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Full Length Research Paper

Bioaccumulation of some heavy metals and histopathological alterations in liver of *Euryglossa orientalis* and *Psettodes erumei* along North Coast of the Persian Gulf

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In order to make evaluation of some heavy metals bioaccumulation and explore their histopathological effects on hepatocytes of oriental sole (*Euryglossa orientalis*) and deep flounder (*Psettodes erumei*), fishes were caught from two areas of north coast of the Persian Gulf, Bandar Abbass and Bandar Lengeh. Concentrations of nickel (Ni) and vanadium (V) in liver of both species in two sampling regions were in the following order: Bandar abbass> Bandar lengeh. Between the two species, these quantities were higher in *P. erumei* than *E. orientalis* in both sampling regions. Histopathology of the liver shows some cellular alterations including degeneration, necrosis and tissue disruption, and histopathological effects were severe in *P. erumei* than *E. orientalis*. Results showed that Bandar Abbass region was more polluted than Bandar Lengeh and because Ni and V were oil pollution indicators and two flat fishes were benthic, they can receive considerable amount of oil pollution through their biological activities like feeding. Also, higher amounts of heavy metal concentrations and major histopathological effects in *E. orientalis* showed strong relationship between benthic habitat of the fish and amounts of received pollutants from water and sediments since *E. orientalis* is more related to the bottom than *P. erumei*.

Key words: Vanadium, nickel flatfishes, Persian Gulf.

INTRODUCTION

Marine pollution is a global environmental problem. Different human activities on land, in the water and air contribute to the contamination of seawater. Sediments as well as organisms releasing potentially toxic substances into the water also contribute their quota to the level of contamination. Contaminants can be natural substances or artificially produced compounds. After discharge into the sea, contaminants can stay in the water in dissolved form or they can be removed from the water column through sedimentation (Oliveira Ribeiro et al., 2002).

Contamination with heavy metals on local, regional and global scales, have been intensively studied in recent years. Due to the fact that metals are persistent and toxic, they tend to bioaccumulate and pose a risk to humans and ecosystems (Szefer, 2002; Rainbow, 2002). The main reason for this is the increasing metal input to the coastal zone from both rivers and non-point sources, especially in developing countries. Metal contamination can have adverse effects on marine organisms only after metal uptake and accumulation (Cajaraville et al., 2000; Funes et al., 2006).

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathologi-

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cal biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death at a cellular level. This manifests as necrosis at the tissue level (Bailey et al., 1996).

Previous studies reported that the exposure of fish to pollutants (agricultural, industrial and sewage) resulted in several pathological alterations in different tissues of fish. Histopathological changes were observed in the muscle of fish as a result of exposure to different toxicants (Braunbeck et al., 1998). The liver, as the major organ of metabolism, comes into close contact with xenobiotics absorbed from the environment and liver lesions are often associated with aquatic pollution.

Vinodhini and Narayanan (2008) studied bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (common carp). In this study, they determined the bioaccumulation of heavy metals in various organs of the fresh water fish exposed to heavy metal contaminated water system. The experimental fish was exposed to chromium (Cr), nickel (Ni), cadmium (Cd) and lead (Pb) at sublethal concentrations for periods of 32 days. The order of heavy metal accumulation in the gills and liver was Cd > Pb > Ni >Cr and Pb > Cd > Ni > Cr, respectively. In the case of kidney and flesh tissues, the order was Pb > Cd > Cr > Ni and Pb > Cr> Cd > Ni, respectively. In all heavy metals, the bioaccumulation of lead and cadmium proportion was significantly increased in the tissues of *C. carpio*.

Pollution studies in the Persian Gulf collectively known as ROPME Sea Area (RSA) are extremely important. The Persian Gulf comprises of a relatively shallow, semi-enclosed with very high evaporation rates and poor flushing characteristic. Thus, pollution discharge into the sea has more limitations for dilution and slower dispersion than what occurs in other open marine systems (De Mora et al., 2004). Two heavy metals studied in present study (Ni and V) are also indicators of oil pollution (a very common contamination in Persian Gulf).

Flat fishes, are benthic fishes in near shore waters. Benthic organisms due to their proximity to sediments encounter more pollutants. This follows that flat fishes can be indicators for pollutants though consumable by humans.

