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Short Communication

Histopathological studies of fish liver as a biomarker of heavy metal pollution in internationally important Harike wetland, India

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ABSTRACT

The histostructure of the liver of fish from Harike wetland was studied in order to detect the effects of heavy metal pollution. Fish samples (n = 10) were collected from two sampling sites i.e. Harike wetland and the river Beas during the breeding season. The river Beas merges with the river Sutlej at Harike wetland and carries pure water as compared to the water of the river Sutlej. This river receives pollutants from its tributaries namely Budha Nallah and East Bein which ultimately pollute the internationally important Harike wetland and resultantly cause morphological alterations in the liver of fish from Harike wetland as compared to that from the river Beas. Fish liver histopathology is a good bioindicator and can be used for the detection of chemical pollution in fish. During this study, the effect of heavy metals present in the Harike wetland i.e. Cadmium (Cd), Zinc (Zn), Chromium (Cr), Lead (Pb), Nickel (Ni) and Copper (Cu) was investigated with the aim of determining the significant histological changes in the liver of the Indian major carp, *Labeo rohita*. The histological changes included hyalinization, vacuolation, cellular swelling and congestion of blood vessels. It can therefore be concluded that heavy metal exposure resulted in histological alterations in the liver of fish. Hence, the liver of *Labeo rohita* can be used as a pollution biomarker.

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KEYWORDS: wetland, heavy metals, histology, bioindicator, pollution

INTRODUCTION

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades [1-3]. The natural aquatic ecosystem may extensively be contaminated with heavy metals released from domestic, mining, agricultural, fuel combustion, industrial and other man-made activities [4-9]. Heavy metal contamination may have devastating effect on the ecological balance of the recipient environment and the diversity of aquatic organisms [10-12]. It appears that the problems of heavy metal accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to the biomagnification potential of toxic metals in human food chain [13-16]. Accumulated heavy metals may lead to morphological alterations in the tissues of fish [17]. Heavy metal pollutants also bring about structural alterations in scales [18, 19], chromatophores [20] and ovary [21] of the fish as revealed in the earlier studies.

Histopathology deals with the study of pathological changes induced in the microscopic structure of the body tissue. Any peculiar type of alteration of cells or disease may indicate the effect of toxic substances. Thus study of histopathology is of prime importance in the diagnosis, etiology and prevention of diseases. In fishes, it is observed that the external organs are affected due to toxic chemicals, causing loss of equilibrium, increase in opercular movements, to and fro irregular vertical movements and finally leading to death. Histopathological study thus gives us useful data regarding significant damage to the internal organs prior to external manifestation.

MATERIALS AND METHODS

Water sampling

Water samples were collected during March 2014 to February 2015 at different seasons of the year from Harike wetland and the river Beas. The surface water samples were thoroughly filtered through a cellulose nitrate filter paper to eliminate suspended solids and stored in plastic bottles with one liter capacity. 1 ml of concentrated nitric acid was added to it for preservation. Heavy metal analysis was done using the atomic absorption spectrophotometer (AAS).

Histopathological analysis

For studying the histopathological changes, samples of the fish species *Labeo rohita* were collected from Harike wetland and the river Beas during the breeding season of the fish. The fish samples were dissected on the spot and the liver tissue was removed immediately and fixed in aqueous Bouin's fluid for 24 hours. This tissue was dehydrated in different grades of alcohol and blocks were prepared in paraffin wax. Sections of 4 to 6 µm were cut and stained with Haematoxylin and Eosin (H & E) and mounted in DPX (mixture of distyrene, plasticizer and xylene). They were examined and photographed under a light microscope unit.

