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RESEARCH ARTICLE

Fish scales as a non-lethal tool of the toxicity of wastewater from the River Chenab

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Abstract Water pollution is gradually increasing in natural waters through anthropogenic activities. This study aimed to use fish scales as a bio-indicator of pollution, along with water quality parameters, and the assessment and detection of selected heavy metals in water samples collected from the River Chenab, including the Chakbandi drain that gathers domestic sewage waste and industrial effluents from Faisalabad and deposits it into this freshwater body. All water quality parameters (pH, total dissolved solids (TDS), total suspended solids (TSS), salinity, conductivity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), phenols and sulphates) and concentrations of selected heavy metals (Cd, Cu, Mn, Pb and Cr) were found to be considerably higher than permissible limits as defined by the WHO, and therefore capable of causing ill health effects in aquatic organisms. Specimens of fish scales from selected fish were described qualitatively and observed quantitatively. In *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*, the scales showed several deformities in shape and different scale structures such as circuli, radii and annuli. In each of the three types of fish, considerable variation in the morphology of their scales was observed in specimens collected from polluted sites.

Keywords Water · Physico-chemical parameters · Heavy metals · Scales · Deformities

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Introduction

Since the twentieth century, thousands of contaminants have been manufactured and, in part, discharged into the atmosphere, with the potential to pose persistent and significant risks for both aquatic and terrestrial environments. Water reservoirs represent the eventual basin for most of these pollutants, either due to direct releases or to hydrology and environmental practises (Stegeman and Hahn 1994). According to a current survey report, many pollutants are present on the surface of water, containing lethal metals, chlorinated organics, polynuclear aromatic hydrocarbons and effluents from the medical industry (Kolpin et al. 2002; Brigham et al. 2009). Among these pollutants, hydrocarbons and pesticides require particular attention because they have unexpected influence at very low concentrations (Triebskorn et al. 2007), and water creatures experience the highest risk because of their long-lasting contact with these substances. Overall, these pollutants are especially toxic and have been associated with many disease outbreaks among all creatures on Earth (Atienzar et al. 2000; Nhapi et al. 2011).

The level of heavy metals and toxic materials in ground water and surface water in Pakistan has been shown to surpass the recommended levels (Atienzar et al. 2000; WHO 2001, 2008; Khan et al. 2012; Ilyas and Sarwar 2003). Human deeds such as the poorly managed disposal of metropolitan and agricultural waste and the unsafe dumping of industrial waste are the main causes of water toxicity in Pakistan (Azizullah 2010). Although heavy metals such as Cu, Co, Zn, Fe and Mn are vital for enzymatic action in humans and other organisms when they exist at low levels, they may become lethal if present at higher levels (Sial et al. 2006). Other metals, meanwhile, such as Cd, Hg and Pb, have no recognized role in living organisms and are extremely lethal even at very low concentrations.

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Fish species have previously been used to assess the biological and biochemical reactions to environmental pollutants (Olaifa et al. 2004; Mahboob et al. 2015). Fish are useful as a study material due to their helpful characteristics like easy adaptation to different environments, rapid growth, inexpensive feeding conditions, their presence in large numbers in natural freshwater and their high financial value (Mahboob et al. 2015). The effects of pollution in fish are assessed mainly by the level of pollution in their water and food (Farkas 2003). One of the most significant features of fish is that their bodies are enclosed within scales, and indeed, fish scales have been used for species identification since the early 1900s (Goodrich 1909), and determination of scale shapes is included as selective features in several keys (Maitland 2004). Since scales are the outermost structures of the fish's body, they are in incessant and direct contact with contaminants presented in the water and can therefore be used as important structures to designate pollution levels in water. The presence of higher level of different heavy metals in water also affects the scale structure in fish because scales are directly in contact with water (Shikha and Sushma 2011). Furthermore, fish scales can be easily studied due to their presence on exposed body parts. Scales contain different parts: the central point of a mature scale is called the focus, from which emanate small circular growth rings are called circuli. The growth rate of fish is higher in summer and lower in winter; so, the circuli formed during summer months are generally more widely spaced than those formed during winter months; indeed, winter circuli can become compacted to form a dark ring called an annulus. The recurrent pattern of these circuli and annuli can be used to determine the age of the host fish (Dua and Gupta 2005). When a fish experiences stressful conditions, its growth stops, and thus, any sudden change in the characteristics of the aquatic environment (e.g. due to metal contamination or any other toxicity) may cause changes and distortions in the shape of the circuli and other structures in the scales of fish (Johal and Dua 1994). Various sorts of aberrations have been documented in scale structures in respect to many sorts of pollutants (metals). For example, when fish scales were exposed to chromium, it was observed that the focus of all the exposed scales was noticeably malformed and that high concentrations had a significant effect on the radii and circuli shape (Dua and Gupta 2005; Chernova 2010). Heavy metal pollution has also been shown to transform the formation of the annulus in the scales due to changes in the feeding pattern of fish (Tandon and Johal 1993). Cadmium and aluminium accumulations also caused fragility in the edge of scales, and circuli were injured in both frontal and subsequent parts of the scales along with calcareous malformations due to different cadmium concentrations (Rishi and Jain 1998). Deformity, thickening of the scales and tears in the lining of the scales have also been reported (Lin-Sun et al. 2009). Overall, these studies suggest that fish scales could be used as bio-indicator for heavy metal contamination and other toxicants existing in the aquatic environment (Darafsh et al. 2008).

Industrial and sewage waste is regularly discharged into Pakistan's river systems. Studies have shown that major industries including ceramics, steel, leather, textiles, pharmaceuticals and fertilizers are the principle reasons for water pollution in Pakistan (PAK-EPA 2010; WHO 1995). As the third largest city in the country, Faisalabad produces significant amounts of such waste, much of which is disposed into the Ravi and Chenab rivers. Faisalabad could be described as the "Manchester" of Pakistan, containing textile industries, dying, finishing, printing, plastic, leather, seizing, sugar mills and many more factories who release a variety of effluents into the River Chenab through the Chakbandi drain at Thatta Muhammad Shah (Ahmed Wala) at latitude 31.570° and longitude 72.534°. With this in mind, the current research was planned to determine the effects of industrial and sewage waste on the water composition of the River Chenab with regard to its physico-chemical parameters and selected heavy metal content. Furthermore, the study sought to test the potential of fish scales of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* as a non-invasive bioindicator of pollution.

Materials and methods

Study area and sampling sites

Faisalabad is the third largest city in Pakistan and is home to 45 % of the country's industry, resulting in the discharge of a large amount of toxic waste into two major rivers, the Chenab and the Ravi. The River Chenab, meanwhile, contains a variety of fish species and provides a source of protein for the population in the vicinity of the river throughout its length. Toxic waste from the Chakbandi drain, however, has drastically reduced the fish population and even made the water so polluted that it has become unsuitable for irrigation purposes (Hussain et al. 2016). Three sites along the length of Chakbandi drain were selected for further investigation, namely Akbar Choak Faisalabad (DS1), 153 JB (DS2) and Ahmed Wala (end of the drain DS3). Six sites were also selected along the length of the River Chenab, three upstream (RS1, RS2 and RS3) and three downstream (US1, US2 and US3) from the exit of the Chakbandi drain, with the latter acting as control sites. A total of nine sites were therefore selected for water sampling (Fig. 1).

