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FISH SCALES - A NON-INVASIVE ASSESSMENT TOOL FOR EVALUATING THE EFFECT OF POLLUTION ON *CIRRHINUS MRIGALA* FROM THE RAVI RIVER, PAKISTAN

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ABSTRACT

The present study evaluated the effects of water pollution on *Cirrhinus mrigala* in the Ravi River by comparing DNA extracted non-invasively from their scales to DNA extracted from the scales of fish collected from controlled fish farm. A single, random sampling was conducted. Fish were broadly categorised into three weight categories: W_1 (500 to 1000 g), W_2 (1001 to 1500 g) and W_3 (1501 to 2000 g). DNA was extracted non-invasively from control and experimental samples. The quantity and quality of DNA from the control and experimental samples were compared. The experimental samples in the W_1, W_2 and W_3 categories had an average DNA concentration (µg/µl) that was lower than the control samples. All control samples had a single DNA band; whereas the experimental samples in W_1 fish had 1 to 2 bands, the experimental samples in W_2 fish had two bands and the experimental samples in W_3 fish had fragmentation in the form of three bands. These bands show the effects of pollution on fish in the Ravi River. We concluded that this non-invasive assessment tool could be successfully used in scale-bearing fish species for assessment of contaminants and damage in the DNA as a rapid, non-lethal and biologically reliable indicator of water quality for the presence of various toxicants in surface water and their effect on the fish health.

Key words: Fish scales, *Cirrhinus mrigala*, heavy metals, non-invasive, DNA fragmentation.

INTRODUCTION

A large number of harmful substances and various types of environmental contaminants are increasing at an alarming rate due to urbanisation and industrial developments that cause water pollution. Human activities mostly affect urban streams (Paul and Meyer 2001). Agricultural, industrial and domestic effluents containing various organic and inorganic pollutants are discharged into small rivers and streams without being properly treated (Pandey *et al*. 2003). Pollution affects a variety of biological organisation levels and its impacts are highly determined by the type of contaminant present (Lawrence and Hemingway, 2003). The chief sources of contaminants are industrial waste discharge, household waste disposal, mining and agriculture and fuel combustion (Patra *et al.* 2005; Saxsena and Garg, 2010).

Fish is an excellent and relatively cheap source of animal protein that may help to bridge the protein gap because of its nutritional quality (Wasko et al., 2007). Food can also be an important source for the accumulation of heavy metals, potentially leading to the biomagnification of such contaminants (Novelli *et al.* 1998). Aquatic organisms have the ability to accumulate heavy metals from various sources, including from sediments, eroded soil, and runoff, air depositions of dust and aerosol and discharges of wastewater (Goodwin *et al*. 2003). Heavy metals have been shown to adversely affect the growth rate of Indian major carps (Hayat *et al*. 2007). The major environmental pollutants, such as metals, are not biodegradable and are considered to cause cytotoxic, mutagenic and carcinogenic effects in animals (More *et al*. 2003).

The water quality of effluent and surface water is monitored with bioassays in aquatic animals. DNA damage in aquatic animals (e.g., fish) is caused by pollution. DNA fragmentation and infertility can occur in response to exposure to environmental or industrial toxins, genetic factors, oxidative stress and other factors (Wang *et al.* 2003; Bian *et al.* 2004). Biomarkers may be used as sensitive and specific early warning signs for pollution in aquatic systems (Strinac and Braunbeck, 2000). The potential utility of biomarkers for monitoring both the environmental quality and health of organisms inhabiting polluted ecosystems has received increasing attention (Gauthier *et al*. 2004).

River Ravi is the smallest among the five main eastern tributaries of the Indus, which takes its origin from India and in Pakistan enters near village Tadyal, Kot Naina, Tehsil Shakargar of Sialkot. After flowing about 560 km, it joins River Chenab near village Sayyal Faqir, Sidhnai, Tehsil Kabirwala. In addition to its surface run-off upstream water, it also receives water from the Qadir Abad link canal and upper Chenab canal between it stretches from Shahdara to Balloki Headworks. Raw

domestic sewage, un-treated industrial effluents originating from the cities of Lahore, Sheikhupura and Kalashahkaku are dumped into the River Ravi by main tributaries of Hudiara drain and Degh Fall. Hudiara drain originates from Batala in Gurdaspur District, India, and enters into Pakistan at village Laloo which is approximately 44 km away from Lahore city (Mahboob *et al.,* 2015).

Different methods exist to evaluate the effect that pollution has on the fish. Our hypotheses were that 1) biomarkers in fish scales can be used as pollutant biosensor and 2) these biomarkers can be used as a reliable contaminant-monitoring tool. The present study was aimed to assess the level of pollution without disturbing the fish through the most exposed part of fish to its environment such as scales. The effect of pollution was determined by estimating the quality and fragmentation of extracted DNA from fish scales using agarose gel electrophoresis.