RESULTS

Heavy metal analysis

Heavy metal pollution of aquatic ecosystems is a serious and widespread environmental problem due their persistent discharge from various sources. They are causing serious health hazards in human beings and posing a threat to the valuable biodiversity due to their non biodegradable and bioaccumulation properties. The mean values of heavy metal concentration detected in the water samples from Harike wetland and river Beas are presented in table 1 and are compared to the WHO maximum permissible limits. Most of the heavy metals in the water samples from Harike wetland were above permissible limits while heavy metals were not detected in the water samples from the river Beas, but a few trace elements were observed which were also below the permissible limits. The heavy metals detected in the water samples from Harike wetland were Copper, Chromium, Nickel, Iron, Cadmium, Lead, Zinc, Manganese and Cobalt. On the other hand, trace elements present in the water samples of the river Beas were Copper, Iron, Zinc and Nickel.

Histopathological observations

Metabolism of food items, their storage and detoxification are the important functions of the liver. Toxic substances, if reaching in limited quantities, are detoxified by the liver, but high load causes damage to the liver. Hence liver is susceptible to a number of toxic substances which cause metabolic disturbances. The liver of fish from river Beas that are not exposed to heavy metals showed

Table 1. Heavy metal concentration (mg/l) in water samples from Harike wetland and Beas river.

Site	Season	Copper (Cu)	Iron (Fe)	Lead (Pb)	Cadmium (Cd)	Zinc (Zn)	Nickel (Ni)	Chromium (Cr)	Manganese (Mn)	Cobalt (Co)
Harike wetland	Summer	0.535	1.995	0.783	0.074	1.907	0.072	0.032	0.086	-
	Monsoon	0.318	1.057	0.265	0.013	0.892	0.028	-	0.042	0.0076
	Winter	0.798	2.043	0.851	0.098	1.842	0.087	0.173	-	0.0094
River Beas	Summer	0.0044	0.974	-	-	0.860	-	-	-	-
	Monsoon	0.0023	0.237	-	-	0.078	0.0036	-	0.0016	-
	Winter	0.0083	0.829	-	-	0.794	-	0.0012	-	-
WHO Permissible Limits (mg/l)		0.02	1	0.05	0.005	1	0.02	0.05	0.05	-

regularly arranged hepatocytes with central vein and sinusoids as shown in fig. 1 (A to F). The histopathological changes observed in the present investigation in the liver of fish *Labeo rohita* after exposure to heavy metal pollutants are depicted in fig. 2 (A to F). The liver of the fish from Harike wetland exposed to heavy metal compounds exhibited marked histopathological alterations which includes

