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Fish Scales as a Bio Indicator of Environmental Pollution in Some Fish Species Collected from Different Areas in Saudi Arabia

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ABSTRACT

The accumulation of heavy metals in different areas is an indicator of toxicity. In this investigation, three fish species; namely, *Scarus pulchellus, Acanthopagrus bifasciatus,* and *Mugil cephalus* were collected from different regions in Saudi Arabia (Jeddah, Tabuk and Al-Jubail). Fish diversity, environmental parameters and scale structure were addressed. For the environmental parameters, some heavy metals (Zn, Mn, Cd and Cu) were estimated in water samples collected from the studied areas. The light microscopic structure of fish scale illustrated the shape of scale and malformations, giving each species its characteristic taxonomical order. The scales showed several deformities in shape and different scale structures such as circuli, radii and focus. With respect to the three fish species, a considerable variation in the morphology of their scales was observed in specimens collected from different sites. Finally, this study revealed important results showing the scale abnormalities as an important indicator that differed with the heavy metal concentrations in different regions. The result recommends setting a decision to prevent industrial and other pollution which led to harmful changes in the tested fishes and subsequently can cause serious effects on human health.

INTRODUCTION

Pollutants may change the properties of water in a way that causes fish death. Heavy metal pollution in aquatic environments occurs as a direct result of atmospheric deposition or industrial waste materials **(Demirak *et al*., 2006; Maier *et al*., 2014)**. Heavy metals are thought to be mostly sourced through industrial effluents, municipal sewage, agrochemical waste products, geological weathering and atmospheric deposition **(Onsanit *et al*., 2010)**. Because of the massive inputs of heavy metals into aquatic ecosystems, fish and other aquatic species can bioaccumulate these contaminants. On the other hand, heavy metal accumulation by organisms can be passive or selective, and differences in this accumulation could be brought on by variations in absorption, egestion or both **(Sneddon *et al*., 2017)**.

Fishes are responsive to alterations occurring in their habitat, particularly escalating water pollution. Fish health examinations may therefore identify alterations to the aquatic ecology. Trace metals in marine environments have been seen to upset the delicate equilibrium of aquatic ecosystems. Before obvious alterations in fish behavior or appearance become apparent, early toxic effects of pollution may be employed as an indicator at the cellular or tissue level **(Galadima & Garba, 2012; Gyampo *et al*., 2013)**. Fish scales have been used for species identification and determination, and they are included as selective features in several keys **(Maitland, 2004)**. Every scale has a "Fields" components, which are the scale's outer surface area, and a "Focus," which is the nucleus, the first central portion of the scale in ontogeny. Scale fields can be classified as anterior or posterior. Circuli, elevated surface patterns that typically take the form of lines that follow the contour of the scale, are usually continuous lines but are frequently broken up by grooves. The term "Radii" refers to grooves that extend outward from the scale margin. In the posterior border, there are extra structures called "cteni" that resemble teeth. The concentration of "Tubercles," or pigmented granules, depends on where the scales are on the fish's body **(Lippitsch, 1990)**.

Since scales are the outermost structures of the fish's body, they are in continuous and direct contact with contaminants presented in the water and can therefore be used as vital structures to mark 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 & Sushma, 2011)**. Furthermore, fish scales can be easily studied due to their presence on exposed body parts. When a fish individual is involved in traumatic conditions, its growth stops, and thus, any abrupt 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. Various sorts of aberrations have been documented in scale structures related 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 the radii and circuli shapes were significantly affected **(Dua & Gupta, 2005; Chernova, 2010)**. In addition, cadmium and aluminium accumulations 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. 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 **(Drafash *et al*., 2008)**.

Specimens of fish scales were described qualitatively and observed quantitatively **(Sultana *et al*., 2016)**. 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.

. **The aim of this investigation is the estimation of:**

1- Different heavy metals concentrations (Cd, Cu, Mn & Zn) in water samples collected from three regions in KSA (Jeddah, Tabuk and Al-Jubail).

2- Effect of heavy metals concentrations on scales morphology of the studied fish species.

MATERIALS AND METHODS

Study areas and fish sampling

Fifteen fishes from each species were collected from three localities (two sites on the Red Sea: Tabuk and Jeddah) and one site on the Persian Gulf coast of Saudi Arabia (Jubail). The three collected species were *Scarus pulchellus*, *Acanthopagrus bifasciatus* and *Mugil cephalus*. The collected fish species were immediately preserved in an ice box and transferred to the laboratory where they were classified and kept frozen at - 20°C until further analysis. Additionally, water samples were collected from each site for further heavy metals analysis.

