

Evaluation of water genotoxicity using comet assay in two freshwater fish species, *Wallago attu* and *Ompok bimaculatus* in Kabul River, Pakistan

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Abstract: The investigation determines the pollution status of river Kabul and its effects on the DNA of *Wallago attu* and *Ompok bimaculatus*. River Kabul gets pollutants from Amanghar industries and sewages from Mardan, Nowshera and Peshawar. Water and fish samples are taken to study heavy metals and physical parameters. The overall sequence of physical parameters in water samples was TDS>TSS>EC>TA>Cl>Na>pH>K, and that of heavy metals was Zn>Cr>Mn>Fe>Cu>Pb>Ni>Cd. The studied parameters in all the water samples except TSS are within the proposed limits of national environmental quality standards. The overall trend of analysed water samples was C>B>A. The study determines the effects of water pollution on the DNA of *Ompok bimaculatus* and *Wallago attu* tissues. Therefore, degrees of DNA damage such as TCS and comet class are studied in gills, liver, muscles, and intestines of *Wallago attu* and *Ompok bimaculatus*. The trend of DNA damage in examined tissues was intestine>skin>liver>gills>muscle, and in studied fish species, it was *Ompok bimaculatus*>*Wallago attu*. More significant DNA damage was observed in the intestine and smaller in the muscle. More DNA damage was found in *Ompok bimaculatus* and less in *Wallago attu*. The investigation recommended detoxifying the effluents and sewages before discharging them in River Kabul.

Keywords: DNA, River Kabul, Comet assay, Physical parameters, Heavy metals, Physical parameters, *Labeo dyocheilus*, *Tor putitora*, Genotoxicity, Water pollution.

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1. Introduction

River Kabul has its originated from the Phaghman mountains of Afghanistan and entered Pakistan at Shalman of Khyber Agency (Gresswell & Huxley, 1965). The water reservoir named Warsak dam was constructed on river Kabul near Warsak. River Kabul is used for both irrigation and electricity purposes. The dam has the same fish population as that of River Kabul (Yousafzai et al., 2008). 54 different fish species are investigated from river Kabul (Rafique, 2001). The most common fish species are *Wallago attu*, *Ompok bimaculatus*, *Labeo rohita*, *Labeo dyocheilus* and *Clupisoma naziri*. The people living in the surroundings are netted and consume these fishes. Most of these fishes are from the carp family. *Botia rostrata* is a popular fish reported from the river Kabul near Warsak Dam (Butt & Mirza, 1981).

Eighty-one different industries are discharging their effluents into the river Kabul resulting in water pollution (IUCN, 1994; Khan et al., 1999). River Kabul is utilized for both commercial and sport fishing. It receives sewage from the different cities of Charsadda, Mardan, Peshawar and Nowshera. Peshawar is a city with a population of 10 million that dumped sewage into the river Kabul. Many industries added their effluents into the river Kabul, causing water pollution (Yousafzai et al., 2008; Fazl-i-Hadi et al., 1988).

Fishes are considered a good source of food for human beings. Fish lipids are beneficial for heart and joint disorders (Shahidi & Botta, 1994). Fatty acids and amino acids of the fish proteins are required for the growth and development of man (Matthew, 1992). Fish is a bio-indicator that plays a vital role in the determination of water pollution because they have a strong response to changes in the aquatic ecosystem (Santhanam et al., 1987). Many diseases and abnormalities of bad impacts of water pollution have been investigated in fish and fish consumers (Bowen, 1979). Contaminated water not only induces different disorders and abnormalities in fish but is also responsible for the transformation of many disorders in humans (Tebbutt, 1983).

Fish are good bioindicators for monitoring water pollution and its impacts, and they are also used to detect water pollution problems. Data collected with fish is beneficial for the investigation of toxic chemicals that cause cancer and other disorders in humans (Matsumoto et al., 2006). Comet assay is a technique which is utilized for the evaluation of DNA damage induced through different chemicals and pollutants (Tice et al., 2000).