MATERIALS AND METHODS

Study area: "The Ravi River is located along the India–Pakistan border and meanders substantially along the alluvial plains of the Amritsar and Gurdaspur districts of Punjab before entering Lahore, Pakistan. After passing through Lahore, the river turns at Kamalia and then debouches into the Chenab River, south of Ahmadpur Sial. In the trans-boundary Ravi River, which meanders in and out of India and Pakistan, and in urban areas of Lahore" (Mahboob *et al.,* 2015), the pollution levels are reportedly very high and are attributed to the careless disposal of large amounts of industrial and agricultural wastewater and the faulty drainage systems in both countries. The water and sediment of a 72-km section of the Ravi River, stretching from the Lahore Siphon to Baloki Headworks, is heavily contaminated with Cd, Cr, Co and Cu. The river sediments are highly contaminated and have become a secondary source of pollution in the river, even though some controls over unauthorized discharges into the river have been put in place. This study was conducted on fish collected from the Ravi River located near Shahdhra, Lahore, Pakistan. The effect of pollution was evaluated on eighteen samples of *Cirrhinus mrigala* (nine experimental and nine control samples).

Sample collection: The fish samples were collected from the Ravi River near Shahdhra, Lahore, Pakistan, and the controls were collected from a fish hatchery located on the Sitiana Road in Faisalabad, Pakistan. A single, random sampling was conducted, and available fish sizes were broadly categorized into three categories: W1 (500 to 1000 grams), W2 (1001 to 1500 grams) and W3 (1501 to 2000 grams).

As a non-invasive method, fish scales were selected as experimental materials because this technique did not disturb the fish. The fish scales were collected by gently scraping the caudal portion of the body with forceps, and the detached scales were collected in polythene bags. The captured fish were returned to the river after they were measured and weighed. Samples were stored in a refrigerator.

DNA extraction: DNA was extracted from fish scales by following the method of Wasko et al., (2003) and Kumar *et al.* (2007) with some modifications. The quantity and quality of the DNA were compared by loading 0.2 µl Lambda Hind III DNA standard marker (by Thermoscientific , UK stock conc. 500 ng/µl) The DNA quantifications were done using extracted DNA samples were then stored at −20°C till their further use.

Statistical Analysis: Data on DNA concentrations in fish scales from control site and the river Ravi was analyzed using two-way classification (factorial experiment). Analysis of Variance and Duncan's Multiple Range tests was performed to analyze differences between the parameters under study.

RESULTS AND DISCUSSION

The control and experimental samples of *Cirrhinus mrigala* were distributed into three weight categories: W_1 (500 to 1000 g), W_2 (1001 to 1500 g) and W_3 (1501 to 2000 g). The mean weights and lengths of both control and experimental specimens from different weight categories were not significantly different ($P>0.5$). The minimum and maximum DNA concentrations of the control samples was recorded as 1.26 ±0.27 and 1.80 ±0.28 µg/µl in fish of weight group W2, whereas the minimum and maximum DNA concentrations of the experimental samples was detected as 0.69 ± 0.08 and 1.48 ± 0.18 µg/µl in fish of weight group W3 and W1 (Table 1). The concentrations of extracted DNA in experimental and control fish were highly significantly different ($P<0.01$). In each of the weight categories, the DNA concentrations of the experimental samples were lower than those of the control samples (Table 2).

In fish, most of the DNA isolation is done from blood. But when small and rare/endangered species from remote/rare location are encountered, it is not desirable to extract blood or sacrifice the fish. Fish fins and scales are a reliable non-destructive source of DNA and these materials have been used to isolate DNA from some species (Shiozawa et al. 1992; Zhang et al. 1994; Estoup et al. 1996; Nielsen et al. 1999; Adcock et al. 2000).

The findings of the present study demonstrate pollution effects, and these findings are in agreement with Tariq *et al.* (1996), Chaudhry, and Jabeen (2010), who reported the effect of pollution in the Indus River. The present

study utilised fish scales to evaluate pollution levels, and the findings reported here are in agreement with Kime's (1999) findings, which indicated that fish are the most

Table 1. Minimum and maximum DNA concentrations observed in *Cirrhinus mrigala* of different weight categories from the Ravi River and a control site

Weight categories	Minimum DNA Conc. (µg/µl)		Maximum DNA Conc. (µg/µl)	
	Control	Ravi River	Control	Ravi River
W1	1.32±0.04	0.88±0.13	1.41±0.12	1.48±0.18
W2	1.26±0.27	0.75±0.16	1.80±0.28	1.33±0.20
W3	1.34±0.23	0.69±0.08	1.54±0.25	1.21±0.10

Table 2. Comparison of means for DNA concentrations (µg/µl) in the scales of *Cirrhinus mrigala* of different weight categories from the Ravi River and a control site.

