# FISH SCALES AS A NONLETHAL SCREENING TOOL FOR ASSESSING THE EFFECTS OF SURFACE WATER CONTAMINANTS IN *Cyprinus carpio*

**Shahid Mahboob[1,2,*], Salma Sultana, Hafiz Muhammad Ashraf[2], Tayyaba Sultana[2], Khalid A. Al-Ghanim[1] and Fahad Al-Misned[1]**

[1]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Saudi Arabia.
[2]Department of Zoology, Government College University, Faisalabad, Pakistan

## ABSTRACT

There is an increasing need for an effective tool to estimate the risks derived from the large number of pollutants released to the environment by human activities. Typical screening methods are highly invasive or lethal to the fish. Recent reports exhibit that fish scales biochemically respond to a wide range of contaminants, including toxic metals, organic compounds, and endocrine disruptors. The present study evaluated the effects of the surface water contaminants on *Cyprinus carpio* in the Ravi River by comparing DNA extracted non-lethally from their scales to DNA extracted from the scales of fish collected from a controlled fish farm. A single, random sampling was conducted. Fish were broadly categorised into three weight groups ($W_1$, $W_2$ and $W_3$). Experimental samples in the weight groups $W_1$, $W_2$ and $W_3$ 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 fish samples in the weight group $W_1$ fish had 1 to 2 bands, the experimental samples in the weight group $W_2$ fish had two bands and the experi-mental samples in the weight group $W_3$ fish had fragmen-tation in the form of three bands. These bands exhibit the effects of pollution on fish in the Ravi River. On the basis findings of this study, we propose that fish scales can be successfully employed as a new non-lethal tool for the evaluation of the effect of surface water contaminants.

**KEYWORDS:** Fish scales, *Cyprinus carpio*, heavy metals, non-invasive, DNA fragmentation

## 1. INTRODUCTION

A wide variety of pollutants have been identified in the environment consequent to urbanization, industrialization and new technological developments [1]. The quality of water of major river systems is getting rapidly degraded due to the massive discharge of industrial waste, domestic sewage, and pollutants of varied origins. Such exploitation has given rise to a long list of challenging problems, especially in Asian countries [2]. In Pakistan increasing human population and establishment of industries in urban areas of the Punjab province, a majority of toxic chemicals and efflux producing industries are situated in and near Lahore. Due to the non-availability of purifying facility in the sys-tem and absence of treatment plants for industrial and met-ropolitan effluents, stable and persistent toxic elements and trace metals discharged into the Ravi River and pollute the aquatic habitat [3]. Fish in the Ravi River at Balloki accu-mulated higher values of Fe and Ni as compared to other locations [4]. It is further stated fish liver and kidney as the highest depository organs in fish from this river. The water and sediment ofu2 this river, stretching from Lahore to Balloki Headworks, was heavily polluted with Cd, Cr, Co, As, Pb, Hg, Ni, and Cu [5].

"There is an increasing demand for development of methods to assess the risks caused by various pollutants released to the environment by various uncontrolled human activities. Environmental toxicology is the quantitative and qualitative study of the harmful effects of various human activities and naturally occurring chemical contaminants". Biological tools like bio- analytical systems, bioassays, biomarkers and biosensors provide us with detection systems for signaling a potential danger to the environment. Early detection will prevent ultimate damage to environmental sources. Ideally, a warning system in ecosystems like biosensors and biochemical responses (biomarkers) as well as the classical effect-related bioassays would not only give us the initial threshold of damage, but these signals will also help to devise strategies to control any such damage and to adopt precautionary measures.

"Biosensors can be classified according to the bioreceptor elements involved in recognition and according to the physicochemical transduction elements. The main classes of bio-receptors used in environmental studies of organic pollution are: enzymes, antibodies, DNA and whole cells. DNA's structure is very sensitive to the influence of environmental pollutants, such as heavy metals [6], polychlorinated biphenyls (PCBs) [7, 8] or polyaromatic compounds

* Corresponding author

(PAHs) [9]. These substances have a great affinity to DNA, and cause mutagenesis and carcinogenesis in fish. So, it is very attractive to use DNA-containing systems, for exam-ple, DNA -based biosensors [10, 11] to perform genotoxic assays, or for rapid testing of pollutants for mutagenic and carcinogenic activity".