MATERIALS AND METHODS

Selection of the study area and samples collection

Fish samples were collected along two coastal area of Hormoz straite, north of the Persian Gulf, in coastal lines of Iran. A total of 24 samples of fish species including *Euryglossa orientalis* and *Psettodes erumei* were caught from April to June 2006. These two species were collected from two fishery regions, Bandar-Abbas and Bandar-Lengeh (Figure 1). The fish species were randomly collected from commercial catches landed at local fishing ports.

Analysis procedure

Immediately after the collection, fish samples were stored on ice in an isolated box (Eaton et al., 1995) and transferred to the environmental laboratory. Body weight and length of fishes were measured and the sexuality determined. Male fishes were then selected and a part of dorsal muscle from each was dissected as a sample. The fish liver tissue was also removed and prepared for processing. All of the samples were dried at 60°C for 48 h in the oven (Gregory et al., 2005).

All pieces of glassware were cleaned by soaking in 10% v/v HNO_3 for 12 h and then rinsed with ultra-pure water. Between 0.2 and 0.4 g of dried sample material were weighed and then digested in acid-cleaned Teflon microwave vessels with 5 ml of ultra-pure nitric acid (65% v/v). A typical microwave digester was operated for 30 -40 min at a target digestion temperature of 200°C after allowing at least 1 h for cooling. The digested samples were transferred to a graduated plastic test tube and brought up to volume (50 ml) with Mili-Q-water (Gregory et al., 2005).

Histopathology

Liver samples were fixed in Bouin's solution for 24 h, dehydrated in a graded series of ethanol and embedded in Paraplast (Merck). Three-micrometer-thick sections were obtained and stained in hematoxylin/fushin for examination by light microscopy (Martoja and Martoja-pierson, 1967).

Statistical analysis

Statistical analyses were done using the Statistical Package for the Social Sciences (SPSS) software (version 11.5). The data were tested to check normality using the Kolmogorov–Smirnov test, which showed normal distribution. Pearson's correlation test was used to assess any significant relationship of heavy metals concentration in tissue with fish length and weight in the two studied regions ($p < 0.05$). In addition, the paired sample t-test was used to compare tissue heavy metals concentrations between stations.

RESULTS

Biometry

The length of *E. oreintalis* recorded in Bandar-Lengeh (station 2) was 41 cm and weight of this sample was 1010 g. *P. erumei* length in station 1 (Bandar-Abbas) was 58 cm and weight was 2650 g (Table 1).

Bioaccumulation

Tables 2 and 3 show the concentration of Ni and V in the liver of the two studied fishes in the sampling areas. These tables show that concentration of Ni and V in *P. erumei* was more than *E. oreintalis*. These results could be due to the habitat and position of *P. erumei* in the food

