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Research Article

Toxicological impact of water pollutants on DNA and tissues of inhabitant fish *Labeo dyocheilus* of River Kabul, Khyber Pakhtunkhwa, Pakistan

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Abstract:
This investigation evaluated pollution in River Kabul and impact on DNA and histology of intestine, gills, liver, and muscle of *Labeo-dyocheilus*. Water and fish samples were collected from non-polluted site of Warsak Dam and polluted sites of Amanghar industrial zone and Nowshera city. Sequence of physicochemical parameters in water samples A, B and C was TDS >TSS >EC >TA >Cl >Na >K >pH and heavy metals was Zn >Pb >Cd >Ni >Fe >Mn >Cu >Cr. The parameters in samples A, B and C except TSS were below NEQS proposed limits. The investigation determined geno-toxicological impact of water pollutants in different tissues. DNA damage cells like TCS and comet classes (0, 1, 2, 3, 4) were determined in intestine, gills, liver, and muscle. Sequence of comet classes in tissues was class 0> class 4> class 3> class 2> class 1. Trend of DNA damage in tissues was intestine >liver >gills >muscle. The study investigated histopathological impacts of water pollutants in intestine, liver, gills, and muscle. Trend of lesions in tissues was liver >intestine >gills >muscle. The study confirmed high levels of pollutants like heavy metals and physicochemical parameters in River Kabul, and geno-toxicological and histopathological disorders of water pollutants in *Labeo-dyocheilus*.

Keywords: DNA, *Labeo dyocheilus*, comet assay, water quality, water pollution, heavy metals, genotoxicity, histopathology.

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1. Introduction

River Kabul has its origination from Afghanistan and enters Pakistan at Mohmand Agency (Gresswell & Huxley, 1965). Warsak dam has been constructed on River Kabul at Warsak, near Mohmand Agency. It irrigates the lands of Charsadda, Peshawar and Nowshera districts (IUCN, 1994). There are about 81 industrial units, which discharge their effluents directly or indirectly into River Kabul (Khan *et al*., 1999). River Kabul received sewages from Charsadda, Mardan, Peshawar and Nowshera districts (Yousafzai *et al*., 2008). The people in the surrounding of River Kabul complained about water pollution in the River Kabul. It was because of increased fish killing in the river, skin problems in humans and maladies in animals. Many people complained about the reduction of crop production as irrigated by the River Kabul (IUCN, 1994). Temperature, pH, salinity, acidity, CO_2, TSS, TDS, TA and heavy metals are physico-chemical parameters that impact the survival of aquatic animals (Chitmanat & Traichaiyaporn, 2010). Pollutants like metals in the aquatic environment are a major problem throughout the world and are a task for environmental agencies (Tay *et al.*, 2009). Heavy metals are discharged into the water bodies through anthropogenic activities, weathering and industrial units which are non-essential to both animals and human beings (Borgmann., 1983). Heavy metals found in the water bodies directly impact on biota and indirectly impact on human beings. Trace metals are common pollutants, which are discharged into aquatic environments from many sources like industrial units, anthropogenic activities, swages and weathering (Merian, 1991).

Fifty-four different fish species are reported in the River Kabul and in its branches (Rafique, 2001). Among these fifty-four species, about thirty fishes are common. *Cyprinus carpio, Wallago attu*, *Clopisoma naziri*. *Ompok bimaculatus*, *Labeo dyocheilus* and *Ctenopharyngodon idella* are commercial fishes. These are eaten by people both locally in villages as well as in towns of Jehangira, Swabi, Charsadda, Mardan, Peshawar and Nowshera districts. *Botia rostrata*has is the only fish that has been investigated in Pakistan from River Kabul (Butt, 1986). Fish is the only living organism, that act as an indicator and helps in the determination and monitoring of water pollution because they are very sensitive and only respond to any kind of changes in the ecosystem (Santhanam *et al*., 1987). Water pollutants are chemicals that contaminate the aquatic environment and result in many disorders and problems in fish as well as in human beings who eat these contaminated fishes (Bowen, 1979; Dix, 1981). Polluted water not only impacts aquatic animals but can also produce different disorders and diseases in humans (Tebbutt, 1983). Fishes are sensitive animals that can be used as bio-indicator for the impact of a water pollutant and are helpful in the determination of water pollution. The fishes can also be used to assess the disorders like teratogenic and carcinogenic in human beings caused by water pollutants (Matsumoto *et al*., 2006).

The comet assay is a sensitive test that can be utilized for the determination of genotoxicity caused by water pollution in fishes and other aquatic organisms (Tice *et al.*, 2000). Comet assay is a test that can be utilized for the investigation of the geno-toxicological effect of water pollutants and other chemicals in aquatic environments. Therefore, fishes are utilized as test animals for the determination of genotoxicity caused by mutagenic and pro-mutagenic chemicals of water in both fresh and marine water. This test is also in practice for the determination of genotoxicity caused by pollutants in the aquatic organisms of rivers and lakes. Similarly, this assay can also be utilized for monitoring genotoxicity in ocean and continental waters and fish from these water bodies are utilized for the determination of DNA damage induced by toxic water pollutants dissolved in the water as well as environmental analysis of water samples (Lee & Steinert, 2003). Similarly, histopathological analysis is the most sensitive technique that can help in the determination of different disorders and abnormalities caused by water pollutants in aquatic organisms including fish (Van der Oost *et al.*, 2003). Histopathological study is tested in the past for the toxicological impacts of different chemicals. This is a very sensitive technique for the determination of pollution in natural water bodies and their impact on the aquatic organisms (Costa *et al.*, 2009).

The current findings meant to assess heavy metal and physico-chemical parameters in the water of River Kabul and their impacts on DNA and tissues of freshwater fish *Labeo dyocheilus* collected from Warsak dam (reference site 1) and Amanghar and Nowshera sites (polluted sites 2 and 3) that receiving industrial effluents and city swages.