Estimation of heavy metals in water samples

Three water samples were collected at a depth of 1.5m from the study regions (Jeddah, Tabuk and Jubail). Four heavy metals (cadmium, copper, manganese and zinc) were estimated in this study. The American Public Health Association's standard techniques were followed for sample preparation and analysis **(APHA, 1998)**. A Shimadzu AA-6701F Atomic Absorption Spectrometer was used for the heavy metal analyses.

Sampling of fish scales

Thirty scales from each fish species from each sampling site were collected using fine forceps. The scales chosen included the dorsal side scales (nearer to the head) and lateral line. In order to remove dirt and mucus, the scales were immersed in ammonium hydroxide for 24h and 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 on room temperature and humidity. Observations were taken using a light microscope **(Esmaeili & Gholami, 2011; Sultana *et al*., 2016)**.

Statistical analysis

Significant differences in metal levels between different areas were identified using one-way ANOVA to compare the metals in a particular area (significant results; $P \geq 0.05$).

RESULTS

1. Heavy metals estimation in surface sea water for the three studied areas

Different heavy metals concentration on water from three locations (Jeddah, Tabuk and Jubail) are recorded and illustrated in Fig. (1). The order of concentration for all heavy metals in all sites was as follows; Zn > Cu > Cd > Mn. In Jeddah site, the highest concentration of metals was zinc with values of 10.516 µg/g, followed by manganese with a value of 7.6 µg/g; the lowest concentration of metals was 2.3 µg/g for copper. At Tabuk site, the order of metals concentration was Zn > Cd > Mn > Cu with values 5.209, 4.974, 0.989 and 0.557µg/ g, respectively. Whereas, at Jubail site, the metals concentration order was Zn > Cu > Mn > Cd, with values 7.879, 7.829, 4.995 and 2.576µg/ g, respectively.