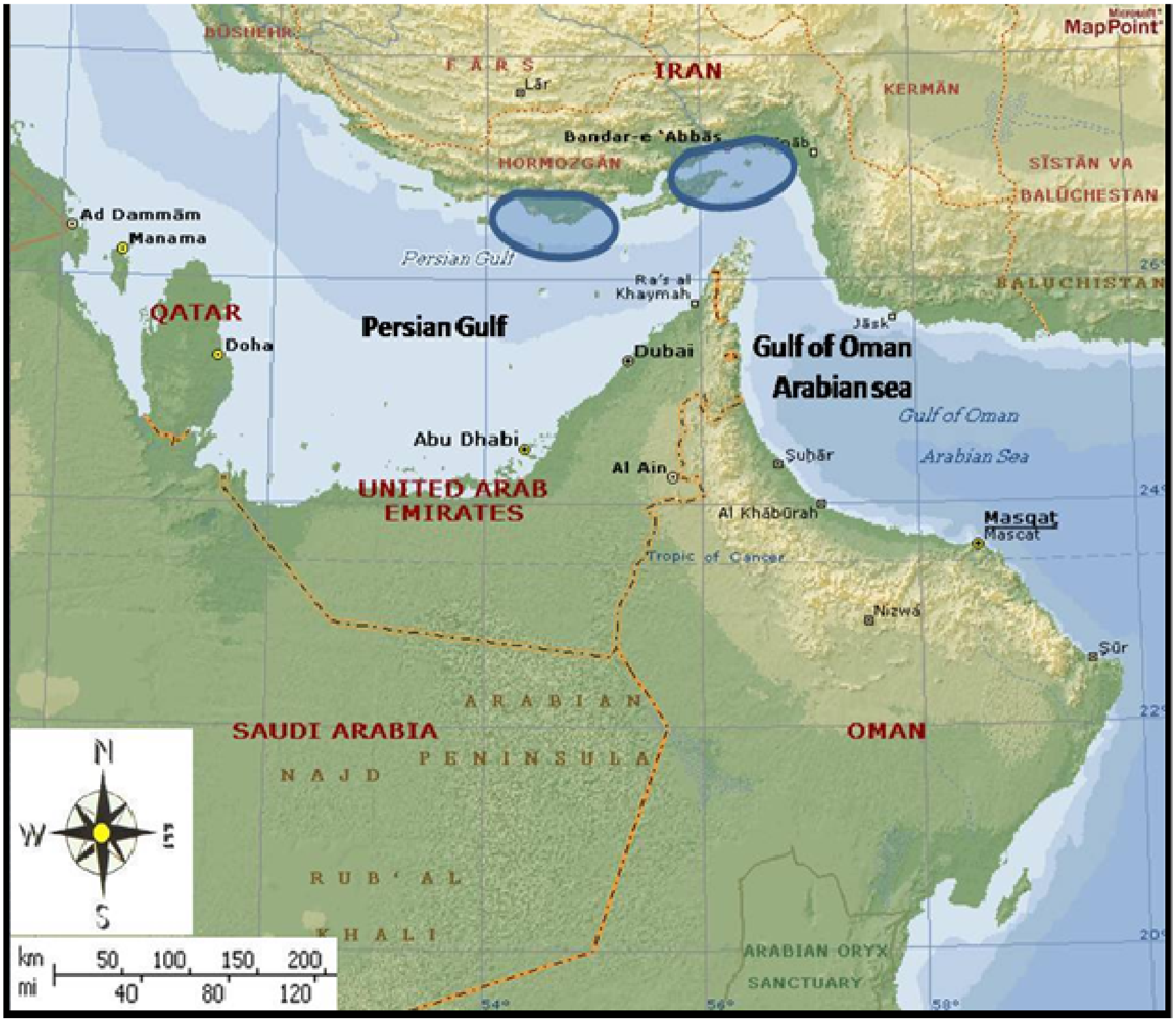

Figure 1. Map showing sampling area.

Table 1. Biometry results for fish samples.

Species	Station	Length		Weight		No. of samples
		Range	Mean	Range	Mean	
E. orientalis	St1	24.5 - 36.5	32.3	240 - 710	585	6
	St2	24 - 41	32.1	209 - 1010	604	6
P. erumei	St1	39 - 58	48.1	754 - 2650	1672	6
	St2	31.5 - 51	43.0	497 - 2286	1309	6

Table 2. Mean and range of concentration of Ni in liver of species (ppm).

Station	Species	Range	Mean	± SD
Bandar Abbass	*P. erumei*	1.02 – 2.20	1.28	0.21
	E. orientalis	1.04 – 1.01	0.89	0.36
Bandar Lengeh	*P. erumei*	1.02 – 2.51	1.12	0.42
	E. orientalis	0.54 – 1.23	0.88	0.28

Table 3. Mean and range of concentration of V in liver of species (ppm).

Station	Species	Range	Mean
Bandar Abbass	*P. erumei*	1.89 – 2.63	2.43
	E. orientalis	1.72 – 2.23	1.89
Bandar Lengeh	*P. erumei*	2.11 – 2.42	2.22
	E. orientalis	1.07 – 1.94	1.54

Table 4. Correlation between length/weight and metal concentration in *P. erumei* in Bandar Abbass.

Metals in tissue	z	Ni
Length	0.927	0.939
Pierson correlation index Sig (2-tailed)	0.001	0.005
Weight	0.960	0.917
Pierson correlation index Sig (2-tailed)	0.002	0.010

Table 5. Correlation between length/weight and metal concentration in *P. erumei* in Bandar Lengeh.

