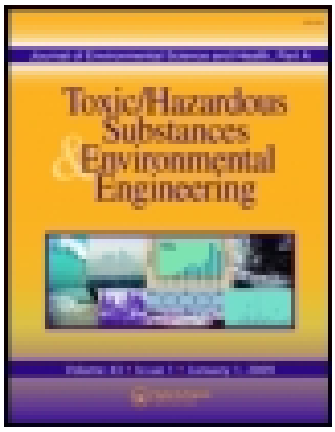

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The ecological complexity of the Thai-Laos Mekong River: III. Health status of Mekong catfish and cyprinids, evidence of bioaccumulative effects

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The ecological complexity of the Thai-Laos Mekong River: III. Health status of Mekong catfish and cyprinids, evidence of bioaccumulative effects

KANTIMANEE PHANWICHIEN[1], APICHART PRADERMWONG[2], HELEN E. KEENAN[3], KAMONPORN MASAWANG[1], NITAYA LAUHACHINDA[1], APISIT SONGSASEN[1], ARTHIT SAKULTANTIMETHA[3], SORNNARIN BANGKEDPHOL[3] and ALEC F. GAINES[3]

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Histopathology of fish organs was used as biomarkers of toxicity from environmental pollutants. A total of 117 fishes comprising of 52 cyprinids and 65 catfishes were randomly collected from the Mekong River from 5 stations: Chiang Rai, Loei, Nongkhai, Nakhon Phanom and Ubonratchathani. The health of the fish collected in December and April, winter and summer respectively, was evaluated. All fish from the 5 stations developed pathologic lesions with the same characteristics in their livers, kidneys and spleen. In the liver, there was vacuolation of hepatocytes, accumulation of brownish-green granules in the cytoplasm of hepatocytes, necrosis of hepatocytes, granuloma formation and angiogenesis. Kidney lesions consisted of glomerular degeneration, necrosis and focal hyperplasia of renal tubules. In the spleen, there were haemorrhage, melanomacrophage centre infiltration and necrosis of the red pulp and white pulp. The pathologic severity of the catfish was found to be more severe than in the cyprinids and the catfish collected in summer were less healthy than the catfish collected in the winter. These histopathological appearances might arise from the fish feeding on the benthos and thereby accumulating toxic pollutants in their organs. The activities of the serum enzymes, Glutamic Oxalacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT), were markedly increased, indicating detoxification activity. The highest activity of GOT found in the cyprinids from Chiang Rai 365.30 U/L whereas in the catfish from Nakhon Phanom the activity was 300.73 U/L. The highest GPT activity found in the cyprinids from Nakhon Phanom was 203.23 U/L where as in the catfish from the same station was GPT 389.77 U/L. According to the results from this study, catfish collected from Chiang Rai, Nakhon Phanom and Ubonratchathani showed more severe pathological changes than catfish from the other stations. Fish organs and river water were analysed for Polyaromatic Hydrocarbons (PAHs) and metals. The fish organs showed bioaccumulation of these toxic pollutants. BioConcentration Factors (BCFs) were calculated. Therefore an attempt is made to correlate these findings to the Mekong study in general.

Keywords: Histopathology, Mekong River, catfish, cyrinids, PAHs, metals, bioaccumulation.

Introduction

The Mekong River represents the international boundary between Thailand and Laos from Chiang Rai to Ubonratchathani. The river is vital resource to human population and important ecological resource of the region. Water quality is affected by natural, agricultural and industrial uses. Erosion process also contributed to high sediment loads in some areas along the Mekong River. The nature of the pollution is diverse. Evaluated concentrations of metals and PAHs have been documented in water, sediments and fishes from the Mekong River in Thailand.[1–3] Among variety of biomarkers adopted in ecotoxicological investigations, there are histopathology and enzymatic activities which used as indicators of exposure to environmental contaminants.

