



## Research Article

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# Impact of Contaminated Water and Sediment on Fish Species of Peshawar Region, KPK, Pakistan

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## Abstract

Rivers in the KPK Peshawar region of Pak are subjected to a variety of anthropogenic impacts, Agricultural operations, industry, and urban garbage are all examples. The purpose of The goal of this research was to find out the degree of water contamination by showing a testing that includes in situ bio-monitoring of water constituents on Genotoxicity in local fish species. Three places in the region were tested for Geno-toxicity using the micronucleus test. At sample locations like the urban perimeter, farmland, and the fertiliser industry, it was interesting to observe if there were any differences between monitored species control and such *Characidium fasciatum*, *Astyanax fasciatus*, *Astyanax altiparanae*. Records showed that the species from sites one, Two, and Three had a considerably greater frequency of Micronucleus when compared to fish from site Four. The frequency of Micronucleus was substantially higher in and *A. altiparanae* and *A. fasciatus* erythrocytes at sites one and two. Micronucleus frequencies were higher at site three in *C. fasciatum*. Micronucleus induction in *C. fasciatum* was associated to Cr levels in water and sediment, whereas *A. altiparanae* and *A. fasciatum* were linked to zinc levels in water and sediment.

**Keywords:** Water Pollution, Fish Species, Water Sampling, Heavy metal

## 1. Introduction

The majority of poisons are thrown into rivers from city and industrialized sources, abandoned armed locations, and agricultural operations, endangering aquatic ecosystems (Kaur and Dua, 2014; Byrne et al., 2015; Pinto et al., 2015; Van der Oost et al., 2003). Heavy metals are a major contaminant that build quickly in the body and take a long time for mammals to metabolize and remove. Heavy metal ion contamination in water is primarily produced by industry, with the most polluting heavy metals being Zn, Cr, Cu, Pb (Noller, 1991; Sabale et al., 2012; Outridge and trbac et al., 2015). The two principal sediment layers are a surface layer and deep layer and in interaction with the ocean. Because of their intimate interaction with metals and ability to tolerate them, sediment contamination contributes to metal deposition in benthic invertebrates (Banks et al., 2013; Kalantzi et al., 2014). Because of these species' tolerance, pollution of additional trophic levels could arise as a consequence of food chain transfer (Weber et al. 2013). Due to these animals' stronger potential to store Zinc and Chromium, heavy metal concentrations in water are lower than in sediments (Wang et al., 2014; Harguinteguy et al., 2014) and higher in fish organs (Dhanakumar *et al.*, 2015; Fatima et al., 2014 Avigliano et al., 2015). As a result, biological monitoring could be utilized to detect pollution and its harmful effects (Gurcu *et al.*, 2010). One of the most used procedures for detecting genotoxic effects linked to environmental contaminants is the micronucleus (MN) test (Peng *et al.*, 2015). This test is more important in aquatic environments because it is the major method for determining Geno-toxic activity of chemical compounds (Ergene *et al.*, 2007). Fish are a good model for identifying Geno-toxic pollutants in aquatic ecosystems because they are sensitive to low amounts of metals in the water, are abundant, and can live in a variety of environments. (Aich et al., 2015; Walia et al., 2015; Souza et al., 2013). (Ali et al., 2008).

The goal of this study was to analyze the ecological situations in relationships of water quality and sediment at 3 sites along the Kabul, Swat, Chitral, and Kunar rivers, which serve the cities of this region of Pakistan's KPK Peshawar province. The Micronucleus test was predicting Geno-toxic responses and pollution in three different fish specie's.