Metals in tissue	V	Ni
Length	0.988	0.968
Pierson correlation index Sig (2-tailed)	0.000	0.002
Weight	0.996	0.988
Pierson correlation index Sig (2-tailed)	0.000	0.000

Table 6. Correlation between length/weight and metal concentration in *E. orientalis* in Bandar Abbass.

Metals in tissue	V	Ni
Length	0.993	0.967
Pierson correlation index Sig (2-tailed)	0.000	0.002
Weight	0.996	0.960
Pierson correlation index Sig (2-tailed)	0.000	0.002

Table 7. Correlation between length/weight and metal concentration in *E. orientalis* in Bandar Lengeh.

Metals in tissue	V	Ni
Length	0.952	0.941
Pierson correlation index Sig (2-tailed)	0.003	0.005
Weight	0.996	0.945
Pierson correlation index Sig (2-tailed)	0.000	0.004

chain and/or pyramid. *P. erumei* is a predator and feeds on small fishes. Tables 4 to 7 show correlation between length/weight and metal concentration in *P. erumei* at the two stations. Figures 2 and 3 compares the concentrations of V in the two flat fishes in the two sampling areas.

Histopathology

Figures 4A and B showed histopathological alterations in the liver of *P. erumei* in Bandar Abbass and Bandar Lengeh, respectively. These alterations include degeneration

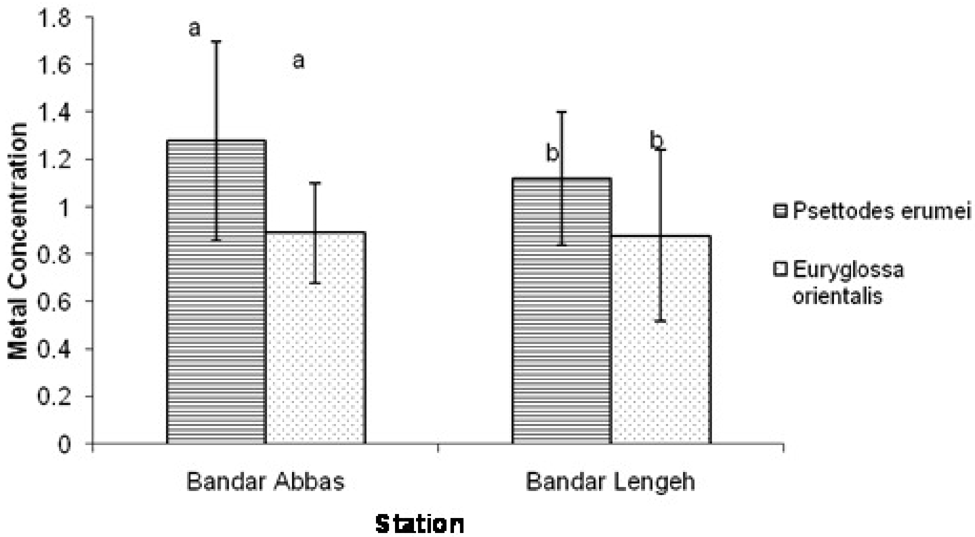

Figure 2. Concentration of V in two flat fishes (*E. orientalis* and *P. erumei*).

