The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River

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Abstract

The Kor River (Fars province) was divided into three parts and water and fish specimens were collected monthly and the concentrations of four heavy metals were determined using the Induction Coupled Plasma Method. As middle sampling zone was the main entrance and discharges of industries into the River, the concentrations of heavy metals in tissue of fish from this zone were significantly higher ($p < 0.05$) than those from the other two sampling zones, whereas no significant differences ($p > 0.05$) were detected between the two sexes and species. Maximum concentrations of lead, mercury and cadmium in fish tissues were higher than the permissible levels for human consumption. Significant changes in the concentrations of steroid hormones were also noticed between the middle and two other sampling zones. Also in middle sampling zone, pathological changes in blood cells, liver, and kidneys of fishes were significantly higher. So heavy metals exposure can effectively decrease estrogenic and androgenic secretion in fish. The results confirmed exposure of fishes to heavy metals not only disrupts reproductive hormones' secretion, but also induces some pathological changes.

Keywords: Exposure, Heavy metals, Hormone, Pathology, Fish, Reproductive system, Kor River

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Introduction

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial wastes, geochemical structure, agricultural and mining activities (Singh, R. K. et al., 2006; Sprocati et al., 2006). All these sources of pollution affect the physiochemical characteristics of the water, sediment and biological components, and thus the quality and quantity of fish stocks (Al-Rawi, 2005; Mantovi et al., 2005; Singh, R. K. et al., 2006). Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein. Heavy metals like copper, iron and zinc are essential for fish metabolism while some others such as mercury, cadmium, arsenic and lead have no known role in biological systems (Sallam Kh et al., 1999; Schmitt et al., 2005; Has-Schon et al., 2007). Studies from the field and laboratory experiments showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period; although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation (Jeffree et al., 2006a; Quan et al., 2006; Singh, R. K. et al., 2006; Has-Schon et al., 2007). Ecological needs, size and age of individuals, their life cycle and life history, feeding habits and the season of capture were also found to affect experimental results from the tissues (Kime et al., 1996; Rurangwa et al., 1998).The obvious sign of highly polluted water, dead fish, is readily apparent, but the sublethal pollution might result only in unhealthy fish. Very low-levels of pollution may have no apparent impact on the fish itself, which would show no obvious signs of illness, but it may decrease the fecundity of fish populations, leading to a long-term decline and eventual extinction of this important natural resource (Krishnani et al., 2003; Burger and Gochfeld, 2005). Such low-level pollution could have an impact on reproduction, either indirectly via accumulation in the reproductive organs, or directly on the free gametes (sperm or ovum) which are released into the water. Control of reproduction in fish is complex and regulated by a wide range of factors and low-level pollution could affect any part of this pathway. Steroid hormones are very important and play essential roles in maintaining reproductive functions (Kime et al., 1996; Rurangwa et al., 1998). The Kor River is one of the longest rivers of northwest Fars province, which originates from Zagros Mountains and joins Sivand River near the city of Marvdasht and finally ends in Bakhtegan Lake. Doroudzan-Dam was built at the starting point of the river and many agricultural lands are being irrigated by this river and many big industries are operating at its vicinity. Increase in the number of industries and factories around the river have increased the potential pollution of the river. Shiraz refinery, Shiraz petrochemical complex, Fars leather, Fars dairy factories, Ab-Barik industrial zone, Sina chemical factory, Fars chemical factory, Rishmak factory plus agricultural runoffs and urban and rural sewages are major polluting sources of the Kor River. The present study has been undertaken to determine the effects of some heavy metals' contamination on reproductive systems of two major fish species from the Kor River.