Comet assay has been utilized for a long time and is a fast protocol for the determination of genotoxicity in aquatic animals. Therefore, fishes are the more sensitive organisms that give more responses to pollutants and are also suitable organisms for the determination of genotoxicity induced by water pollution (Lemos et al., 2005). Comet assay is also utilized for the investigation of the genotoxicity of different water bodies like rivers and lakes. Therefore, this tool is also proposed as a sensitive protocol for the determination of genotoxicity in fishes and other aquatic animals induced by contaminated waters of different water bodies (Lee & Steinert, 2003).

The present findings aimed to assess the pollution status in river Kabul and their genotoxicity in two freshwater fishes, *Wallago attu* and *Ompok bimaculatus* netted from control and contaminated water of river Kabul that received effluents and city swages by using alkaline comet assay.

2. Materials and methods

2.1. Sampling sites

Both fish and water samples were collected from reference and contaminated sites of river Kabul. One sample (Water and fish) was collected near Amangarh industrial estate (site 2). The second one was collected near Nowshera (site 3). The third sample was collected near Warsak dam (site 1), probably sixty kilometres away from polluted parts of river Kabul.

2.2. Water collection

Water was taken 70 cm deep in plastic bottles from reference and contaminated localities of river Kabul. For both metal and physicochemical parameters, water samples were taken in separate plastic bottles. For heavy metals analysis, 5 ml nitric acid was added in one of the bottles. The samples then were shifted to the laboratory.

2.3. Field study

In the field, pH was found through pH meter, EC through conductivity meter and TDS through TDS meter.

2.4. Laboratory study

Total suspended solvent (TSS) was measured with the help of filter paper, total alkalinity (TA) and chloride (Cl) were measured through PC multi direct spectrophotometer. Na, K⁺ and metals Zn, Ni, Cr, Cu, Pb, Cd, Fe and Mn were studied through atomic absorption spectrometry (Spectra-AA-700).

2.5. Fish samples collection

Fish samples were collected from reference and contaminated belts of river Kabul. Fishes are included *Wallago attu* and *Ompok bimaculatus* were collected through local fishermen. They use the gills net for the collection of fish samples.

2.6. Dissection of fishes

The collected fish species were dissected for skin, gills, muscle, liver and intestine tissues. The tissues then rinsed with tap water and kept in the refrigerator for analysis of DNA damage.

2.7. Comet Assay

Tissues like skin, gills, muscle, liver, intestine and blood were processed through comet assay as described by Singh *et al* (1988). The tissue was ground, and 1mL HBSS (20 mM ethylene diamine tri acetic acid/ 10% dimethyl sulphur oxide) was added to the ground suspension. 10 µl of ground tissues were mixed with 75 µl of low melting agarose layered on pre-coated slides with normal melting agarose and placed on an ice bag for 20 mins. After 20 mins of solidification, a third layer of 85 µl of normal melting agarose was spread on the top and again placed on the ice bag for 20 minutes. The slides were then kept in freshly lysing solution (2.5

M Na, 100 EDTA, 10 Tris, 1% Triton X-100, 10% DMSO, pH 10.0) for lysis of cells and kept the slides in the refrigerator at 4°C overnight. After this, slides are kept in a gel box containing buffer solution (1mM ethylene diamine tri acetic acid 300 sodium hydroxide, pH 13.0) for 20 minutes for unfolding of DNA. Electrophoresis was conducted by setting the power supply at 20V and 300 mA for 20 minutes, and it turned out. Thereafter, the slides were treated with a neutralization solution for 15 minutes and then kept in ethanol for drying. Slides then are dipped in 20 µg/ml acridine orange for staining.

2.8. Scoring of slides for DNA damage

The slides were seen through a fluorescent microscope, and photographs were taken. 100 cells were selected from every slide. Four comet classes (0,1,2,3,4) were counted on the basis of comet tail length. Total Comet Score (TCS) was determined by formula like $TCS = 0(n) + 1(n) + 2(n) + 3(n) + 4(n)$. n showed the number of cells in every class.

2.9. Statistical analysis

Mean and standard error values of the data were found by using SPSS software. Value ($P < 0.05$) was considered for significance.