## 2. Material's and Methods

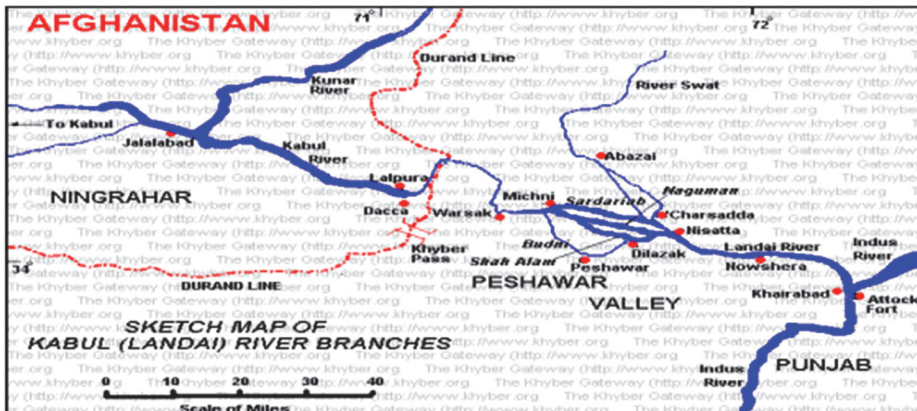
Area for research The Kabul's basin It is the primary river in eastern Afghanistan, with a tributary to the right in Pakistan's Khyber Pakhtunkhwa region. The Swat River is a perennial river in Pakistan's Khyber-Pakhtunkhwa Province's northern section Chitral, also known as Chitral is a town in Pakistan's northern province of Khyber Pakhtunkhwa.(Rome et al., 2008).

The Kunar River (also known as the Chitral River) runs through a valley that is 2 miles (3 kilometres) wide and Four thousand nine hundred feet (one thousand four

hundred ninety metres) above sea level. It first appears in the upper Chitral District of Peshawar just south of the Broghil Pass, near the Afghan border. The basin is Twenty-Six Thousand km<sup>2</sup> in size (10,000 sq mi) and 480 km long (300 mi)

Water samples were taken at four different locations. Site 1 was described as the Kabul River near the Cement Industry (18° 10' 58.69" S, 47° 54' 28.46" W). The Swat River is a perennial river in Pakistan's Khyber-Pakhtunkhwa Province's northern section. Chitral, also called Chitral, is a town in Pakistan's northern Khyber Pakhtunkhwa province, located at (18° 11' 24.18" S, 47° 55' 17.45" W). It is a tributary to the right of the main river in eastern Afghanistan and the Khyber Pakhtunkhwa province of Pakistan (18° 14' 04.46" S, 47° 49' 18.78" W). Due to its water quality, the Kunar River (also known as the Chitral River) was designated as site 4 (19° 11' 38.64" S, 48° 54' 53.18" W) in a valley Two miles (Three km) wide and at an elevation of around Four thousand nine hundred feet (1,490 metres) above sea level (Abbott et al., 1879).

Samples of water for this investigation, 1-Lamber glass bottles were used, which had previously been labelled with information about the sampling place, date, and time of collection, as well as the identity of the person in charge of the sampling. The tests were carried out in the University of Peshawar KPK Department of Chemistry.



**Figure 1:** Map of the Kabul region of Pakistan, illustrating the study's collecting sites. **Source:** Swat River (Site 1), Kabul River (Site 2), Chitral River (Site 3) and the Location Site (Site 4). [www.researchgate.net](http://www.researchgate.net)

### 3. Sediment Samples

Following the collection of a water sample, around Two hundred g of bottom sediments were together at each sampling site. Plastic containers were used to store the sediments. with lids and labelled with the following data: All samples were kept refrigerated in an isothermal box until the chemical analysis at the Chemistry Laboratory could begin.

