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Effects of Heavy Metal Accumulation on the Variation of Glutathione S-transferases (GSTs) Activity in some Economic Fishes in Nhue-Day River Basin

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Abstract: The aim of this study was to investigate the effects of metal accumulation on the variation of glutathione S-transferase (GST) activities in some fishes (Cyprinus carpio L, Hypophthalmichthys molitrix, and Oreochromis niloticus) in Nhue-Day river basin. Samples for analysis were taken four times from September 2012 to July 2013. The heavy metals were deposited mostly in kidney and liver of all studied fishes by the following order: Zn > Cu > Pb > Cd. Their accumulated patterns in tissues are ranked as: liver >>[1] kidney > gill for Cu; accumulation patterns are similar for Zn, Pb and Cd, accumulated more in kidneys than in liver and gills but at the different extents: kidney > liver ≥ gills for Zn; kidney >> liver > gills for Pb, and kidney > liver >> gills for Cd. GSTs activities in tissues of common carp, silver carp and tilapia were in the following order: liver > kidney > gill. Effects of heavy metal bioaccumulation to the variation of GSTs activity in fish tissues are reflected by the correlations between heavy metal bioaccumulation in fish tissues and GSTs activities observed in respective tissues. In general, metal accumulation in fish tissues showed that Nhue-Day river water was polluted with heavy metals and this influences physiological health of fishes which are reflected by the changes of GSTs in fish tissues. The results of this research help to establish background data for management of aquaculture practices and environmental protection of Nhue-Day river basin.

Keywords: Nhue-Day river basin, heavy metals, GSTs activity, common carp, silver carp, tilapia.

1. Introduction

The water quality degradation of rivers is one of the most concerns in Vietnam, especially with rivers run through big cities. The increase in population and rapid growth of economy are considered as major causes leading to this degradation (Hiep and Truong, 2003). Nhue-Day river basin is located in the socio-economic center of the northern Vietnam and plays a vital role in the socio-economic development of the region. However, recent studies showed that the water quality of Nhue-Day river is extremely polluted by organic and inorganic substances due to the effluents from residences, industrial

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zones, craft villages, etc., discharge to surface waters. This problem is even more severe in Nhue river section flows through Hanoi city with levels of DO, COD, BOD_5, NH_4^+, PO_4^{3-}, H_2S, NH_3 and heavy metals (Pb: 0.035 mg/L, Hg: 0.0018 mg/L; As: 0.025 mg/L) exceeded the Vietnamese standards for water quality type A2 (for conservation of aquatic animals and plants). Among water pollutants, heavy metals are recently caught the public attention because of their high toxicity and persistent (Ololade *et al*, 2008) [1]. The contamination of heavy metals in water, even at levels as low as in the natural environment, may cause a chronic stress (Ngo *et al*, 2011a,b,c) [2-4], directly affecting the aquatic organisms, especially fish (Khayatzadeh and Abbasi, 2010) [5]. Fish is usually consumed by many people, especially in developing countries, as a main source of protein and nutrients. However, fishes are also considered as good indicators of trace metal contamination in aquatic systems (Moiseenko *et al*, 2008) [6]. They may absorb dissolved elements and trace metals such as Cu, Zn, Pb, Cd and then accumulate them in various tissues, i.e. gills, livers, kidneys and muscle. The bioaccumulation of heavy metals in tissues varies from metal to metal as well as from different fishes. Heavy metals are transferred into fish through gills, intestine or skin to the circulatory system and then transferred to the target organs of detoxification including livers, spleens and kidneys (Health, 1987) [7]. When humans use these fishes as a food, heavy metals bioaccumulated in fishes can be harmful to their health. However, Fish is an important link in the food chain, and one of the best biological markers to assess the level of heavy metal pollution in the river basin. Therefore, the use of biomarkers to study and evaluate the effects of heavy metals on fish has received an increasing concern. Glutathione-S-transferases (GSTs; EC 2.5.1.18) are an intracellular family of Phase II detoxification enzymes. The changes in GSTs activity in fish represent as the response of the organism to the environmental contamination has been extensively studied in recent years. Most results showed that, to a certain extent, when being exposed to heavy metals, one of the very early responses of fish is inducing the production of GSTs activity in some specific organs, i.e., liver, kidney and gills, in order to cope with the stress condition.

In this study, three important fishes such as common carp (*Cyprinus carpio L*), silver carp *(Hypophthalmic molitrix)* and tilapia (*Oreochromis niloticus)* were collected along the river basin to investigate the impacts of heavy metals (Zn, Cu, Pb, Cd) on the variation of GSTs activities. In order to answer that question, the relationship between the accumulation of Zn, Cu, Pb, Cd and the variation of GSTs activities in their respective organs were examined. The result will also reflect the effects of metal pollution on the physiological health of fishes.

2. Material and methods

2.1. Study area and sampling

The study area is located along Nhue river, from Ha Noi to Ha Nam province, and the downstream of Day river from Ha Nam, Ninh Binh to Nam Dinh province, has the geographic coordinates of 20° - 21°20' North latitude and 105° - 106°30' East longitude (Fig. 1).