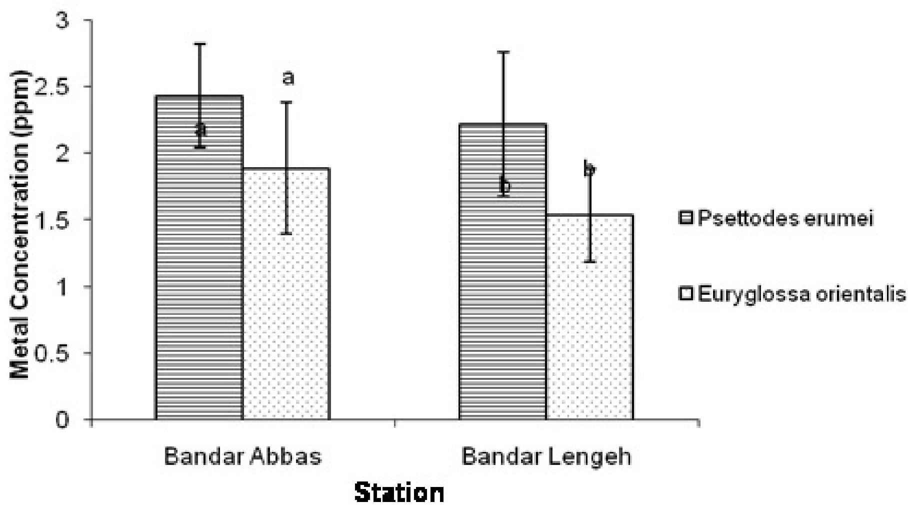

Figure 3. Concentration of Ni in two flat fishes (*E. orientalis* and *P. erumei*).

and necrosis of hepatocytes and tissue disruption and hemorrhagia. Figures 5A and B showed histopathological alterations in the liver of *E. orientalis* in Bandar abbas and Bandar lengeh; cellular alteration were same as the *P. erumei* but more severe. Cellular alterations were severe in Bandar abbass than in Bandar lengeh.

DISCUSSION

Results of the concentrations of Ni and V in the two studied species *P. erumei* and *E. orientalis* with same living conditions (benthic) showed that fishing grounds of Bandar Abbass were more polluted than Bandar lengeh's fishing grounds. Since Ni and V were indicators of oil pollution, it shows that transportation of ships and freighters, especially those carrying oil and fuels were the major sources of pollution in the area. Also, results showed that the concentration of these two heavy metals was significantly related with length and weight of the fishes; this result confirms the bioaccumulation of heavy metals in organisms.

Heavy metals concentration between the two studied species (*P. erumei* and *E. orientalis*) in the two sampling

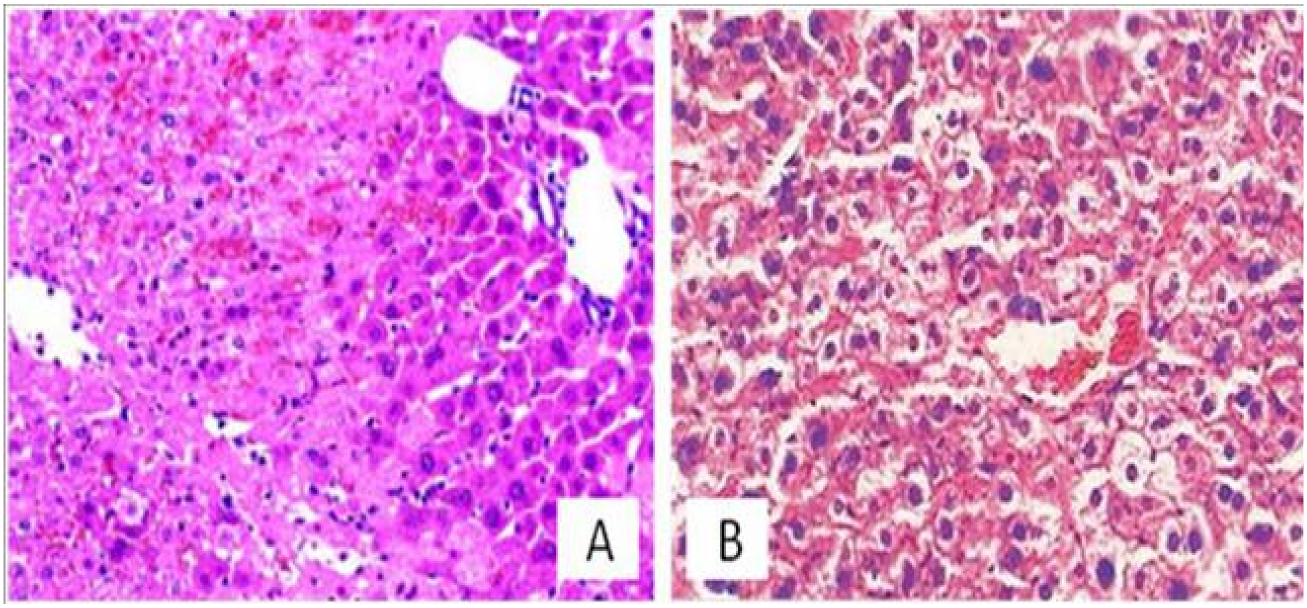

Figure 4. Liver histopathology of *P. erumei* in Bandar Abbass (A) and Bandar Lengeh (B). Hemorrhagia, cell necrosis and degeneration are the most histopathological alterations observed in the liver of *P. erumei* which is like the alterations that are observed in *E. orientalis* but more milder.