**Table 1:** Shows the averaged over-all features of the four sampling places for the rainy and dry seasons

Water physicochemical characteristics (in situ)	Site 1		Site 2		Site 3		Site 4		CONAMA	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	357/05	WHO
Temperature	22.75	25.50	22	23.50	20	22.50	20.5	23.50	NE	NE
pH	6.82	6.80	6.55	6.67	6.63	6.57	6.81	6.85	6.0–9.0	6.0–8.5
Dissolved oxygen (mg/L)	6.85	6.65	7.13	6.96	12.35	12.30	7.00	6.85	>4	>5
Conductivity (IS/cm)	166.63	170.88	89.00	85.50	14.5	15.50	82.25	75.50	NE	NE
Turbidity (NTU)	21.25	22.00	56.00	21.00	20.60	19.10	24.25	21.75	100	29
Iron (mg/L)	0.24	0.23	0.22	0.21	0.18	0.13	0.23	0.22	5.0	0.3
Phosphate (mg/L)	1.61 <sup>a</sup>	1.82 <sup>a</sup>	0.73 <sup>a</sup>	0.70 <sup>a</sup>	0.19 <sup>a</sup>					
	0.14 <sup>a</sup>	0.41 <sup>a</sup>	0.35 <sup>a</sup>	0.025	NE					
Ammonia nitrogen (mg/L)	0.55 <sup>b</sup>	0.64 <sup>b</sup>	0.22 <sup>b</sup>	0.25 <sup>b</sup>	0.17 <sup>b</sup>					
	0.19 <sup>b</sup>	0.19 <sup>b</sup>	0.19 <sup>b</sup>	13.3	<0.02					
Sulphate (mg/L)	18.4	11.60	4.26	4.26	0.2	0.20	3.1	3.10	250	200
Potassium (mg/L)	4.67	5.03	3.18	3.11	1.37	2.89	2.89	3.69	NE	NE
Zinc (mg/L)	0.11	0.11	0.04	0.08	0	0.01	0.02	0.05	5.0	5.0
Chromium (mg/L)	0.01	0.01	0.00	0.02	0.04	0.07 <sup>ab</sup>	0.05	0.02	0.05	0.05

#### 4. Capture of Fish Species

At the four sampling sites, Fifty *Astyanax fasciatus* (tetra) specimens were collected in total. A total of Forty-three *Astyanax altiparanae* (yellow-tail tetra) specimens were also obtained. At sites 1, 3, and 4, 38 *Characidium fasciatum* specimens were also collected. Despite the fact that these species were chosen for the MN test because to their vast distribution in the Kunar river During the study, no *Characidium fasciatum* specimens were caught at site 2 or its tributaries. Fine mesh nets, a fine-mesh cast net, a 70-cm-diameter sieve with 4-mm mesh apertures, and a reed with a line and hook were used to capture fish specimens. the next day. Because each fish species must be kept alive for MN analysis, several capture procedures were chosen.

#### 5. Micronucleus Test

Smears were created as soon as possible by depositing a drop of blood on microscope slides and drying them for 24 hours (Starling, 2001 and Grisolia ). The cells were then fixed for 10 minutes in hundred% methanol. The slides were stained with a 1:10 ratio of Giemsa stain and phosphate buffer solution (pH 6.8) for 10 minutes, then rinsed with D.W and air-dried. 4 slides were made for each animal, totaling Four Thousand erythrocytes analyzed using Light microscopy (1,000 magnifications, immersion oil) according to the Schmid method (1976).

#### 6. Statistical Analysis

The researcher to used ANOVA to check each fish species sample city site industry site fertilizer site .Pearson’s correlation was used to establish the sensitivity of the Micronucleus test for each species in terms of the presence and concentration of Chromium and Zinc. p.05. was used as the significant threshold.

## 7. Literature Review

Noreen *et al.*, 2019 showed that Physico-chemical parameter changes in water and employees' blood will have a substantial effect on freshwater resources and Public health in the study location. Furthermore, an education Programme should be launched to inform marble manufacturing unit employees, as well as the general public, about water pollution and heavy metal's bioaccumulation caused by marble manufacturing units, as well as mitigation measures to protect themselves and oceanic animal in the study area from the disturbing effects of water contamination.

Sthanadar *et al.*, 2013 investigated that in comparison to other heavy metal's, Cadmium was the most accumulated heavy metal in fish species gill's. Our data imply that fish gills have a higher proclivity for bio-accumulating Cadmium than other heavy metal's such as Zinc, Chromium, and Nickel.

