Effects of Humic Acid and Water Hardness on Acute Toxicity and Accumulation of Cadmium in the Freshwater Fish (*Puntius gonionotus* Bleeker*)*

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ABSTRACT   The effects of humic acid and water hardness on acute cadmium toxicity and accumulation were determined, using *Puntius gonionotus* Bleeker. The toxicities were expressed as the median lethal concentration (LC_{50}) and the 96-h LC_{50} value of cadmium was 2.30 mg/L. Three different types of water hardness were used: soft, moderately-hard and hard, with total hardness concentrations of 55, 113, and 225 mg/L $CaCO_3$, respectively. The acute cadmium toxicity and accumulation significantly decreased by increasing water hardness concentration. An increase in humic acid concentrations (0.5, 5.0, and 50 mg/L) also decreased cadmium toxicity and accumulation. Moreover, simultaneous increases in humic acid and water hardness concentrations also resulted in a decrease in cadmium toxicity. However, they were less effective in decreasing cadmium accumulation than the effect of water hardness or humic acid alone.

KEYWORDS: *Puntius gonionotus*, cadmium, toxicity, accumulation, humic acid, water hardness.

INTRODUCTION

Water hardness is among the well-known factors that modify the toxicity of trace metals in many species of fish (Calamari et al, 1980; Carol et al, 1979; Pascoe et al, 1986). The results from previous studies indicated that the toxicity of metals was hardness-dependent. Cadmium has frequently been shown to be less toxic with an increase in water hardness. Calamari et al (1980) found that the acute toxicity of cadmium to *Salmo gairdneri* increased as the water hardness concentration decreased. They also reported that the addition of calcium hardness decreased the cadmium toxicity in brook trout, *Salvelinus fontinalis.* Furthermore, Pascoe et al (1986), using rainbow trout, reported that cadmium was less toxic in hard water. Davies et al (1980) also reported that hardness reduced the acute toxicity of cadmium to the rainbow trout, *Oncorhynchus mykiss,* through an antagonistic mechanism. They concluded that the antagonistic properties of hardness were primarily controlled by calcium, while magnesium played a minor role. Borgmann et al (1991) reported that a ten-fold reduction in hardness caused a two-fold decrease in the 6-week EC_{50} of cadmium in *Hyalella azteca.*

Unlike cadmium, reports of the influence of humic acid on cadmium toxicity are contradictory. Stackhouse and Benson (1988) suggested that the acute toxicity of cadmium was increased, decreased, or not influenced by humic acid depending on humic acid concentrations and the time-point examined. They also found that humic acid reduced cadmium accumulation in barnacle, *Semibalanus balanoides*, over a 7-day exposure period, but had no effects over 15- or 30-day exposure periods. Moreover, Penttinen et al (1995) reported that the uptake rate of cadmium in *D. magna* was two times faster in humic lake water than in humic-free water.   Thus, it was concluded that the acute toxicity of cadmium was enhanced by humic acid.

In contrast, Hansten et al (1996) reported that humic acid decreased cadmium toxicity in the clam, *Anodonta anatina.* Hollis et al (1996) also suggested that the addition of dissolved organic carbon decreased cadmium toxicity in small rainbow trout (*O. mykiss*) and metal-dissolved organic carbon complexes did not become appreciably stronger with time.

The interactive effects of humic acid and water hardness on cadmium toxicity are still unclear. Winner (1984) found that 0.75 mg/L of humic acid

had no effects on chronic toxicity of cadmium to *Daphnia magna* in either moderately-hard and hard water, while 1.50 mg/L of humic acid resulted in an increase in the cadmium chronic toxicity. Similarly, Winner and Gause (1986) also found that humic acid in soft, medium, and hard water increased chronic cadmium toxicity to *D. magna*. However, Penttinen et al (1995) reported that the uptake rate of cadmium in humic-lake water by *D. magna* was almost equal in water hardness of 0.1 and 0.5 mM/L; a further increase of water hardness from 0.5 to 1.5 mM/L decreased the uptake rate by 80%. Thus, an increase in water hardness in humic-lake water resulted in a decrease in cadmium chronic toxicity in *D. magna*.