Some physical agents, such as high temperature, UV rays and other radiations, can induce DNA damage. For example, UV rays can induce thymine dimerization to pro-duce thymine dimmers. There is many chemicals which are the major source of water pollution that can cause DNA damage, such as the base molecular isomerization, $O_2$, $H_2O_2$. Furthermore, trace metals, medicine, pesticide and their metabolites, may also affect the fish and other aquatic animals and ultimately cause DNA damage. Heavy metal may have no direct damage to DNA, but they can cause oxidative damage of DNA [12, 13]. Alkylating agent can also cause serious DNA damage because of the alkylation of bases [14]. "DNA's structure is very sensitive to the influence of environmental contaminants, such as trace met-als [6, 14], polychlorinated biphenyls [7] and polyaromatic compounds [9]. So, it is very convenient to use DNA- based biosensors [10] to perform genotoxicity assays or for rapid identification and presence of contaminants for mutagenic and carcinogenic activity".

Traditional methods for detecting pollutants in fish tissues are based on the sacrifice of fish in order to assess the bioaccumulation of trace metals in various tissues to know risk if any for the human population on consumption of fish as food. These conventional test methods are though very practical, they can be laborious. The sensitivity of fish to trace concentrations of contaminants is well documented. There is great need for non-invasive, biologically relevant biosensors for the determination of the effects of surface water pollutants on fish species. Traditional screening methods are highly invasive or lethal to the fish. Recent studies exhibited that fish scales biochemically respond to a wide variety of contaminants, including toxic metals, organic compounds, and endocrine disruptors

Fish are present at the highest trophic level of the food chain, and therefore, may bio magnify contaminants from the food and in addition, they can bio accumulate pollutants from the water [15, 16]. The aim of this experiment was to use fish scales as non -lethal biosensors for various heavy metal contaminants. 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.

## 2. MATERIALS AND METHODS

### 2.1 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, 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 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". The influence of water pollution was assessed on 18 samples of *Cyprinus carpio* (nine experimental and nine control samples). The experimental samples were collected from the Ravi River near Shahdhra, Lahore, Pakistan, and controls were collected from a fish hatchery located on the Sitiana Road in Faisalabad, Pakistan. A single, random sampling was conducted, and available fish samples were generally categorized into 3 categories: W1 (500 to 1000 g), W2 (1001 to 1500 g) and W3 (1501 to 2000 g).

### 2.2 Water Sampling

The water samples were collected in polypropylene bottles. Before sample collection, all bottles were washed (with dilute nitric acid and then with River water). All bottles were labeled with date and sampling station for heavy metal analysis. Replicated water samples of about one liter were collected from about 30 cm depth of surface water from the predetermined locations. The water samples were preserved in 55% $HNO_3$ and stored at 4 ºC in the refrigerator.

Standard methods as described by [17] were followed to determine various parameters of these water samples. All chemicals were of analytical grade and bought from Merck. The blanks and calibration standard solution were also analyzed in the similar manner as for the samples. The instrument calibration standards were made by diluting standard (1,000 ppm) supplied by Merck, Germany. A known 1,000 mg/L concentration of all the above men-tioned metal solution was prepared from their salts. All re-agents used were of analytical grade.

### 2.3 Analytical procedures and analysis of fish muscles:

Fish muscles were chopped into approximately 10-g pieces. Whole-body muscles were digested using the wet digestion method according to the procedure described by [18].

### 2.4 Sample collection of fish scales

As a non-invasive method, fish scales were selected as experimental materials because this technique did not harm the fish. The fish scales were collected in polyethylene bags by gently scraping the caudal portion of the body with forceps. The captured fish were released back into the river after measuring size and recording body weight. The samples were stored in a refrigerator for further analysis.