3. Results

Table-1 shows the mean values of physical parameters of water sample A from reference site 1 (control) and water samples B and C from site 2 (contaminated/polluted) of river Kabul. According to investigation, most of the parameters of water samples B and C were higher than those of water sample A. All physical parameters except TSS were below the permissible limits of NEQS, where only TSS showed more value than NEQS permissible values.

Table-1: Physical parameters (Mean $\pm$ standard deviation) of water samples

Water samples	Sampling Sites	pH	TSS	TDS	EC	Cl	K ⁺	Na	TA
Sample A	Site 1	7.2 $\pm$ 1.6	581.1 $\pm$ 5.9	418.8 $\pm$ 9.9	229.1 $\pm$ 5.7	15.7 $\pm$ 5.1	1.1 $\pm$ 1.6	12.8 $\pm$ 6.3	113.5 $\pm$ 4.0
Sample B	Site 2	7.3 $\pm$ 2.0	881.3 $\pm$ 4.0	567.3 $\pm$ 6.8	297.1 $\pm$ 1.7	25.0 $\pm$ 8.1	5.8 $\pm$ 2.2	16.0 $\pm$ 7.9	122.3 $\pm$ 4.4
Sample C	Site 3	7.4 $\pm$ 3.4	962.3 $\pm$ 2.6	615.0 $\pm$ 2.4	345.7 $\pm$ 1.1	30.7 $\pm$ 8.2	5.7 $\pm$ 2.2	22.3 $\pm$ 7.0	205.3 $\pm$ 2.7

NEQS permissible limits 6-10 150 3500 NA 100 NA NA NA
Abbreviations: NEQS: National environmental quality, NA: Not available TSS: Total suspended solvent, TDS: Total dissolved solvent, EC: Electrical conductivity, K⁺: Potassium, Na: Sodium, TA: Total alkalinity

Table-2 demonstrates the mean values of heavy metal in water sample A from the reference belt (site 1) and in water samples B and C from contaminated belts (sites 2 and 3) of river Kabul, respectively. Mean values of the metals from various sites of river Kabul highlighted that Zn was found to be the highest metal and Cu was the lowest metal in all water samples A, B, and D of river Kabul. This study also investigated that all the metals showed fewer mean values than the permissible limits of national environmental quality standards.

Table-2: Heavy metals contents (Mean $\pm$ standard deviation) of water samples

Water samples	Sampling sites	Zn	Ni	Cr	Cu	Cd	Pb	Fe	Mn
Sample A	Site 1	66.3 $\pm$ 69.9	7.7 $\pm$ 10.2	31.3 $\pm$ 15.5	16.7 $\pm$ 10.1	4.0 $\pm$ 45.0	8.3 $\pm$ 6.5	21.0 $\pm$ 10.5	23.3 $\pm$ 11.5
Sample B	Site 2	230.3 $\pm$ 61.4	21.3 $\pm$ 16.8	54.3 $\pm$ 8.8	26.0 $\pm$ 8.3	18.7 $\pm$ 12.8	25.3 $\pm$ 3.0	38.3 $\pm$ 28.3	42.7 $\pm$ 9.7
Sample C	Site 3	254.7 $\pm$ 35.1	25.7 $\pm$ 16.3	65.7 $\pm$ 17.1	29.0 $\pm$ 8.8	23.0 $\pm$ 8.8	28.7 $\pm$ 3.1	47.0 $\pm$ 8.9	55.0 $\pm$ 12.1

NEQS permissible limits 500 1000 1000 1000 500 100 8000 1500

Abbreviations: Zn: Zinc, Ni: Nickel, Cr: Chromium, Cu: Copper, Cd: Cadmium, Pb: Lead, Fe: Iron, Mn: Manganese