It appears that the roles of humic acid and water hardness and the mechanisms involved in modifying cadmium toxicity are not clearly understood. Therefore, the roles of humic acid and water hardness on cadmium toxicity and accumulation were investigated in the present study.

MATERIALS AND METHODS

Experimental waters

Each assay was conducted in a glass aquarium (30 cm x 30 cm x 25 cm) containing five liters of dilution water. The dilution water was dechlorinated tap water which had been aerated overnight. Cadmium used in these experiments was of a reagent grade $CdCl_2.H_2O$. A stock solution of cadmium was freshly prepared in distilled water for each experiment. Experimental solutions were then prepared by diluting the stock solution to the required concentrations. Humic acid, obtained from Aldrich Chemical Company, was prepared by dissolving it in distilled water and the concentration was expressed as ash-free dry weight. The stock solution of humic acid was then filtered through a 1.2 μm membrane filter to remove remaining particulates before use (Brockaert et al, 1989). The final humic acid concentrations used were 0.5, 5.0, and 50 mg/L. Three different levels of water hardness, designated as soft, moderately-hard, and hard water, with the average total hardness concentrations of 55, 113, and 225 mg/L as $CaCO_3$, respectively, were used. The hardness concentrations were tested by the EDTA titration method (APHA, 1995). Control is the experiment with no humic acid or hardness added. The experimental water temperature was maintained at around 25°C throughout the study.

Experimental animals

Juvenile common silver barb (45 days old), *Puntius gonionotus* Bleeker, with an average length of 3.21 ± 0.24 cm and an average weight of 0.47 ± 0.19 g, were obtained from a local hatchery of the Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. They were kept in stock tanks filled with local tapwater, which was aerated vigorously for about three days before use. Prior to performing each experiment, they were acclimated to laboratory conditions for at least seven days. During the acclimatization period, they were fed daily with commercial fish feed. The experimental fish were starved two days prior to use. Excess food and metabolic wastes were removed from the aquaria every day by siphoning. The fish were observed closely and frequently for signs of stress, unusual behavior, parasites or diseases, changes in color, or failure to eat. Dead and abnormal individuals were immediately removed.

Cadmium toxicity

The acute toxicity of cadmium was determined by median lethal concentration (LC_{50}) bioassays using *P. gonionotus*. For each experiment, a preliminary assay consisting of five concentrations (1, 2, 4, 8, and 16 mg Cd/L) was first conducted to establish the critical range of cadmium toxicity. Then, the definitive assay was performed to determine the concentration at which a particular end point occurred. This assay served as the control for the other experiments. Ten randomly selected fish from an acclimated stock tank were simultaneously exposed to each concentration for 96 hours without aeration or feeding. Each assay was replicated three times. Fish were checked daily for mortality. Dead fish were removed as soon as observed and recorded.

The first series of experiments was set up to study the cadmium toxicity. After acclimatization, ten randomly selected fish were simultaneously exposed to each of five cadmium concentrations (1, 2, 4, 8, and 16 mg/L) and a control for 96 hours without aeration or feeding. In the second series of experiments, the effects of water hardness concentrations on the acute cadmium toxicity were evaluated. Three water hardness concentrations (soft, moderately-hard and hard) were prepared by adding the hardness ions as described previously.

In the third series of experiments, the influence of humic acid on acute cadmium toxicity was assessed using three humic acid concentrations (0.5, 5.0, and 50 mg/L).

In the fourth series of experiments, the interactive effects of humic acid and water hardness on acute cadmium toxicity were determined. The experiments on the combination of three water hardness concentrations (soft, moderately-hard and hard) and three humic acid concentrations (0.5, 5.0, and 50 mg/L) were performed in order to investigate the combined effects of humic acid and water hardness concentrations on the cadmium toxicity. Tested fish were simultaneously exposed to different combinations of humic acid and three water hardness concentrations as described earlier.