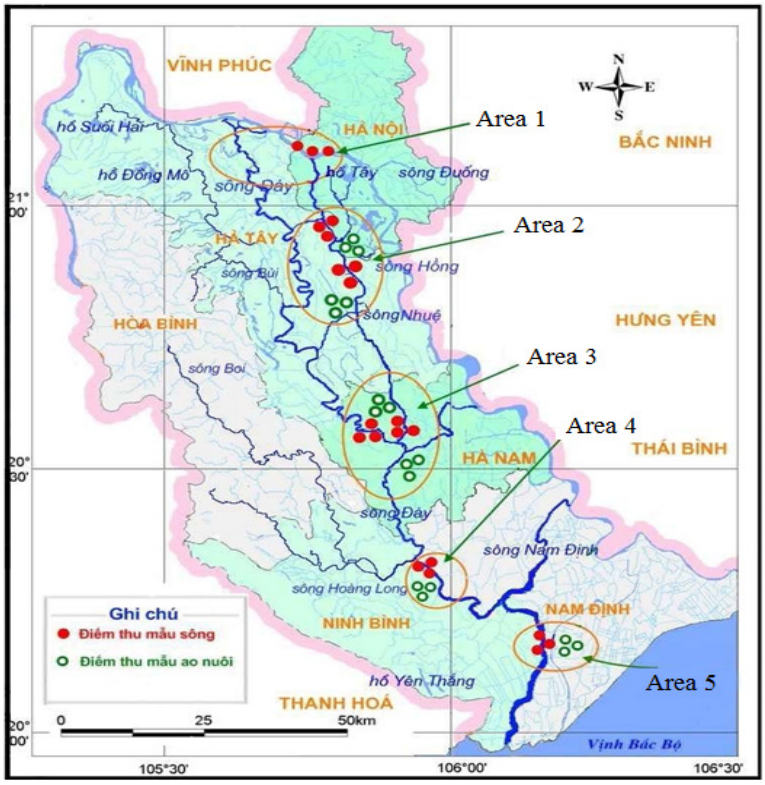

Figure 1. Study area and sampling sites.

A total of 140 fish samples including common carp, silver carp and tilapia were collected in five areas along the Nhue-Day river and during four seasons from September 2012 to July 2013 (Fig. 1). Fishes were collected from Nhue-Day river and aquaculture ponds which used the water from these rivers. They were transported alive to the laboratory in the rich-oxygen containers and were anaesthetized before sampling of gills, livers, and kidneys.

2.2. Sample preparation and analyses

Sample preparation:

The anaesthetized fish were dissected and gill (10-20 mg w. wt.), liver and kidney (5-10 mg w. wt.) samples were taken into 2 mL-eppendorf containing 300 µl Dulbecco's Phosphate Buffered Saline (DPBS) and then stored at -80°C for GSTs activity quantification. A portion of about 20-100 mg each was also taken into another test-tube for heavy metal determination.

Heavy metal determination:

Tissue samples were digested in 4:1 HNO_3 65% and 30% HCl. One blank (only reagents) and one reference material were included in each sample batch. Briefly, 2 ml of 65% HNO_3 and 0.5 ml of 30% HCl are added into each test-tubes containing sample and kept at room temperature for 24 hours. Then, 200 µl H_2O_2 was added into each sample and left at room temperature for another 5 hours before being digested in a digestion box (bio-carrier) at 120°C for at least 5 hours until the sample is completely digested. Then the digested samples were diluted with bidest water up to 20 mL, filtered through a cellulose membranes syringe filter with a pore size of 45 µm. Samples were then ready for measuring heavy metals by inductively coupled plasma mass spectrometry (ICP-MS, ELAN 9000; Perkin-Elmer SCIEX, Waltham, MA, USA); detection limits for Cu

and Zn was 1 µg/L, for Cd, Pb was 0,001 µg/L, respectively. The analytical method was validated with certified standard reference materials from oyster and fish liver (Graham B. Jackson Pty Ltd, Dandenong, Victoria, Australia). Recoveries were within the certification range, i.e., 93% for Cd, 90% for Pb, and 92% for Cu and Zn. Procedural blanks consisting of aqua regia were below detection limits. The results were reported in mg/ kg for fish wet weight. All reagents used were of analytical grade (Merck, Darmstadt, Germany).

GSTs activity assays

GSTs activity was determined by the method of Habig *et al*. (1974) [8] using 1 chloro 2,4 dinitrobenzene as substrate. Samples were defrosted on ice, homogenized and centrifuged twice at 9205 rpm at -4^{o}C for 15 min. Combined supernatants were collected for the assay. The reaction solution (substrate) was a mixture of 100 mM DPBS buffer (pH 6.5), 200 mM GSH and 100 mM CDNB. The reaction was started by mixing 0.98 or 0.95 mL reaction mixture with 0.02 or 0.05 mL sample, respectively and the absorbance was measured every one minute for 8 min at 340 nm using a Thermo Sciencetific^{TM} Biomate spectrophotometer. A blank sample (containing 1 mL of substrate) was measured for each sample batch. The specific activity of GSTs activity was calculated and expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein.