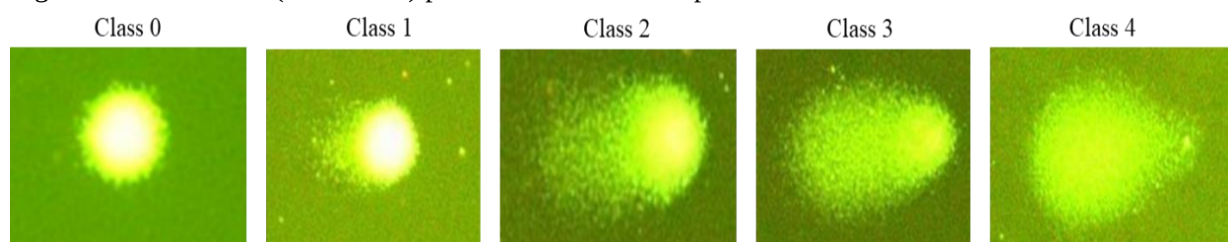
The mean values of comet class 0, 1, 2, 3, 4 and TCS of *Wallago attu* and *Ompok bimaculatus* netted from reference and contaminated belts of river Kabul are shown in Table-3. The skin, gills, muscle, liver and intestine tissues of *Wallago attu* and *Ompok bimaculatus* from contaminated water had more degrees of comet classes (0,1,2,3,4) and TCS than the reference water of river Kabul. Higher mean values of comet classes and TCS are found in the intestine than in its skin, liver, and gills tissues and lower mean values in the muscle of both studied fishes. Overall, the order showed that a greater degree of comet classes and TCS were studied in *Ompok bimaculatus* and smaller in *Wallago attu*. Similarly, more degrees of DNA damage cells are investigated in the intestine, while less in muscle. The intestine came first, and muscle came last for DNA damage cells.

Table-3: Degree of comet class 0, 1, 2, 3, 4 and TCS in skin, gills, muscle, liver and intestine of *Wallago attu* and *Ompok bimaculatus* collected from site 1 (Reference) and sites 2 and 3 (contaminated)

Tissues	Fish species	Sampling sites	Class 0	Class 1	Class 2	Class 3	Class 4	TCS
Skin	<i>Wallago attu</i>	Site 1	90.6±3.6	2.0±2.1	4.3±3.1	3.0±1.0	3.1±3.6	23.1±8.6
		Site 2	49.3±5.5	5.4±1.0	7.5±3.1	16.6±2.3	18.6±2.6	152.4±4.5
		Site 3	35.6±3.2	8.6±0.5	11.6±0.5	21.0±2.0	23.0±1.0	187.0±1.1
	<i>Ompok bimaculatus</i>	Site 1	82.1±2.5	5.1±2.1	6.1±1.0	3.1±1.0	4.1±1.0	40.0±9.1
		Site 2	27.1±6.1	7.5±2.3	13.0±2.0	25.2±2.1	27.2±3.1	217.1±2.5
		Site 3	13.6±2.5	12.3±0.5	15.6±0.5	28.0±1.0	30.3±0.5	249.0±6.5
Gills	<i>Wallago attu</i>	Site 1	91.6±4.0	3.0±1.0	2.0±1.0	2.0±1.0	13±1.5	18.3±1.0
		Site 2	55.6±4.0	6.0±1.0	5.0±1.0	16.0±1.0	17.3±1.5	133.3±1.0
		Site 3	43.6±4.0	8.0±1.0	10.0±1.0	17.3±1.5	21.0±1.0	164.0±1.5
	<i>Ompok bimaculatus</i>	Site 1	85.1±2.3	3.1±2.1	5.2±3.1	3.1±1.1	4.1±2.1	36.2±6.5
		Site 2	40.2±3.6	9.2±1.0	7.2±3.1	20.3±1.5	23.2±4.1	179.1±4.7
		Site 3	29.1±3.4	9.0±1.0	13.0±1.0	23.0±1.0	26.0±1.0	208.0±8.7
Muscle	<i>Wallago attu</i>	Site 1	93.0±4.0	2.0±1.0	3.0±1.0	1.0±1.0	1.0±1.0	15.0±1.0
		Site 2	58.6±4.1	5.0±1.0	6.0±1.0	15.0±1.0	15.3±1.5	123.3±1.1
		Site 3	50.6±4.5	7.0±1.0	9.0±1.0	16.0±1.0	17.3±1.5	135.6±8.0
	<i>Ompok bimaculatus</i>	Site 1	88.0±3.2	3.0±1.0	2.6±0.5	3.0±1.0	3.0±1.0	29.3±8.0
		Site 2	40.3±3.7	9.0±1.0	11.0±1.0	19.6±1.5	20.0±1.0	170.0±1.1
		Site 3	30.0±3.4	10.3±0.5	13.0±1.0	3.0±1.0	23.6±1.5	203.3±9.2
Liver	<i>Wallago attu</i>	Site 1	92.0±5.0	3.0±1.0	2.0±1.0	1.3±1.5	1.6±1.5	17.6±13.5
		Site 2	53.0±4.3	6.0±1.0	6.3±1.5	17.0±1.0	17.6±1.5	140.3±11.3
		Site 3	42.0±4.5	8.3±4.5	9.0±1.0	19.3±1.5	21.3±1.5	169.6±13.2
	<i>Ompok bimaculatus</i>	Site 1	87.6±2.0	4.0±1.0	3.3±0.5	3.0±1.0	2.0±1.0	27.6±6.6
		Site 2	33.0±5.5	8.0±1.0	10.3±1.5	23.3±1.5	25.3±1.5	200.0±14.7
		Site 3	20.3±3.7	11.3±0.5	14.3±0.5	26.0±1.7	28.0±1.0	230.0±10.5
Intestine	<i>Wallago attu</i>	Site 1	86.6±3.7	4.0±1.0	3.3±1.5	3.0±1.0	3.0±1.0	31.0±9.4
		Site 2	48.3±3.7	4.0±1.0	6.6±1.0	21.0±1.0	21.0±1.0	161.3±9.2
		Site 3	35.0±4.0	7.0±1.0	10.0±1.0	25.0±1.0	25.0±1.0	196.0±1.0
	<i>Ompok bimaculatus</i>	Site 1	76.0±4.0	7.0±1.0	6.0±1.0	5.0±1.0	6.0±1.0	58.0±1.0
		Site 2	20.0±3.5	9.6±0.5	13.0±1.0	27.0±1.5	29.0±1.5	238.6±6.4
		Site 3	12.0±3.6	12.0±1.0	15.0±1.0	29.0±1.0	32.0±1.0	257.0±8.7