During the experimental period, the behavior and physiology of the tested fish were also observed. Measurements of the length and weight of each fish were also performed after each experiment. For any experiment, if the death of untreated (control) fish exceeded 10%, the experiment was repeated.

Data analysis

The acute toxicity of cadmium was determined as the median lethal concentration (LC_{50}), with the 95% confidence intervals, using the Probit Analysis Program (Raymond, 1987). The 96-h LC_{50} values of cadmium in each concentration of water hardness, humic acid, and the combination of each concentration of water hardness and humic acid, were compared with the 96-h LC_{50} value of cadmium alone by calculating 95% confidence intervals for the difference between each pair of means as described by Finney (1971). Comparisons between the interactive effects of humic acid and water hardness on acute cadmium toxicity and the effect of humic acid or water hardness alone were also performed. The least-square regression equation and coefficient of determination were calculated for the effects of each concentration of water hardness, humic acid, and the combination of each concentration of water hardness and humic acid on acute cadmium toxicity.

Cadmium accumulation

Four sets of experiments were conducted by using the same procedures as those described previously in sections 2.1 and 2.2. Ten randomly selected fish were exposed to the 96-h LC_{50} value of cadmium (2.3 mg/L) in each experimental condition. Cadmium accumulation was determined at different time intervals (1, 2, 3, and 4 days). In the first set of experiments, the rates of cadmium accumulation were determined. The results of this experiment formed the control for other experiments. In the second set of experiments, the cadmium accumulation in fish exposed to three water types (soft, moderately-

hard, and hard water) were determined. Similarly, in the third set of experiments, the cadmium accumulation was also determined in fish exposed to three different concentrations of humic acid which were 0.5, 5.0, and 50 mgHA/L (HA = humic acid). In the fourth set of experiments, the interactive effects of humic acid (0.5, 5.0, and 50 mgHA/L) and water hardness (soft moderately-hard, and hard) concentrations on cadmium accumulation were evaluated. All four sets of experiments were performed in triplicate.

After the exposure of fish to cadmium, the fish were removed at each selected time interval and rinsed three times with distilled water. Thereafter, they were prepared for whole-body cadmium residue analysis by using the nitric acid digestion method (APHA, 1995). After digestion, cadmium concentration was determined using a flameless atomic absorption spectrophotometer (Spectral AA Model 640). The results of the accumulation experiments were reported as concentration of cadmium per weight of fish (μg Cd/g wet weight of fish).

RESULTS

Cadmium toxicity
Acute toxicity of cadmium

During the experimental period, the swimming behaviors of *P. gonionotus* were observed after being exposed to cadmium to provide an index of sublethal toxicity and subsequent mortality. First, the speed in movement of the fish decreased. Then, the swimming posture changed from normal to head-up swimming or, sometimes, spiral swimming due to the loss in equilibrium. Thereafter, most fish became hypoactive and remained at the bottom of the container. Finally, the fish convulsed and consequently died. Dead fish were rapidly removed and recorded.

The acute toxicity of cadmium was then determined from the dose-response curve over the 96-hour exposure time (Fig 1). The LC_{50} values for 24, 48, 72, and 96 hours of exposure were 2.74, 2.53, 2.44, and 2.30 mg/L, respectively. The 96-h LC_{50} value of cadmium (2.3 mg/L) was used as a control in the comparisons of the means from the experiments on combination effects.

Effects of water hardness on cadmium toxicity

Increases in water hardness concentrations resulted in a statistically significant decrease in the LC_{50} value of the acute toxicity of cadmium ($P<0.05$). The LC_{50} values were 4.17, 4.35, and 5.05 mg/L for soft, moderately-hard and hard water, respectively (Fig 2).