The effect of certain metals and xenobiotics results in the increase of xenobiotic enzymes[4] as well as histopathological alterations.[5] Since information on histopathology of the Mekong fish is not available, the objective of this report is to describe the microscopic findings in cyprinids and catfish in the Mekong River, Thailand. This data could provide

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an important baseline for future studies. Moreover, genotoxic pollutants that affect the aquatic ecosystems can also bioaccumulate and biomagnify via food chains resulting in greater levels in human far beyond the immediate environment. The result of this study will give an early awareness of potential adverse exposure from fish consumption due to a contaminated aquatic environment.

Material and methods

Fish sampling

A total of 117 fishes comprising of 52 cyprinids and 65 catfishes were randomly collected from the Mekong River from 5 selected stations out of 10 stations from the Mekong study (part 1): namely station 1 Chiang Rai, station 3 Loei, station 5 Nongkhai, station 8 Nakhon Phanom and station 10 Ubonratchathani (Fig. 1). The sampling stations were chosen on the basis of previous chemical analyses of fish tissues.[2,3] The health of the fish collected in December and April, represented winter and summer respectively, were evaluated for the years 2001–2004. Directly after capture, each fish was anaesthetized by 99.5% pure MS-222 (FINQUEL, USA). The fish were then weighed and measured, and immediately their blood was drawn by caudal vein punctuation to get the serum. All serum samples were stored in liquid nitrogen for further processing.

Histopathology

Fish were dissected immediately to separate livers, kidneys and spleens. Tissues were immediately placed in 10% neutral buffered formalin. For consistent histopathological evaluation, the portion of the middle-most section of the liver, posterior kidney and spleen were processed routinely into paraffin, sectioned at 5 μm, and stained with hematoxylin (Merck, Darmstadt, Germany) and eosin (Merck). Stained sections were analysed by light microscope and digital images were taken. Histopathological interpretations from all tissues were recorded and compared between catfish and cyprinids, as well as between seasons.

Enzyme activities

Serum GOT and Serum GPT activities were measured from the fish serum. Only the clear-yellow serum were analyzed using the method of IFCC (1986a, 1986b).[6,7] The mixture was measured spectrophotometrically at 340 nm, 25°C.

PAHs and metals

PAHs

Catfishes were collected from 4 stations: 1, 3, 5 and 10 in summer. Liver and belly muscle of each fish were dissected and directly stored in liquid nitrogen before transported to the laboratory. In the laboratory, the tissue samples were totally dried by lyophiliser (Heto, FD3) and homogenized before analysis. Each freeze-dried fish tissue (1.0 g liver and 0.5 g muscle) was extracted by ultrasonic extraction with 20 ml analytical grade hexane (Merck) for 30 minutes. The hexane extracted sample was evaporated in a rotary evaporator. Each dried sample was adjusted to 2 ml final volume with HPLC grade acetonitrile (BDH, Pool, England). The extract was filtered through a 0.2 μm PTFE syringe membrane filter before HPLC analysis.

A stock solution of 200 mgL^{-1} mixed standards was prepared in absolute ethanol (analytical grade, Merck). The mixed standard comprised of naphthalene, phenanthrene, anthracene, pyrene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perilene and benzo[b]fluoranthene produced by BDH, acenaphthylene (BDH), acenaphthene (Merck), fluorene