2. Materials and methods

2.1. Sampling sites

Both fish and water samples were taken from low and highly polluted belts of River Kabul and compared with reference samples of Warsak dam. One sample (Water and fish) were taken from the area of Amangarh industrial zone (site 2). It receives effluents from Amangarh industries and factories. The second sample (Water and fish) was collected near Nowshera city about 4 km downstream Amangarh industrial zone (site 3). This site receives sewages from Nowshera city, Mardan, Risalpur and other adjacent towns. The third sample (Water and fish) was taken from reference site 1 (Warsak dam) about 60 km upstream from polluted parts of River Kabul.

2.1.1. Water samples collection

Water samples were collected at 70 cm depth from both reference and polluted localities of River Kabul in one-litre plastic bottles. For heavy metals determination, water samples were taken in separated one-litre plastic bottles and added 5 ml of nitric acid for preservation.

2.1.2. Field observations

In situ, pH was determined by pH meter, electrical conductivity (EC) by conductivity meter and total dissolved solids (TDS) by TDS meter.

2.1.3. Laboratory analysis

In the laboratory, TSS was determined by using the filter paper, total alkalinity and chloride by PC multi-direct spectrophotometer. Sodium, potassium and heavy metals (Zn, Ni, Cr, Pb, Cd, Fe, Mn) in water samples were analyzed using the atomic absorption spectrometry (Spectra-AA-700).

2.2. Collection of fish samples

Fishing was done with the help of fishermen. The gill net was used for the netting of fish, with the help of four fishermen and a wooden boat that usually operated a single pati. Fish species included *Labeo dyocheilus.*

2.3. Collection and preservation of fish tissues

The netted fish was dissected for collection of different tissues like gills, muscle, liver and intestine. The tissues were washed and stored in the refrigerator (-20°C) for determination of DNA damage and histopathological studies.

2.4. Comet Assay

This technique was performed according to the protocol of Singh *et al.* (1988). Tissue such as gills, muscle, liver and intestine were homogenized in 1 mL HBSS (20 mM EDTA/ 10% DMSO). 10μl of cell suspension were mixed in 90μl of LMA layers pre-coated slides with NMA. After 20 mins, 120μl of LMA was again layered on the slides and left at 4°C again for 20 mins. The cells for lysis were then kept in lysing solution (2.5M NaCl, 100mM EDTA, 10mM Tris, 1% Triton X-100, 10% DMSO, pH 10.0) at 4°C. After this slide were washed with cold water and then kept in gel box and filled the box very gently with electrophoresis buffer (1mM EDTA 300mM NaOH, pH 13.0) and allowed the slides for 20 min for DNA unwinding. After 20 mins the gel box was kept in the refrigerator and set the power supply at 24V and 300mA current for 30 minutes. Thereafter, the slides were washed with neutralization buffer for 15 mins, kept in ethanol for dehydration. The slides were stained with 70μl of 20μg/ml in acridine orange.

2.5. Scoring of slides for DNA damage

100 cells were selected from each slide and images were taken by fluorescent microscope. Comet tail lengths were scored visually into 5 comet classes like comet class 0, 1, 2, 3 and class 4. A final overall total comet score for all 100 cells was obtained by a formula such as TCS = 0(n) + 1(n) + 2(n) + 3(n) + 4(n), Where (n) indicates the number of cells in each class.

2.5.1. Histopathological examination

The tissues like gills, muscle, liver and intestine were fixed in formaline solution for 24 hrs. For dehydration, the tissues were then treated with ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-6 µm thick were cut with a microtome machine and stained with haematoxylin and eosin. They were then examined under a complex Olympus light microscope and photographed by a camera.

2.6. Statistical analysis

Statistical analysis was done by using ANOVA software for windows. The mean and standard deviation values of the data were determined.

3. Data and results

3.1. Physico-chemical parameters

Table-1 reveals the contents of physico-chemical parameters in water sample A (control) and samples B and C (contaminated) from polluted areas of River Kabul. It is clear that water samples B and C collected from polluted localities had greater contents of different parameters as compared with reference water sample A of River Kabul. On comparison with NEQS recommended values, all physico-chemical parameters except total suspended solid (TSS) were within recommended minimum limits.

3.2. Heavy metals parameters

Table-2 highlights that more mean contents of metals were also found in water samples B and C from polluted sites 2 and 3 of River Kabul than those in water sample A from reference site 1 respectively. Mean values of all studied metals in water samples A, B and C revealed that Zn was the abundant metal in water whereas Cr was the least metal. All the metal parameters were below the permissible limits laid down by NEQS.

3.3. DNA damage

Mean values of comet classes (0,1,2,3,4) and total comet score (TCS) of *Labeo dyocheilus* collected from sites 2 and 3 (polluted) and site 1 (reference) of River Kabul water are shown in Table-3. Different comet classes and TCS in gills, intestine, liver and muscle tissues of fish netted from polluted sites were greater as compared control site 1 of River Kabul. Highest values of comet classes and TCS were observed in the intestine followed by the gills and liver and the lowest values in the muscle of *Labeo dyocheilus.*

Table-1: Physico-chemical parameters of water sample A from control site 1 (Warsak dam) and samples B and C from polluted sites 2 and 3 (Amanghar and Nowshera) of River Kabul

Water samples	Sampling sites	pH	TSS	TDS	EC	Cl	K^+	Na	TA
Sample A	Site 1	7.3±1.7	418.8±398.9	581.1±255.9	343.1±111.7	12.8±6.2	7.5±0.7	10.8±8.5	108.5±55.0
Sample B	Site 2	7.4±1.0	543.8±189.3	793.1±353.4	453.0±125.1	32.1±12.4	8.2±2.2	36.3±19.2	207.8±112.7
Sample C	Site 3	7.5±2.0	549.7±228.7	830.0±482.4	473.7±102.0	39.0±14.4	9.2±1.8	75.5±65.5	302.1±54.2
NEQS permissible limits		6-10	150	3500	NA	100	NA	NA	NA