Effects of humic acid on cadmium toxicity

It appeared that cadmium toxicity decreased as humic acid concentration increased. The addition of 0.5 mg/L of humic acid resulted in an increase in the LC_{50} values from 2.30, for the control, to 3.04 mg Cd/L (Fig 3). A further increase of humic acid to 5.0 and 50 mg/L resulted in an increase in the LC_{50} values to 4.17 and 4.33 mg Cd/L, respectively
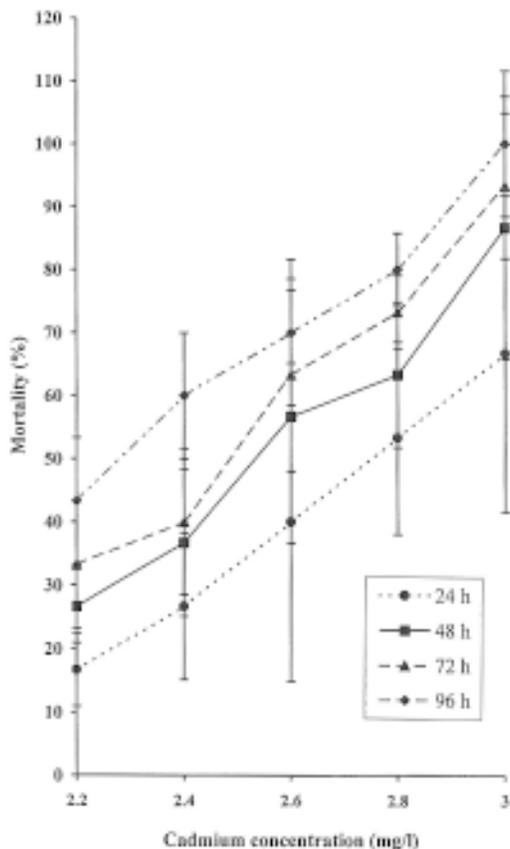
(Fig 3). The LC_{50} of the treatments with 5.0 and 50 mg/L humic acid was statistically greater than that of the control ($P<0.05$). However, with 0.5 mg/L of humic acid, there was no statistically difference between the experimental and control groups ($P>0.05$).

Interactive effects of humic acid and water hardness on cadmium toxicity

An increase in humic acid concentration for each water hardness concentration resulted in an increase in the 96-h LC_{50} value. Moreover, an increase in water hardness concentration for each humic acid concentration also resulted in an increase in the 96-h LC_{50} value of cadmium (Table 1).

The 96-h LC50 value of each combination of water hardness and humic acid concentration was compared with that of the control (2.30 mg/L; cadmium alone), using 95% confidence intervals. In soft water, humic acid at 50 mg HA/L resulted in a

Fig 1. The dose-response curves of *Puntius gonionotus* exposed to cadmium for different time periods.

Table 1. Interactive effects of water hardness and humic acid on the 96-h LC_{50} of cadmium in *Puntius gonionotus*.

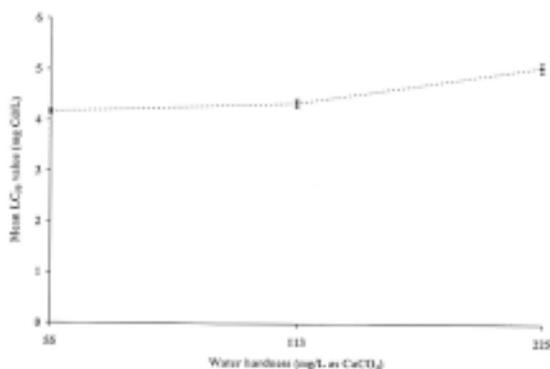| Water hardness concentration (mg/L as CaCO₃)	96-h LC_{50} of cadmium with 95% confidence interval (mg/L)			
	Humic acid concentration (mg/L)			
	0	0.5	5.0	50
Control	2.30	3.04	4.17	4.33
	(2.19-2.40)	(2.81-3.24)	(3.98-4.53)	(4.10-4.54)
Soft	4.17	2.43	2.84	3.32
	(4.01-4.53)	(1.96-2.73)	(2.67-2.96)	(3.20-3.50)
Moderately-hard	4.35	2.71	2.92	3.35
	(4.14-5.15)	(2.57-2.83)	(2.56-3.17)	(3.05-3.50)
Hard	5.05	3.05	3.32	3.49
	(4.87-5.21)	(2.80-3.19)	(3.13-3.48)	(3.33-3.62)