Ahmad *et al.*, 2014 studied that the physicochemical parameters of Panjkora water were also shown to change as a result of sewage disposal. The water and fish species of the river Panjkora are currently safe to consume, but further sewage dumping could cause problems in the future.

Siraj *et al.*, 2014 suggested that in natural settings, omnivore fish bio accumulate more heavy metals than carnivorous fish. Although muscles accumulated the least amount of heavy metals compared to other tissues, levels of Nickel, Chromium, Cadmium, and Lead in this tissue surpassed US (RDA) limits, posing a health risk to these fish species consumers'. The study's goal was to assess the metal buildup pattern of two fish species with distinct feeding patterns in the same natural habitat, as well as any potential concealed dangers to fish consumers.

Yaqoob *et al.*, 2020 showed that Ravi's water is unfit for marine life or irrigation. Metal concentrations in fish species meat are higher than (RDA) limits, effect a major health risk to both fish and humans.

Kakar *et al.*, 2020 studied that The Gadani shipbreaking area, on Pakistan's coast, is a major fish production location. Heavy metal concentrations in eatable muscle tissues of all examined fish species from the Gadani seaside were found to be safe for human consumption after comparison to maximum allowed limits.

Munir *et al.*, 2021 studied The multiple linear regression revealed that eating fish on a daily or weekly basis increases Hg buildup in human scalp hair. Fruit's and cruciferous and leafy vegetables were reported to lower Mercury toxicity in the population when consumed regularly. More research is needed to determine the sources of Mercury and the impact of fish species pollution on the Swat fishing community's welfare.

Qasim *et al.*, 2019 reported that Concerned authorities should take the initiative to raise knowledge about fish conservation and ensure that fishing laws and regulations are strictly followed.

Haseeb *et al.*, 2015 concluded that The Tanda Lake in the Kohat district has ideal

conditions for supporting a rich fish fauna. As a result, our research will provide useful information about the Tanda Dam's fish species fauna, which will be useful in systematics, fisheries management, and conservation.

## 8. Results and Discussion

The frequencies of Micronucleus detected at sites one, two and three were significantly higher than the rate detected at site 4, the control site, as shown in Table 2. The frequency of Micronucleus (MN) erythrocytes rose in *A. altiparanae*, and *C. fasciatum* *A. fasciatus*. However, no major differences amongst the 3 Fish species were discovered. Among the sampling locations examined, sites 1 and 2 had the highest rates of Micronucleus in *A. altiparanae* cells and *A. fasciatus*. At site 3, the most Micronucleus cells were found in *C. fasciatum*. Although genotoxic events were reported to be more common at sites monitored during the rainy season, Micronucleus was found to be more common in animals collected during the dry season. Tables 1 and 2 describe the physico-chemical analyses conducted at all of the monitored sampling sites. The consequences of physicochemical and biological tests revealed the existence of heavy metal Geno-toxins in water, which are known to damage organisms. Co-relations were constructed between Micronucleus frequencies recorded in the three bio indicator species and Chromium and Zinc concentrations in water and sediment to determine the sensitivity of various species. (Table 4). Garutti and Britski have proposed a new species designation for *Astyanax altiparanae*, previously known as *A. bimaculatus* (2000). (Gustavino et al., 2001). When compared to *C. fasciatum*, the frequency of Micronucleus in *A. fasciatus* and *A. altiparanae* specimens at sampling sites one and two which are sites with Zn contamination, was equal (Table 2).

**Table 2:** Shows the micronucleus frequency of *A. altiparanae*, *A. fasciatus* and *C. fasciatum* Reinhardt obtained during biomonitoring sessions at Sites 1–4