Table-2: Heavy metal parameters of water sample A from control site 1 (Warsak dam) and samples B and C from polluted sites 2 and 3 (Amanghar and Nowshera) of River Kabul

Water samples	Sites	Zn	Ni	Cr	Cu	Pb	Cd	Fe	Mn
Sample A	Site 1	54.8±52.9	19.4±14.9	5.0.5±9.3	8.0±9.2	50.3±3.11	16.7±6.7	15.0±7.0	13.7±10.1
Sample B	Site 2	226.1±39.5	46.3±10.1	23.0±14.5	23.8±17.6	70.8±17.5	57.7±13.2	40.8±15.2	38.5±9.1
Sample C	Site 3	247.7±36.4	60.0±24.2	23.7±14.8	29.8±20.0	77.7±22.7	65.0±14.6	52.7±25.2	43.7±12.
NEQS permissible limits		500	1000	1000	1000	500	100	8000	1500

Table-3: Degree of comet classes (0, 1, 2, 3 and 4) and total comet score (TCS) in gills, intestine, liver and muscle tissues of *Labeo dyocheilus* collected from control site 1 (Warsak dam) and polluted sites 2 and 3 (Amanghar and Nowshera) of River Kabul

Tissues	Sampling sites	Class 0	Class 1	Class 2	Class 3	Class 4	TCS
	Site 1	89.3±4.1	3.0±1.0	3.0±1.0	2.6±1.5	2.0±1.0	25.0±11.1
Gills	Site 2	50.6±5.8	6.3±1.5	6.3±1.5	17.3±1.5	19.3±1.5	148.3±14.9
	Site 3	37.0±5.0	8.3±1.5	11.6±1.5	19.0±1.0	24.0±1.0	184.6±11.5
	Site 1	84.0±2.6	5.0±1.0	4.0±1.0	3.0±1.0	4.0±1.0	38.0±7.0
Intestine	Site 2	39.0±3.6	5.3±0.5	9.0±1.0	22.0±1.0	24.0±1.0	186.0±8.7
	Site 3	27.0±4.0	9.0±1.0	12.0±1.0	25.0±1.0	27.0±1.0	216.0±10.0
	Site 1	92.0±3.6	3.0±1.0	2.0±1.0	2.0±1.0	1.0±1.0	17.0±9.1
Liver	Site 2	44.0±4.5	7.3±0.5	8.0±1.0	19.3±1.5	21.3±1.5	166.6±13.2
	Site 3	31.3±2.8	9.3±0.5	12.3±0.5	23.0±1.0	24.0±1.0	199.0±7.8
	Site 1	90.0±5.0	2.3±1.5	3.0±1.0	2.6±1.5	2.0±1.0	24.3±12.0
Muscle	Site 2	54.6±4.1	6.0±1.0	6.3±1.5	19.3±4.9	17.0±1.0	134.6±10.5
	Site 3	42.6±3.2	8.0±1.0	10.0±1.0	19.0±1.0	20.3±0.5	166.3±7.5

3.4. Histopathological lesions

Histological study of different tissues of *Labeo dyocheilus* shows the presence of different histopathological alterations among examined gills, intestine, liver and muscle. In polluted areas, histopathological examination of sampled *Labeo dyocheilus*, the intestine tissue revealed alterations like degeneration of cilia, coagulative necrosis and inflammation (Figures 1, 2 and 3). Gills from the polluted areas have presented desquamation of gills epithelium, non-specific inflammation in gills lamellae and necrosis in primary gills lamellae (Figures 4,5 and 6). Liver from polluted areas has shown histopathological disorders like coagulative necrosis, non-specific inflammation and liquefactive necrosis (Figures 7, 8 and 9). Muscle tissues of *Labeo dyocheilus* from polluted sites revealed pathological abnormalities like inflammation and coagulative necrosis (Figures 10 and 11).

4. Findings and discussion

Industrial units, weathering and sewage are the main sources that lead to aquatic pollution probably in each and every country (Adeogun, 2012). Mostly the pollutants released into the water bodies impact the aquatic environment and aquatic organism in an ecosystem (Adeogun, 2012). High contents of physico-chemical parameters found in River Kabul revealed that pollutants are the chemicals that change water quality. Bad impact of pollutants added into the river was verified by more levels of all physico-chemical parameters. pH plays a vital role in the balance of aquatic ecosystem (Osman *et al.*, 2010). A little more value of pH of River Kabul water may be correlated to large amounts of pollutants that are released into this river. Higher contents of TSS impact fish life, which results in different disorders and lesions (Packman *et al.*, 1999). This parameter was observed in greater content in River Kabul water, which is attributed to sewages and industrial effluents, as reported in past studies by Yousafzai *et al.* (2008). The increase in total suspended solid contents in the water of River Kabul is an indicator of the high pollutants that are discharged into River Kabul by sewage, industrial units and agriculture-runoff. Furthermore, the presence of total dissolved solids in water also impacts water quality (Bruvold & Ongerth, 1969). Physico-chemical parameter like TDS was investigated in water that led to genotoxicity (Burton & Cornhill, 1977) and heart diseases (Schroeder, 1966).