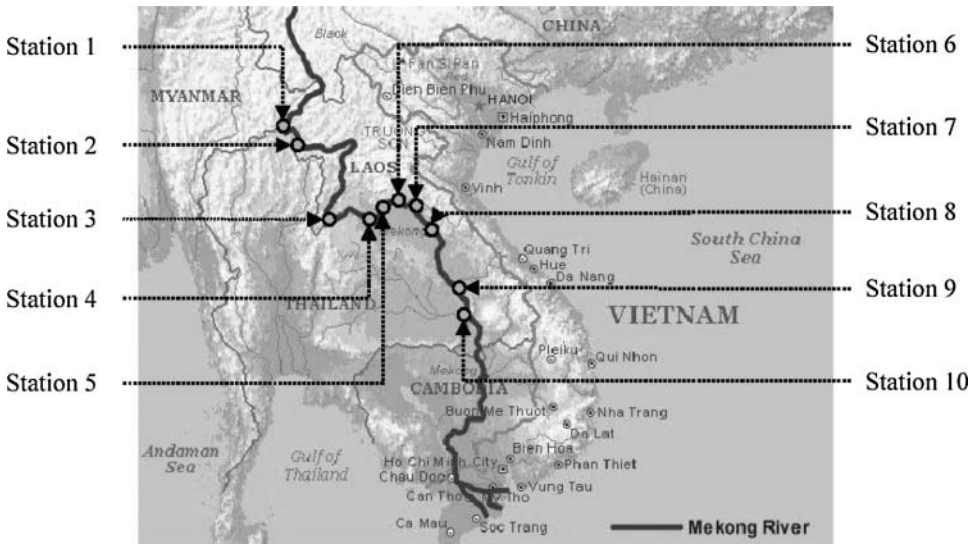

Fig. 1. Map shows the area of sampling fish from Mekong River.

and indeno(1,2,3-cd)pyrene (Chem Service, West Cherter, USA), and fluoranthene (Sigma-aldrich, St. Louis, USA).

Working solutions in the range 0.1, 0.25, 0.50, 0.75 and 1 mg L^{-1} were prepared by serial dilution in acetonitrile (HPLC grade, BDH). The calibration obtained was linear of the range with resolution of all 15 standards. Qualitative analysis was obtained by the retention time and quantitative analysis by peak area ($MVsec^{-1}$). All analysis were carried out in triplicate (n = 3) and an average value obtained ($\bar{X}$).

HPLC instrumentation and conditions were an Agilent High Performance Liquid Chromatographic system. The reconstituted sample was spiked with a known amount of standard then injected into the HPLC system (Agilent, 1100 series) by means of a rheodyne value fittered with a 30 μl loop. The separation was achieved using gradient elution (Chromspher C_{18} PAH 250 × 4.6 mm ID), mobile phase water:acetonitrile 50–100 %, 25 mins held 25 min. Flow rate was 1 ml min^{-1}. The column temperature was constant at 25°C. The photodiode array detector was fixed at 254 nm.

Metals

Catfishes were collected in summer (April) from all 5 stations. The entire of posterior kidney, belly muscle and blood were sampled, stored in liquid nitrogen and transported to laboratory. All samples were dried by lyophiliser (Heto FD3) and homogenized before analysis. Then, each sample (0.5–1 g) was digested with 10 ml, 65% analytical grade nitric acid (Merck) followed by Microwave digestion system (CEM model MARS5) at the temperature of 210°C, 200 PSI pressure, for 20 min. After digestion, the samples were cooled to room temperature and diluted to 10 ml with deionised distilled water. Metal concentrations (mg g^{-1} dry weight) were measured using Atomic Absorption Spectrophotometer (Perkins Elmer Model 1100B). Three replications of each sample were done (n = 3) and an average value obtained ($\bar{X}$). Calibration was linear, the concentration of each sample was determined from the Calibration Graph.

Results

Histopathological examination

Histopathological interpretation from fish liver, kidney and spleen of cyprinids showed minor change whereas the tissues of catfish from all stations revealed more severe damage. Therefore, this paper will report only the results from the catfish.