### 2.4 DNA extraction

"DNA was extracted from fish scales by following the method of [19, 20]. Approximately 50 mg scales were taken

from each sample, dried on filter paper. Scales were cut into small pieces and placed into 2 ml Eppendorf tube con-taining 940 µl Lysis buffer (200 mMTris-HCl, pH 8; 100 mM EDTA, pH 8 250 mM NaCl), 30 µl Proteinase K. (10 mg/ ml), 30 µl RNAse (10 mg/ml), and 30 µl 20% SDS solution. The contents of the tubes were incubated at 48 °C for 45-50 minutes using water bath (Mummers, WB 14). The appropriateness of the incubation temperature was studied in a separate experiment, by incubating scale samples from *C. carpio* at different temperatures viz. 42°, 44°, 46 °, 48°, 50°, 52° and 54°C. After incubation, an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the tubes containing list scale cells. Then the contents were mixed by gently inverting the tubes for 10 minutes to precipitate pro-teins and the other contents of nucleic acid. Then the tubes were centrifuged at 12000 rpm for 10 minutes using a cen-trifuge machine Hermle Labortechnik GmbH (type: Z216). The top aqueous layer was transferred into 1.5 ml Eppen-dorf tube without disturbing interphase and lower phase. The DNA was precipitated by adding an equal volume of isopropanol and 0.2 volumes of 10 M ammonium acetate solution, inverting the tubes gently several times. Then the tube was centrifuged at 14350 rpm for 10 minutes. The su-pernatant was removed by pouring, taking care to avoid the loss of DNA pellets. The pellets were washed with 500 µl chilled 70% ethanol; air dried and re-suspended in 200 µl TE buffer pH 7.5".

After ensuring complete solubility of DNA, the purity factor (A260/A280 nm) was measured spectrophotometri-cally and its integrity was checked by loading 10 µl DNA preparation (2 µl extracted DNA, 2 µl dye and 6 µl sterile water) on 0.7% agarose gel and stained with eth-idium bromide by the following method. 40 ml 1XTris-ac-etate-EDTA (TAE) buffer and the 0.28g agarose were added in the flask. Heated the solution in the microwave oven for 30 seconds, swirl and reheat before its boiling. When starts boiling then swirl again to dissolve the aga-rose. The flask placed in a 55-65° C on a stir plate to cool. The gel apparatus prepared for casting the gel while the agarose is cooling. Just prior to pouring the gel, add Eth-idium bromide to dissolve in agarose and swirl to mix. Poured the gel into the casting tray and adjust the comb to keep the wells perpendicular and waited to cool and harden the gel (20-30 minutes). The quantity and quality of the DNA were compared by loading 0.2 µl Lambda Hind III DNA standard marker (by Thermoscientific, UK stock cans. 500 ng/µl).