2.3. Data processing and analyses

Data were processed by Excel software and statistical analyses were performed using biostatistical software of Graphpad Instat (San Diego, CA). Two-way analysis of variance was used to determine whether differences in metal accumulation and enzyme activities among tissues and sampling seasons were significant. If the significant difference was detected then the Student-Newman-Keuls multiple comparisons test was applied. Correlations between variables (heavy metal concentration and GSTs activities in tissues of fishes) were tested with the nonparametric correlation (Spearman r) test. Statistical significance was assigned at $P < 0.05$.

3. Results and discussion

3.1. Metal bioaccumulation in fish tissues

Accumulation patterns of Zn, Cu, Pb and Cd were significantly different in different fishes and different tissues ($p < 0.05$); however, in terms of different metals, all fishes and tissues accumulated in the order of Zn > Cu > Pb > Cd (Table 1). Zn and Cu are both essential metals, in contrast to Cd and Pb, thus they are accumulated in the higher concentration in all investigated tissues and fishes.

Accumulation patterns in tissues are similar for Zn, Pb and Cd, accumulated more in kidneys than in liver and gills, but at the different extents: kidney > liver ≥ gills for Zn; kidney >> liver > gills for Pb, and kidney > liver >> gills for Cd (Table 1). In contrast, Cu tended to concentrate more in liver than in kidney and gills (liver >> kidney > gills). The differences in metal concentration for the three species are likely due to their different feeding habits, ages, and sizes (Linde *et al.* 1998; Canli and Atli 2003) [9,10]. Zn in tissues of common carp (190 mg/ kg w. wt in gills, 120 mg/kg w. wt in liver, 250 mg/ kg w. wt in kidney) were much higher than those in tissues of other fishes ($p < 0.001$) and no difference ($p > 0.05$) was found between tilapia and silver carp (common carp >> tilapia ≥ silver carp). However, Cu, Pb and Cd tended to highly accumulate in tissues of tilapia ($p < 0.05$) compared to those in common carp and silver carp (for Cu and Pb: tilapia >> common carp ≈ silver carp; for Cd: tilapia ≥ common carp >> silver carp).

Table 1. Means and standard errors of metal accumulation in gills, livers, kidneys of common carp, silver carp and tilapia (mg/kg w. wt.) over 1 year

	Zn			Cu		
	Gill	**Liver**	**Kidney**	**Gill**	**Liver**	**Kidney**
Common carp	***190 ± 16***	***120 ± 23***	***250 ± 24***	***2.4 ± 0.4***	***20 ± 1.6***	***6.9 ± 1.8***
Autumn	151 ± 28	88 ± 15	190 ± 38	1.5 ± 0.15	19 ± 7.8	4.1 ± 0.41
Winter	201 ± 23	109 ± 13	236 ± 35	2.1 ± 0.44	24 ± 5.3	5.2 ± 0.87
Spring	227 ± 29	189 ± 60	304 ± 71	2.5 ± 0.85	16 ± 7.1	6.2 ± 1.5
Summer	183 ± 23	96 ± 16	270 ± 57	3.5 ± 0.21	21 ± 4.7	12 ± 1.9
Silver carp	***29 ± 2.2***	***53 ± 6.6***	***56 ± 17***	***2.4 ± 0.8***	***27 ± 3.2***	***6.7 ± 2.6***
Autumn	22 ± 1.8	70 ± 25	29 ± 5.1	0.94 ± 0.08	23 ± 7.9	2.4 ± 0.76
Winter	32 ± 8.8	58 ± 6.6	49 ± 10	1.3 ± 0.19	21 ± 6.8	3.6 ± 0.68
Spring	30 ± 6.5	44 ± 6.7	107 ± 69	2.0 ± 0.37	34 ± 14	6.7 ± 3.9
Summer	30 ± 3.2	40 ± 7.7	39 ± 7.6	5.5 ± 0.89	31 ± 6.7	14 ± 5.4
Tilapia	***35 ± 5.8***	***42 ± 6.0***	***82 ± 13***	***3.8 ± 0.94***	***133 ± 39***	***11.4 ± 3.7***
Autumn	27 ± 2.3	32 ± 2.8	77 ± 22	6.3 ± 3.9	80 ± 18	13 ± 4.5
Winter	52 ± 11	589 ± 14	107 ± 21	2.4 ± 0.51	101 ± 10	6.5 ± 0.98
Spring	32 ± 4.3	35 ± 4.5	47 ± 4.1	2.4 ± 0.14	249 ± 56	5.2 ± 0.48
Summer	28 ± 1.6	42 ± 4.7	98 ± 27	4.1 ± 0.56	100 ± 13	21.3 ± 6.4
		Pb			**Cd**	
Common carp	***0.59 ± 0.062***	***0.45 ± 0.10***	***0.96 ± 0.31***	***0.020 ± 0.010***	***0.10 ± 0.007***	***0.36 ± 0.054***
Autumn	0.52 ± 0.08	0.34 ± 0.09	0.51 ± 0.13	0.009 ± 0.002	0.09 ± 0.05	0.46 ± 0.22
Winter	0.48 ± 0.1	0.31 ± 0.08	0.33 ± 0.05	0.004 ± 0.002	0.09 ± 0.05	0.34 ± 0.15
Spring	0.73 ± 0.11	0.39 ± 0.05	1.6 ± 0.36	0.006 ± 0.003	0.10 ± 0.04	0.22 ± 0.07
Summer	0.67 ± 0.11	0.75 ± 0.06	1.4 ± 0.24	0.060 ± 0.004	0.12 ± 0.02	0.44 ± 0.07
Silver carp	***0.61 ± 0.19***	***0.73 ± 0.30***	***0.87 ± 0.34***	***0.020 ± 0.013***	***0.057 ± 0.014***	***0.20 ± 0.048***
Autumn	0.32 ± 0.05	0.29 ± 0.07	0.33 ± 0.09	0.009 ± 0.004	0.03 ± 0.01	0.11 ± 0.03
Winter	0.28 ± 0.04	0.28 ± 0.04	0.27 ± 0.08	0.006 ± 0.004	0.05 ± 0.03	0.31 ± 0.09
Spring	1.10 ± 0.34	0.76 ± 0.12	1.2 ± 0.56	0.004 ± 0.002	0.05 ± 0.03	0.12 ± 0.05
Summer	0.74 ± 0.16	1.6 ± 0.57	1.7 ± 0.63	0.060 ± 0.010	0.10 ± 0.04	0.25 ± 0.09
Tilapia	***0.97 ± 0.39***	***0.92 ± 0.24***	***1.6 ± 0.41***	***0.026 ± 0.016***	***0.20 ± 0.038***	***0.37 ± 0.061***
Autumn	0.61 ± 0.08	0.52 ± 0.1	1.7 ± 0.62	0.025 ± 0.010	0.11 ± 0.03	0.28 ± 0.08
Winter	0.38 ± 0.08	0.63 ± 0.14	0.72 ± 0.16	0.002 ± 0.0008	0.17 ± 0.03	0.27 ± 0.07
Spring	2.1 ± 1.21	0.93 ± 0.14	1.3 ± 0.27	0.004 ± 0.002	0.26 ± 0.06	0.37 ± 0.07
Summer	0.77 ± 0.15	1.6 ± 0.27	2.67 ± 0.82	0.071 ± 0.007	0.27 ± 0.04	0.54 ± 0.17