Water sampling

Water sampling was performed between September 15, 2014 and July 15, 2015 (autumn, winter, spring and summer). Water sample was collected 20–30 cm under the water surface with a 5-L water sampler. Representative water samples were

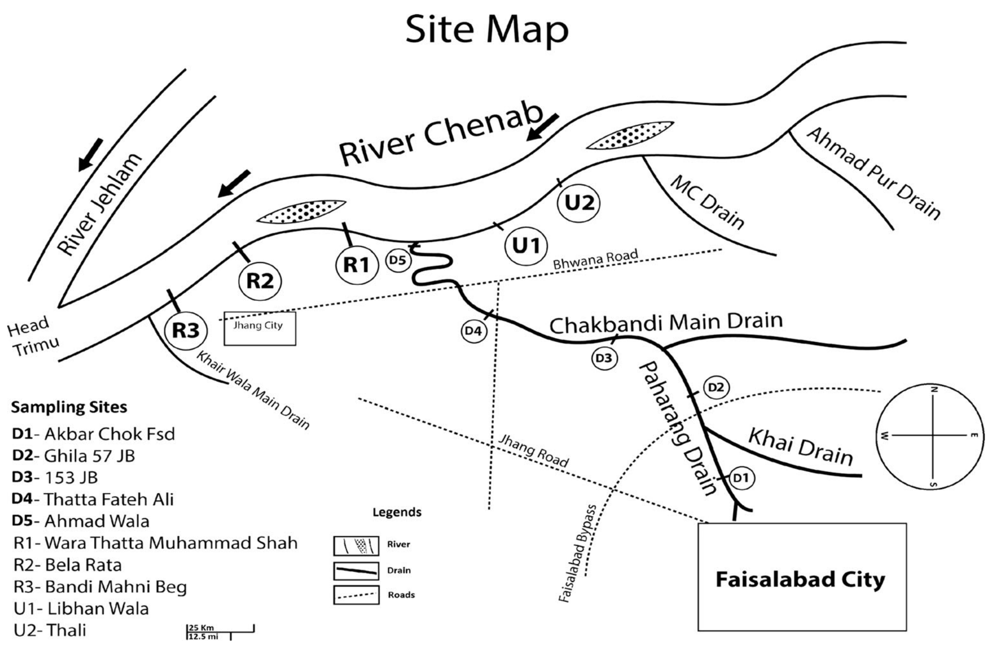

Fig. 1 Site map of the study area indicating experimental

collected from the sampling sites in 1.5 l polypropylene bottles with polyethylene caps. In winter, there is little water flow through the river, and therefore, the majority of the water in the river is contributed by drainage from the areas surrounding the river. This is due to the fact that water from River Chenab is diverted into a network of channels for irrigation purposes.

Water analysis

Samples were analysed immediately after collection. The water samples from the River Chenab were taken from every point of the fish harvest and analysed for selected heavy metals and other water quality parameters (WQPs) defined by the Environmental Protection Agency of Pakistan. The WQPs were analysed following the method of Boyd (1981) for biological oxygen demand (BOD) and chemical oxygen demand (COD). COD was also measured with the help of a COD cell test kit. Among other WQPs, pH was measured using a portable pH meter (pH meter 1,722,431, India) in the field during sample collection, whereas total dissolved solids (TDS), salinity and electrical conductivity were measured with the help of an EC meter (Model 3084 Amber Science, Inc. Eugene, Oregon). Total suspended solids (TSS) were measured following the method defined by Boyd (1981). Phenols were measured with the phenol test kit (Model PL-1 HACH, USA). Sulphates were measured with the help of a sulphate test kit (Model SF-1HACH, USA).

Heavy metal analysis

The heavy metals analysed were cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and chromium (Cr). These heavy metals were estimated using Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) using an air-acetylene flame with a digital readout system. Analytical analysis of Cd, Cu. Mn, Pb and Cr was performed following the method and conditions described in AOAC (1990). "The same procedure was used for blanks and calibration standard as for the water samples. The instrument calibration standards were made by diluting standard (1,000 ppm) supplied by MERRCK, Germany. A known 1,000 mg/L concentration of all the above mentioned metal solution was prepared from their salts as already described by Mahboob et al. (2014)". The level of accuracy for studied heavy metals was 87–96 %. The limits of detections for the heavy metals were 0.008, 0.003, 0.005, 0.009 and 0.006 mg l^{-1} for Cd, Cu, Mn, Pb and Cr, respectively.

Sampling of fish scales

Catla catla (weight 1030.40 ± 27.80 g and length 43.18 ± 1.12 cm), *Labeo rohita* (weight 1015.80 ± 29.5 g and length 44.77 ± 2.18 cm) and *Cirrhina mrigala* (weight 1075.60 ± 32.25 g and length 45.44 ± 2.76 cm) were collected from upstream and downstream areas once a month between September 15, 2014 and July 15, 2015 (autumn, winter, spring and summer). Thirty scales from each fish species from each sampling site were collected using fine forceps and the morphological variations and distinguishing characteristics, such as the scale size, scale type, overall scale shape, the shape of the margin, and the position and appearance of the focus, annulus, circuli and radii were described. The scales chosen included the dorsal side scales (nearer to the head) and posterior dorsal side scales (nearer to the tail), ventral scales (nearer to the middle abdomen) and lateral line, from the area midway between the dorsal fin and lateral line (this area is less variable in shape and size compared to other parts of the body). The scales were collected in labelled (date, locality, species, etc.) envelopes, measuring 49 × 28 cm. In order to remove dirt and mucus, the scales were immersed in ammonium hydroxide for 24 h and then further cleaned with a soft bristle brush. Each scale was then whole mounted with araltide on a 3 × 1 glass slide and left to dry. Drying took between 1 and 7 days depending upon room temperature and humidity. Observations were taken by studying under a light microscope.

Statistical analysis

The data obtained was subjected to appropriate statistical analysis. One-way analysis of variance was performed using the Minitab program. Mean and standard error were worked out. The data were further analysed using Duncan's multiple range test. Probability values of $p < 0.05$ were considered significant. Microsoft Excel was used for graphical representations. The correlation coefficient was used to estimate the strength of the association between freshwater pollution and scale damage in fish species using SPSS 9 for PC.

Results

Water quality parameters

The pH value of the water was found to increase gradually downstream along the length of the drain. The highest pH value (11.40 ± 0.10) was recorded at polluted site DS1. The pH value also gradually decreased in the river at sites RS1, RS2 and RS3 (upstream). Significant differences were found in the pH values of the samples from the drain, river and the control sites. The control sites (US1 to US3) showed gradually decreasing pH values while increase notices along River Chenab sites (RS1, RS2 and RS3) (Table 1).