Materials and methods

Doroudzan Reservoir, Band-e-Amir village, and Korak village were three sampling sites along the Kor River; they represent the upper, middle and lower reaches, respectively. The control site (upper zone) was chosen because no agricultural and industrial activities exist behind the Doroudzan Dam. Sewage and wastes from the main industries enter the river at the middle zone, and the lower zone was at the junction where the Kor River enters Bakhtegan Lake. Water samples were taken in one month intervals from April 2005 to January 2006 using 250 ml bottles (precleaned with polyethylene and acidified with 5 ml of 1M sulfuric acid, washed at least 10 times with distilled water and once with double distilled water) and stored in a -20°C freezer. Fish samples: A total of 225 adult fish specimens (75 samples from each zone, roughly about 20 samples collected monthly) of each of the two species, *Cyprinus carpio* and Capoeta sp. were caught with a cast net by professional fishermen and transferred alive in a cooled ice box (4°C) to the laboratory of the Physiology Department, School of Veterinary Medicine, Shiraz University, Iran. Various attributes, species, weight (three different weight groups, 7.5–20 g, 20.1–100 g, and 100.1–600 g), length, and sex of each fish were recorded. Blood samples were taken and centrifuged, and serum was extracted and kept in a -20°C freezer, before killing fish. Steroid hormones (estradiol, progesterone, and testosterone) in adult female and male fish were measured following previously described methods (Ebrahimi, 2004). 500 mg of muscle, liver, kidney, gonad, and brain tissues from each fish were dissolved in HCl (98%) and nitric acid (65%) solutions (3:7) and left for 16 hours in a water bath (100 °C); then deionized water was added to bring the volume up to 1ml, and the samples were kept in the freezer until the heavy metal assay. Ten microliters of each sample defrosted at room temperature and then Mercury (Hg), Arsenic (As), Cadmium (Cd) and Lead (Pb) were assayed by Induction Coupled Plasma (ICP-OES) method, which makes it possible to assay many heavy metals at the same time with small amounts of samples. About 200 mg of liver and kidney from each fish specimen were fixed in 10% formaline solution for histological analysis; samples were prepared in blocks of paraffin, sliced in 5 μm sections, placed on slides, and stained so that pathological changes in the tissues could be examined under a light microscope.

SPSS 13 for Windows software was used for the statistical analysis of those four heavy metal contents in different fish tissues and different sampling sites by using multivariate ANOVA (95% significancy level).

Results

In fish samples, 59% of both species were male and 41% were female, with 77 grams (7.5–600 g) and 15 cm (6–58 cm) of weight and length averages, respectively. Cd, Pb, Hg, and As average concentrations in water in the upper zone were 0.44±0.14, 2.83±0.8, 0.006±0.001, and 0.8±0.17 mg/L, respectively; in the middle zone 4.51 ±0.94, 7.49±1.85, 0.036±0.012, and 1.8±0.19 mg/L, and in the lower zone 1.73±0.48, 4.68 ±0.92, 0.011±0.001, and 1.1±0.76 mg/l. The quantity of heavy metals in both fish and water samples was significantly greater (P < 0.05) at the middle sampling zone (Band-e-Amir) compared to the two other areas; however, no significant differences were observed between male and female fishes (P > 0.05) (Table 1).In female fish from the upper sampling zone, the concentration of estradiol (13.4 ± 2.13, mean ± SE) was significantly (P < 0.05) higher than the concentrations from the other sampling zones and the concentration of the same hormone was significantly (P < 0.05) higher in lower sampling zone (5.72 ± 1.93, mean ± SE) than middle sampling zone (1.93 ± 0.65, mean ± SE) and no significant differences (P > 0.05) was found in male and female fishes from the middle sampling zone. Progesterone concentration in male fishes from middle sampling zone (0.77 ± 0.015, mean ± SE) was significantly (P < 0.05) different from the other two zones and the same differences were noticable between female and male fish from the upper sampling zone, but no differences were found between sexes in the middle and lower sampling zone. The concentration of testosterone in male fish was significantly higher in fishes from the upper sampling zone (1.74 ± 0.32, mean ± SE) comparing to the other sampling zones.

Table 1: Pb, Hg, Cd and As concentrations assayed in three sampling sites (Doroudzan-Dam, Band-e-Amir and Korbal Village) of two species; Copeta and *Cyprinous carpio*, male and female. Data shown as Mean ± SE of each hormone

	Copoeta				*Cyprinus carpio*			
	Lead concentration							
Sampling site	Female		Male		Female		Male	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Doroudzan- Dam	0.38	0.16	0.28	0.11	0.47	0.27	0.32	0.22
Band-e-Amir	0.59	0.18	0.40	0.12	1.24	0.22	1.16	0.11
Korbal Village	0.25	0.10	0.56	0.20	0.29	0.10	0.82	0.25
Mercury concentration								
Sampling site	Female		Male		Female		Male	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Doroudzan- Dam	0.08	0.04	0.11	0.05	0.15	0.10	0.32	0.07
Band-e-Amir	0.67	0.63	0.41	0.30	1.03	0.62	1.92	1.09
Korbal Village	0.22	0.23	0.74	1.01	0.29	0.62	0.84	0.80
Cadmium concentration								
	Copoeta				Common Carp			
Sampling site	Female		Male		Female		Male	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Doroudzan- Dam	0.11	0.00	0.11	0.00	0.11	0.01	0.11	0.01
Band-e-Amir	0.06	0.03	0.03	0.00	0.05	0.02	0.03	0.02
Korbal Village	0.61	0.03	0.11	0.01	0.55	0.02	0.11	0.00
Arsenic concentration								
	Copoeta				Common Carp			
Sampling site	Female		Male		Female		Male	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Doroudzan- Dam	0.20	0.02	0.29	0.05	0.20	0.05	0.12	0.07
Band-e-Amir	0.61	0.20	0.24	0.08	0.68	0.20	1.25	0.09
Korbal Village	0.47	0.18	0.56	0.20	0.28	0.12	0.17	0.03