## 3. RESULTS

In this experiment, the concentration of various metal Cd, Cu, Pb, Hg, Ni, As and Zn were assessed in *Cyprinus car-pio* collected from the Ravi river. The concentration of Cd, Cu, Pb, Hg, Ni, As and Zn in water samples from the Ravi river and a control pond and was determined as 0.023±0.003, 0.45±0.001, 0.013±0.001, 0.056±0.001, 0.11±0.002, 0.035±0.002 and 0.098±0.001; 0.012±0.002, 0.20±0.001, 0.006±0.001, 0.023±0.001, 0.06±0.002, 0.016±0.001 and 0.036 ±0.001mg $l^{-1}$, respectively. The concentration recorded from water analysis reflected the order of occurrence of heavy metals to be Cu > Zn > Ni >Hg > As> Cd >Pb (Table 1). The levels of selected trace metals, including Cd, Cu, Pb, Hg, Ni, As and Zn in the mus-cle tissue of *Cyprinus carpio* under investigation in this study are presented in the Table 1. *Cyprinus carpio* captured from the Ravi River and control site was examined for the selected metal concentration. The ranking order of the heavy metals in the fish muscle from the river Ravi was as: Zn (434.88±5.66), Cu (55.70±2.35), As, (8.55±1.10), Ni (7.84±0.88), Pb (4.88±0.48), Cd (2.81±0.12), Hg (2.33±0.26) (mean; mg/kg dry wt.). The concentration of Cd, Cu, Pb, Hg, Ni, As and Zn in the muscle of *Cyprinus carpio* captured from the control site was detected as 1.10±0.27, 20.66±1.80, 2.03±0.16, 1.01±0.11, 2.26±0.45, 3.12 ±0.66 and 190.11±6.23 mg/kg dry wt. , respectively. The concen-tration of Cd, Cu, Pb, Hg, Ni, As and Zn in the gills, liver and kidney in *Cyprinus carpio* captured from the Ravi river were determined as: gills (0.31±0.11, 2.14±0.56, 0.10±0.04, 0.07±0.03, 1.76±0.23, 1.02±0.001 and 10.17±1.06 mg/kg dry wt., respectively), liver (4.67±0.44, 73.77±2.15, 7.79±1.19, 5.85±0.53, 13.71±2.23, 11.94±0.89 and 457.24±5.67 mg/kg dry wt., respectively) and kidney (3.35±0.65, 52.71±2.91, 5.95±0.71, 4.96±0.52, 9.91±1.11, 8.51±0.78 and 392.71±4.65, respectively). The level of Cd, Cu, Pb, Hg, Ni, As and Zn in the gills, liver and kidney in *Cyprinus carpio* procured from the control site were determined as: the gills 0.12±0.05, 0.42±0.12, 0.003±0.00, 0.003±0.00, 0.35±0.0.07, 0.20±0.00 and 4.44±0.56 mg/kg dry wt., respectively), liver (1.89±0.0.34, 7.91±2.13, 4.71±0.62, 2.62±0.24, 4.88±0.45, 0.81±0.11 and 200.75±4.81 mg /kg dry wt., respectively) and kidney (1.52±0.23, 20.71±1.26, 6.11±0.58, 3.38±0.42, 4.06±0.71, 0.43±0.03 and 14.55±3.45 mg/kg dry wt., respectively) (Ta-ble 2). There existed significant differences among the fish organs for the bioaccumulation of all heavy metals (Table 2). Accumulation of Zn in the 4organs of fish exhibited the high-est level. Cu, Ni, Pb and As was also accumulated in signifi-cantly higher (P≤ 0.01) concentration in muscles, gills,

**TABLE 1 - Metal concentration in water from River Ravi and control site**

| | Cd | Cu | Pb | Hg | Ni | As | Zn |
|---|---|---|---|---|---|---|---|
| River Ravi Surface Water $mgl^{-1}$ | 0.023±0.003b | 0.45±0.001a | 0.013±0.001a | 0.056±0.001d | 0.11±0.002c | 0.035±0.002e | 0.098±0.001 |
| Control site Surface Water $mgl^{-1}$ | 0.012±0.002b | 0.20±0.001a | 0.006±0.001a | 0.023±0.001d | 0.06±0.002c | 0.014±0.001e | 0.036±0.001 |

Means with similar letters in a row are statistically similar at $P \leq 0.05$.

**TABLE 2 - Metal concentration (mg/kg dry wt.) in tissues of *Cyprinus carpio* from River Ravi and control site**

| Fish Organs | Sampling Site | Cd | Cu | Pb | Hg | Ni | As | Zn |
|---|---|---|---|---|---|---|---|---|
| Muscles | River Ravi | 2.81±0.12e | 55.70±2.35b | 4.88±0.48 | 2.33±0.26e | 7.84±0.88d | 8.55±1.10c | 434.88±5.66a |
| | Control site | 1.10±0.27e | 20.66±1.80b | 2.03±0.16 | 1.01±0.11e | 2.26±0.45d | 3.12±0.66c | 190.11±6.23a |
| Gills | River Ravi | 0.31±0.11d | 2.14±0.56b | 0.10±0.04e | 0.07±0.03f | 3.74±0.23c | 3.02±0.002g | 10.17±1.07a |
| | Control site | 0.12±0.05d | 0.42±0.12b | 0.003±0.00f | 0.003±0.00e | 0.35±0.07c | 0.20±0.00e | 4.44±0.56a |
| Liver | River Ravi | 4.67±0.45f | 73.77±2.15b | 7.79±1.19e | 5.85±0.53f | 13.71±2.23c | 11.94±0.89d | 457.22±5.67a |
| | Control site | 1.89±0.0.35d | 7.91±2.13b | 4.71±0.21c | 2.62±0.24e | 4.88±0.45c | 0.81±0.11f | 200.75±4.81a |
| Kidney | River Ravi | 3.35±0.65e | 52.71±2.91b | 5.95±0.71e | 4.96±0.52f | 9.91±1.11c | 8..51±0.78d | 392.71±4.65a |
| | Control site | 1.52±0.23e | 20.71±1.25b | 6.11±0.58c | 3.38±0.42f | 4.06±0.71d | 0.43±0.03f | 14.55±3.44a |