The mean concentration of TDS was recorded as 1766.89 ± 3.54 mg l^{-1}. TDS concentration was high at site DS2 (2934.29 ± 12.75) and decreased at DS3 (2484.00 ± 1.90) which were the polluted (drain sites) (Table 1). The TDS concentration then further decreased gradually at sites RS1 to RS3 located in the River Chenab (upstream). The downstream sampling sites (US1 to US3) showed readings of 308.43 ± 0.75, 321.14 ± 0.77 and 320.10 ± 0.76 mg l^{-1}, respectively (Table 1).

Mean total suspended solids (TSS) were recorded as 323.34 ± 4.37 mg l^{-1} during the current study period. TSS decreased downstream through the length of drain starting from DS1 to DS3. The values of TSS were further decreased in the river sites (RS1, RS2 and RS3). The control sites showed gradually decreasing TSS. The lowest (166.50 ± 4.33 mg l^{-1}) TSS was therefore recorded at site US3.

Table 1 Comparison of means for various water quality parameters from different sites of River Chenab

Water sampling sites	pH	Total dissolved solids (mg l^{-1})	Total suspended solids (mg l^{-1})	Salinity (mg l^{-1})	Electrical conductivity (mS/m)	Biochemical oxygen demand (mg l^{-1})	Chemical oxygen demand (mg l^{-1})	Phenols (mg l^{-1})	$SO^{2-}4$ (mg l^{-1})
DS1	10.10 ± 0.085bcd	2796.00 ± 2.13b	682.00 ± 3.07a	3314.29 ± 14.29a	2.42 ± 0.093d	237.96 ± 2.07a	481.14 ± 1.87a	1.22 ± 0.018d	304.71 ± 2.860d
DS2	11.40 ± 0.102a	2934.29 ± 12.75a	478.71 ± 4.69b	3385.71 ± 14.24a	4.88 ± 0.040a	194.06 ± 3.95b	343.57 ± 11.1b	2.75 ± 0.059a	485.71 ± 1.973a
DS3	11.11 ± 0.055a	2484.00 ± 1.90c	424.86 ± 6.84c	2742.86 ± 20.20b	4.13 ± 0.049b	152.61 ± 2.00c	227.86 ± 7.27c	2.77 ± 0.025a	444.14 ± 4.877b
RS1	10.39 ± 0.103b	2397.86 ± 1.24d	308.29 ± 4.13d	1942.86 ± 20.10b	3.17 ± 0.061c	78.56 ± 1.22d	195.43 ± 1.48d	2.19 ± 0.012b	435.00 ± 2.717b
RS2	10.30 ± 0.022bc	2269.00 ± 11.3e	261.00 ± 2.53e	1771.43 ± 18.44c	3.08 ± 0.041c	67.47 ± 1.9d	183.00 ± 2.88d	1.91 ± 0.014c	420.71 ± 1.409c
RS3	10.06 ± 0.087cde	2071.14 ± 0.26f	242.29 ± 4.47ef	1414.29 ± 14.29d	2.81 ± 0.061d	55.43 ± 1.04e	174.00 ± 1.40d	1.80 ± 0.018c	410.57 ± 4.407c
US1	7.80 ± 0.146 g	308.43 ± 0.75 h	176.71 ± 5.59 g	114.29 ± 14.25f	0.21 ± 0.016e	37.77 ± 0.70f	70.14 ± 1.14e	0.10 ± 0.005e	81.14 ± 1.580e
US2	7.76 ± 0.06 h	321.14 ± 0.77 g	169.71 ± 3.69 h	214.29 ± 14.04e	0.31 ± 0.02ef	33.89 ± 0.66f	60.00 ± 2.24f	0.13 ± 0.003e	76.02 ± 0.951e
US3	7.74 ± 0.05hI	320.10 ± 0.76 g	166.50 ± 4.33 h	200.15 ± 13.44e	0.25 ± 0.041f	33.76 ± 0.43f	55.18 ± 2.24f	0.11 ± 0.004e	72.16 ± 0.87e
Mean	9.63 ± 0.079	1766.89 ± 3.54	323.34 ± 4.37	1677.80 ± 15.97	2.36 ± 0.045	99.06 ± 1.552	198.92 ± 3.501	1.44 ± 0.018	303.35 ± 2.405

Means sharing similar letters in a row or in a column are statistically non-significant ($P > 0.05$). Experimental site details are as follows: DS1 to DS3 are drain sites; RS1 to RS3 are river sites; US1 to US3 are control

The salinity was highest at site DS2 (polluted), 3385.71 ± 14.29 mg l^{-1} and then decreased downstream through the drain and upstream river sites (RS1, RS2 and RS3). The control sites (US1 to US3) showed the 114.29 ± 14.29, 214.29 ± 14.29 and 200.15 ± 13.44 mg l^{-1}, respectively. The lowest salinity was recorded at US1, 214.29 ± 14.29 mg l^{-1} (Table 1).

The conductivity was also highest at DS2 before gradually decreasing downstream through the length of the drain and River Chenab sites (RS1, RS2 and RS3). There was a significant difference in the conductivity between DS3 (4.13 ± 0.049) and RS1 (3.17 ± 0.016). The control sites US1 to US3 showed salinities of 0.21 ± 0.01, 0.31 ± 0.020 and 0.25 ± 0.041 mS/m, respectively. The low electrical conductivity was from site US1 (Table 1).

The BOD was high at DS1, decreased downstream throughout the length of the drain and again decreased in the downstream River Chenab sites (RS1, RS2 and RS3). The BOD in water samples collected from the control sites (US1 to US3) was recorded as 37.77 ± 0.70, 33.89 ± 0.661 and 33.76 ± 0.43, respectively. The chemical oxygen demand (COD), meanwhile, was highest at site DS1, and then decreased downstream through the length of the Chakbandi drain, and further decreased in the River Chenab sites (RS1, RS2 and RS3) and the downstream of the river (US1, US2 and US3) (Table 1).

The phenol concentration increased in the drain water downstream along the length of the drain. The concentration of phenol was highest (2.77 ± 0.025 mg l^{-1}) at polluted site DS3 where the drain entered into the River Chenab and then gradually decreased at the river sites RS1, RS2 and RS3 (Table 1). The downstream sites showed a further decreasing trend, except for US2 (0.13 ± 0.003 mg l^{-1}), with the lowest concentration of phenol being found at site US1 (0.10 ± 0.005 mg l^{-1}).