In the current study, relatively higher amount of TDS was determined than other studied parameters in River Kabul. This could be associated with dumping of sewages and effluents into River Kabul. The present findings are agreed with the studies of Yousafzai *et al.* (2008), who have also investigated higher TDS contents from the same water body. The increase in total dissolved solid levels in water is an indicator of pollution in River Kabul. The relatively high electrical conductivity levels found in water of River Kabul suggested that this may attributed with run-off from agriculture lands, industrial units and sewages. This is in agreement with the report of UNESCO (1996), which investigated that anthropogenic activities are the main source of conductivity. Currently more electrical conductivity was observed in water from polluted sites of River Kabul than reference site. This is because of high level of chloride concentration in water, which is received at these sites from city sewages and industrial effluents. This study found minimum level of chloride from this point as compared to the previous finding reported by WHO (1993). More chloride contents are dangerous to aquatic organisms (Hunt *et al*., 2012). More intaking of chloride in drinking-water may lead to high blood pressure (Fadeeva, 1971).

In the present investigation, this parameter was reported in high concentration form polluted sites than reference site of River Kabul. An increase in chloride level is indicator of water pollution in River Kabul due to run-off, animal feeds and industrial effluents in the surrounding of River Kabul. Drinking water containing potassium has a bad health impact on healthy people. A large single dose of potassium usually induces vomiting (Gosselin *et al*., 1984). In current findings, this parameter was observed in more concentration in water from polluted portions than the control site of River Kabul. This could be because of the dumping of industrial effluents and city sewages into River Kabul. The present result reported more concentration of potassium as compared to the investigation by Powell *et al.* (1987). The presence of high content of potassium is an indicator of pollution in water of River Kabul. Presence of normal content of Na in drinking water has no health problem however, excess amount may be related to health problem (Sayyed & Bhosle, 2011). In present study, sodium from polluted sites showed an increasing tendency as compared to the reference site of River Kabul. This is correlated to sewages and industrial effluents discharging at polluted sites of River Kabul. Therefore, this result is in agreement with the finding of Sayyed and Bhosle (2011).

More levels of alkalinity in an aquatic environment make the ecosystem unsuitable for living organisms (Raju *et al*., 2014). Currently, the higher level of this parameter was observed in water from polluted sites than the reference site of River Kabul. This could be because of dumping industrial effluents and sewages into River Kabul. This parameter showed maximum content than Cl, K^+, pH and Na and less concentration than TSS, TDS and EC. The increase of alkalinity concentration in the water of River Kabul is an indicator of the presence of pollution. It was found that most physico-chemical parameters showed higher contents in the water of polluted sites of River Kabul as compared to water of reference site but generally, all physico-chemical parameters except TSS in water from polluted and control areas are below the NEQS limits. Different sources like weathering, industries or sewages added tonnes of toxic metals into water bodies, which led to water pollution and impact aquatic organisms (Rajeshkumar & Munuswamy, 2011). Current study found all metals in River Kabul within permissible limits of NEQS. These results are agreed with the finding of El Bouraie *et al.* (2010), who have investigated metals in drain outfalls of Egyptian within permissible limits. Similarly in another study Lasheen *et al.* (2012) have also investigated metals in El-Moheet drain within the permissible range. Therefore, greater contents of TSS and smaller levels of pH, TDS, EC, Cl, K^+, Na and TA and trace metals in water of River Kabul comparing to the Warsak dam water verified and confirmed the presence of the large number of pollutants in the River Kabul. This could be because of dumping of domestic, agricultural and industrial effluents.

Fish are sensitive organisms that can be utilized as a tool for investigating the geno-toxicological impacts of water pollution (Souza & Fontanelli, 2006). Water pollutants not only affect the growth and survival of the fish but also damage DNA, which results in cancer (Russo *et al.*, 2004). In the current study overall result showed that DNA damage like comet class 0,1,2,3,4 and TCS in gills, intestine, livers and muscle tissues of *Labeo dyocheilus* collected from polluted sites was greater than those collected from the reference site of River Kabul. This is evidence of the response of fish to the pollutants. The increase in DNA damage is strongly attributed to elevated levels of physico-chemical and heavy metal parameters as a result of pollution stress in River Kabul. Overall DNA damage in examined tissues of *Labeo dyocheilus* was highest in the intestine and lowest in the muscle. This could be because of the exposition of this tissue to water pollutants for long time and low detoxification mechanism. The results suggested that physico-chemical and heavy metal parameters have the potential to lead DNA damage in various tissues of fish at very low concentrations. Histopathological examination of different tissues of fish help to study the toxic impact of pollutants on aquatic organisms and protect the environmental ecosystem from degradation (Costa *et al*., 2011).

Different tissues of fish like intestine, digestive tract, liver, gills and kidneys are the target organs that are more exposed to water pollutants and can be studied through their histopathology easily to assess the toxicity of the pollutants (Au, 2004). In the current investigation, histological changes in gills, intestine, muscle and liver of *Labeo dyocheilus* netted from River Kabul may attribute to physico-chemicals and heavy metals as reported by Tayel *et al.* (2007). Histopathlogical lesions like necrosis and inflammation are most visible damages in tissues caused by pollutant (Rabitto *et al*., 2005). In the current investigation, the histopathological findings in intestine of *Labeo dyocheilus* collected from River Kabul are in agreement with Gardner and Yevich (1970), Gaber *et al*. (2013) and Gutierrez *et al.* (1978), who have also reported nearly the same lesions in the intestine of fish after exposure to cadmium chloride. Establier *et al*. (1978) have investigated intestinal toxic lesions in the intestine of *Mugil auratus* after exposure to metal like mercury. Sastry and Gupta (1978) have studied pathological alterations in the intestine of *Channa punctatus* after exposure to mercuric chloride.