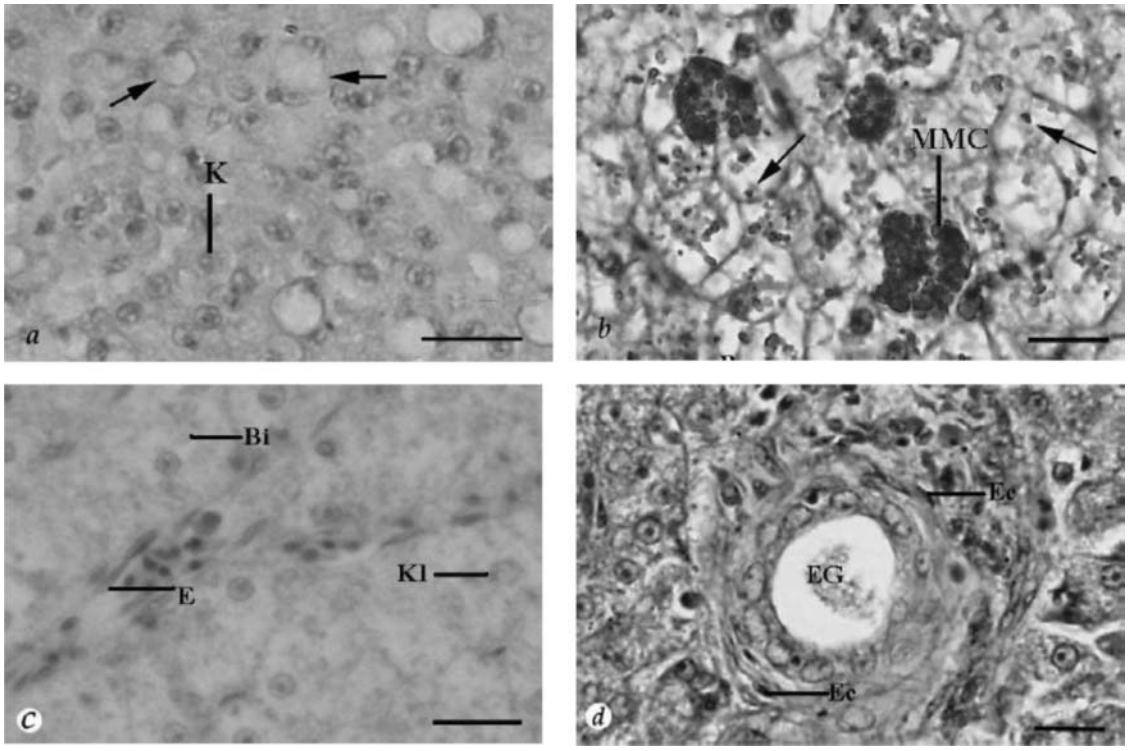

Fig. 2. Livers of catfish sampled from station 1 in summer. (a) Cytoplasmic vacuoles (arrows) are clear with well-demarcated margins. Some cells contain multiple vacuoles and fragment of the nucleus (K) is noted. (b) Melanomacrophage centers (MMC) are presented with an accumulation of brownish-green pigments in the cytoplasm (arrows). (c) New capillary is formed by lining of the endothelial cells (E) into blood vessel. Nuclear lysis (Kl) of the hepatocyte is seen. (d) Early granuloma (EG) is frequently observed with epithelioid cell lining (Ec) as a wall. Scale bars are 50 μm.