The initial concentration of sulphate ($SO^{2-}4$) at Chakbandi polluted site DS1 was 304.71 ± 2.860 mg l^{-1} before increasing at site DS2 (485.71 ± 1.973 mg l^{-1}) and decreasing at site DS3 (444.14 ± 4.877 mg l^{-1}). The concentration of SO_4 then decreased gradually downstream in river sites RS1, RS2 and RS3 due to the mixing of drain water into the river. The concentrations of sulphate at the control sites US1, US2 and US3 were determined as 81.14 ± 1.58, 76.02 ± 0.951 and 72.16 ± 0.87 mg l^{-1}, respectively (Table 1).

Detection of selected heavy metals

The concentration of Cd increased between polluted sites of Chakbandi drain DS1 and site DS2 (0.227 ± 0.007 mg l^{-1}) and then decreased at site DS3 where the drain entered into the River Chenab. The concentration of Cd then further decreased gradually downstream in the river sites RS1, RS2 and RS3. The level of Cd at the control sites, US1, US2 and US3, was detected as being 0.008 ± 0.002, 0.007 ± 0.002 and 0.007 ± 0.003 mg l^{-1}, respectively, indicating that there is a considerable difference upstream and downstream of the entrance of the Chakbandi drain into the River Chenab. There was a significant difference in the concentration of Cd between DS3 (0.218 ± 0.005) and RS1 (0.183 ± 0.005). The lowest concentration of Cd was found at downstream site US2 of the river, 0.007 ± 0.002 mg l^{-1} (Table 2). The concentration of Cu was increased at polluted site DS2 (1.988 ± 0.031 mg l^{-1}) and, but again increased at site DS3 (1.974 ± 0.027 mg l^{-1}) where the drain enters into the River Chenab. The concentration of Cu then decreased gradually downstream in river sites RS1 (1.670 ± 0.020 mg l^{-1}), RS2 (1.622 ± 0.038 mg l^{-1}) and RS3 (1.557 ± 0.020 mg l^{-1}) due to the mixing of drain water into the river water. The concentration of Cu at control sites US1, US2 and US3 was further decreased as 0.043 ± 0.006, 0.033 ± 0.006 and 0.03 ± 0.006 mg l^{-1}, respectively (Table 2). The concentration of Mn was high at DS2 (2.05 ± 0.019 mg l^{-1}) and decreased gradually downstream through the length of the drain and the River Chenab at sites RS1, (2.12 ± 0.025 mg l^{-1}), RS2 (2.02 ± 0.037 mg l^{-1}) and RS3 (1.86 ± 0.040 mg l^{-1}). The concentration of Mn at the control sites of the River Chenab US1, US2 and US3 was recorded as 0.43 ± 0.01, 0.40 ± 0.023 and 0.36 ± 0.022 mg l^{-1}, respectively, exhibiting a further decreasing trend (Table 2). The concentration of Pb was increased at polluted sites DS2 and DS3, being highest at site DS3 (2.956 ± 0.003 mg l^{-1}) where the drain enters into the River Chenab. The concentration then decreased gradually downstream in the river sites RS1 (2.043 ± 0.014 mg l^{-1}), RS2 (1.749 ± 0.094 mg l^{-1}) and RS3 (1.729 ± 0.035 mg l^{-1}). The concentration of Pb at the control sites US1, US2 and US3 was detected as 0.054 ± 0.008, 0.080 ± 0.007 and 0.070 ± 0.006 mg l^{-1}, respectively (Table 2). The initial concentration of Cr at the first water sampling site of the drain was 0.246 ± 0.015 mg l^{-1}, but it increased at polluted site DS2 (0.840 ± 0.034 mg l^{-1}) then again decreased at site DS3 (0.644 ± 0.004 mg l^{-1}). The concentration of Cr was therefore highest at site DS2 (0.840 ± 0.034). The concentration of Cr then decreased gradually downstream in the river sites RS1 (0.527 ± 0.023), RS2 (0.431 ± 0.011) and RS3 (0.357 ± 0.013) (Table 2). The maximum permissible limits copper (Cu), Mn, Pb and Cr concentration were found to be high in the drain and the river, and to exceed the WHO (2013) standards.

Fish scale studies

Generally, all the scales of fish species found in the different regions of the fish body have a focus, a radius, a circulus, radius, a lateral field, a posterior field and an anterior field. A normal scale structure has circuli (C) present all over and evenly. All the parts of the scale were normal in terms of their basic architecture, i.e. foci, radii, circuli and annuli. The anterior and posterior margins possessed normal outlines. Few (less than 10 % of scale samples) deformations were observed on the

Table 2 Comparison of means for Cd, Cu, Mn, Pb and Cr from different sites of the River Chenab

Water sampling sites	Cd (mg l^{-1})	Cu (mg l^{-1})	Mn (mg l^{-1})	Pb (mg l^{-1})	Cr (mg l^{-1}))
DS1	0.130 ± 0.009c	0.880 ± 0.002e	1.58 ± 0.057f	1.377 ± 0.016f	0.246 ± 0.015f
DS2	0.225 ± 0.007a	1.988 ± 0.031a	2.84 ± 0.012a	2.910 ± 0.037ab	0.840 ± 0.034a
DS3	0.218 ± 0.005a	1.974 ± 0.027a	2.65 ± 0.061b	2.956 ± 0.003a	0.644 ± 0.004b
RS1	0.183 ± 0.005b	1.670 ± 0.020c	2.12 ± 0.025c	2.043 ± 0.014c	0.527 ± 0.023c
RS2	0.182 ± 0.001b	1.622 ± 0.038 cd	2.02 ± 0.037 cd	1.749 ± 0.094de	0.431 ± 0.011d
RS3	0.180 ± 0.001b	1.557 ± 0.020d	1.86 ± 0.040e	1.729 ± 0.035de	0.357 ± 0.013e
US1	0.008 ± 0.002d	0.043 ± 0.006f	0.43 ± 0.010 g	0.054 ± 0.008 g	0.027 ± 0.005 g
US2	0.007 ± 0.002d	0.033 ± 0.006f	0.40 ± 0.023 g	0.080 ± 0.007 g	0.027 ± 0.007 g
US3	0.007 ± 0.003d	0.029 ± 0.004f	0.36 ± 0.022 g	0.070 ± 0.006 g	0.025 ± 0.006 g
Mean	0.127 ± 0.004	1.088 ± 0.017	1.58 ± 0.032	1.441 ± 0.024	0.347 ± 0. 013

Means sharing a similar letter in a row or in a column are statistically non-significant ($P > 0.05$). Experimental site details are as follows: DS1 to DS3 are drain sites; RS1 to RS3 are river sites; US1 to US3 are control

scales of the selected control groups. In case of control *Catla catla* fish, the body was covered with noticeably large cycloid scales, the head was devoid of scales while the lateral line possessed 40 to 43 scales. *Labeo rohita* is a freshwater fish of the carp family Cyprinidae. During the early stages of its lifecycle, it consumes mostly zooplankton, but as it grows, it prefers phytoplankton, and it is an herbivorous column feeder as a juvenile and as an adult consumes mainly phytoplankton and submerged vegetation. The scales of the *Labeo rohita* were of moderate size, and there were 40–42 scales along lateral line. Hatchlings of *Cirrhinus mrigala* usually stay in the surface or sub-surface waters, while fry and fingerling are likely to move to deeper water. Adults are bottom dwellers, feeding on detritus, decayed vegetation, phytoplankton and zooplankton. There are 40–45 scales on each lateral line, and lateral transverse scale rows vary between 6 and 7/6 between the lateral line and pelvic fin base (Fig. 2). When the scales of control fish species viz. *C. catla*, *L. rohita* and *C. mrigala* were studied, it was observed that scales were normal in shape and the structures of the foci, annuli, circuli radii, anterior margins and posterior margins were all normal. Specifically, few (less than 10 %) morphological deformities (scale disorientation) were observed in the foci, annuli, circuli, radii, anterior margins and posterior margins (Figs. 2 and 3).