Categories	Treatment					
	Control Site			River Ravi		
W_1	1.36	±	0.12c	1.31	±	0.23c
W_2	1.53	±	0.35a	1.04	±	0.15c
W_3	1.44	±	0.42b	1.37	±	0.22c

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

suitable aquatic organisms with which to assess environmental pollution. The DNA concentrations in the control samples were higher than the DNA concentrations in the experimental samples. These findings are in agreement with Tripathi *et al.* (2003), to the extent that the pollutants such as pesticides appears as potential inhibitor of DNA synthesis. Although Tripathi *et al*. (2003) isolated DNA from gonad tissue (1mg/ml), however, in the experiment 50 mg of scale samples was processed for both the control and experimental fish. In this study, better results were achieved mixing the scales with a cell lysis solution containing urea. DNA fragmentation has an integrated response to the impact of multiple toxic and environmental factors. However, this response depends on both the toxicant concentration and the duration of exposure. Because of electrophilic nature, the pollutant compounds may attack many enzymes responsible for normal metabolic pathway. Thus, it is possible that these pollutants of River Ravi will have inhibited the enzyme necessary for DNA synthesis. In the present study, larger fish had more bands, representing greater fragmentation and demonstrating that these fish had experienced a longer exposure to the polluted environment. These findings are agreeing with Steinert (1999), who reported that pollutant exposure leads to corresponding increases in DNA damage. These findings are also in agreement with Connell *et al*. (1999), who suggested that changes in DNA might have long-lasting effects but that the self-repairing capability of DNA may affect the precise interpretation of the relevant bioassays.

The effect of habitat on the quality of DNA was assessed by running the extracted DNA on an agarose gel. The DNA extracted from the control samples showed its integrity by appearing as a single band in all of the samples, whereas the DNA extracted from the experimental samples appeared in more than one band, showing fragmentation. DNA fragmentation was assessed by considering the number of bands, the size of the fish and indirect exposure of the fish to its habitat.

All control samples had a single band (Fig. 1), whereas samples from the Ravi River in weight group W_1 had 1 to 2 bands. The fish in weight group W_2 had two bands. The fish in weight group W_3 from the Ravi River had a variable number of bands (2-3). Fish that were less than 1800 grams had two bands, and fish that weighed between 1900 grams and above had three bands, showing fragmentation (Fig. 2).

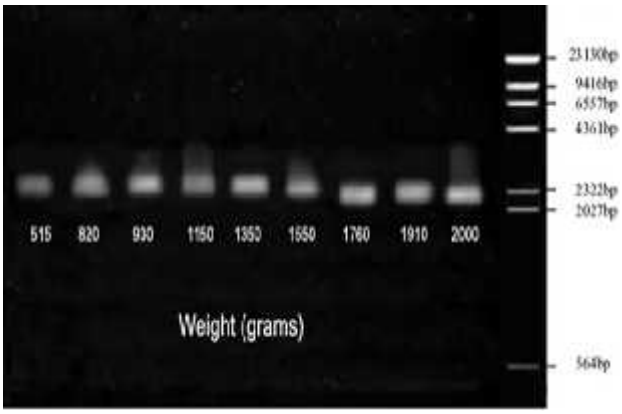

Fig. 1: Agarose gel electrophoresis of *Cirrhinus mrigala* from the control site of different weights from Lane 1-9 And Lane 10 (marker)

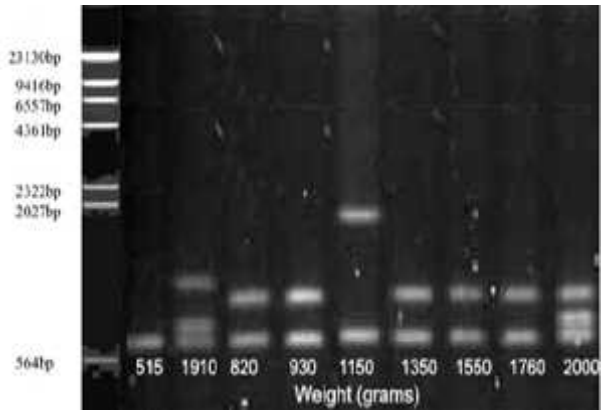

Fig. 2: Agarose gel electrophoresis of *Cirrhinus mrigala* from the Ravi River Lane 1 (Marker) Lane 2-10 (samples).

When the qualities of the samples were observed, fragmentation was found in all of the experimental samples but not in any of the control samples. These findings are in agreement with Malins *et al.* (2004), who reported that damaged DNA is a promising marker for identifying contaminant effects in fish because greater fragmentation is associated with longer periods of exposure. The present findings regarding fragmentation are also in agreement with Flammarion *et al.* (2002), who studied the level of DNA damage in cells, and Czene *et al.* (2002), who determined that the state of DNA fragmentation in cells was affected by pollution. The DNA fragmentations observed in the present study were caused by pollution in the Ravi River. Several scientists have reported that metals may induce genotoxicity in fish inhabiting polluted water bodies (Naga *et al*. 2005). Ahmad *et al.* (2008) noted that arsenic is a pollutant involved in the fragmentation of DNA in *Channa punctatus.* The findings of the present study are substantiated by the findings of Rauf *et al.* (2009), Obasohan *et al.* (2010), Kaoud and El-Dahshan (2010) and Kumar and Singh (2010), all of whom have demonstrated that heavy metal pollution affects the health of fish, which can ultimately be transferred to humans by way of the food chain.

Conclusion: The extent of damage that results from the intake of heavy metals may increase with increasing body weight in fish. The findings of the present study suggest that fish scales can be used as non-invasive bioindicator for assessing heavy metal pollution.

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