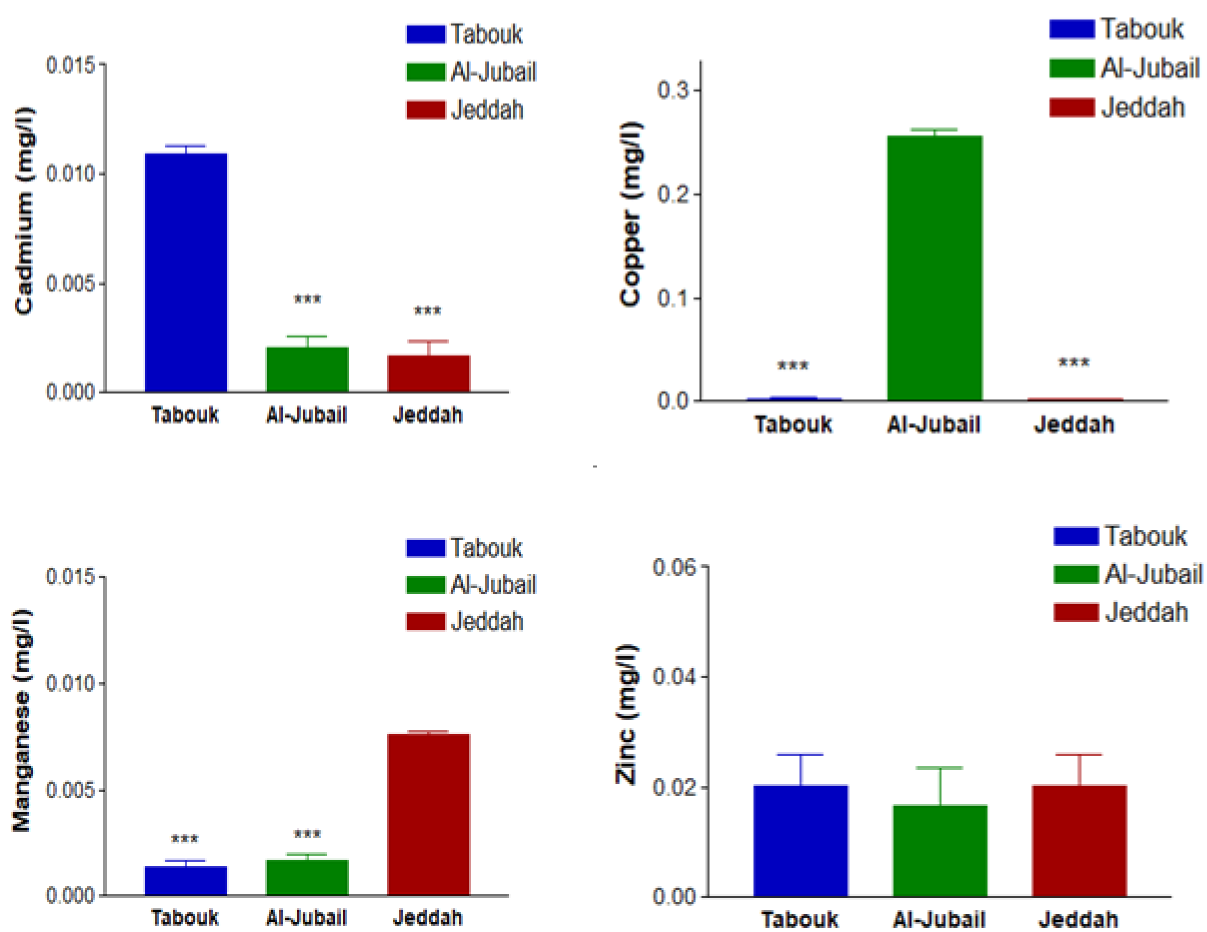

Fig. 1. Heavy metals concentrations in water samples of the three studied areas
(***) indicates significant differences between different heavy metal concentrations at $P < 0.001$.

2. Scale characteristics of different fish species

The scales maintain the same morphological proportions located on the different parts of the body. The scales present below the dorsal fin, above the lateral line, are the largest. As the scale from this region depicts all the features; this scale has been designated as the "key scale" of the fish. The scale shapes of three studied fishes are illustrated in Fig. (2). The scales are large and cycloid for *S. pulchellus,* but for *A. bifasciatus,* they are small convex with e projections that may be straight. In contrast, the scale of *M. cephalus* was ctenoid.

The normal scale of each studied species can be divided into anterior or rostral field, posterior or caudal field, lateral fields and the focus. There is no cteni at the posterior part of the scales of *S. pulchellus*, and *A. bifasciatus*; hence, they are cycloid scales (Figs.2 a & b). But the scales of *M. cephalus* are ctenoid scales (Fig.2 c). The anterior field is embedded in the skin and overlapped by the posterior side of the proceeding scale. The ventral surface of the scales is shiny and smooth while the dorsal is rough, convex and has distinct structures, consisting of ridges and radii forming nearly circular rings around the central focus and granules (tubercles).

A lateral line scale is also divided into anterior and posterior parts. The focus is absent in the lateral line scale. This scale has a canal which, characteristically, lies along the anterior-posterior axis, slightly towards the posterior part with two openings. The posterior opening lies towards the posterior margin, while the anterior opening lies towards the anterior part of the scale (Fig. 2d - f).