Similarly, Smith and Piper (1975) studied different pathological disorders in the intestine of fish exposed to ammonia for a long period. The present histological examination of the gills of *Labeo dyocheilus* in polluted water revealed more histological lesions, including desquamation of gills epithelium, non-specific inflammation in gills lamellae and necrosis in primary gills lamellae than reference water of River Kabul. It is possible that the damage to the gills may be attributed to direct exposition to water pollutants (El-Bakary *et al*., 2011). Histological examination of the liver showed increased coagulative necrosis, non-specific inflammation and liquefactive necrosis, which could be correlated with water pollutants. These data consist of many past investigations (Abd El-Gawad, 1999). In addition, pathological liver necrosis was investigated by Badr *et al.* (2014), accompanied by additional feature such as vacuolar degeneration. Similarly, histological examination of muscle showed lesions like inflammation and coagulative necrosis, which may be because of water pollutants like physico-chemical and heavy metal parameters. These results agreed with the investigation of Badr *et al.* (2014), who have investigated perivascular edema and hemorrhagic myositis in the muscle of *Oreochromis niloticus* after collection from polluted water of the River Nile Basin, Egyptian.

5. Conclusion

In this study, heavy metals (Zn, Ni, Cr, Cu, Pb, Cd, Fe, Mn) and physico-chemical parameters (pH, TSS, TDS, EC, Cl-, K+, Na+ and TA) were determined in water from three sites. The outcomes of the investigation clearly demonstrated that River Kabul is polluted with physico-chemical and heavy metal parameters due to the continuous dumping of industrial and sewage discharges into it. It was also determined that these parameters induce DNA damage and cause several histopathological changes in the intestine, gills, liver and muscle of *Labeo dyocheilus.* This is because River Kabul received industrial effluents from Amanghar industrial zone and sewages from Peshawar, Nowshera and Mardan cities. Consequently, different authorities are needed to protect the River Kabul from pollution and reduce environmental risk.

Declaration of conflict of interest

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References

Abd El-Gawad, A. M. (1999). Histopathological studies on the liver and gills of Nile tilapia,(Oreochromis niloticus) exposed to different concentrations of lead acetate and zinc sulphate. *Journal of the Egyptian German Society of Zoology, 30*, 3-22.

Adeogun, A. O. (2012). Impact of industrial effluent on water quality and gill pathology of Clarias gariepinus from Alaro stream, Ibadan, Southwest, Nigeria. *European Journal of Scientific Research*, *76*(1), 83-94.

Au, D. W. T. (2004). The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. *Marine Pollution Bulletin, 48*(9-10), 817-834. https://doi.org/10.1016/j.marpolbul.2004.02.032

Badr, A. M., Mahana, N. A., & Eissa, A. (2014). Assessment of heavy metal levels in water and their toxicity in some tissues of Nile Tilapia (Oreochromis niloticus) in River Nile basin at Greater Cairo, Egypt. *Global Veterinaria*, *13*(4), 432-443.

Borgmann, U. (1983). Metal speciation and toxicity of free metal ions to aquatic biota [Algae and bacteria, invertebrates and fish]. *Advances in Environmental Science and Technology (USA)*, *13*, 47-72.

Bowen, H. J. M. (1979). *Environmental chemistry of the elements*. Academic Press.

Bruvold, W. H., & Ongerth, H. J. (1969). Taste quality of mineralized water. *Journal-American Water Works Association*, *61*(4), 170-174. https://doi.org/10.1002/j.1551-8833.1969.tb03732.x

Burton A.C., & Cornhill J. F. (1977). Correlation of cancer death rates with altitude and with the quality of water supply of the 100 largest cities in the United States. *Journal of Toxicology and Environmental Health, 3*(3), 465-478. https://www.cabdirect.org/cabdirect/abstract/19782701142

Butt, J. A. (1986). Fish and fisheries of North West Frontier Province (N.W.F.P.), Pakistan. *Biologia (Pakistan), 32*(Special Issue), 21-34.

Chitmanat, C., & Traichaiyaporn, S. (2010). Spatial and temporal variations of physical-chemical water quality and some heavy metals in water, sediments and fish of the Mae Kuang River, Northern Thailand. *International Journal of Agriculture and Biology*, *12*(6), 816-820.

Costa, P. M., Caeiro, S., Lobo, J., Martins, M., Ferreira, A. M., Caetano, M., ... & Costa, M. H. (2011). Estuarine ecological risk based on hepatic histopathological indices from laboratory and in situ tested fish. *Marine Pollution Bulletin, 62*(1), 55-65. https://doi.org/10.1016/j.marpolbul.2010.09.009

Costa, P. M., Diniz, M. S., Caeiro, S., Lobo, J., Martins, M., Ferreira, A. M., ... & Costa, M. H. (2009). Histological biomarkers in liver and gills of juvenile Solea senegalensis exposed to contaminated estuarine sediments: a weighted indices approach. *Aquatic Toxicology*, *92*(3), 202-212. https://doi.org/10.1016/j.aquatox.2008.12.009

Dix, H. M. (1981). *Environmental pollution.* John Wiley and Sons.

El-Bakary, N. E. R., Said, S. B., & El-Badaly, A. (2011). Using Oreochromis niloticus for assessment of water quality in water treatment plants. *World Applied Sciences Journal*, *12*(9), 1455-1463.

El-Bouraie, M. M., El-Barbary, A. A., Yehia, M. M., & Motawea, E. A. (2010). Heavy metal concentrations in surface river water and bed sediments at Nile Delta in Egypt. *Suo*, *61*(1), 1-12. https://www.cabdirect.org/cabdirect/abstract/20103219704

Establier, R., Gutierrez, M., & Arias, A. (1978). Accumulation and histopathological effects of organic and inorganic mercury to the Lisa Mugil auratus. *Scientia Marina: Investigación Pesquera*, *42*(1), 65-80.

Fadeeva, V. K. (1971). Effect of drinking water with different chloride contents on experimental animals. *Gigiena i Sanitariya*, (6), 11-15. https://www.cabdirect.org/cabdirect/abstract/19721490992

Gaber, H. S., El-Kasheif, M. A., Ibrahim, S. A., & Authman, M. (2013). Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish. *World Applied Sciences Journal*, *21*(3), 329-341.