No significant difference was detected in concentration of testosterone among female fish from the three sampling zones while significant difference ($P < 0.05$) of testosterone concentration was observed between male and female sexes from the lower sampling zone (Table 2). Pathological changes such as vacuolar degeneration, biliary canaliculi dilation, hemosiderosis, prevascular edema, and melanomacrophage hyperplasia were detected in 70% of samples from the middle sampling zone; and in just 20% of samples from the lower sampling zone, vascular degeneration and edema were found in liver tissues. Cell swelling, hyperemia, edema, urinary tubular vacuolar degeneration, hemorrhage, and sedimentation of oxalate crystals and hyaline casts were found in kidneys of 80% and 70% of fish samples from the middle and the lower sampling zones. No pathological lesions were found in samples from the upper sampling zone. In 20% of samples from lower sampling zone, hyperemia, cellular degeneration and vacuolation were noticed in livers and the same pathological changes plus deposition of larger amounts of hemosiderin in Kupfer cells, vascular edema (Fig. 1) and increase in melanomacrophage (Figure 2) were observed in 70% of samples from the middle sampling zone. In all renal samples from the middle and in just 70% of renal samples from the lower sampling zones, hyperemia, vascular edema, hyaline casts, hemosiderosis, cellular degeneration and cellular vacuolization were observed (Fig. 3); while no pathological changes were observed in kidneys of fishes from the upper sampling zone. Muscular degeneration in 10% and 20% of samples from the middle and the lower sampling zones were found but there were no pathological changes in muscle tissues of fish samples from the upper sampling zone found (Fig. 4).

Table 2: Concentrations of three steroid hormones (estradiol, progesterone and testosterone) in three sampling sites (Doroudzan-Dam, Band-e-Amir and Korbal Village) of male and female fish.

	Estradiol *(Sampling sites)*					
	Doroudzan-Dam		Band-e-Amir		Korbal Village	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Male	7.23	0.91	6.38	0.82	6.98	0.93
Female	13.4	2.13	1.93	0.65	5.72	1.93
	Progesterone*(Sampling sites)*					
	Doroudzan-Dam		Band-e-Amir		Korbal Village	
	Mean	SE	Mean	SE	Mean	SE
Male	0.09	0.01	0.77	0.015	0.25	0.05
Female	0.26	0.86	0.45	0.085	0.22	0.13
	Testosterone *(Sampling sites)*					
	Doroudzan-Dam		Band-e-Amir		Korbal Village	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Male	1.74	0.32	0.44	0.22	0.91	0.15
Female	0.54	0.15	0.63	0.13	0.56	0.31

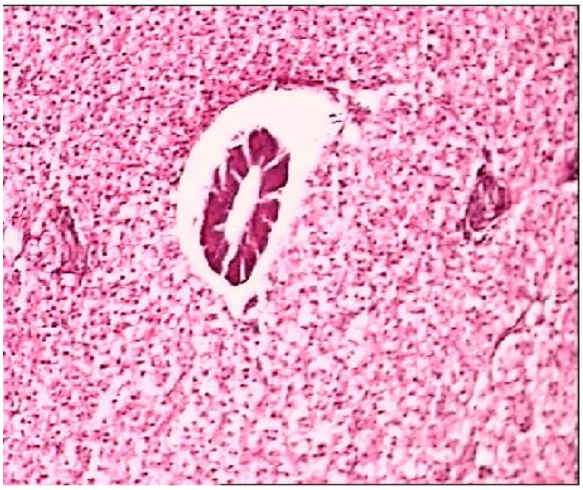

Figure 1: Histological section of liver with vacuolar degeneration and lipid infiltration in samples from middle sampling zone (H&E, x160)