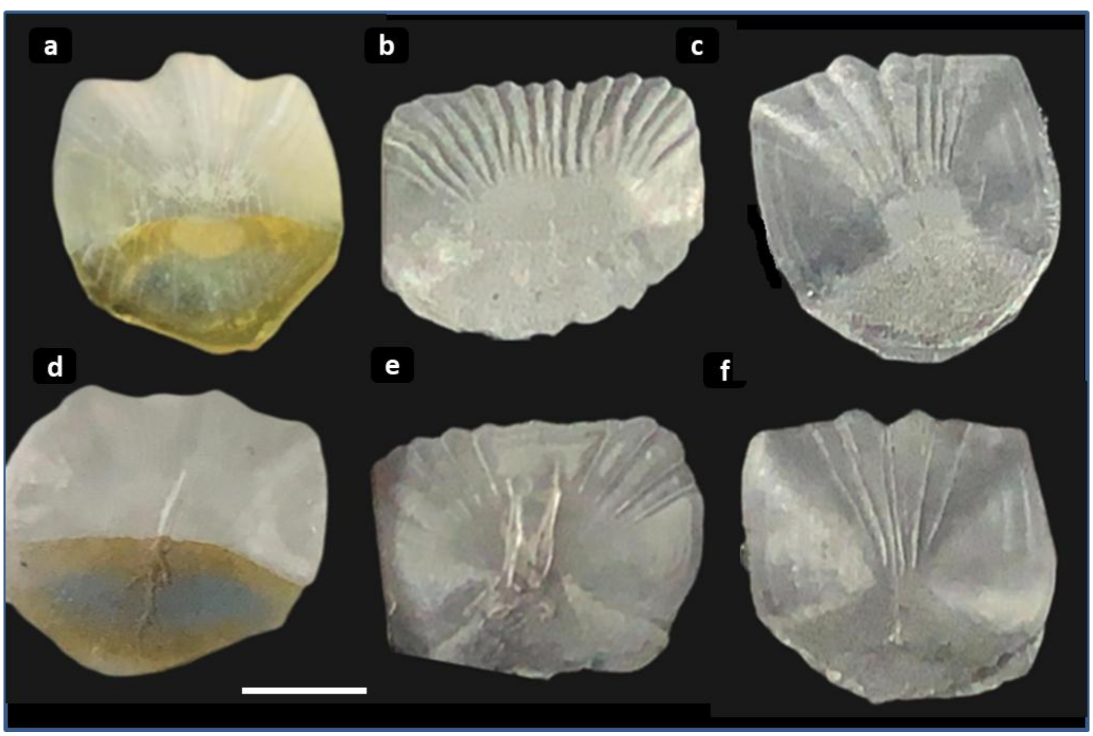

Fig. 2. The scale features of different studied species, **(a - c)** the normal scales of *S. pulchellus, A. bifasciatus* and *M. cephalus*. **(d - f)** the lateral line scales with lateral line canals of *S. pulchellus, A. bifasciatus* and *M. cephalus*. Scale bar equal to 1cm.

2.1. Radial grooves

In the anterior parts of the scale, the circuli are partitioned by deep and narrow grooves that run radially towards the focus; they are called radii. The radial groove of normal scales for all studied fish species is shown in Fig. (3). For *S. pulchellus*, the radial groove of the scales is narrow. For *A. bifasciatus,* the inter-circular grooves in the rostral field are flattened. For *M. cephalus*, the radial grooves are deep with wide and regular split. In terms of vulnerability to pollutants, whether petroleum oils or heavy metals, it was observed that there are some sediments and pigmented granules in the scales of different types of fish, especially those collected from Jeddah (Fig. 3g – i). The most affected species with these granules is the *A. bifasciatus* (Fig. 3h).