Gardner, G. R., & Yevich, P. P. (1970). Histological and hematological responses of an estuarine teleost to cadmium. *Journal of the Fisheries Board of Canada*, *27*(12), 2185-2196. https://doi.org/10.1139/f70-248

Gosselin, R. E., Smith, R. P., Hodge, H. C., & Braddock, J. E. (1984). *Clinical toxicology of commercial products*. Williams & Wilkins.

Gresswell, R. K., & Huxley, A. (1965). *Standard encyclopedia of the world's rivers and lakes*. Weidenfield and Nicholson.

Hunt, M., Herron., E., & Green, L. (2012). Chlorides in fresh water. *URI Watershed Watch, Cooperative Extension College of the Environment and Life Sciences, Department of Natural Resources Science, Coastal Institute in Kingston, 1 Greenhouse Road, Kingston, Rhode Island*. https://cels.uri.edu/docslink/ww/water-quality-factsheets/Chlorides.pdf

IUCN. (1994). Pollution and the Kabul River, an analysis and action plan. *Environmental Planning and Development Department, NWFP, Pakistan*.

Khan, A. R., Akif, M., Khan, M. U. M. T. A. Z., & Riaz, M. (1999). Impact of industrial discharges on the quality of Kabul River water at Amangarh, Nowshera (Pakistan). *J. Chem. Soc. Pak*., *21*(2), 97-105. https://jcsp.org.pk/ArticleUpload/1778-7971-1-PB.pdf

Lasheen, M. R., Abdel-Gawad, F. K., Alaneny, A. A., & El-Bary, H. M. H. A. (2012). Fish as bio indicators in aquatic environmental pollution assessment: a case study in Abu-Rawash area, Egypt. *World Applied Sciences Journal*, *19*(2), 265-275. https://www.cabdirect.org/cabdirect/abstract/20123329244

Lee, R. F., & Steinert, S. (2003). Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutation Research/Reviews in Mutation Research*, *544*(1), 43-64. https://doi.org/10.1016/S1383-5742(03)00017-6

Matsumoto, S. T., Mantovani, M. S., Malaguttii, M. I. A., Dias, A. L., Fonseca, I. C., & Marin-Morales, M. A. (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticus and chromosome aberrations in onion root-tips. *Genetics and Molecular Biology*, *29*, 148-158.

Merian, E. (1991). *Metals and their compounds in the environment. Occurrence, analysis and biological relevance*. UCH, Wienheim-New York- Basel-Cambridge.

Osman, A. G. M., Al-Awadhi, R. M., Harabawy, A. S. A., & Mahmoud, U. M. (2010). Evaluation of the use of protein electrophoresis of the African catfish Clarias gariepinus (Burchell, 1822) for biomonitoring aquatic pollution. *Environmental Research Journal*, *4*(3), 235-243. https://www.cabdirect.org/cabdirect/abstract/20103322344

Packman, J., Comings, K., & Booth, D. (1999). Using turbidity to determine total suspended solids in urbanizing streams in the Puget lowlands. *Canadian Water Resources Association Annual Meeting*.

Powell, P., Bailey, R. J., & Jolly, P. K. (1987). Trace elements in British tap-water supplies. *WRC Report*.

Rabitto, I. S., Costa, J. A., De Assis, H. S., Pelletier, E., Akaishi, F. M., Anjos, A., ... & Ribeiro, C. O. (2005). Effects of dietary Pb (II) and tributyltin on neotropical fish, Hoplias malabaricus: histopathological and biochemical findings. *Ecotoxicology and Environmental Safety*, *60*(2), 147-156. https://doi.org/10.1016/j.ecoenv.2004.03.002

Rafique, M. (2001). Fish fauna of the Himalayas in Pakistan with comments on the origin and dispersal of its high Asian elements. *Pakistan Journal of Zoology*, *33*(4), 279-288.

Rajeshkumar, S., & Munuswamy, N. (2011). Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (Chanos chanos) of Kaattuppalli Island, South East Coast, India. *Chemosphere*, *83*(4), 415-421. https://doi.org/10.1016/j.chemosphere.2010.12.086

Raju, P. A. R. K., Reddy, M. S. R., Raghuram, P., Suri Babu, G., Rambabu, T., & Jeevan Kumar, J. (2014). Alkalinity and Hardness variation in ground waters of east Godavari district due to Aquaculture. *International Journal of Fisheries and aquatic studies*, *1*(6), 121-127.

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. https://doi.org/10.1016/S0147-6513(03)00027-7

Santhanam, R., Sukumaran, N., & Natarajan, P. (1987). *Manual of fresh-water aquaculture*. Oxford & IBH Publishing.

Sastry, K. V., & Gupta, P. K. (1978). Effect of mercuric chloride on the digestive system of Channa punctatus: A histopathological study. *Environmental Research*, *16*(1-3), 270-278. https://doi.org/10.1016/0013-9351(78)90161-5

Sayyed, J. A., & Bhosle, A. B. (2011). Analysis of chloride, sodium and potassium in groundwater samples of Nanded City in Mahabharata, India. *European Journal of Experimental Biology*, *1*(1), 74-82.

Schroeder, H. A. (1966). Municipal drinking water and cardiovascular death rates. *Journal of the American Medical Association*, *195*(2), 81-85.

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. https://doi.org/10.1016/0014-4827(88)90265-0

Smith, C.E, R.G, Piper. (1975). Lesions associated with chronic exposure to ammonia. In W. E. Ribelin, & G. Migaki, G. (eds.), *The pathology of fish* (pp: 497-514). University of Wisconsin.