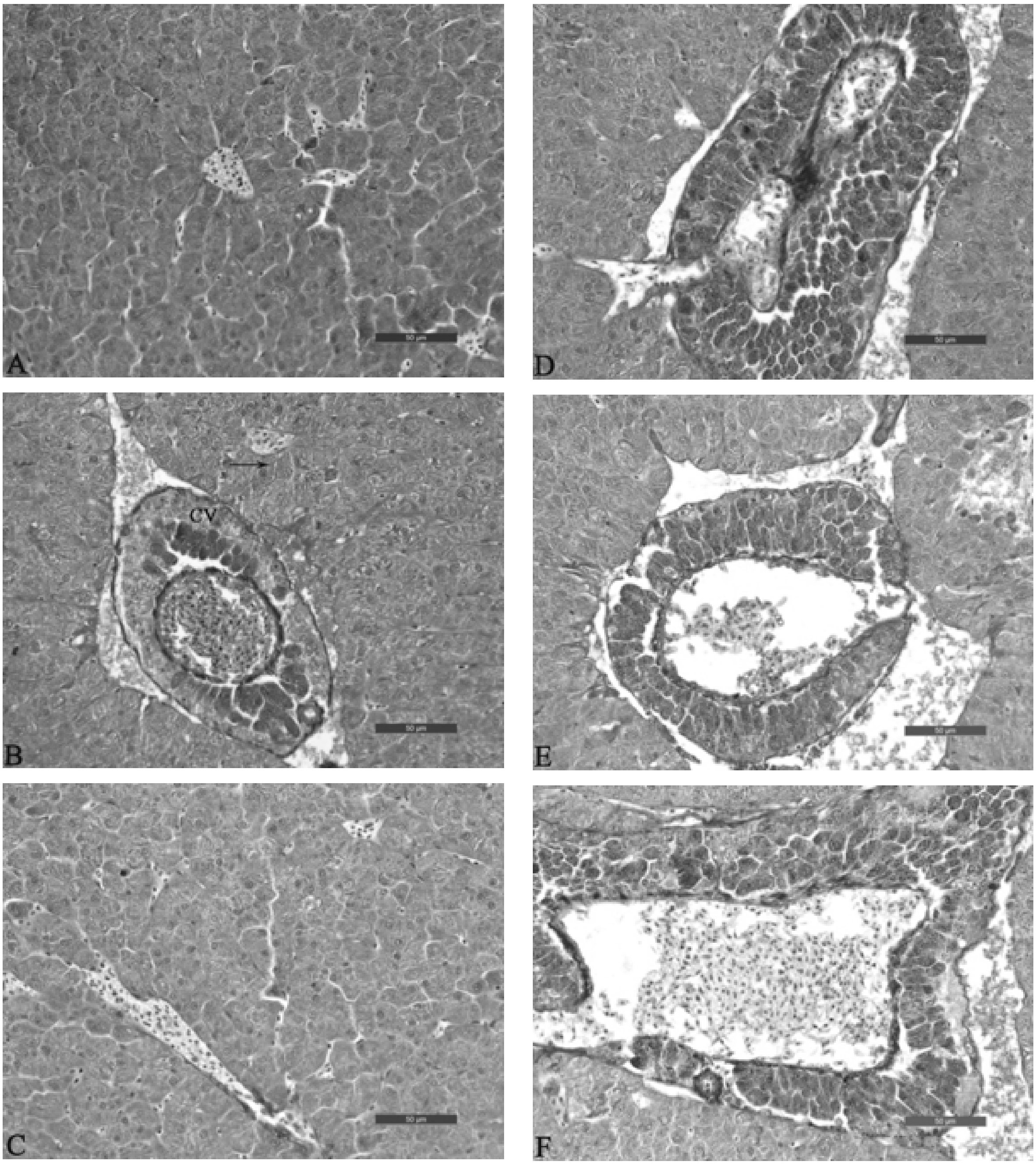

Fig. 1. Histological structure of liver of *Labeo rohita* from river Beas, not exposed to heavy metals. A: Histological appearance of normal cells in the liver tissues; B: Liver showing the hepatocytes arranged around central vein (black arrow); C: Regularly arranged hepatocytes with nucleus; D&E: Section of hepatopancreas with hepatocytes; F: Pancreatic acinus lined with large cells.