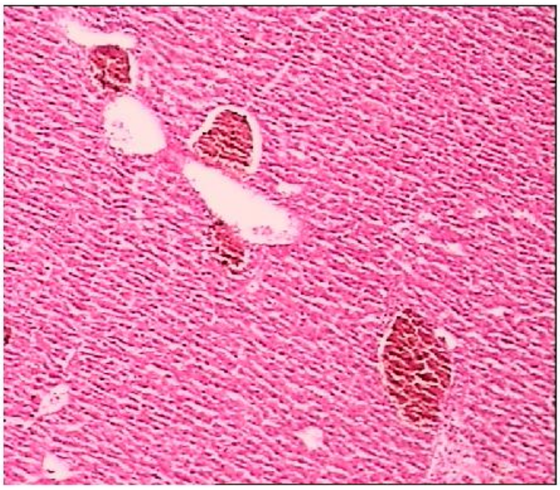

Figure 2: Increase in melanomacrophage centers in liver parenchyma of fish samples from middle sampling zone (H & E, x 90)

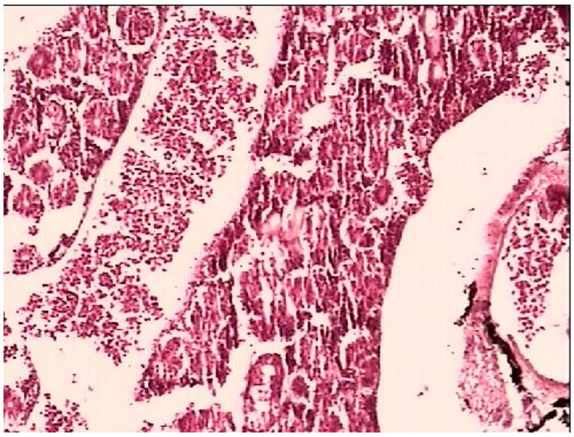

Figure 3: Cellular lesions and vascular hemorrhage in kidney of fish sample from middle sampling zone (H& E, x160)

Figure 4: Cross section of muscles of fish samples from middle sampling zone showing muscular degeneration (H& E, x160)

Discussion

During the last two decades, per capita consumption of fish in Iran has increased sharply from 2 to 7 kg and the demand for fish as a source of protein is on constant rise, especially in some inland areas that are far away from the Caspian Sea and Persian Gulf. In some inland areas, such as Kamfirouz and Korbal (along the Kor River), utilizing stock of freshwater fishes from the rivers has become a full-time job for newly emerging fishermen. Therefore, it is vital to make sure that consumers are using healthy fishes. Kor River has played a vital role in development of agro-industrial sections in Fars province and now is serving as a potential source of fish food in the area with fingerling stockings of the River.

Bioaccumulation of metals occurs when organisms incorporate and retain them from the surrounding environment (Jeffree et al., 2006a), which includes water, sediments, suspended solids, and prey organisms. If incorporation of the chemical outpaces metabolism or excretion of the chemical, then bioaccumulation occurs (Dural et al., 2006). Therefore, tissue analysis can reveal the presence of contaminants that may not be detected otherwise (Dural et al., 2006). While long-term exposure of fish to low-level pollutants might not show any obvious or visible effect on the fish itself, it could exert deleterious effects on the reproductive organs leading to a decline in numbers of offspring and hence to eventual extinction of fish stocks (Kime, 1995). Pituitary damage, testicular degeneration and decrease in fry numbers after heavy metals exposure have already been reported due to exposure to heavy metals (Fernicola et al., 1985; Popek et al., 2006). The concentrations of different heavy metals in five tissues of two cyprinid species collected from the upper, middle, and lower parts of the Kor River, Iran, were determined using the ICP method. The goal was to determine whether or not these fish are suitable for human consumption.

In both fish and water samples the concentrations of heavy metals from middle sampling zone were significantly higher than the concentrations at the two other sites. These findings are consistent with the geographical distribution of the polluting industries, as they are mainly based around the middle sampling zone. Fars petrochemical complex is one of the main pollutant industries in this zone, dealing with heavy metals and discharging its wastes (and sometimes untreated wastes) into the river. Interestingly, no significant differences ($P>0.05$) were observed in the contamination of heavy metals of Doroudzan reservoir (the upper sampling zone) and Korbal village (the lower sampling zone); as it was expected to detect more heavy metals at the Korbal sampling point, because it receives all polluted water from the middle sampling zone before it enters Bakhtegan Lake. The recycling of heavy metals in the river and their deposition into the sediments could be the main cause of sharp decline of heavy metals in water and fish samples from the lower sampling zone (Johnson et al., 2005; Tipping et al., 2006). Although heavy metals pollution in water samples followed the same patterns as fish samples, the concentrations of Pb, Cd and As were much higher in water than fish samples; while Hg concentration was higher in fish samples which could be due to active absorption and accumulation of this metal in fish tissues.