Means with similar letters in a row are statistically similar at $P \leq 0.05$.

liver and kidney in fish procured from the Ravi river. The fish liver and kidney accumulated higher quantities of Cu, followed by Cu, Pb and Hg.

Control and experimental samples of *Cyprinus carpio* were distributed in three weight groups; $W_1$, $W_2$ and $W_3$. The mean weights and lengths of both control and experi-mental specimens from different weight categories were not statistically different (P>0.5). DNA concentrations of the control samples ranged from 1.24 to 1.79 µg/µl, whereas, DNA concentrations of the experimental samples ranged from 0.82 to 1.51 µg/µl (Tables 3 and 4). The comparison of the means showed non- significant differences in the DNA concentration in scales of different weight categories of fish collected from the Ravi River (Table 5).

**TABLE 3 - DNA concentrations (µg/µl) extracted from *Cyprinus carpio* of different weight categories from the Ravi River and a control site.**

| Controlled samples (gm) | DNA Conc. µg/µl | Experimental samples (gm) | DNA Conc. µg/µl |
|---|---|---|---|
| 510.00 | 1.44 | 525.0 | 1.32 |
| 905.00 | 1.39 | 880.0 | 1.12 |
| 990.00 | 1.34 | 940.0 | 1.04 |
| 1180.00 | 1.24 | 1160.0 | 0.71 |
| 1240.00 | 1.32 | 1370.0 | 1.21 |
| 1490.00 | 1.76 | 1495.0 | 1.41 |
| 1715.00 | 1.74 | 1730.0 | 1.30 |
| 1910.00 | 1.79 | 1920.0 | 1.06 |
| 2000.00 | 1.39 | 2005.0 | 0.78 |

**TABLE 4 - Minimum and maximum DNA concentrations observed in *Cyprinus carpio* 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 | 1.34 | 0.82 | 1.44 | 1.32 |
| W2 | 1.24 | 0.71 | 1.76 | 1.41 |
| W3 | 1.39 | 0.78 | 1.79 | 1.36 |

The concentrations in experimental and control fish were highly signifi-cantly different (P<0.01)

**TABLE 5 - Two-way AVOVA analysis testing the effect of pollution and interaction (treatment x weight) for DNA concentrations (µg/µl) in the scales of *Cyprinus carpio* of different weight categories from the Ravi River and a control site.**

| Effect | Df | F | p |
|---|---|---|---|
| **Treatment** | 1 | 14.08 | ≤ 0.01 |
| **Weight** | 2 | 0.52 | ns |
| **Treatment x Weight** | 2 | 0.64NS | ns |
| **Error** | 12 | | |
| **Total** | 17 | | |

The F and p values are given for each variable, ns-not significant

**Comparison of means**

| Categories | Treatment | |
|---|---|---|
| | Control Site | River Ravi |
| $W_1$ | 1.39±0.16c | 1.32±0.20c |
| $W_2$ | 1.44±0.35b | 1.41±0.24a |
| $W_3$ | 1.79±0.42a | 1.36±0.28b |