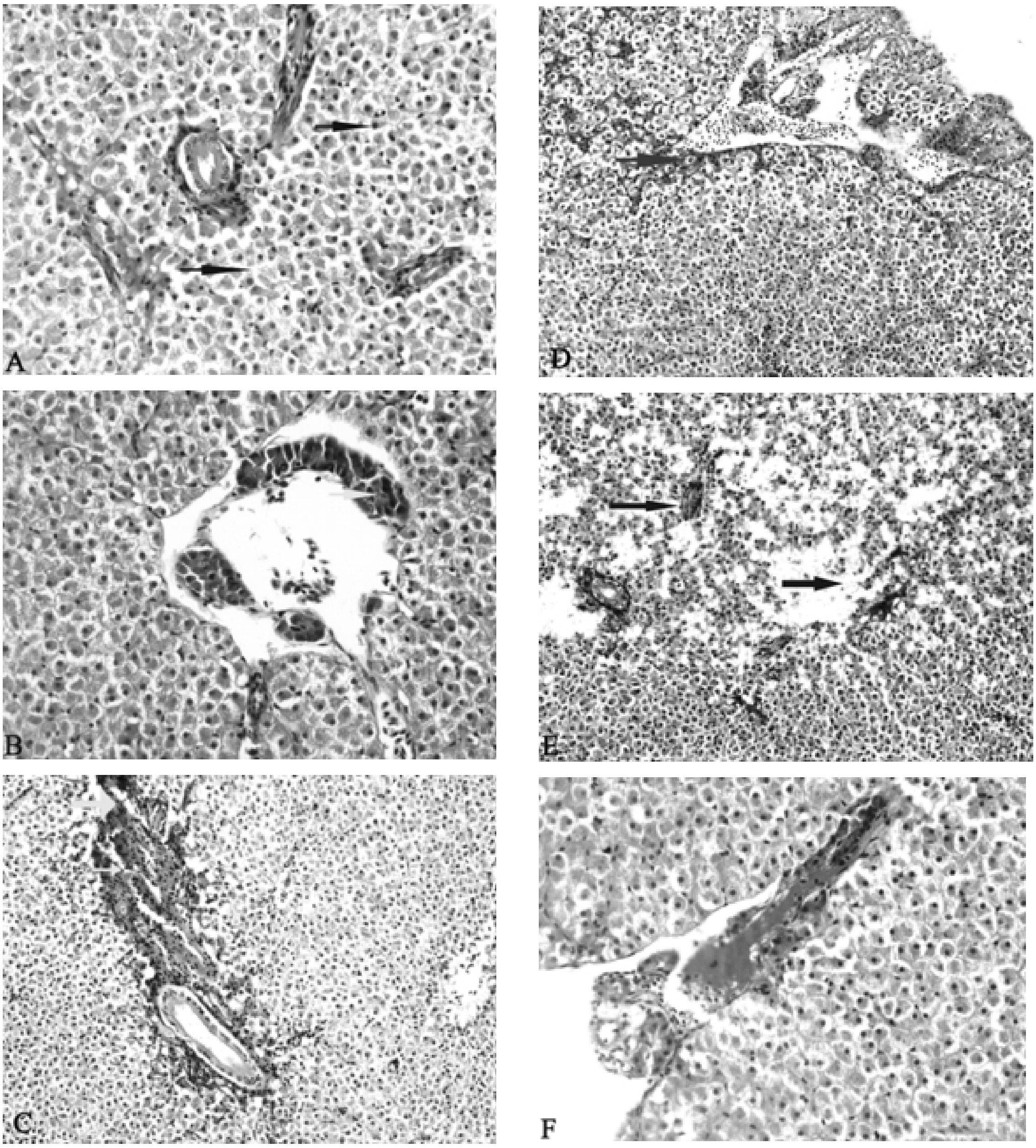

Fig. 2. Histological structure of liver of *Labeo rohita* showing histopathological alterations due to exposure of heavy metals. A: Liver section from a fish, showing the increased vacuolation in hepatocytes (black arrows); B: Liver section shows congestion of central vein and dilated sinusoids, degenerated nuclei and vacuolation (white arrows); C: Erythrocyte infiltration into blood sinusoids and degenerated nuclei (white arrow); D: Sections from fish characterized by erythrocyte infiltration into blood sinusoids (black arrow); E: Increased haemorrhage and vacuolation in hepatocytes (black arrow); F: Infiltration of sinusoids with leukocytes.

vacuolation in the cytoplasm, degeneration of nuclei, vacuolation in stroma, cloudy swellings of the cells with large vacuoles, pycnotic nuclei, prominent necrosis, rupture of blood sinusoids, disorganised hepatic cords and degeneration of hepatocytes.

DISCUSSION

The occurrence of heavy metals in the environment as a result of both natural processes and human activities is posing serious health hazards [22]. These metals in trace amount may play an important role in the biochemical processes of the life of aquatic organisms [23]. However, their high concentration becomes lethal to fish and other aquatic organisms when the duration of exposure to these metals is prolonged [24]. Harike wetland receives heavy metal pollutants from various sources which persist throughout the year. As observed during the present investigation the level of some highly toxic metals is above permissible limits. The high concentration is posing a big threat to the valuable flora and fauna existing there. Similar observation has been reported [25] in water samples from Lake Uluabat where Zn and Cu concentrations were significantly higher due to the industrial and domestic discharge. The same observations have been found in inland waters of Hong Kong [26].