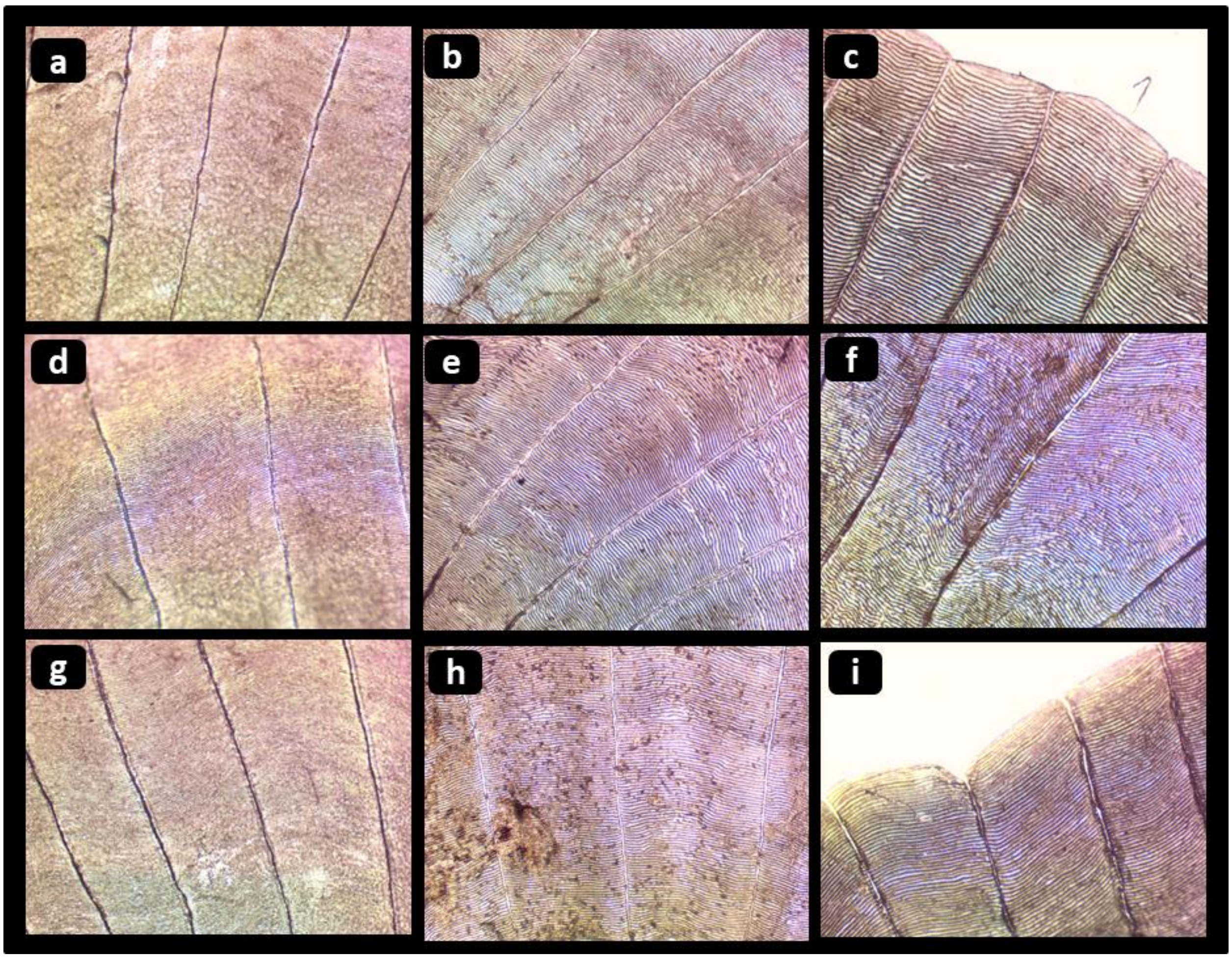

Fig. 3. Photographs of the anterior field with radii and radial groove of the scales of studied species, **(a - c)** the scale of *S. pulchellus*, *A. bifasciatus* and *M. cephalus* from Tabuk. **(d - f)** the scale of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Al-Jubail. **(g - i)** the scale of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Jeddah.

2.2. Focus region

The central part of the focus in each scale of studied species has no circuli. However, the scales of *S. pulchellus* have tubercular ridges around the center of their focus (Fig. 4a, d & g). But, the focus region in *A. bifasciatus* appear circular with rounded to oval granules arranged in circular rings around the focus (Fig. 4b, e & h). Moreover, *M. cephalus* scale has focus region similar to that of the *A. bifasciatus* one. But, the granules of *M. cephalus* focus are small and numerous (Figs.4 c, f & i).

For the focus region, it was observed that, there are some sediments and pigmented granules in addition to malformation in the scales of different types of fishes collected from Jeddah (Fig. 4g – i). The most affected species with these granules and malformed is the *A. bifasciatus* (Fig. 4h).