Seasonal variations were found for Cu, Pb and Cd in all fishes and tissues (Table 1) with higher levels in summer and spring and lower levels in autumn and winter ($p < 0.05$); especially, this is clearly seen in silver carp, i.e. Cu in silver carp kidney: 14 ± 5.4 mg/ kg w. wt in summer in comparison with 6.7 ± 3.9 (spring), 3.6 ± 0.68 (winter) and 2.4 ± 0.76 mg/

kg w. wt (autumn). However, no variation in terms of sampling times was observed for Zn in common carp and silver carp, with similar accumulation patterns in all tissues and season; the only variation was seen in tilapia with higher level of Zn in winter in comparison with other seasons (52 ± 11, 589 ± 14 and 107 ± 21 mg/ kg w. wt in gills, liver and kidney, respectively; Table 1). There is only little fluctuation among Zn accumulation in different tissues and also at different season. The reason might be that Zn is essential element for the hydroxylation and other enzymatic reactions in organisms; therefore the internal concentrations of Zn tend to be tightly regulated by fish (Bury *et al.* 2003) [11].

Zn is essential to many enzymes that influence cell division and regulate cell proliferation. However, these enzymes only work well in certain limitation of Zn concentration. The specific metabolism process and coenzyme catalyzed reactions taking place in kidney that Zn involved could be used to explain for the high Zn concentration in kidney (Jaffar and Pervaiz 1989) [12]. Differently, Cu concentration was found to be the highest in fish livers ($p < 0.01$; common carp: 24 ± 5.3, silver carp: 34 ± 14 and tilapia: 249 ± 56 mg/ kg w. wt). Cu is one of the most important elements involved in many processes supporting life, participates in destruction of free radicals by cascading enzyme systems. The presence of Cu and Zn cofactors reduce superoxide radicals to hydrogen peroxide through superoxide dismutase. And the liver is an important organ in the body which performs multiple critical functions to keep the body pure of toxins and harmful substances. The Cu as well as Pb and Cd concentrations in liver were higher than those in other organs which can be explained by the storage and detoxification functions of liver.

3.2. Variation of GSTs activity in fish tissues

Significant differences of GSTs activities among three fishes were observed in liver and kidney tissues, especially in autumn with the higher levels found in common carp and tilapia compared to that of silver carp ($p < 0.05$; fig. 2). In all three species, liver GSTs activity tends to be the highest one, follow by kidney and then the gill GSTs; especially the significant differences among these tissues were found in winter samples ($p < 0.05$).

For common carp, the significant differences in GSTs activities of three investigated tissues in each season as well as GSTs activities of each organ among four seasons were found ($p < 0.001$, fig. 2a). Average value of liver GSTs activity (1.14 ± 0.24 µmol/ mg protein/ min) was significantly higher ($p < 0.01$, fig 2a) than those in gills (0.31 ± 0.08 µmol/ mg protein/ min) and kidney (0.45 ± 0.23 µmol/ mg protein/ min). The highest level of GSTs was observed in liver of this species in autumn (2.97 ± 0.75 µmol/ mg protein/ min) and the lowest value was found in the gills during summer (fig. 2a). In gills, GSTs activity level (0.60 ± 0.06 µmol/ mg protein/ min) was higher in spring in comparison to the winter and summer ($p < 0.05$, fig. 2a) but not difference with autumn ($p > 0.05$). Both in the liver and kidney of common carp, GSTs levels in autumn were significantly higher than those in other seasons ($p < 0.05$, fig. 2a).