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

**TABLE 6 - Two way AVOVA analysis, testing the effect of pollution and interaction (treatment x height) for DNA concentrations (µg/µl) in the scales of *Cyprinus carpio* of different weight categories from the Ravi River and a control site.**

| Effect | Df | F | P |
|---|---|---|---|
| **Treatment** | 1 | 13.06 | ≤ 0.01 |
| **Weight** | 2 | 0.49 | ns |
| **Treatment x Weight** | 2 | 0.61NS | ns |
| **Error** | 12 | | |
| **Total** | 17 | | |

The F and p values are given for each variable, ns-not significant

**Comparison of means**

| Categories | Treatment | |
|---|---|---|
| | Control Site | River Ravi |
| $W_1$ | 1.38±0.12c | 1.33±0.23c |
| $W_2$ | 1.55±0.35a | 1.06±0.15c |
| $W_3$ | 1.46±0.42b | 1.39±0.22c |

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

The effect of habitat on the quality of DNA was deter-mined 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 Ravi River samples appeared in more than one band, exhibited fragmentation. DNA fragmentation was determined by counting the num-ber of bands, the size of the fish and indirect exposure of

the fish to its habitat. In each of the weight groups, the DNA concentrations of the fish samples from the river were lower than those of the control fish samples (Table 6).

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

## 4. DISCUSSION

The results of this study, exhibit pollution effects, and these results are in agreement with [21] and [22], who also reported the effect of pollution in the Indus River. In this study we utilised fish scales to evaluate pollution levels, and the findings reported here are in conformity with [23], which indicated that fish are the most suitable aquatic organisms with which to evaluate environmental pollution. The metal accumulation trends in fish organs at two sam-pling sites varied significantly. However, the fish at the Ravi River showed the higher accumulation of metals. The heavy metal bioaccumulation in fish comes from two dif-ferent routes of intake; free ions and simple compounds dissolved in water or taken up directly through the epithe-lium of the skin, gills and the alimentary canal while others, accumulate in food organisms on which fish feed in their habitat [4]. Thus, the heavy metals when released into the river may enter the food chain (plankton) and accumulate in the fish body as assessed during this experiment.

The DNA concentrations in the fish scales procured from the control site were higher than the DNA concentrations in fish scale samples from the Ravi River. These findings are in agreement with [24], to the extent that the pollutants such as pesticides appears as potential inhibitor of DNA synthesis. Although [24] isolated DNA from gonad tissue (1mg/ml), however, in this experiment 50 mg of scale samples was processed for both control and exper-imental fish. In this study better findings were achieved