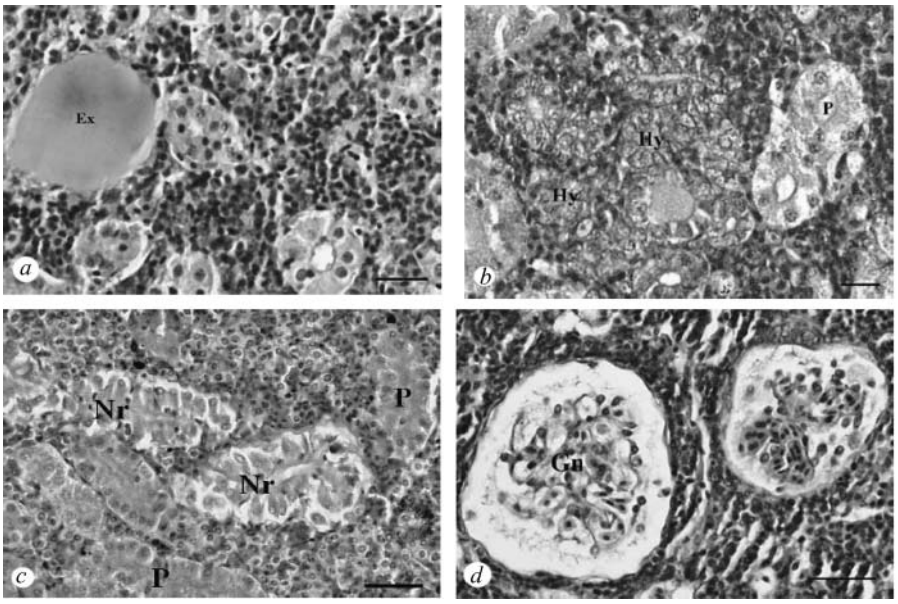

Fig. 3. Kidneys of catfish sampled from station 1 in summer. (a) Accumulation of eosinophilic fibrous material (Ex) in a damaged glomerulus and the proximal tubule (P) are seen. (b) Proliferation of renal tubule (Hy) is found in foci accompanied with the necrosis of proximal tubules (P). (c) The necrotic proximal tubules (Nr) are revealed in comparison to the normal tubules (P). (d) Severe glomerulus destruction (Gn) is observed. Scale bars are 50 μm.

Liver

Hepatocyte cytoplasmic vacuolization was the most obvious pathological alteration. Hepatocyte swelling and an intracytoplasmic accumulation of bile within the hepatocytes, cholestasis, recognized by the appearance of brownish-green pigments were observed. Necrosis of single hepatocyte was observed generally with nuclear lysis (karyolysis). In the most severe situations necrotic cells underwent cytolysis (Figure 2). The regular appearance of large pigmented macrophage aggregates, the melanomacrophage center (MMC), was detected. Additionally, new capillaries were formed within the liver tissues. The most severe histopathological alteration was found in the catfish from station 1 in summer (Figure 2). The findings for the cyprinids showed very slight damage in comparison to the catfish.

Kidney

Considering the renal corpuscle, dilation of blood capillaries and necrosis of their endothelium were observed on the glomeruli. An increased Bowman's space and apparent glomerular destruction was observed in all fish. Considering the renal parenchyma and tubules, the proliferation of renal parenchyma and of distinguish renal tubules in foci were observed. An accumulation of fibrous material was often seen within Bowman's space and tubules (Figure 3). The most severe histopathological alteration was also found in the catfish from station 1 in summer.

Spleen

Changes in the spleen were increase in MMCs, reduction in the red pulp, necrosis and the infiltration of fibroblasts (fibrosis) (Figure 4). Many foci of early granuloma were observed. These pathological lesions suggest that fish exposed to environmentally relevant levels of contamination. The histological alterations lead to anemia and immunosuppression that indicate an initial defense mechanism of the fish against concentration and duration of contamination exposure.

The serum GOT and GPT activities

The activities of the serum enzymes, GOT and GPT, of cyprinids and catfish were markedly increased. From Table 1, the highest activity of GOT found in the cyprinids from station 1 was 365.30 U/L and the activity of GOT of the catfish from station 8 was 300.73 U/L. From Table 2, the highest GPT activity was 203.23 U/L which found in the cyprinids from station 8 and the activity of GPT of the catfish from the same station was GPT 389.77 U/L. According to the results from this study, catfish collected from station 1, 8 and 10 showed more severe pathological changes than the ones from the other stations. An attempt is made to correlate these findings to the other environmental parameters of the Mekong study in general.