For silver carp, GSTs activity in gills, livers and kidneys were also different from each other and from different seasons ($p < 0.05$; fig. 2b). The average value of GSTs activity in liver (0.6 ± 0.17 µmol/ mg protein/ min) was significantly higher ($p < 0.05$) than that in the kidney (0.34 ± 0.11 µmol/ mg protein/ min) and in the gills (0.29 ± 0.16 µmol/ mg protein/ min). There were significant differences between GSTs of different tissues from the same season ($p < 0.05$, fig. 2b). Different trend was found in spring time with lower level of GSTs in the kidney in comparison to those in the liver and gills.

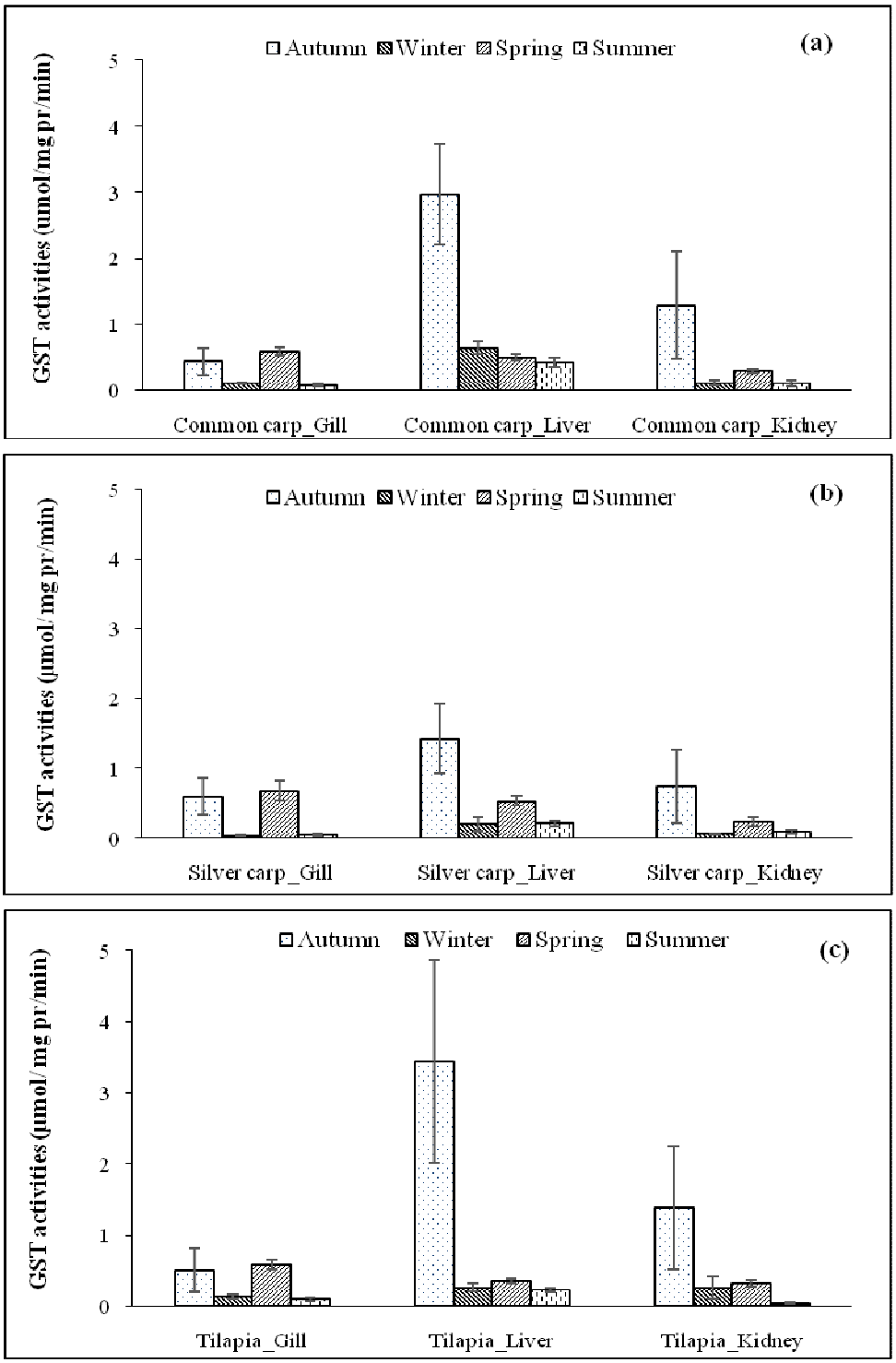

Figure 2. GSTs activities (µmol/ mg protein/ min) in gills, livers and kidneys of fishes sampled in different seasons: Common carp (a), Silver carp (b), Tilapia (c). Values are the means ± SEM of 5-12 samples.