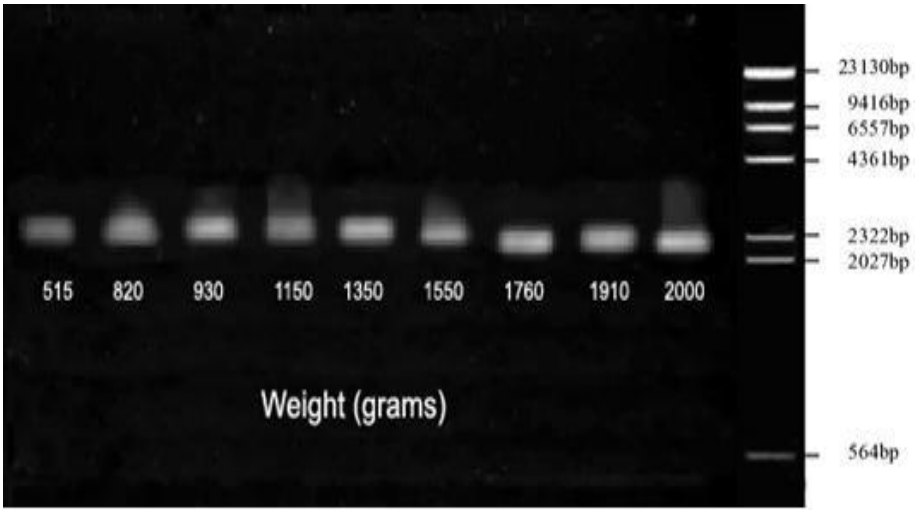


**FIGURE 1- Agarose gel electrophoresis of *Cyprinus carpio* from the control site.**

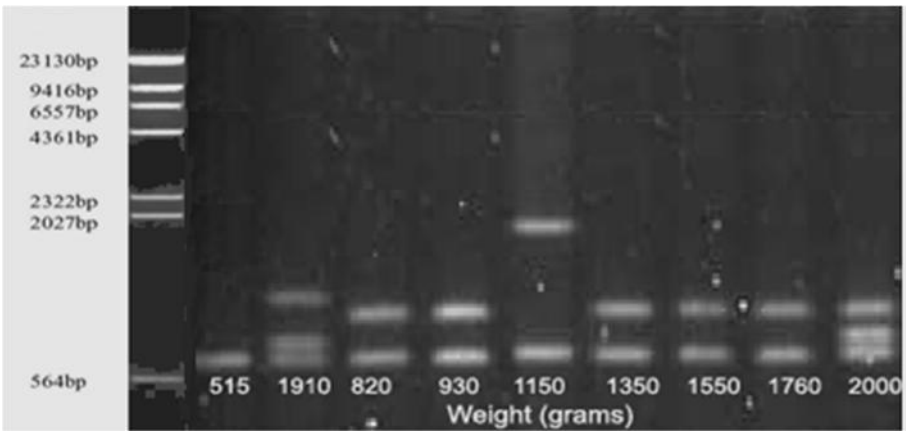


**FIGURE 2 - Agarose gel electrophoresis of *Cyprinus carpio* from the Ravi River.**

mixing the scales with a cell lysis solution containing urea. Thus, it is possible that these pollutants of the River Ravi 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 longer exposure to the polluted environment. These findings are agreeing with [25], who reported that pollutant exposure leads to corresponding increases in DNA damage. These results are also in agreement with [26], 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.

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. Our results were in line with the findings with [27]. The present results regard-ing fragmentation are also in agreement with [28], who re-ported the level of DNA damage in cells, and [29], who assessed that the state of DNA fragmentation in cells was affected by pollution. The DNA fragmentations deter-mined 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 [30]. Arsenic is a pollutant involved in the fragmen-tation of DNA in *Channa punctatus* [31]. The findings of the present study are substantiated by the findings of [5, 32-34], 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.

Finally, at the sites under study, there has been observed alarming levels of some toxic metals like As, Cd, Pb, and Hg, which are needed to be monitored regularly. Previous studies on heavy metal contamination of fish species from River Ravi were limited, but the results obtained in this study indicate that this fish species contain danger-ous levels of heavy metals. The results of this study exhib-ited that fish scales biochemically responded to toxic met-als. We observed that fish scales can be easily collected non-lethally from fish to evaluate fish scales as businesses for different contaminants in freshwater ecosystems. We are of the view screening for contaminants using fish scales provides a rapid, inexpensive, non- lethal and biologically relevant first pass indicator of water quality for sensing the presence of bioactive chemicals in surface water and the exposure to such compounds by endangered and threatened fish species. This technique could be used with any scale-bearing fish species. Further studies are required to validate the use of fish scales as a non-lethal tool for assessing the effect of surface water contaminants.

## 4. CONCLUSION

The extent of damage caused from the bioaccumulation may enhance with an increase in body weight of the fish. In the Ravi river at Shahdrah, there has been detected an alarming level of heavy metals which are needed to be monitored regularly. We are of the view fish scales can be successfully employed as non-lethal tool for assessing a damage caused by pollutants in aquatic ecosystems.

## ACKNOWLEDGEMENTS

The authors would like to their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. PRG-1436-11.

*The authors declare that they do not have any kind of conflict of interests and/or any financial benefit from any trademark mentioned in the paper.*

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**Received:** December 27, 2014
**Revised:** April 13, 2015
**Accepted:** May 18, 2015

## CORRESPONDING AUTHOR

**Shahid Mahboob**
Department of Zoology
College of Science
King Saud University
P.O. Box 2455
Riyadh-11451
SAUDI ARABIA

Phone: +966-1-4675925
Fax: +966-1-4678514
E-mail: shahidmahboob60@hotmail.com

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