Bioaccumulation of PAHs and metals

Fifteen PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene,fluoranthene,

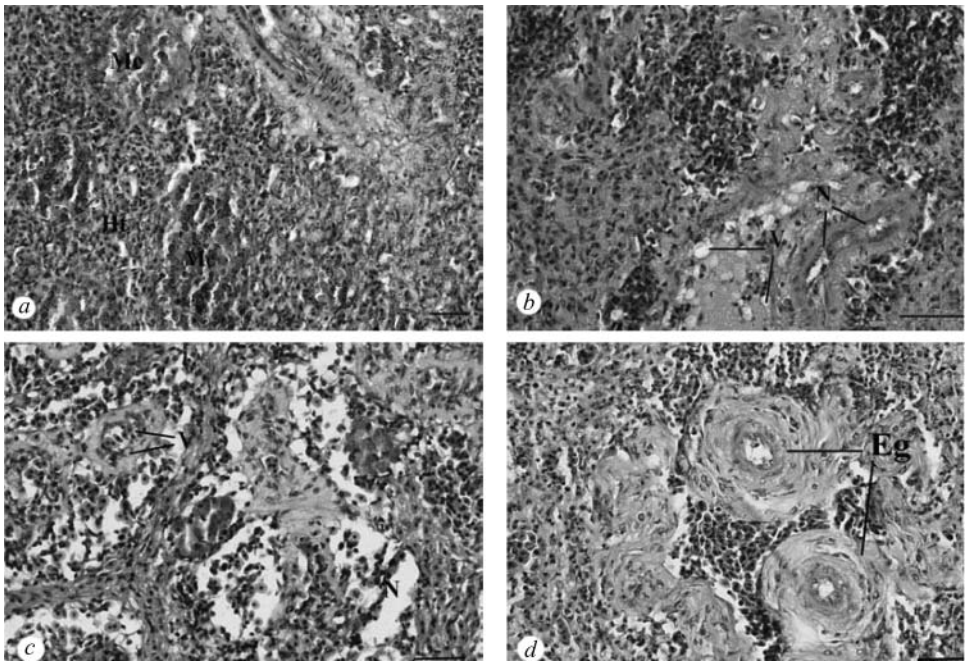

Fig. 4. Spleens of catfish collected from station 1 in Thailand in summer. (a) Macrophages (Mc) with small amount of brown pigment in cytoplasm aggregate in many foci among the hemopoietic tissue (Ht). (b) Severe necrosis (N) of blood vessels with eosinophilic fibrous material is observed. (c) Necrotic foci (N) of red pulp and damaged blood vessel (V) are seen. (d) Early granulomas (Eg) are presented surrounding damaged blood vessels. Scale bars are 50 μm.

pyrene, chrysene, benzo(a)anthracene, benzoty (b) fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, benzo[g,h,i] perylene and indeno(1,2,3-cd)pyrene were identified and quantified from all samples in the range of 0.0014–11.5944 mg kg^{-1} dry weight. The total PAHs ($\sum$PAHs) concentration in the liver of catfish from station 5 was higher than the other stations (see Table 3). The total PAHs concentration was generally found to be higher in the liver than in the muscle.

From Table 4, the highest cadmium concentration was detected in the kidney of catfish, 6.766 mg kg^{-1} dry weight, from station 1and the lowest concentration was detected in the muscle, 0.159 mg kg^{-1} dry weight, from station 10. Concentration of copper was found highest in the kidney of catfish, 22.347 mg kg^{-1} dry weight, collected from station 1 and the lowest was found in the muscle, 7.589 mg kg^{-1} dry weight, from station 3. Concentration of lead was high in the muscle of catfish, 34.590 mg kg^{-1} dry weight, from station 8 and the lowest was found in blood, 5.440 mg kg^{-1} wet weight, from the station 10.

It appeared that the concentration of cadmium analysed from kidney, muscle and blood of catfish from almost all stations were higher than that of ISQG value in the freshwater sediment (600 μg kg^{-1}). For copper and lead concentrations, they were still lower than those of ISQG value in the freshwater sediment, 35,700 and 35,000 μg kg^{-1}, respectively.

Evidence of bioaccumulation in fish was calculated in this study. Water and fish samples were taken from station 1 in 2004 (summer) and analyzed for metals and PAHs.[8] From this, the BioConcentration Factors were determined in Equation 1.

$$\text{BCF} = [\text{fish}] / [\text{water}] \quad (1)$$

Table 1. The average serum GOT activity (U/L) of cyprinids and catfish.

Station	*Cyprinids*	*Catfish*
1	365.30	296.50
3	76.12	175.37
5	216.70	261.80
8	282.70	300.73
10	212.00	289.30

Table 2. The average serum GPT activity (U/L) of cyprinids and catfish.

Station	*Cyprinids*	*Catfish*
1	202.70	362.14
3	92.71	274.12
5	147.71	273.53
8	203.23	389.77
10	202.91	388.74

Table 3. The concentrations of PAHs in the liver and muscle of catfish from the Mekong River in summer (2004).