Abbreviation: TCS: Total comet score

Figure 1: Comet class (0, 1, 2, 3,4) produced due to water pollution in various tissues of fishes



4. Discussion

During the electrophoresis process, the breaking of DNA strands shows movement from cathode to anode and results in comet tail formation (Singh *et al.*, 1998). This technique has the great power to determine the environmental impact on the DNA of aquatic animals and can also utilized for the impact of other toxic chemicals on the DNA of living things (Singh, 2000; Abd-Allah *et al.*, 1999). It is considered the most sensitive tool to investigate the effect of water contaminants on the DNA of fishes (Nagarani *et al.*, 2012), as it is utilized for the determination of genotoxicity of toxic pollutants on the DNA of aquatic animals. It imagines one of the fast and quick protocols for the study of genotoxicity as induced in the red blood cells by toxic chemicals (Kim *et al.*, 2002). The present results found that *Ompok bimaculatus* showed considerably higher DNA damage than *Wallago attu*.

In current years, it is necessary to determine the impact of toxic chemicals on fish health because there is dumping of sewage and industrial wastes into the different water bodies that result in various disorders and abnormalities in the fish. Toxic chemicals of water impact the DNA of fishes and are utilized as an indicator of DNA damage in contaminated aquatic environments (Jha, 2008). Fish are suitable animals that help in the determination of the genotoxicological impact of toxic pollutants in water because they can accumulate toxic chemicals in their different tissues (Elliott *et al.*, 1988). In the current investigation, the comet assay was utilized for the genotoxicological impact of water burn chemicals in the river Kabul, which was more helpful in the determination of DNA damage in fish from different aquatic sites of River Kabul.