In Tilapia, overall mean of GSTs activity in liver (1.07 ± 0.38 µmol/ mg protein/ min) was higher ($p < 0.05$; fig. 2c) than those in the kidney (0.50 ± 0.27 µmol/ mg protein/ min) and gills (0.33 ± 0.11 µmol/ mg protein/ min). Liver GSTs activities tend to be higher than those in kidneys and gills in all seasons, except for spring. When comparing GSTs activity of different tissues in one season, significant differences were detected in autumn ($p < 0.001$) and summer ($p < 0.05$) with distinctive higher level in livers compared to those in kidneys and gills.

3.3. Effects of metal accumulation on GSTs activity in fish tissues

As the key intracellular enzymes of the second phase of detoxification processes, GSTs involved in both detoxification of various xenobiotic chemicals and endogenous reactive compounds of cellular metabolism. Fish tissues are endowed with antioxidant defense systems consisting of many enzymes, i.e. catalases, GSTs, Glutathione (GSH), superoxide dismutase (SOD), etc. and their changes reflects the presence and impacts of heavy metals on the fish physiology (Farombi *et al*, 2007) [13]. Among those enzymes, GSTs play a vital role in protecting fishes from oxidative stress caused by metals; therefore these enzymes also have been popularly used as biomarkers to detect stress. The relationship between heavy metals accumulation and GSTs activity in organs of different animals has been assessed by many researchers (Stone *et al*, 2002; Zawisza-Raszka *et al*, 2010) [14,15]. This relationship have been studied in liver, kidney, and gill tissues of different fish species in laboratory and under field conditions (Mani *et al*, 2014; Romeo *et al*, 1994) [16,17]. The result showed the gradual increase of GSTs enzyme activities in the liver and kidney of Cd treated *A. arius* to reach a peak after 72 hrs exposure and then it gradually declined until 96 hrs (Mani *et al.*, 2014) [16]. The common carp exposed to the waterborne Cd and Pb at a sub-lethal level for 32 days in laboratory showed the increase trend of enzyme GSTs in the liver; however, slowly increased in the kidney and after that decreased on the 32nd day like other antioxidants. The higher GSTs activity observed in the liver of the carp after exposure indicated an augmented detoxification activity in the liver tissue. The kidney also showed a prominent response in GSTs activity, but at a lesser extent compared to the liver (Vinodhini and Narayanan, 2009) [18]. However, results from laboratory tests do not always coincide with results obtained under the field conditions. The differences may be due to the fact that fish are exposed to a constantly changing composition of different chemical substances under natural conditions.

In this study, for Cu, only one correlation between Cu concentration and GSTs activity of common carp kidney was found in spring with $p = 0.035$, $r = 0.74$ (fig. 3a). No correlation in organs of other fishes was detected. The Cu levels in tissues of silver carp and tilapia are too high (27.1, 6.7 and 2.4 mg Cu/kg w. wt in silver carp liver, kidney and gill, respectively; and 133, 11.4 and 3.78 mg Cu/kg w. wt in tilapia liver, kidney and gill, respectively) so that a severe dysfunction of fish liver, kidney and gill might be occurred and therefore those organs cannot induce GSTs synthesis anymore to cope with this highly stress condition. As a consequence, no correlations were found in these two fish. In contrast, Cu level were lower in common carp (common carp liver, kidney and gill: 20.1, 6.9 and 2,4 mg/ kg w. wt, respectively), and this is a strong fish in comparison with silver carp, therefore, one of these organ, kidney, still can be functioning in inducing GSTs synthesis to detoxify Cu intoxication, and this result in a tight positive correlation between Cu level and GSTs activity in kidney.