	Conc. of PAHs in dry weight sample (mg/kg)											
	%Recovery		*Station 1*		*Station 3*		*Station 5*		*Station 8*		*Station 10*	
PAHs	*L*	*M*	*L*	*M*	*L*	*M*	*L*	*M*	*L*	*M*	*L*	*M*
1	61.1056	190.3402	0.1991	–	1.1871	–	10.4719	–	0.4052	0.3960	0.2478	0.1240
2	3585.2187	105.5347	0.3336	–	0.2420	–	*	–	0.3425	*	0.7088	0.0573
3	2378.1296	68.0889	0.1080	–	0.1780	–	0.2736	–	11.5944	3.3840	*	*
4	94.1554	96.3806	0.0806	–	0.0613	–	0.0896	–	0.0829	0.0542	0.0867	0.0115
5	96.3027	111.4303	0.0478	–	0.0530	–	0.0102	–	0.0149	0.0183	0.0152	0.0047
6	92.2488	98.939	0.3113	–	0.6831	–	1.9587	–	0.0407	0.0513	0.1680	0.0434
7	118.3494	110.7862	*	–	0.0733	–	0.0856	–	0.0390	0.0801	*	0.0232
8	209.546	66.4976	1.7223	–	0.0638	–	*	–	0.0963	*	1.4305	1.1293
9	57.0809	100.5518	0.0014	–	0.0158	–	*	–	*	*	0.0124	0.0051
10	67.9507	96.2866	0.0010	–	0.0029	–	0.0170	–	*	*	0.0205	0.0099
11	*NT*	*NT*	0.0184	–	0.0089	–	0.0282	–	0.0235	*	*	0.0187
12	184.7651	99.2083	0.0610	–	0.0114	–	0.0956	–	*	*	0.1164	0.0465
13	59.0336	79.0877	0.0107	–	*	–	0.0098	–	*	*	0.0115	0.0101
14	116.9015	89.5989	*	–	*	–	0.1995	–	*	*	*	*
15	*NT*	*NT*	0.0066	–	*	–	1.2361	–	*	*	0.0337	*
	∑PAHs		2.9018	–	2.5806	–	14.4757		12.6393	–	2.8515	1,4838

Actual concentration = Result × %Recovery/100.
NT= not tested.
–= no sample.
*= below detection limited, L = liver, M = muscle.
PAHs species:
1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benzo(a)anthracene; 10 = chrysene; 11 = benzo(b)fluoranthene; 12 = benzo(k)fluoranthene; 13 = benzo(a)pyrene; 14 = benzo(g,h,i)perylene; and 15 = indeno(1,2,3-cd)pyrene.

Apart from showing that the default model for PAHs is underestimated. This study also shows that even levels of contaminants that are below the detection limits of the analytical methods are significant and can lead to bioaccumulation with the accompanying adverse physiological effects.

Discussion

Previous studies by the research group[2,3] have shown that the catfish collected from station 1 accumulated higher PAHs (∑PAHs = 180.4242 mg kg^{-1} dry weight in the liver) and metals (cadmium = 10.316 mg kg^{-1} dry weight in the kidney). Catfish is the bottom feeder and it appears that they must ingest the contaminated sediment with food. The study group has measured metals and PAHs at levels exceeding current Environmental Quality Standards (EQS)[8] which are then bioaccumulated in the fish. As the levels found are greater than that measured in sediments and considering that the diet of catfish, in that they are mostly bottom feeders, this is evidence of bioaccumulation and biomagnification.

The increased density of hepatic MMCs generally found in this study reflected important liver lesions associated with a degenerative-necrotic condition. A similar study has

Table 4. The heavy metal concentrations in different organs of catfish from the Mekong River in summer (2004).

	Cadmium (mg/kg dry weight)			*Copper (mg/kg dry weight)*			*Lead (mg/kg wet weight)*		
Stations	*kidney*	*muscle*	*blood*	*kidney*	*muscle*	*blood*	*kidney*	*muscle*	*blood*
S1	6.766	1.946	1.231	22.347	*	*	19.150	18.710	5.730
S3	3.051	0.850	*	13.898	7.589	–	19.800	18.060	–
S5	1.713	2.331	*	19.933	19.382	–	18.110	20.580	–
S8	0.694	2.733	0.704	4.702	18.784	–	13.450	34.590	6.120
S10	0.508	0.159	1.148	19.786	14.442	–	14.100	14.380	5.440

*= below detection limited.
–= no sample.