The scales of experimental fish species viz. *C. catla*, *L. rohita* and *C. mrigala*, meanwhile, showed several deformities in shape and different scale structures. The focus showed most deformities (76.19 %) in *L. rohita* whereas these were less (66.67 %) in *C. catla.* In respect to the annuli, most deformities (66.67 %) were observed in *C. mrigala* and the least (47.62 %) in *C. catla*. In respect to the circuli, the highest proportion of deformities (76.19 %) was evident in *C. mrigala* and the least (71.43 %) in both *C. catla* and *L. rohita.* In respect to the radii, meanwhile, the highest proportion of

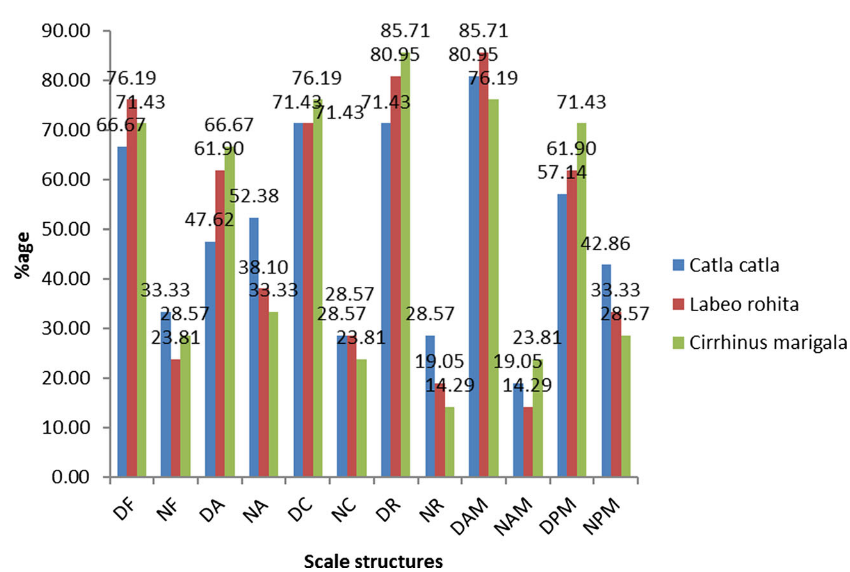

Fig. 2 Comparative structral details of scale in *Catla catla*, *Labeo rohita*, and *Cirrhinus marigala* collected from River Chenab. Scale structures were as follows: *NF* normal focus, *DF* deformed focus, *NA* normal annuli, *DA* deformed annuli, *NC* normal circuli, *DC* deformed circuli, *NR* normal radii, *DR* deformed radii, *NAM* normal anterior margins, *DPM* deformed posterior margins

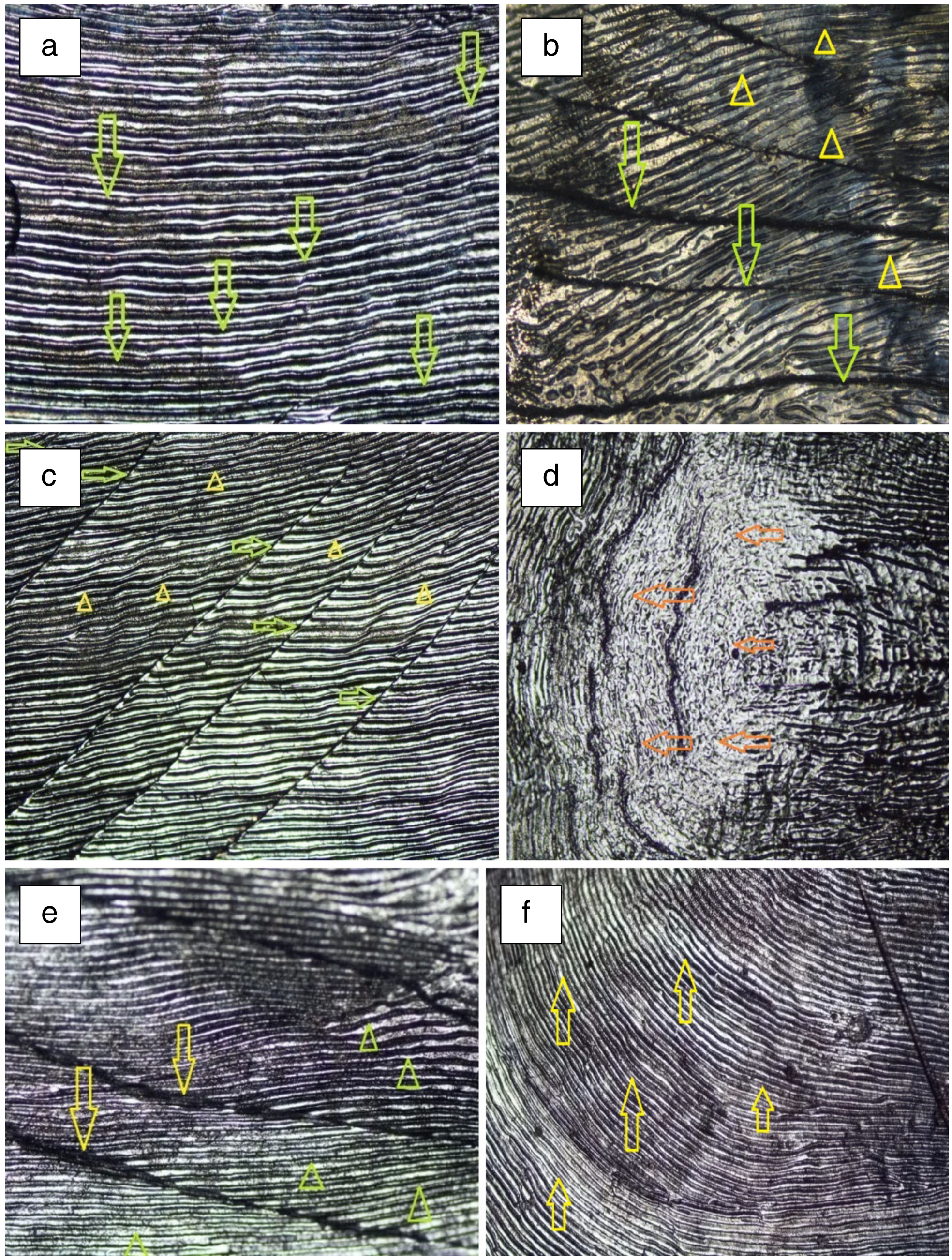

Fig. 3 Details of scale structure in fish from the control group shown by *arrows* in **a** (well-organized circuli), **b** (well-organized circuli and Radii) in *Catla catla*, c (well-organized circuli and radii), **d** (well-organized focus) in *Labeo rohita*, **e** (well-organized circuli and radii), **f** (well-organized circuli and focus) in *Cirrhinus mrigala*