It was investigated that all physical parameters from polluted sites of River Kabul showed higher contents as compared to those of the control site, and River Kabul received these parameters from surrounding industrial units and city sewages (Siraj *et al.*, 2022). Present results showed that most of the physico-chemical parameters except TSS in river Kabul water were found within the permissible limits as compared to national environmental quality standards. The data of the current study is agreed with the findings of Yousafzai *et al.* (2008), who had also determined the same parameters in the water of river Kabul from the same sites and found the contents of these parameters below the limits of NEQS. Similarly, Lasheen *et al.* (2012) also investigated the same physical parameters in drain water and found the contents of this parameter within limits. Generally, higher levels of TSS and lower content of pH, TDS, EC, Cl, K⁺, Na and TA in the water of river Kabul compared to reference water confirmed and verified a greater amount of organic and inorganic pollution in the river Kabul. This may be attributed to the discharging of a greater number of sewages and effluents into the Kabul River. Water samples B and C from contaminated belts showed significantly greater contents of physical parameters than water samples A from the control belt of river Kabul. This could be attributed to the discharging of huge amounts of city sewages and industrial effluents into river Kabul at polluted sites.

Different sources like industries, city sewages, leaching from landfills, and anthropogenic activities are adding tonnes of heavy metals into the aquatic environments (Rajeshkumar & Munuswamy, 2011). The current investigation highlighted that all the metals except Pb in the water of river Kabul were found within the permissible limits as compared to national environmental quality standards. The current findings are in agreement with El Bouraie *et al.* (2010), who had also studied the same metals in drain water in Egypt and observed the mean values of metals below the limits of Egyptian law. Similarly, Lasheen *et al.* (2012) also studied

the same metals in drain water and found the mean values of these metals below the proposed limits of Egyptian law. Generally, higher levels of metals in the water of River Kabul confirmed and verified a greater amount of metal pollution in the river Kabul. This may be attributed to the dumping of a greater number of sewages and effluents into river Kabul. Water samples B and C from polluted belts showed significantly greater contents of metal parameters than non-polluted belts of river Kabul. This may be because of the discharging of huge amounts of domestic, agricultural and industrial effluents into river Kabul at polluted sites.

The skin of the studied fish species showed more DNA damage than that of the liver, gills and muscle and less than the intestine and blood because it is the prime part of the body that is exposed to water pollution. Accordingly, DNA damage is comparatively more in the skin than in other tissues. The current study investigated significantly ($P > 0.5$) higher DNA damage (TCS) in the skin of all fishes from contaminated water than in reference water of river Kabul. The study indicates that the skin of *Ompok bimaculatus* had greater DNA damage (TCS) compared to *Wallago attu*. This may be related to more exposition of this fish to contaminated water for a long time. Our data is strongly supported by Abdel-Gawad *et al.* (2011), who have also investigated the same result in the fish and insects of the river Nile. The current result verified and confirmed genotoxicity in different tissues of *Ompok bimaculatus* and *wallago attu* caused by water pollution.

Gills are the target site for bioaccumulation of pollutants, and more accumulation of pollutants in gills may result in the death of the fish due to damaging of gills through water pollution (Elahee & Bhagwant., 2007). In the current investigation, significantly ($P > 0.5$) more DNA damage (TCS) was found in the gills of both the fishes from contaminated water than in non-contaminated water. This could be because of water pollution at polluted sites of river Kabul. Gills showed more DNA damage than muscle and less than other examined tissues. In the current investigation, we observed more DNA damage in the intestine and less in the muscle of both studied fish species as compared to other examined tissues. The present result also showed lower DNA damage in *wallago attu* and higher in *Ompok bimaculatus*. We observed more DNA damage cells in the gills of *Ompok bimaculatus* and less in *Wallago attu*. The current study showed that the main cause of DNA damage in the gills of studied fishes is water pollution in river Kabul, which receives industrial effluents from the Aman Ghar industrial zone and sewages from Nowshera city. The present result strongly agrees with Malins *et al.* (2004), who have also investigated the same data for DNA damage (TCS) in the gills of Puget Sound fish.