Site	Species	Dry MN frequency (mean ± SD)	Rainy MN frequency (mean ± SD)
Site 1	<i>A. fasciatus</i>	0.10 ± 0.03*	0.08 ± 0.02*
	<i>A. altiparanae</i>	0.13 ± 0.04*	0.08 ± 0.03*
	<i>C. fasciatum</i> Reinhardt	0.06 ± 0.02*	0.04 ± 0.01*
Site 2	<i>A. fasciatus</i>	0.09 ± 0.02*	0.08 ± 0.03*
	<i>A. altiparanae</i>	0.14 ± 0.04*	0.12 ± 0.03*
	<i>C. fasciatum</i> Reinhardt	ND	ND
Site 3	<i>A. fasciatus</i>	0.04 ± 0.02*	0.03 ± 0.01
	<i>A. altiparanae</i>	0.08 ± 0.03*	0.07 ± 0.02*
	<i>C. fasciatum</i> Reinhardt	0.13 ± 0.05*	0.07 ± 0.02*
Site 4	<i>A. fasciatus</i>	0.01 ± 0.01	0.01 ± 0.01
	<i>A. altiparanae</i>	0.01 ± 0.02	0.01 ± 0.01
	<i>C. fasciatum</i> Reinhardt	0.01 ± 0.01	0.01 ± 0.01

A higher occurrence of Micronucleus was found in *A. bimaculatus* than in *H. malabaricus* in Micronucleus studies (Pantaleo et al., 2006). Omar et al. (2012) found a higher sensitivity in the incidence of Micronucleus in a nektonic species, *Oreochromis niloticus*, compared to *Mugil cephalus*, a benthic species, in a study assessing heavy metal pollution with Zn, Pb, and manganese. Entirely sampling locations, including the control, had chromium in their sediments. However, only site 3 had Chromium levels higher than the national and international water quality requirements during the rainy season, according to the water study (Table 3).

**Table 3:** Averaged Physicochemical Analyses of Sediment During Rainy and Dry Seasons

Sediment chemical analysis	Site 1		Site 2		Site 3		Site 4		CONAMA 357/05	WHO
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy		
Sulfate (ppm)	0.2	0.2	0.2	0.2	0.16	0.2	0.18	0.18		
Phosphorus (ppm)	5.5 <sup>a</sup>	18.62 <sup>a</sup>	6.25 <sup>a</sup>	5.50 <sup>a</sup>	0.15	0.45 <sup>a</sup>	3.3 <sup>a</sup>	2.30 <sup>a</sup>	0.15	
Volatile solids (%)	3.80	4.61	2.97	3.73	2.60	3.56	3.14	3.26		
Total solids (%)	96.19	95.38	95.52	96.26	97.60	95.44	96.84	96.73		
Potassium (ppm)	510	231	296	1016	785	761	698	1507		
Zinc (ppm)	15.85 <sup>ab</sup>	9.18 <sup>ab</sup>	10.01 <sup>ab</sup>	10.96 <sup>ab</sup>	10.5 <sup>ab</sup>	3.00	5.98 <sup>ab</sup>	9.34 <sup>ab</sup>	5.0	5.0
Chromium (ppm)	10.88 <sup>ab</sup>	5.82 <sup>ab</sup>	9.75 <sup>ab</sup>	8.77 <sup>ab</sup>	21.5 <sup>ab</sup>	18.83 <sup>ab</sup>	6.58 <sup>ab</sup>	7.56 <sup>ab</sup>	0.05	0.05
Iron (ppm)	30438	17713	16527	468	3322	1347	13133	2822		

**Table 4:** The Pearson correlation values for heavy metal element concentrations and the micronucleus test in fish species cells are shown in Table 4

Species	Heavy metal							
	Chromium				Zinc			
	Sediment		Water		Sediment		Water	
	r <sup>2</sup>	p	r <sup>2</sup>	p	r <sup>2</sup>	p	r <sup>2</sup>	p
<i>A. fasciatus</i>	.17	.31	.16	.06	0.31	0.14	.56	.25
<i>A. altiparanae</i>	.03	.63	.03	.10	0.37	0.11	.31	.15
<i>C. fasciatum reinhardt</i>	.86	.01*	.65	.29	0.14	0.45	.28	.14

Note. \*p > 0.01.