deformities (85.71 %) was evident in *C. mrigala* and the least (71.43 %) in *C. catla*. The anterior margins showed most deformities (85.71 %) in *L. rohita* and the least (76.19 %) in *C. mrigala*. Finally, the posterior margins showed most deformities (71.43 %) in *L. rohita* and the least (57.14 %) in *C. catla* (Fig. 2). Overall, *C. mrigala* showed the highest % age of deformities in deformed annuli (DA), deformed circuli (DC), deformed radii (DR) and deformed posterior margin (DPM) whereas *L. rohita* showed the highest in deformed focus (DF) and deformed anterior margin (DAM) (Fig. 2).

The scales of the experimental *C. catla* showed several deformities in shape and different scale structures. The foci showed 66.67 % deformities, the annuli 47.62 % deformities, the circuli 71.43 % deformities and the radii 71.43 % deformities. The anterior margins, meanwhile, showed 80.95 % deformities and the posterior margins 57.14 % (Fig. 4).

The scales of experimental *L. rohita* collected from the River Chenab also showed deformities in shape and different scale structures: 76.19 % for the foci, 61.90 % in the case of the annuli, 71.43 % for the circuli, 80.95 % for the radii, 85.71 % for the anterior margins and 61.90 % for the posterior margins (Fig. 5). The scales of experimental *C. mrigala* collected from the River Chenab, meanwhile, showed 71.43 %

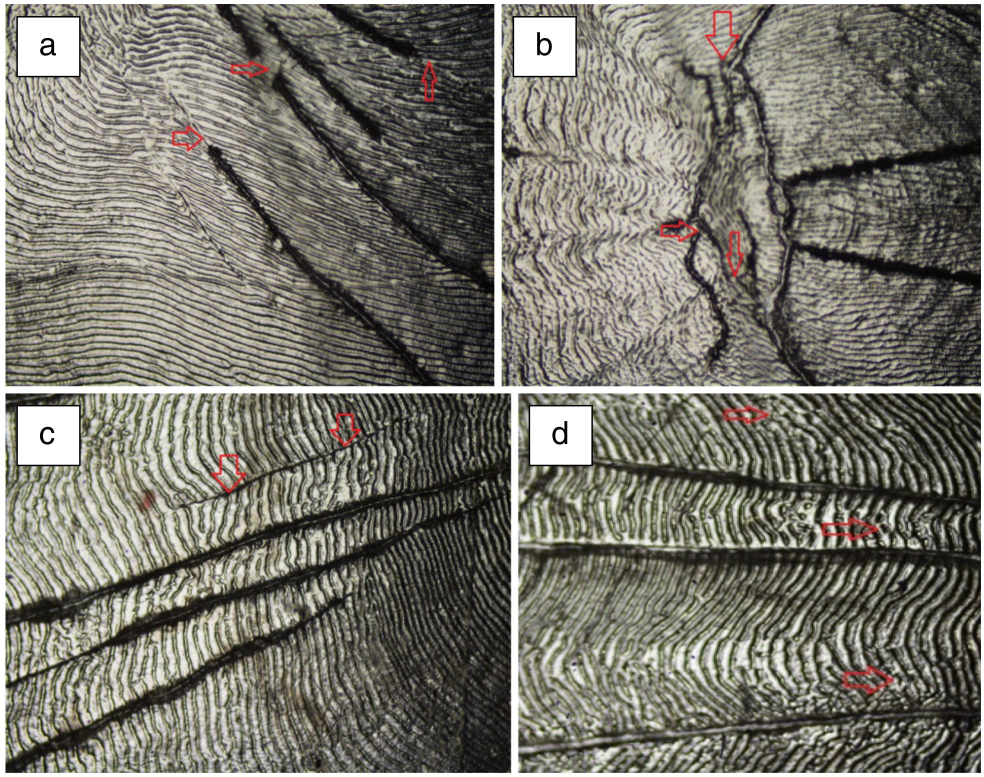

Fig. 4 Photographs of scale structural anomalies as shown by *arrows* in **a** (damaged radii and distorted circuli), **b** (anomalies of radii, annuli, circuli and focus), **c** (damaged circuli and calcified and incomplete radii), **d** (non-symmetrical/distorted circuli in scales of *Catla catla* collected from the polluted sites of River Chenab)

deformities in the foci, 66.67 % in the annuli 66.67, 76.19 % in the circuli, 85.71 % in the radii, 76.19 % in the anterior margins and 71.43 % in the posterior margins from studied scale samples (Fig. 6).

Discussion

pH is quite an important measurement among the other water quality parameters since it can be affected by chemicals in the water. Pawar (2012) and Nhapi et al. (2011) reported similar findings for the pH of the water of Nyabugogo River system. In this study, the drain site had an alkaline pH that increased the alkalinity downstream (Muhirwa et al. 2010; Hussain et al. 2016). Salinity, meanwhile, has an important role in understanding many water characteristics, especially the chemistry of natural waters and the biological processes occurring in it. The salinity of a water body is enormously important for all the organisms living in and around it: since a minor change in salinity can cause an aquatic organism either to float too much (high salinity) or not enough (low salinity). In this study, all physicochemical parameters showed a significant difference between the DS3 and RS1 sites. This difference can be attributed to the dilution of the drain wastewater by the water of the Chenab River. The highest concentration of dissolved solids was due to the effluent received from the industry of the city of Faisalabad. In the current study period, the upstream river sites showed TDS values almost six times less than the river sites RS1–RS3.

Total suspended solids (TSS), also known as non-filterable residue, are those solids that remain trapped in a 1.2-μm filter (USEPA 2002). TSS includes a variety of materials, such as silt, decaying animal and plant matter, sewage and industrial waste. High concentrations of suspended solids result in many problems for the health of water bodies and aquatic organisms by reducing water clarity, degrading habitats, clogging fish gills, decreasing photosynthetic activity and causing an increase in water temperatures, etc. Considerable differences in TSS have been reported between DS3 and RS1. Upstream sites showed TSS values that exceeded the permissible limits. Similar findings were also reported by USEPA (2002) and Prasanna and Ramesh (2013).