Fish is a suitable organism that can be used as an indicator of the genotoxic impact of aquatic pollution (Kumar *et al.*, 2009). In current findings, muscles of *Wallago attu* and *Ompok bimaculatus* from contaminated water showed more frequency of DNA damage than muscles from non-polluted water of river Kabul, which strongly agreed with Siraj *et al.* (2018), who have also studied more DNA damage in the muscle of *Cyprinus carpio* from the same water body. This could be because of high water pollution in the Kabul River. In this study *Ompok bimaculatus* showed more DNA damage (TCS) in its muscle than *Wallago attu*. This may be because of the exposition of this fish to polluted water for more time. As a whole, muscle came last for DNA damage (TCS) after the intestine, skin, liver and gills. In the present study, we found more TCS in the intestine and less in the muscle of both *Ompok bimaculatus* and *wallago attu* as compared to its other tissues. These results also agreed with the past findings, which also investigated more DNA damage in the muscle of *Channa*

punctatus, *Cirrhinus mrigala* and *Catla catla* from contaminated water as compared to non-contaminated water (Javed *et al.*, 2016).

Contaminated water with genotoxic potential is a serious problem for aquatic organisms. Therefore, it leads to DNA damage in the liver of fish (Ohe *et al.*, 2004). Genotoxicity in the liver of *Clarias gariepinus* was investigated and increased statistically in the liver from contaminated water of El-Fayoum drainage canals of Egyptian (Zaghloul *et al.*, 2020)). In the current findings significantly more DNA damage was found in the liver of both *Wallago attu* and *Ompok bimaculatus* from contaminated water as to non-contaminated water. This may be correlated to water pollution at sites 2 and 3 of river Kabul. The liver came fourth after the intestine and skin for TCS, followed by gills and muscle. More TCS was found in the liver of *Ompok bimaculatus* and less in *Wallago attu*. This may be due to the exposition of this fish to contaminated water for more time. In another finding, Pandey *et al.* (2003) have also determined greater DNA damage (TCS) in the liver of *Wallago attu* collected from the contaminated river of Yamuna. Our results are further in agreement with the findings of Gyimah *et al.* (2020) as they also have determined more DNA damage in the liver of zebrafish. Similarly, Siraj *et al.* (2018) investigated more TCS in the liver of *Cyprinus carpio* from the same water resource. The current study proved and confirmed the genotoxicity caused by water pollution.

Contaminated water often induces genotoxicity in aquatic organisms. The intestine of fish is indirectly exposed to water pollutants with a genotoxic potential and finally binds with DNA molecules and provokes genotoxicity (Ohe *et al.*, 2004). In the current findings, the overall intestine of both examined fish species showed more DNA damage (TCS) than skin, liver gills and muscle. More DNA damage (TCS) was observed in the intestine of *Ompok bimaculatus* than *Wallago attu*. This may be correlated with exposition of this fish to contaminated water for more period. In a previous finding, Lima *et al.* (2006) investigated a greater degree of TCS in the intestine of *Oreochromis niloticus* as collected from pollutant water. Fatima *et al.* (2014) have also investigated the higher frequency of TCS in the intestine of commercial fish species in an Urban Reservoir. Anyhow, in the current investigation, a greater frequency of DNA damage was investigated in the intestine of *Ompok bimaculatus* and smaller in *Wallago attu*. This could be because of the high pollution in the river Kabul. As a whole, the trend of TCS in different tissues was intestine > skin > liver > gills > muscle, and in studied fishes was *Ompok bimaculatus* > *Wallago attu*. This demonstrated that more DNA damage (TCS) was observed in the intestine and less in the muscle. Similarly, a higher TCS was found in *Ompok bimaculatus* and lower in *Wallago attu*.

5. Conclusion

Results of the present study clearly showed that river Kabul is contaminated with physical and heavy metal parameters due to the discharging of effluents and sewages into it. These parameters induce DNA damage in skin, intestine, gills, liver and muscle of *Ompok bimaculatus* and *Wallago attu*. It concluded that by utilization of comet assay, *Ompok bimaculatus* and *Wallago attu* can be utilized as indicators of water pollution in water bodies. The present investigation will also preserve the fish fauna in Pakistan. Consequently, great efforts are also needed to protect the river Kabul from contamination. This can be achieved by detoxifying the sewages and industrial effluents before discharging them into River Kabul.

Declaration of conflict of interest

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