More than 80% of the water samples from the Ganga river had Cd and Ni levels above the recommended maximum permissible concentration [27]. Similar results were reported in Ona river [28]. During the present course of observations in the Harike wetland, the concentrations of Cd and Ni were found to be above the maximum permissible concentration recommended by the WHO.

The toxicity effect of heavy metals on liver has been studied by many workers. Liver cord disarray, shrinkage in the liver cells, degenerated nuclei and focal necrosis in *Channa punctatus* due to lead intoxication has been reported [29]. Cytoplasmic vacuolation has been observed in hepatocytes of liver of *Ictaburus nebulous* due to copper pollution [30]. Vacuolation within and outside the hepatocytes, severe necrotic changes in liver, breakdown of cellular boundary, and vacuolation have been recorded in liver of *Puntius conchonius* induced by copper and zinc intoxication [31]. Necrosis, hypertrophy and atrophy in the liver tissues, loss of polygonal shape of liver cells, splitting of the cells and formation of spaces in the tissues after exposure of *Cyprinus carpio* to lethal and sublethal concentration of copper and cadmium have been noticed [32]. Similar effects which predominantly include cellular damage, nuclear hypertrophy of hepatocytes, vacuolation and necrosis leading to lysis, and increase in blood sinuses, bile canliculi and bile pigments have been reported in *Gambusia affinis* exposed to mercury chloride [33]. Hyperplasia, nuclear pycnosis, fatty necrosis, degeneration of hypatocytes leading to tumor and Sycytium formation, blood vessel congestion, oedema, and marked reduction in hypatosomatic index have been reported in *Channa punctatus* exposed to mercurial fungicide [34]. The study of the liver of *Sartherodon mossambicus* observed necrosis, fatty degeneration, red blood cell occlusion in portal vessels, engorged blood vessel congestion, and vacuolar degeneration of hepatocytes [35]. Cellular necrosis, clumping of chromatin and its aggregation at the centre, loss of nuclear membrane of hepatocytes in liver have been observed in fishes after exposure to copper [36-38]. Alterations in hepatic capillaries of liver of *Rutilus rubiliohridanus* and *Barbus meridionalis* from heavy metal-contaminated lakes have been observed [39, 40]. Similar histological alterations in the liver of *Labeo rohita* on exposure to heavy metal pollutants from Harike wetland were found during the present course of work.

CONCLUSION

The present study is an attempt to detect the heavy metal pollution in the water of Harike wetland. If the declining trend of fish population is allowed to continue unabated, it is most likely that the food web complexes in this wetland might be at the highest risk due to heavy metal-induced contamination. Such alarming concentration may also create discomfort to the people living in the vicinity of this wetland. Hence, strict management actions should be taken in order to protect the ecological sustainability of this wetland.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Vutukuru, S. S. 2005, Int. J. Environ. Res. Public Health, 2(3), 456.
2. Dirilgen, N. 2001, Turk. J. Chem., 25(3), 173.
3. Voegborlo, R. B., Methanani, A. M. E. and Abedin, M. Z. 1999, Food Chem., 67(4), 341.