Souza, T. d. S., & Fontanetti, C. S. (2006). Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *605*(1-2), 87-93. https://doi.org/10.1016/j.mrgentox.2006.02.010

Tayel, S. I., Ibrahim, S. A., Authman, M. M. N., & El-Kashef, M. A. (2007). Assessment of Sabal drainage canal water quality and its effect on blood and spleen histology of Oreochromis niloticus. *African Journal of Biological Sciences*, *3*(1), 97-107.

Tay, C. K., Asmah, R., & Biney, C. A. (2010). Trace metal levels in water and sediment from the Sakumo II and Muni Lagoons, Ghana. *West African Journal of Applied Ecology*, *16*(1), 75-94. https://doi.org/10.4314/wajae.v16i1.55870

Tebbutt, T. H.Y. (1983). *Principles of water quality control* (3rd ed.). Pergamon Press.

Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., ... & Sasaki, Y. F. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis*, *35*(3), 206-221.

UNESCO. (1996). *Water quality assessments - A guide to use of biota, sediments and water in environmental monitoring* (2nd ed.). University Press Cambridge.

Van der Oost, R., Beyer, J., & Vermeulen, N. P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, *13*(2), 57-149. https://doi.org/10.1016/S1382-6689(02)00126-6

WHO. (1993). Guideline for drinking water quality. *World Health Organization*.

Yousafzai, A. M., Khan, A. R., & Shakoori, A. R. (2008). An assessment of chemical pollution in river Kabul and its possible impacts on fisheries. *Pakistan Journal of Zoology, 40*(3), 199-210.

Appendices:

Figure 1: Degeneration of cillia. in intestine of *L. dyocheilus*

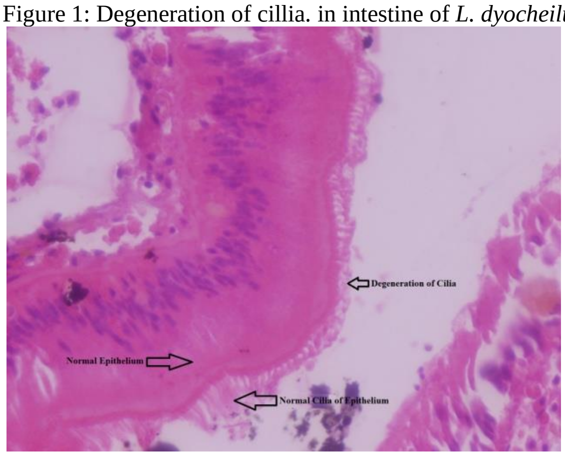

Figure 2: Coagulative necrosis intestine of *L. dyocheilus*

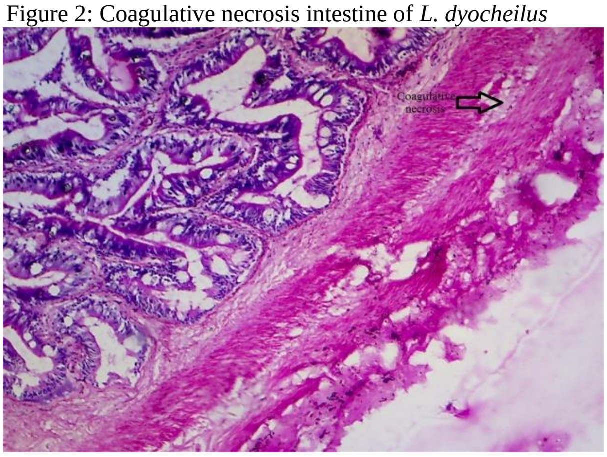

Figure 3: Inflammation in intestine of *L. dyocheilus*

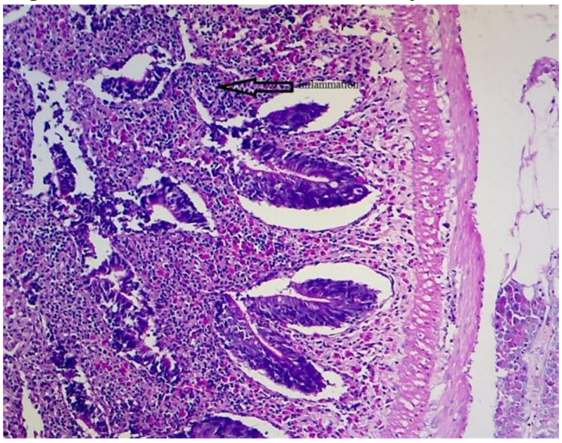

Figure 4: Desquamation of gills epithelium in intestine of *L. dyocheilus*

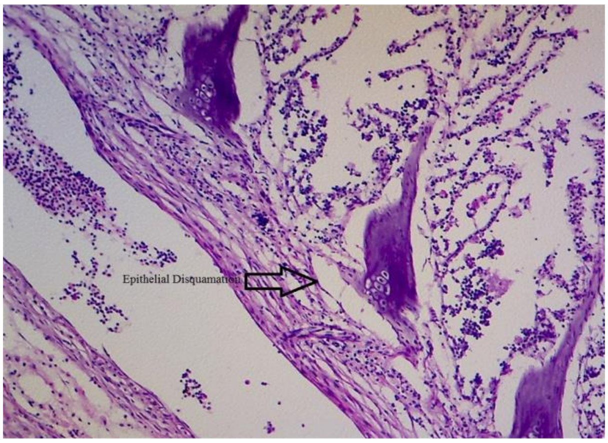

Figure 5: Non-specific inflammation in gills lamellae of in gills of *L. dyocheilus*