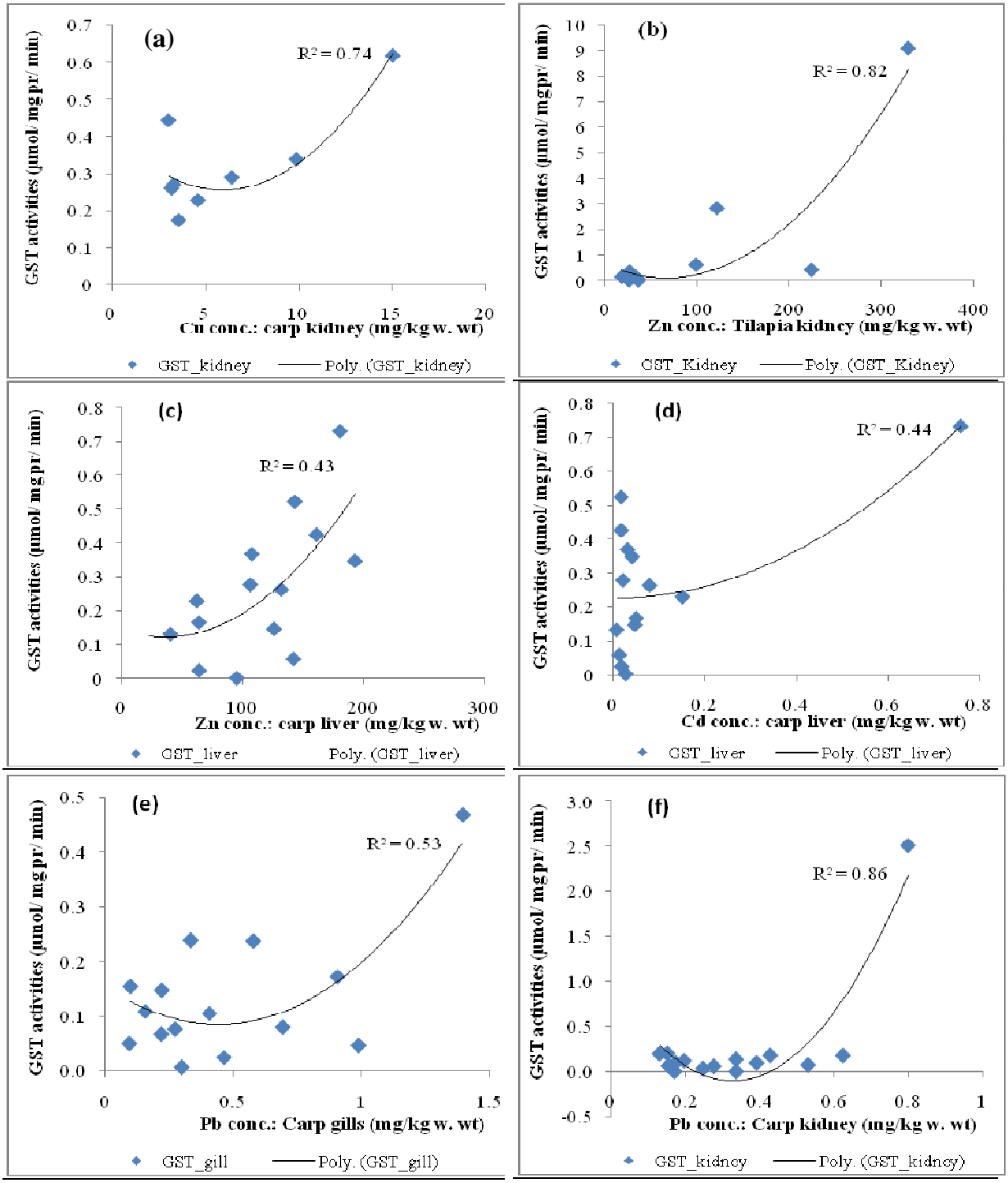

Figure 3. Correlation between heavy metal accumulation (mg/kg w. wt.) and GSTs activities (µmol/ mg protein/ min) in fish tissues: Cu and GSTs in common carp kidney (a); Zn and GSTs in tilapia kidney (b) and in common carp liver (c); Cd and GSTs in common carp liver (d); Pb and GSTs in common carp gills (e) and kidney (f).

The results showed that Zn accumulation in fish tissues exerts effects on the levels of GSTs activity. Two correlations between Zn accumulation and GSTs activity in tissues were observed in autumn and winter ($p < 0.05$), but not in summer and spring ($p < 0.05$). The relationship was observed in tilapia kidney in autumn with $p = 0.0016$, $r = 0.83$ (fig. 3b) and in common carp liver in winter with $p = 0.016$, $r = 0.63$ (fig. 3c). In accordance with our results, Saliu and Bawa-allah (2012) [19] reported the increase of GSTs activity in fishes exposed to $ZnCl_2$ in comparison with control. Significant relationships between GSTs activity and Zn concentrations in fish stomach was also observed at all sampling sites in the Pote River by (Muposhi *et al*, 2015) [20]. Even though GSTs are not sensitive to low Zn exposure (Liu *et al*, 2005) [21] and Zn is an essential metal of organisms, but in this study, Zn concentration in fish tissues are very high so that it can influence GSTs activity in those tissues which resulted in some correlations of Zn accumulation to GSTs activity (fig. 3b&c). This might be explained by the conclusion that GSTs level was significantly enhanced with dietary Zn levels up to a certain point (Wu *et al*, 2014) [22].

There was only one correlation between Cd concentration and GSTs activity found in common carp liver in winter with $p = 0.012$, $r = 0.65$ (fig. 3d). No such relationships were found in the organs of tilapia and silver carp in all four seasons. The Cd concentration in kidney and liver were much higher than in the gills and muscle in this study because kidney and liver are major targets for Cd accumulation and distribution (Mani *et al*, 2014); although, Cd is firstly absorbed by gills that act as a transient store for Cd accumulation. Cd induced enzymatic defenses that means damage could occur as the enzyme activities are inhibited (Crupkin and Menone, 2012) [23]. The results of this study also showed the higher values of GSTs activity in liver and kidney compared to those in gills because the liver and kidney are particularly rich in GSTs, especially liver (Nimmo, 1987) [24]. Mani *et al* (2014) also showed that during 72 hrs of exposure to Cd (15 mg/l), GSTs in liver and kidney gradually increased and reached the peak of 7.3 ± 0.45 (μM/ min/ mg protein) in liver, 5.7 ± 0.32 (μM/ min/ mg protein) in kidney and then gradually decreased till 96 hrs of exposure, while after 48 hrs of exposure, GSTs level in gills gradually decreased. Significant relationships between GSTs activity and Cd levels in fish stomach were also observed at all sampling sites in the Pote River (Muposhi *et al*, 2015). The correlation between Cd accumulation and GSTs activity in liver of common carp revealed the stronger influence of Cd in common carp compared to other fishes in this river basin. This might be that Cd concentration in some organs of tilapia and silver carp are not high enough and in other organs are too high (tilapia liver, kidney and gill: 0.2, 0.37, 0.026 mg/kg w.wt, respectively; silver carp liver, kidney and gill: 0.05, 0.18, 0.02 mg/kg w.wt, respectively) to induce more production of GSTs for the purpose of detoxification, and as a consequence, no correlation was found for these two fish.