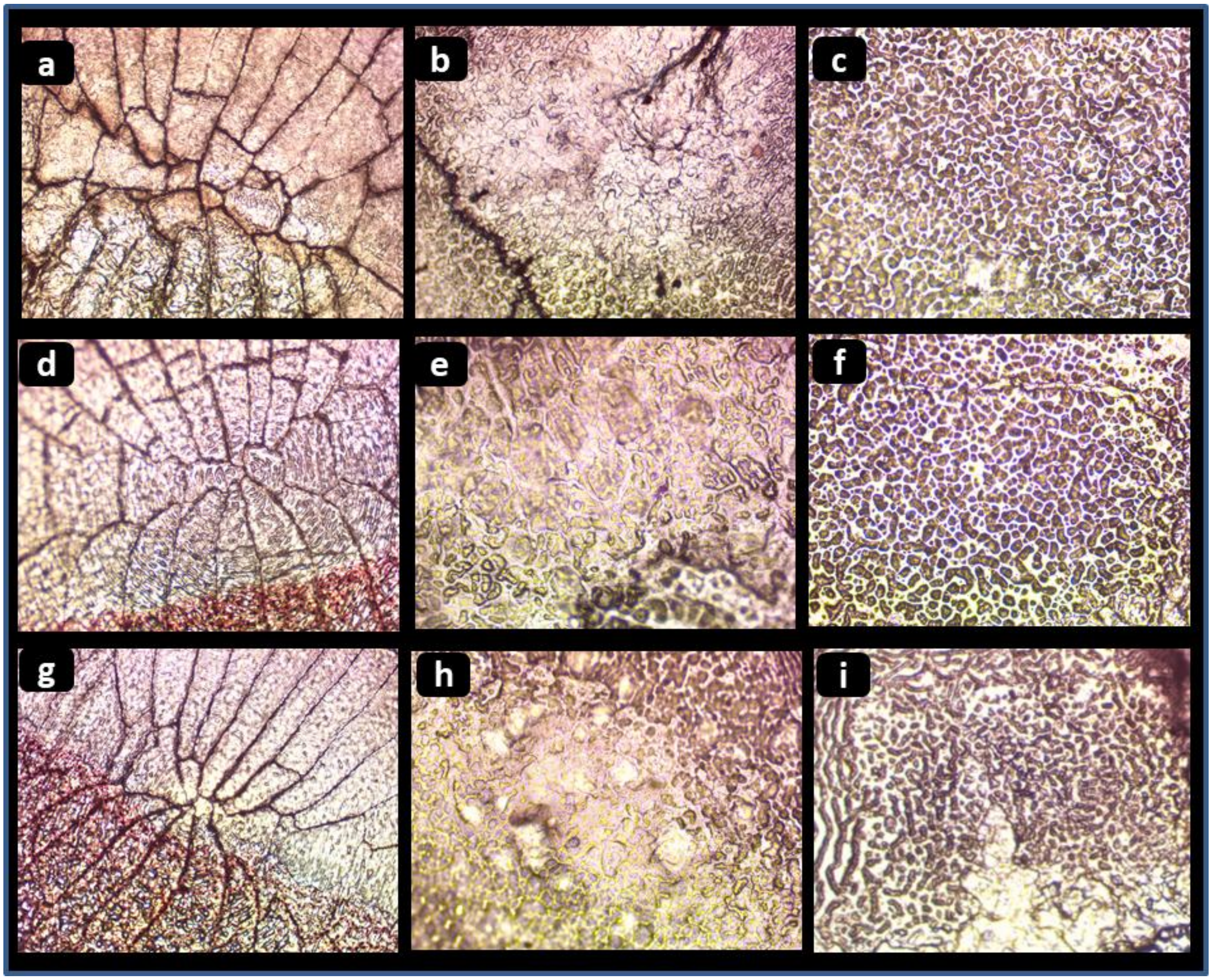

Figure 4. Photographs of the focus region of the scales of studied species, **(a - c)** the scale focus of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Tabuk. **(d - f)** the scale focus of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Al-Jubail. **(g - i)** the scale focus of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Jeddah.

2.3. Caudal field

In the external posterior part of the scale, the circuli lose their characteristic features. In this part, the scale is covered with epidermis and has several rows of rough tubercles. The tubercles impart a specific color of fish as they contain chromatophores in the outer surface. The caudal region of *M. cephalus* scales (ctenoid scales) have tooth like additional structure found in the posterior margin, known as cteni arranged in definite semi-circular rows (Fig. 5c, f & i).

In addition, some sediments and pigmented granules wee detected in addition to malformation in the scales of different types of fishes collected from Jeddah. Hence, all the species were affected with these granules and malformed (Fig. 5g – i).