The concentration of heavy metals showed no significant differences between the two sexes or between the two fish species from the different sampling sites, which could be due to a similar degree of accumulation in both sexes and species, as their feeding habits and habitats are similar (Jeffree et al., 2006b; Singh, R. K. et al., 2006). Residues of heavy metals in fish tissues captured from control or upper sampling zone with no industrial or agricultural activity in this area, could be due to the deposition of heavy metals from the atmosphere and polluted air from nearby large cities (Ettler et al., 2005).

Maximum value for Pb in fish muscles measured in this study was higher than 2 mg/kg maximum allowance set by the European Union (Biggeri et al., 2006) and the mean concentrations of this metal in water samples taken from upper, middle and lower sampling zones were lower than the lethal concentrations of Pb in water for cyprinids (100 mg/liter), although low levels of Pb pollution could cause some adverse effects on fish health and reproduction (Delistraty and Stone, 2007). The highest concentrations of Hg and Cd in fish tissues from the Kor River were higher than the maximum allowed concentrations (MACs) of Hg and Cd in fish meat according to the standards stipulated by the Environmental Protection Agency (EPA) of the United States [0.2 and 1 ppm (mg/kg), respectively] (Cech et al., 2006; Sun et al., 2006); while the concentration of As was lower than the maximum allowed (5 mg/kg). Because the concentrations of Hg, Pb, and Cd in edible muscles exceeded the maximum levels allowed, these fish were unsuitable for human consumption (Ettler et al., 2005).

As mentioned earlier (see Introduction), heavy metals may insert their deleterious effects on fish reproduction and gamete development via disruption of the endocrine system and the inhibition of hormone production, such as disruption of hypothalamic-pituitary system (Kime, 1995). Significant decrease in plasma estrogen of Atlantic croaker has been reported after exposing fish to lead (Thomas, P. and Trant, 1989; Thomas, P., 1990), or after exposing Asian swamp eel (Monopterus albus) to cadmium (Saxena et al., 1989). On the contrary, in another study it has also been shown that cadmium can increase transcription of the progesterone receptor but decrease the estrogen receptor gene in humans (Garcia Morales et al., 1994). Depression of plasma androgens in females by lead (Thomas, G. M. et al., 1989) or cadmium exposure (Singh, H., 1989) have been reported. In another study, we showed exposure to various heavy metals decrease the fecundity of fish populations, either indirectly via accumulation in the reproductive organs or directly by acting on sperm and ova (Rurangwa et al., 1998). So it would be reasonable to suggest that increase in heavy metal exposure can disrupt the balances of sex hormones in fishes from the Kor River, as confirmed again here. In heavy polluted sampling zone (middle site), the concentration of estradiol in female fish was significantly lower than at the other two sampling sites; showing the direct effect of heavy metal contamination on steroidogenesis in female fish, either due to the harmful effects of metals on either the hypothalamus–pituitary axis (Kime, 1995; Kime et al., 2001; Song et al., 2002) or on the germinal cells capacity of estradiol production (Kime, 1995; Drevnick and Sandheinrich, 2003; Webb et al., 2006; Hinck et al., 2007). The same disruptive effect was noticed in male fish; the concentration of testosterone hormone in control sampling zone (upper site) was higher than the two other areas.

More pathological lesions were found in samples from the middle sampling zone, which is in line with the ICP and hormonal findings. The lesions confirmed that exposure to heavy metals can induce histological and pathological changes, as already mentioned in other studies (Damek-Poprawa and Sawicka-Kapusta, 2003; Zhang et al., 2005; Martin-Diaz et al., 2006; Raldua et al., 2007). The same type of damage has been reported in liver and kidneys of fish due to subchronic exposure to Hg (Dua and Gupta, 2005; Devlin, 2006; Raldua et al., 2007), Pb (Damek-Poprawa and Sawicka-Kapusta, 2003; Liu, 2003; Chetty et al., 2005; Goswami et al., 2005), As (Graeme and Pollack, 1998; de Burbure et al., 2006), and Cd (Buchet et al., 1980; Barregard et al., 1999; Damek-Poprawa and Sawicka-Kapusta, 2003).

In conclusion, this study showed that heavy metal contamination not only directly affects fish health, but it can also disrupt the normal steroidogenesis pattern in fish, leading to impaired hormone production in both male and female fish, and decrease the quality and quantity of sperm and ova production. Extinction of the native fish stocks of the Kor River in the near future would not be surprising unless urgent protective actions are taken to stop river pollution and boost the stock by artificial propagation. This study also showed that the industrial activities around the Kor River (especially from the midpoint downward) have already polluted the river, and Pb, Hg, and Cd concentrations in the Kor River were higher than TSE-266, WHO, EPA, and EC-1998 standards (Enrique et al., 2002; Munoz et al., 2005).

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