Correlations between Pb accumulation and GSTs activity in fish tissues were found in fishes taken in autumn, winter and summer ($p < 0.05$), but not in spring. Only one correlation between Pb concentration and GSTs activity in liver of silver carp ($p = 0.014$, $r = 0.74$) taken in autumn and one correlation in gills of tilapia taken in summer with $p = 0.013$, $r = 0.62$ were observed (data not shown). However, in common carp collected in winter, two correlations were found in gills and kidney with $p = 0.028$, $r = 0.57$ (fig. 3e) and $p = 0.007$, $r = 0.67$ (fig. 3f), respectively. The study of Awoyemi *et al* (2014) [25] revealed the significant increase of GSTs activity in *C. gariepinus* exposed to Pb. Another research also found that Pb concentration in fish liver can positively impacted GSTs activity (Napierska and Podolska, 2008) [26], while the

expression of this enzyme can be modulated by trace metals, i.e. Hg, Pb and Cu (Korashy and El-Kadi, 2006) [27]. Besides, the change in the specific isoenzyme pattern of GSTs in the livers of chubs exposed to metal pollutants from industrial areas also observed by Lenártová *et al* (2000) [28]. In contrast, Saliu and Bawa-allah (2012) revealed the decrease of GSTs in fishes exposed to $Pb(NO_3)_2$ compared to control. Significant relationships between GSTs activity and Pb concentrations in fish stomach were observed at all sampling sites in the Pote River by Muposhi *et al.* (2015). Results from this study suggested that Pb accumulation in fish tissues affect the expression of GSTs activity in tissues of fishes in the Nhue-Day river basin as the Pb concentrations in fish tissues increase, GSTs activities also increases.

4. Conclusion

In summary, levels of Cu, Zn, Pb and Cd accumulated in fishes in Nhue-Day river basin showed that the water quality of Nhue-Day river is extremely degrading by wastewater from domestic activities of residential areas, industrial zones, craft villages…etc. Some correlations between GSTs activity and metal bioaccumulation in fish tissues taken in Nhue-Day river basin were observed; this proved the impacts of heavy metal accumulation on variation of GSTs activity, especially in kidney and liver. The physiological health of fishes was affected by heavy metal contamination in water as well as by their accumulation in fish tissues.

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Ảnh hưởng của sự tích tụ kim loại nặng lên biến động của hoạt tính enzim glutathione S-transferase (GST) ở một số loài cá kinh tế trong lưu vực sông Nhuệ - Đáy

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Tóm tắt: Mục đích của nghiên cứu này nhằm nghiên cứu những ảnh hưởng của sự tích lũy kim loại lên sự biến động của hoạt tính enzim glutathione S-transferase (GST) trong một số loài cá kinh tế (*Cyprinus carpio L, Hypophthalmichthys molitrix, và Oreochromis niloticus*) trong lưu vực sông Nhuệ-Đáy. Mẫu phân tích được thu bốn lần, từ tháng 9/2012 đến tháng 7/2013. Trong tất cả các loài cá nghiên cứu, các kim loại nặng được tích tụ chủ yếu ở thận và gan theo trình tự sau: Zn> Cu> Pb> Cd. Sự tích tụ của các kim loại trong các mô được xếp theo thứ tự: gan >> thận> mang đối với Cu; Các kim loại Zn, Pb và Cd có kiểu tích tụ tương tự nhau, tích tụ nhiều trong thận hơn trong gan và mang nhưng ở mức độ khác nhau: thận> mang ≥ gan đối với Zn; thận >> gan> mang đối với Pb, và thận> gan >> mang đối với Cd. Hoạt tính của GSTs trong các mô của cá chép, cá mè, cá rô phi tuân theo thứ tự sau: gan> thận> mang. Ảnh hưởng của sự tích lũy sinh học của kim loại nặng đối với sự biến động của hoạt tính GSTs trong mô cá được phản ánh bởi các mối tương quan giữa sự tích tụ sinh học của kim loại nặng trong các mô cá và hoạt tính của GSTs trong các mô tương ứng. Nhìn chung, sự tích tụ kim loại trong các mô cá cho thấy nước sông Nhuệ-Đáy đã bị ô nhiễm kim loại khá nặng nề và điều này ảnh hưởng đến sức khỏe sinh lý của các loài cá, được thể hiện bởi những biến động của hoạt tính GSTs trong mô cá. Các kết quả của nghiên cứu giúp cho việc thiết lập nguồn dữ liệu nền cho việc quản lý nuôi trồng thủy sản và bảo vệ môi trường lưu vực sông Nhuệ-Đáy.

Từ khoá: Lưu vực sông Nhuệ-Đáy, Kim loại nặng, Hoạt tính của GSTs, cá chép, cá mè, cá rô phi.