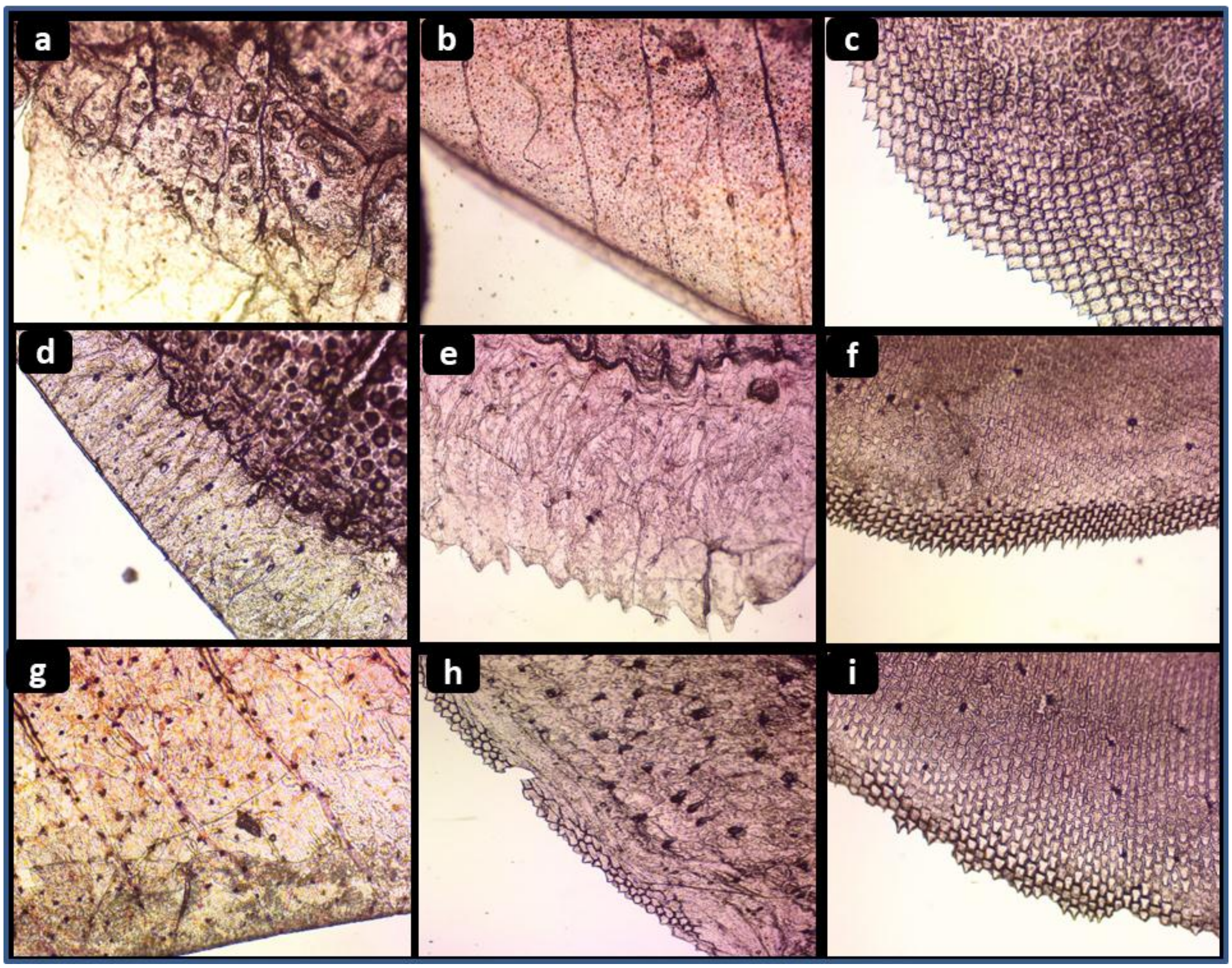

Figure 5. Photographs of the caudal region of the scales of studied species, **(a - c)** the scale caudal region of *S. pulchellus*, *A. bifasciatus* and *M. cephalus* from Tabuk. **(d - f)** the scale caudal region of *S. pulchellus*, *A. bifasciatus* and *M. cephalus* from Al-Jubail. **(g - i)** the scale caudal region of *S. pulchellus*, *A. bifasciatus* and *M. cephalus* from Jeddah.

2.4. Lateral line scales

The lateral line scales are also divided into anterior and posterior parts, while the focus is absent. The lateral line canal of scales is considered as a simple tube embedded within the matrix of the scale (Fig. 6). For scales of *S. pulchellus* such canal is long, wide and may slightly deviate from the anterior-posterior axis of the scale. Otherwise, for *A. bifasciatus* scale the canal is short and wide. But, the canal appeared narrower and more regular in the scales of *M. cephalus*. The anterior opening of the lateral line canal vary in shape from narrow rounded (*S. pulchellus*), wide curved (*A. bifasciatus*) and pointed (*M. cephalus*).

It has been noted that, there are many oily spots inside the channel of the lateral line, especially in the scales of fishes collected from the Jeddah and Al-Jubail, especially in *S. pulchellus* and *A. bifasciatus* (Fig. 6d, e, g & h). However, pigmented granules, oily spots in addition to malformation were recorded for *M. cephalus* scales, especially those collected from the Jeddah and Al-Jubail (Fig. 6f, i).