4. Velez, D. and Montoro, R. 1998, J. Food Protection, 61(9), 1240.
5. Conacher, H. B., Page, B. D. and Ryan, J. J. 1993, Addit. Contam., 10(1), 129.
6. Woodling, J. D., Brinkman, S. F. and Horn, B. J. 2001, Arch. Environ. Contam. Toxicol., 40, 381.
7. Patra, R. C., Swarup, D., Naresh, R., Puneet, K. and Shekhar, P. 2005, Bull. Environ. Contam. Toxicol., 76(4), 1092.
8. Swarup, D., Patra, R. C., Naresh, R., Puneet, K., Pallav, S. and Balagangatharathilagar, M. 2006, Small Ruminant Res., 63(3), 309.
9. Sexsena, R. and Garg, P. 2011, GERF Bull. Biosci., 1(1), 1.
10. Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R. 2007, Int. J. Environ. Res. Public Health, 4(2), 158.
11. Vosyliene, M. Z. and Jankaite, A. 2006, Ekologija, 4, 12.
12. Ashraj, W. 2005, Environ. Monit. Assess., 101(1-3), 311.
13. Das, S. and Kaviraj. 2000, Geobios., 27, 69.
14. Laxi, R. 2005, Himalayan J. Environ. Zool., 19.
15. Jayakumar, P. and Paul, V. I. 2006, Veterinarshki Archiv., 76, 167.
16. Kumar, P., Prasad, Y., Patra, A. K. and Swarup, D. 2007, Bull. Environ. Contam. Toxicol., 79, 396.
17. Monteiro, S. M., Mancera, J. M., Fernandes, A. F. and Sousa, M. 2005, Comp. Biochem. Physiol., 141C, 375. doi:10.1016/j.cbpc. 2005.08.002
18. Brraich, O. S. and Jangu, S. 2013, Trends Fish. Res., 2(2), 23.
19. Jangu, S. and Brraich, O. S. 2014, Int. J. Sci. Res., 3, 392.
20. Kaur, R. and Dua, A. 2012, Environ. Monit. Assess., 184, 2729.
21. Brraich, O. S. and Jangu, S. 2015, Int. J. Fish. Aquacul., 7(2), 9.
22. Jordao, C. P., Pereira, M. G., Bellato, C. R., Pereira, J. L. and Matos, A. T. 2002, Environ. Monit. Assess., 79(1), 75.
23. Tay, C. K., Asmah, R. and Biney, C. A. 2009, West African J. Appl. Ecol., 16, 75.
24. Deekay, S. N., Abowei, J. F. N., Alfred Ockiya, J. F. 2010, Res. J. Environ. Earth Sci., 2, 208.
25. Elmaci, A., Teksoy, A., Topac, F. O., Huseyin, N. and Kaya, S. 2007, African J. Biotechnol., 6, 2236.
26. Zhou, J. L., Huang, P. L. and Lin, R. G. 1998, Environ. Pollut., 101, 67.
27. Panday, J., Shubhashish, K. and Richa, P. 2010, Trop. Ecol., 51, 365.
28. Adefemi, S. O. and Awokunmi, E. E. 2010, African J. Environ. Sci. Technol., 4(3), 145.
29. Sastry, K. V and Gupta, R. K. 1978, Bull. Environ. Contam. Toxicol., 19, 549.
30. Benedetti, I. L., Benedetti, A. M., Biolognani, F., Marini, M. and Ottavini, E. 1981, Riv. Idro. Biol., 20(3), 611.
31. Kumar, S. and Pant, S. C. 1981, Ind. J. Exp. Biol., 19, 191.
32. Dalela, K., Kumar, A. and Sharma, R. B. 1984, National Symposium on assessment of Environmental Pollution due to industrialization and Urbanization at Aurangabad, India, 37.
33. Bakre, V. P. 1985, Role of environmental variables on the biology of aquatic vertebrates in relation to mercury pollution, Ph.D Thesis, University of Rajasthan, Jaipur, India.
34. Ram, R. N. and Sathyanesan, G. 1987, Environ. Pollut., 47(2), 135.
35. Ramalingam, K. 1988, Environ. Ecol., 6(3), 761.
36. Khangarot, B. S. 1992, Ecotoxicol. Environ. Saf., 23(3), 282.
37. Figueiredo-Fernandes, A., Ferreira-Cardoso, J. V., Garcia-Santos, S., Monteiro, S. M., Carrola, J., Matos, P. and Fonjtainhas-Fernandes, A. 2007, Pesq. Vet. Bras., 27(3), 103.
38. Roncero, V., Duran, E., Soler, F., Masot, J. and Gomez, L. 1992, Environ. Res., 57(1), 45.
39. Roganovic-Zafirova, D. and Jordannova, M. 1998, Macedonian Ecol. Scciety, 51(1-2), 530.
40. Roganovic-Zafirova, D., Jordannova, M., Panov, S. and Velkova-Jordanoska, L. 2003, Folia Veterinaria, 47(1), 35.