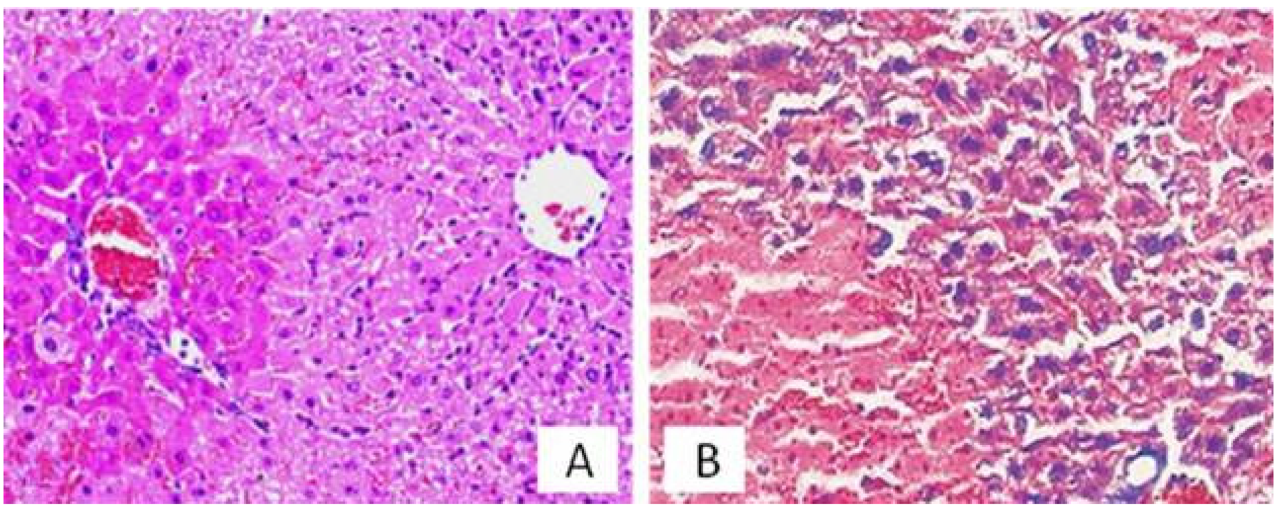

Figure 5. Liver histopathology of *E. orientalis* in Bandar Abbass (A) and Bandar Lengeh (B). Hemorrhagia, cell necrosis and degeneration are the most histopathological alterations observed in the liver of *E. orientalis.* Hepatocytes alterations were severe in Bandar Abbass than Bandar Lengeh.

area (Bandar abbass and Bandar lengeh) showed no significant difference ($p < 0.05$), which may be as a result of the same living conditions for both species.

Vanadium and nickel are present as porphyrins in crude oils. Data concerning V in seafood are scarce, but bioaccumulation of V in mollusks has been reported (Edel and Sabbioni, 1993). Ni can cause toxicity if its levels exceed the regulated values in foods. The WHO recommends 100 – 300 µg of Ni for daily intake. The lowest and highest V contents were found as 0.82 µg/g in hake and 5.14 µg/g in cockle. For Ni, the minimum content is found in mussel (2.94 µg/g) and the maximum in cockle (46 µg/g) (WHO, 1992).

The biological and ecological responses to certain pollutants (organic and inorganic) may vary from changes at the population/community level, organ/tissue, and even at the molecular level (Bailey et al., 1996). Since histological and histopathological changes produced by pollutants in organs and tissues can occur before they produce irreversible effects on the biota, histological methods can be used in conjunction with other parameters and/or ecotoxicological bioindicators as an early warning system for the survival of the species, as well as for environmental protection.

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