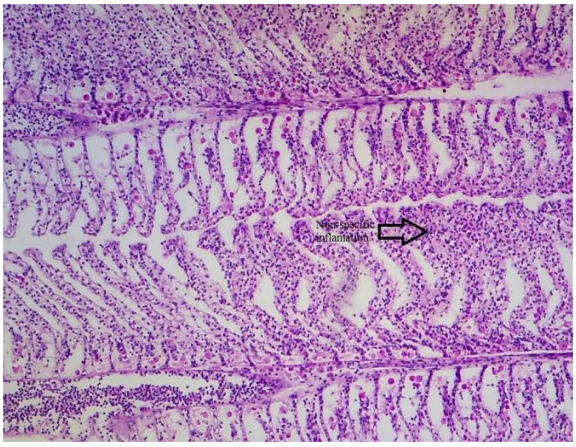

Figure 6: Necrosis in primary gills lamellae in gills of *L . dyocheilus*

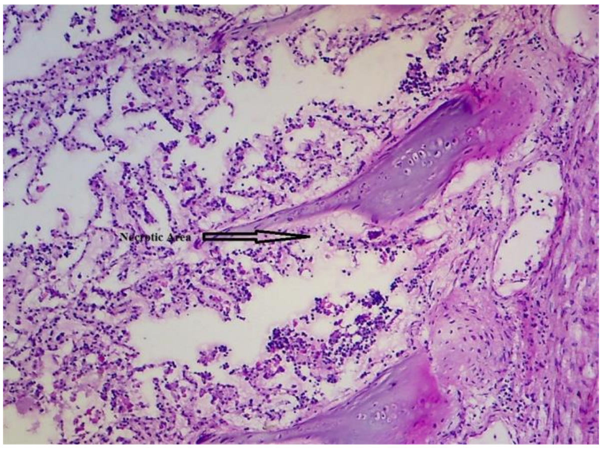

Figure 7: Coagulative necrosis in liver of *L. dyocheilus*

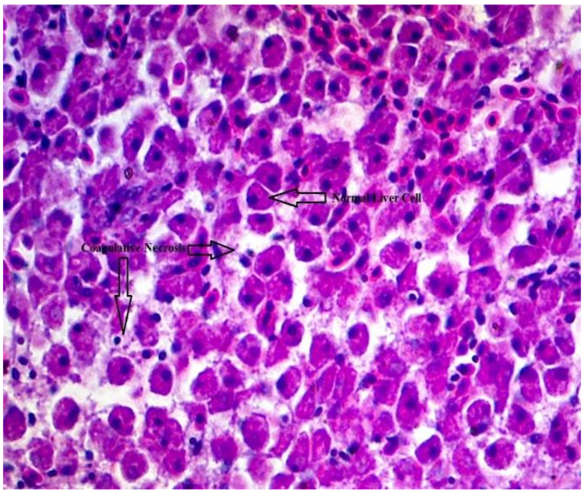

Figure 8: Non-specific inflammation in liver of *L. dyocheilu*

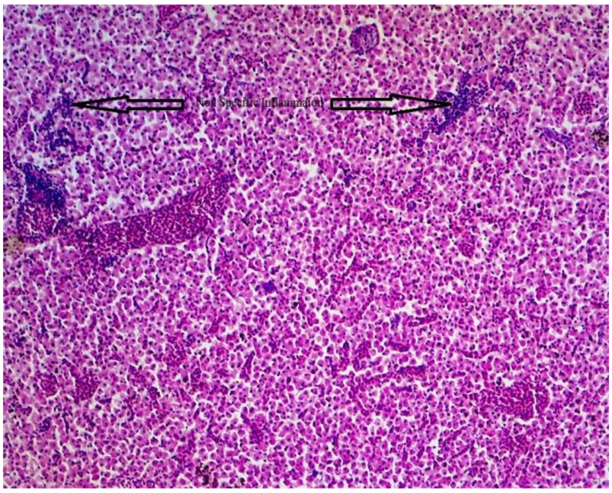

Figure 9: Liquefactive necrosis in liver of *L. dyocheilus*

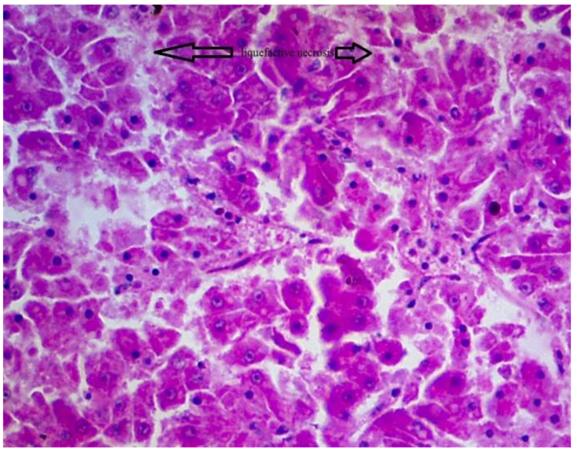

Figure 10: Inflammation in muscle of *L. dyocheilus*

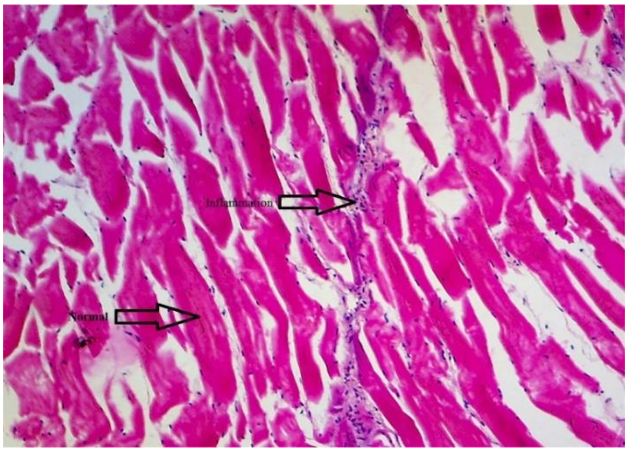

Figure 11: Coagulative necrosis in muscle of *L. dyocheilus*