Table 5. BioConcentration of metals and PAHs in Mekong Catfish at Station 1 (2004).

	Pyrene	*Anthracene*	*Pb Kidney*	*Pb Muscle*	*Pb Blood*
Catfish hv(mg/kg)	1.72	0.31	19	34	6
Water (μg/L)	0.26	0.09	< dl 5	< dl 5	< dl 5
BCF Experimental	6615 (∼>6 dv)	3762 (∼>7 dv)	> 3,800	> 6,800	>1,200
*Default	1100	530	na	na	na

*EPI suite (Keenan, 2007) dv = default value na = not available hv = highest value.

been reported in fish exposed to the PAHs anthracene and acenapthene.[9] Therefore, it appeared that bioaccumulation of PAHs in Mekong catfish contributed in some way to the physiological damage observed. The accumulation of brownish-green granules or bile salts (cholestasis) recognizable in the liver of all catfish is a manifestation of a pathophysiological condition, attributable to failure of the metabolism or excretion of bile pigments.[10] The liver vacuolization detected in all liver tissues shows an intracellular degenerative process from metabolism disorders.[11,12] Prolonged exposure to potentially toxic agents causes chronic inflammation of the liver. The liver tissues of many catfish show chronic inflammation by the formation of granulomas. These granulomas are formed by the epithelioid cells and fibroblasts to limit the injuried foci. Histologically, the proliferation of new small blood vessels occurs to provide nutrient and oxygen during the tissue repairing process.[13]

The renal injury observed in the catfish were the loss of endothelial cells lining the glomerulus and histological alterations in the proximal tubules. These lesions frequently occur due to xenobiotic injury.[14] The proliferation of proximal tubules found in Mekong catfish kidney has never previously been detected or reported in the literature. Indeed, this adverse physiological reaction has never been reported for any species. The pathological accumulation of fibrous material in the renal tubules and within the Bowman's space of fish was observed similar to the observation of Gul et al. and Simpson et al.[5,15] This fibrous material may consist of small molecular weight proteins and is further evidence of physiological damage by exposure to pollutants.[16]

The pathological lesions found in the spleen suggest that fish have been exposed to pollutants. The histological alterations observed can lead to anemia and immunosuppression. This weakens the defense mechanism and may lead to illness and fatality.[17,18]

The serum GOT and GPT activities from both cyprinids and catfish were markedly high when compared to a study of *Anabas testudineus* [19] and *Ictalurus punctatus* [20] in culture ponds. But when compare to the fish from the polluted natural water, the GOT and GPT activities were in the same ranges as fish reported by Lenhardt in 1992, and Zikic et al. in (1997).[21,22] Although this study compared to the intensive farming of fish to river fish, the serum GOT and GPT activities were in the same range. This suggests physiological evidence of exposure to the same levels of pollution.

Conclusion

The effects on the liver, kidney and spleen were related to the high activities of serum enzymes of both cyprinids and catfish. The fish were initially able to homeostatically regulate and detoxify toxic chemicals. However further exposure or increasing bioconcentration of pollutants results in irreversible physiological damage and the ultimate decline of fish populations. This would be concomitant with a reduction of fish available for human consumption and increasing biomagnification of pollutants to the detriment of all top predators. Thus impacting directly on the health and resources that are available for those > 25 million denizens of the Mekong River. Based on these findings and in order not to exacerbate an already precarious situation it is recommend that there is an immediate response to toxicant spills and that EQS values based on toxicological data are defined for this area. Consideration of potential interactions of multiple stressors and investigation of additive and synergistic effects of major pollutants also requires urgent attention.

Bioaccumulation may be dangerous to the consumer even if it appears to be safe for animal itself. This was observed by the emergence of Minamata disease in Japan, whereby mercury accumulation in fish resulted in many human fatalities.

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