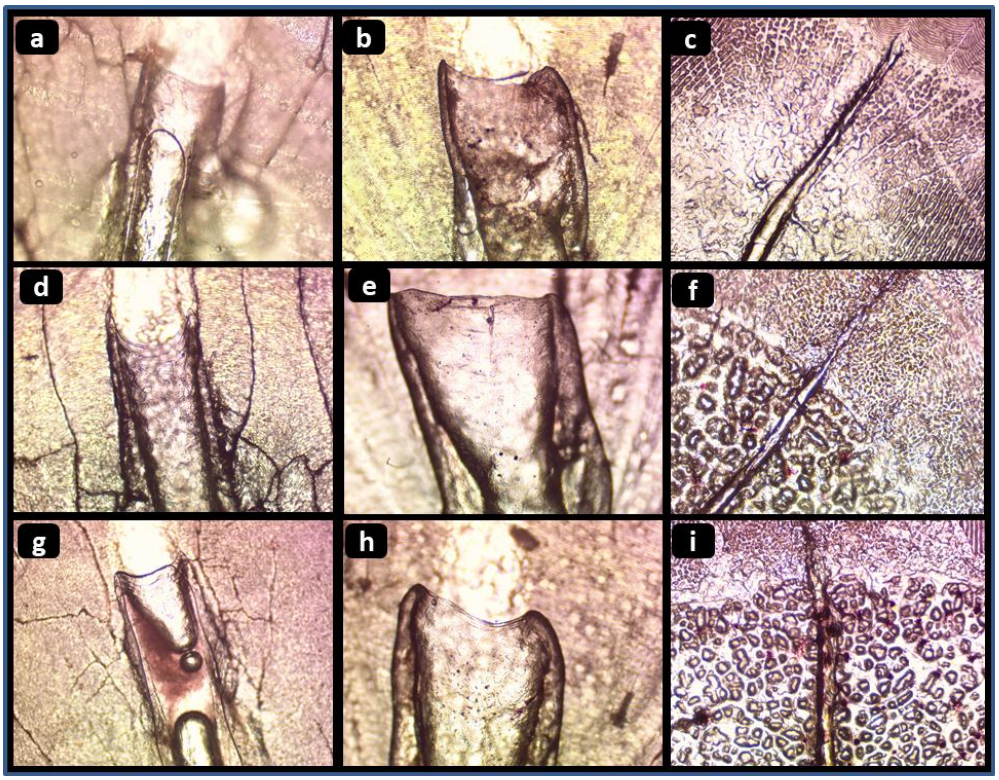

Figure 6. Photographs of the anterior part of lateral line scales of studied species, **(a - c)** the anterior part of lateral line scales of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Tabuk. **(d - f)** the anterior part of lateral line scales of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Al-Jubail. **(g - i)** the anterior part of lateral line scales of *S. pulchellus, A. bifasciatus* and *M. cephalus* from Jeddah.

DISCUSSION

Heavy metals concentrations varied with sites, which indicates that Cd concentration was high in the drain and the river, exceeding the tolerable limits set by the WHO standards. Copper (Cu), Mn, Pb and Cr concentrations were high in the drain and the river and exceeded the **WHO, (2013)** standards, indicating the release of the Cu and Mn from the sediment and its convenience by water currents. These higher concentrations of heavy metals as pollution indication was reinforced in the study of **Yousafzai *et al*. (2008)**.

This study examined the levels of different heavy metals in water taken from three regions. The highest concentration of heavy metals in water for three regions was that of Zn (8.89 µg/g), followed by Cd (3.11 µg/g) and Cu (2.81 µg/g); these results are consistent with those of **Elnabris *et al*. (2013)** and **Baki *et al*. (2020)**.

Fishes 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)**; at different cadmium concentrations, the circuli were damaged in the anterior parts of the scales. **Çoban *et al*. (2013)** reported deformities in focus region of scales in *Cyprinus carpio* after exposure to heavy metals, and it increased with increasing the concentration in water. Furthermore, more damage was noticed in anterior region compared to the posterior region of the scales. In this context, **Kaur and Dua (2015)** reported alterations in the scale morphology due to exposure of scales to pollution. The combination of these results with our own results suggests that fish scales can be used as bio-indicator for heavy metal pollution.

The stability of the surface structure and ornamentation of the rostral and caudal field of scales has been researched by several authors **(Jawad, 2005, Harabawy *et al*., 2007, Reza *et al*., 2009, Harabawy *et al*., 2012; Zubia *et al*., 2015)**. Only basic ctenoid scales with distinct ctenii from the scale body are found on *M. cephalus* **(Zubia *et al*., 2015)**. In this study, the complete ctenoid type of scales were found in the three varieties of studied *M. cephlus*.

CONCLUSION

The results of this study concluded that, fish scales can be successfully used as a biomarker of heavy metal contamination in sea water. It was observed that, the interaction with the pollutants and the survival of the fish depended on the biological state of the water, the level of different chemical components and the type of toxicity. These pollutants accumulate on the outer most protective layer of the scales, entering the integration system through absorption and gradual erosion of the superstructure of the scales in various ways. Therefore, extensive deformations and progressive damage to the fully developed scale structures are associated to pollutants.

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