Variations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) among the sampling sites were insignificantly different, but even upstream sites of the River Chenab showed higher values than the permissible limits. Drainage and the addition of waste in water can affect the composition of the river water, as demonstrated in the current findings that indicated high BOD in the river even before the Chakbandi drain water was mixed in. Similar differences in different months were reported by Nhapi et al. (2011), although the differences in BOD between the upstream and downstream sites were statistically insignificant at the 5 % level and were within the permissible limits, contrary to the current findings where all the values exceeded the

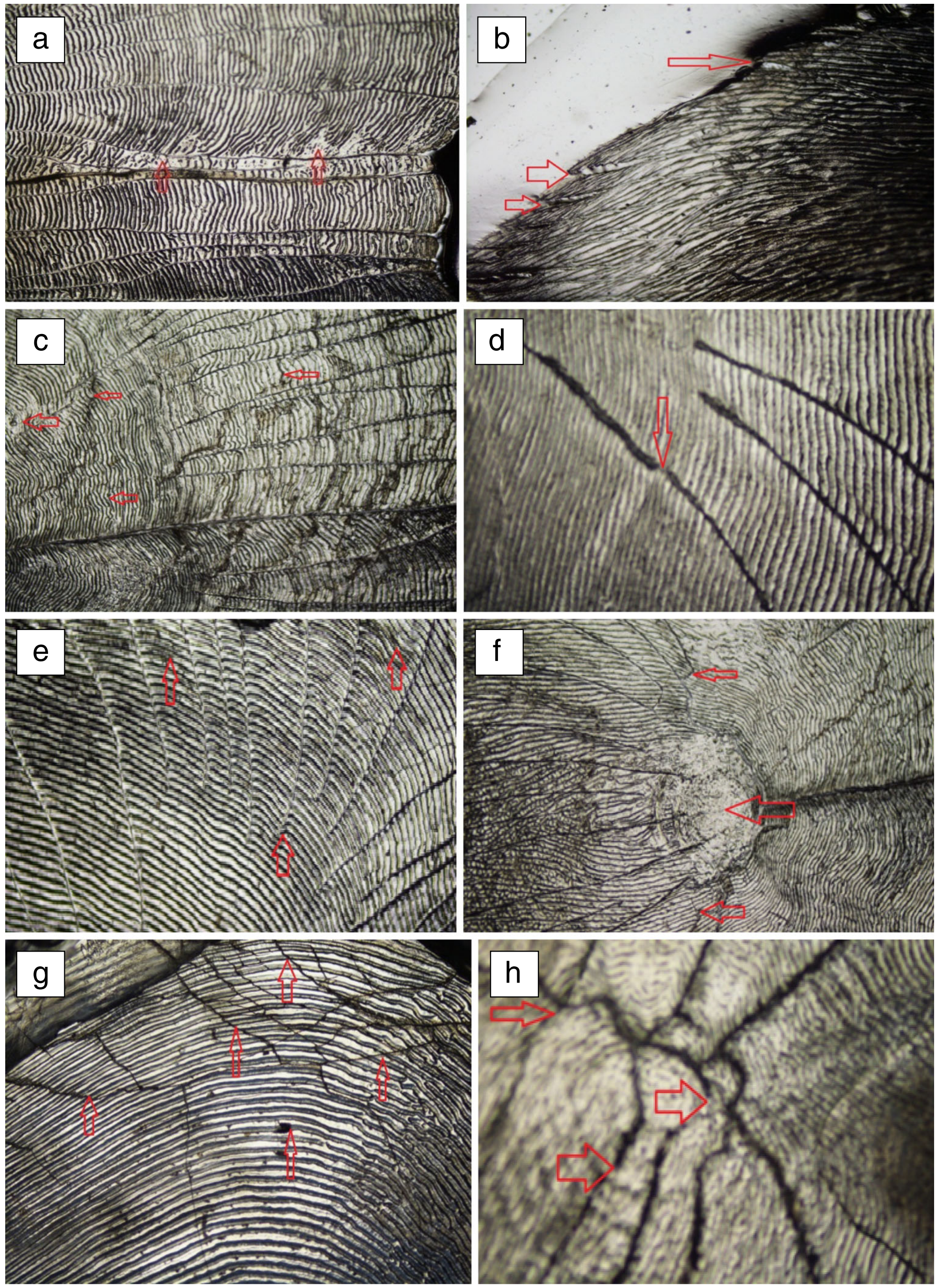

Fig. 5 Photographs of scale structural anomalies as shown by *arrows* in **a** (damaged circuli), **b** (damaged anterior margin of scale), **c** (damaged circuli and distorted radii), **d** (damaged/ interrupted radii), **e** (damaged circuli), **f** (interrupted annuli, distorted radii and focus), **g** (severe distortion of radii) and **h** (severe distortion of radii and focus) in a scale of *Labeo rohita* collected from the polluted site of River Chenab

permissible limits. Multiple drains, like Ahmadpur, MC, Chakbandi and Khairwala drains, are polluting the River Chenab and raising the BOD and COD values to acute levels, and these findings are also supported through several studies (Amneera et al. 2013).

Phenols were reported in sufficiently high concentrations that cause DNA damage in fish. (Hussain et al. 2016). Phenols were present in high concentrations in the majority of the sampling sites, even at the entrance of the Chakbandi drain, where the water current is high and there is considerable mixing of river water and sediments. These higher concentrations of phenols were due to the large amount of industrial waste in this drain, which caused noticeable changes in the river water (Sprynsky et al. 2007). Zhong et al. (2013) mentioned that higher concentrations of phenols resulted in potential stress to aquatic environment of three rivers. Phenols and their derivatives commonly exist in the environment and are often used in drugs, dyes, polymers and other such kinds of organic substances. The occurrence of phenols in aquatic ecosystems is also linked with industrial and municipal waste and in the production and degradation of numerous pesticides. Several studies have reported that phenols are

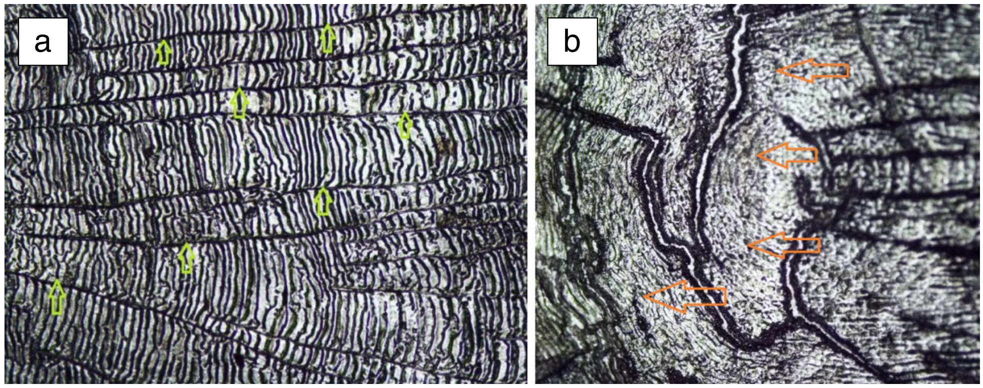

Fig. 6 Photographs of scale structural anomalies shown by *arrows* in **a** (damaged circuli and distorted radii), **b** (severe distortion of radii and focus) in a scale of *Cirrhinus mrigala* collected from the polluted site River Chenab

among harmful exotoxins, being mutagenic, haematotoxic and carcinogenic to humans and other living organisms (Dû-Lacoste et al. 2012), and confirm the present findings relating to high levels of phenols.