Due to the impossibility to obtain free Chromium in nature, the bulk of Chromium is deposited in stream and river sediments, resulting in greater amounts of Chromium in sediments. Long-term exposure to high levels of Chromium causes genotoxic and carcinogenic consequences (Kumar et al., 2012; avas and Ergene-Gözükara, 2005). Krumschnabel and Nawaz (2004) found that when *Carassius auratus* hepatocytes were exposed to Two hundred fifty M Chromium (VI) for brief periods of time, their viability was reduced. The increased generation of reactive oxygen species causes this compound's toxicity (ROS). In mammals, Chromium (VI) exposure is connected to the development of intestinal cancers (Shietal.,1999; Thompson et al., 2012).

The release of industrial effluents and sewage which include larger amounts of

sulphate under anaerobic circumstances, is the principal source of sulphide in natural waters, as found at sample sites one and Two. (Table 1).

Biological processes could convert sulphates to sulphides, even while the levels were below the legislative bodies' standards. Phosphate levels are also connected to proximity to urban and industrial areas, and the main sources of are detergents and phosphates untreated waste from fertilizer factories (Ghose et al., 2009).

However, in developing nations, the regulations on its usage are less stringent, and water quality is less closely monitored. As a result, poor countries' rivers and streams are more polluted than developed countries' (Sikder et al., 2013). The measured heavy metal levels did not surpass the national and international regulatory organisations' limits, however the examined fish species are more prone to pollutants. Brito and colleagues studied waterways in developing countries (2012)

MN analysis in fish erythrocytes is a key approach for demonstrating the activities of genotoxic substances present in water as a biomonitoring tool, in addition to its low cost and convenience of application (Kumar et al., 2013; Holth et al., 2011). The ichthyofauna can be utilized to detect urban, agricultural, and industrial impacts on rivers because of their characteristics and sensitivity to changes in water and sediment, as indicated in this study.

The Previous literature reported that Ahmad et al., 2015 In both tissues, the concentrations of Cr, Ni, Cu, Zn, and Pb were higher, but the concentration of Cd was lower. Metal concentrations, on the other hand, were surpassing the RDA of the United States) standards. As a result, regular fish eating may cause health difficulties for customers. The findings of this study are disturbing, and they propose enacting environmental legislation and launching a river biomonitoring Programme.

**Table 5:** The atomic absorption spectrophotometer standard to employ for analysing the variable (Spectra-AA-700)

Element	Wavelength (nm)	Flame gases (A-AC air acetylene)	Lamp current (m-ampere)	Band pass
Cr	357.9	A-AC	10	0.5
Cu	324.7	A-AC	5	0.5
Pb	283.3	A-AC	10	0.5
Ni	232.0	A-AC	15	0.2
Zn	213.9	A-AC	8	0.5

Rajeshkumar et al., 2018 reported that showed that Both fish species' edible parts had much lower amounts of Cadmium, Copper, Chromium and Lead than the Chinese Food Health Criteria (1994). However, significant variances in the four metal content examined among the 2 Fish species and unlike tissues were discovered, by means of



significant changes. Pb levels were highest in the liver's of the fish Species, Cadmium levels were about identical in all fish organs, Cr levels were mostly concentrated in the kidney and liver, and Cu levels were highest in the gills. The liver, gills, and muscle, on the other hand, had the highest overall metal bioaccumulation. When compared to C. carpio, P. fluvidraco exhibited the largest total accumulations.

## 9. Conclusions

The species A. fasciatus and A. altiparanae are sensitive to Zinc contamination, while C. fasciatum Reinhardt is extremely prone to Chromium contamination, according to the data. Benthic and nektonic species have variable sensitivity to heavy metal ions, thus they should be used together in water biomonitoring, according to the findings. Given that pollutants have genotoxic effects on native ichthyofauna even at low concentrations, it is critical to restructure land-use planning and include evaluations of other variables such as MN in formulating tougher water-use legislation. The result of the present study suggest that the heavy metals are accumulated into the water that heavy metal damage of the living organism in water. The heavy metals are Zinc, Chromium, Mercury bio accumulate in the fish species these heavy metal metabolized in the living organism.

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