 ± 0.21 mg/kg and it was below the maximum permissible limit. Cr is a hazardous metal notified by the USFDA (1993) and the maximum permissible food consumption limits for Cr is 2 mg/kg. In this study, the Cr level is very low (0.20 ± 0.34 to 0.33 ± 0.29 mg/kg) and it due to the quick elimination of Cr through faeces from the fish body (Mishra and Mohanty, 2008). Cr is an imperative element that helps the body to use sugar, protein, and fat although excess amounts encompass adverse effects on the fish body (Akan et al., 2009). Cu is an important part of several enzymes and important for the synthesis of hemoglobin mainly in marine organisms which contains appropriate mechanisms to standardize its concentrations in their tissues (Thiyagarajan et al., 2012). The permissible food consumption limits of hazardous substances for aquatic products established a limit for Cu as 50.0 mg/kg (Table 5) and in the current study, the concentration of Cu varied between 0.18 ± 0.12 – 0.52 ± 0.20 mg/kg which is very low to the permissible limit. The present result is found to be in line with Stokes (1979) who pointed out that the high Cu exposure to fish in the external environment hardly shows its accumulation in the body.

The micronucleus test was an important parameter to assess the mutagenic properties of contaminants in water bodies (Dourado et al., 2017). The induction of micronuclei in river fish

Table 5
Permissible limits of heavy metals in fish.

Heavy metals	Concentration (mg/kg)
Pb	2.0
Cu	50
Cr	2
Cd	0.1

might be a mutagenic effect of metals of contaminated water. The micronucleus assay is a more sensitive and simple technique because it takes less time and not dependent on the karyotyping of the chromosome (Heddle et al., 1991). The induction of MNi in an aquatic organism and fish indicates the mutagenic damage and scoring of MNi is a more reliable and faster method in comparison of chromosomal aberration counting (Hartwell et al., 2000). Researchers have been reported that the genotoxic effect of environmental pollutants; pharmaceuticals could be measured by comet technique and this technique is thought of as a reliable, highly sensitive method for determining genotoxicity in prokaryotic and eukaryotic cells (Ali et al., 2008; Ullah and Zorriehzakra, 2015). So, in the current study, MNi and alkaline single cell gel electrophoresis were applied to detect a mutagenic and genotoxic effect of environmental pollutants (Andrade et al., 2004). The contamination of metals in the fish body cause alterations in condition factor and this parameter used as a biomarker of environmental pollution (Yi and Zhang, 2012; El-Serafy et al., 2013).

The present finding demonstrated Pb, Cr, Cu, Cd concentrations in fish and water may cause a dangerous effect on human's health due to their excessive consumption. Heavy metals of polluted river water-induced mutagenic and genotoxic effects in aquatic organisms. Hence, there is an urgent need to enhance information broadcasting, monitoring programs to improve environmental protection awareness for sustainable management of river water.

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.

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