Sulphate (SO_4^{2-}) is commonly present in natural waters in concentrations from a few to several hundred milligrammes per litre (WQSR 2009) and is also a part of naturally occurring minerals in soil and rock formations (USEPA 2013). The highest concentration of SO_4 in this study was in line with Nhapi et al. (2011), who reported the highest level of sulphates in the Mpazi River (20.50 ± 23.72 mg l^{-1}) in dry and rainy seasons. The concentration of all the heavy metals decreases from site DS2 to DS3, indicating that along the length of the drain, concentration was low at site DS1 perhaps because this site is upstream to the point at which the Main Paharang drain, which mainly comprises the industrial wastes, joins the Chakbandi drain. Cd concentration was found to be high in the drain and the river, even exceeding the permissible limits set by the WHO standards. These findings are in line with the findings of Bentivegna et al. (2013). Copper (Cu), Mn, Pb and Cr concentrations were found to be high in the drain and the river and to exceed the WHO (2013) standards. The Cu concentration increased at DS3 due to the slope and increased water movement at the mouth of the drain, indicating the release of the Cu and Mn from the sediment and its availability by water currents. Several authors reported higher concentrations of Cu as a pollution indication, supporting the present study findings (Yousafzai et al. 2008).

The river sites also showed a considerable amount of Mn, with upstream sites on the river also showing the highest concentrations of Mn. Its concentration in the Chakbandi Drain indicated large amount of manganese release into the environment (Nhapi et al. 2011). Cr concentration was found to exceed both WHO (2013) standards in the Chakbandi drain and the River Chenab. Although Cr was found to be lower at the DS1 site compared to DS2, this is perhaps due to the domestic sewage waste at DS1 in contrast to the entrance of industrial waste at DS2. The level rises slightly again at DS3 due to the high flow of the water at the mouth of the drain (Nhapi et al. 2011), before steadily decreasing downstream in the River Chenab.

Overall, therefore, the present study revealed that the quality of the water in the River Chenab was adversely affected by the presence of large amounts of heavy metals and phenolic compounds. All the water quality parameters studied were found to be higher than the WHO permissible limits. The River Chenab can therefore be considered to be highly polluted by the industrial activity in the Faisalabad city, and this is a matter of concern given the large local population depending on water from the river for many purposes, but especially agriculture and fishing.

Our findings also indicated the effect of pollution on the structure of fish scales from *C. catla*, *L. rohita* and *C. mrigala* collected from polluted sites. The scale structure of fish collected from the control area in this study exhibited normal patterns in line with those reported in Dua and Gupta (2005). Since fish grow faster in summer and slower in winter, the circuli formed during the summer are more widely spaced than their winter equivalents, where the lines can be so closely spaced as to form a dark ring called an annulus. Fish collected from polluted sites exhibited changes and deformations in the shape of the circuli and other structures in the scales that may be due to the experience of stress due to metal pollution or other toxic exposure. The present findings are in line with findings of Khanna et al. (2007) who reported that the frequency and severity of damage were negligible or nil in the scales of fishes in less polluted areas. Shikha and Sushma (2011) argued that contaminants accumulate on the outer most protective layer of scales and then enter to the integumentry system after absorption and slowly erode the ultra-structure of scales in different ways in *Oreochromis mossambica*. It has caused ultra-scale deformities, gradual damage of the structure and block of the scale function. These contaminants first affect the fully developed scale and gradually affect the dermis region. Resultantly soft, calcified scale structures were destroyed. Disruption/dislocation and the loosening of scales because of that was found to increase from the middle of the summer and in the monsoon season when the total solids, silts and particulate suspended in the river increased due to runoffs, soil erosion and landslides. The qualitative study shows that exposure to such extreme conditions for prolonged periods resulted in partial or complete damage to the scales of fish in the river. Dua and Gupta (2005) reported that fish scales exposed to chromium and mercury exhibited greatly deformed foci in exposed scales and that high concentrations affected the shape of the radii and circuli. Rishi and Jain (1998), meanwhile, reported that cadmium and aluminium accumulation also caused brittleness in the margin of scales and at different cadmium concentrations; the lepidonts and circuli were damaged in both the anterior and posterior parts of the scales along with deformities in the calcareous parts. Malformation and thickening of the scales and tears in the edges of the scales were reported by Lin-Sun et al. (2009). Çoban et al. (2013) reported deformities in focus region of scales in *Cyprinus carpio* after exposure with chromium, and it increased with increasing Cr concentration in water. They further reported that radius, circulars and lepidonts on scale damaged at various rates depending on level of Cr. Furthermore, more damage was noticed in anterior region compared to the posterior region of the scales. Kaur and Dua (2015), meanwhile, examined the effect of municipal wastewater on scale morphology of the freshwater fish *Labeo rohita.* They reported alterations in the scale morphology due to exposure of scales to pollution. It was found that the focus parts of all the exposed scales were greatly deformed as well as dissections in the radii and circuli. Lin-Sun et al. (2009) determined malformation, thickening of the scales and tears in the edges of the scales of *Oreochromis* spp. from rivers with high heavy metal levels. The combination of these results with our own strongly suggests that fish scales can be used as bio-indicator for heavy metal pollution (Darafsh et al. 2008; Lin-Sun et al. 2009; Mahboob et al. 2015). Heavy metals accumulate and affect the scales more than other organs because they are directly exposed to pollution and environmental conditions. This accumulation effects change according to the time period and concentration level. Heavy metals and organic pollutants of water and sediments may be the major reason behind the scale deformities in fishes (Middaugh et al. 1990). Scale deformity and disorientation were also reported by Okunola et al. (2005) and Bukola et al. (2015).

Conclusions

The findings of this study suggest that fish scales can be successfully used as bio-indicator for heavy metal contamination in river system. It was noticed that the reaction to pollutants and survival of fish depend not only the biological state of water and on the level of various chemical constituents, type of toxicity and duration of exposure. Hence, these contaminants accumulate on the outer most protective layer of scales and enter to the integumentry system through absorption and gradually erosion to the ultra-structure of scales in various ways. Consequently, ultra-scale deformities are observed, gradual damage of the structure and block of the scale function. The pollutant primarily affects the completely developed scale and slowly affect the dermis region. We concluded that there is a dire need for non-lethal, biologically relevant screening tools to assess the effects of surface water contaminants on threatened or endangered fish species.

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