Fig 2. Mean 96-h LC_{50} values for *Puntius gonionotus* exposed to cadmium in soft, moderately-hard, and hard water (55, 113, and 225 mg/L as CaCO₃).
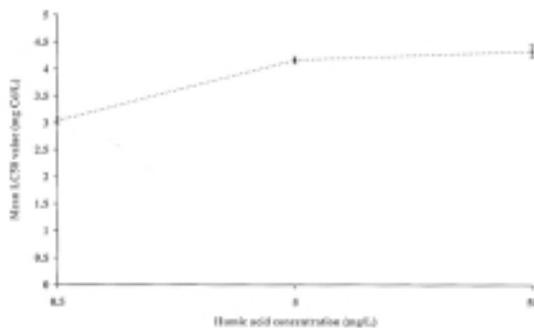

Fig 3. Mean 96-h LC_{50} values for *Puntius gonionotus* exposed to cadmium with the addition of humic acid (0.5, 5.0, and 50 mg/L).

statistically significant increase in the 96-h LC_{50} value (3.32 mg Cd/L) (P<0.05). However, with 0.5 and 5.0 mg/L of humic acid, the 96-h LC_{50} values (2.43 and 2.84 mg/L, respectively) increased but did not significantly differ from those of the control (P>0.05). In moderately-hard water, humic acid at 0.5 and 5.0 mg HA/L (LC_{50} of 2.71 and 2.92 mg Cd/L, respectively) did not have any significant effect on the 96-h LC_{50} values (P>0.05). Conversely, 50 mg HA/L caused a statistically significant increase in the LC_{50} value to 3.35 g/L (P<0.05). In hard water and at 0.5 mg/L of humic acid, the increase in the LC_{50} value (3.05 mg/L) was not statistically significant (P>0.05). In contrast, with 5.0 and 50 mg/L of humic acid (LC_{50} = 3.32 and 3.49 mgCd/L, respectively), the increases in the LC_{50} values were statistically significant (P<0.05).

The least square regression equations were also calculated for each of the relationships between water hardness concentrations to cadmium toxicity and humic acid concentration to cadmium toxicity. It appeared that the degree of correlation between water hardness concentration and the LC_{50} value of cadmium ($R^2 = 0.89$) was very close to the correlation between humic acid and the LC_{50} value of cadmium ($R^2 = 0.84$). Fig 4 illustrates the relationship between the combined effects of each water hardness with each humic acid concentration on the LC_{50} value of cadmium. A combination of the three water hardness concentrations and 0.5 mg/L of humic acid resulted in the perfect correlation (R^2 value of 1.0), while combinations of the three water hardness concentrations and 5.0 and 50 mg/L of humic acid resulted in the R^2 values of 0.87 and 0.8, respectively.

Cadmium accumulation

Sensitivity of *Puntius gonionotus* to cadmium

The rate of cadmium accumulation was high during the first 12-hour exposure period (Fig 5), after which cadmium accumulation slowly increased until it reached 72-hour exposure and then flattened off slightly.

Effects of water hardness on cadmium accumulation

The accumulation of cadmium in all water types was significantly decreased with time as compared to the water with no hardness ions added. The relationship between the exposure time and the amount of cadmium accumulation in all water hardness concentrations were negative (Fig 6). The results in Table 2 also show that increasing water hardness concentrations decreased cadmium concentration accumulation. There is some relationship between cadmium toxicity and its accumulation. The cadmium accumulation found in fish was less in the conditions that made it more toxic than in the conditions where it was less toxic. For example, when humic acid was 0.5 mg/L the cadmium ac-
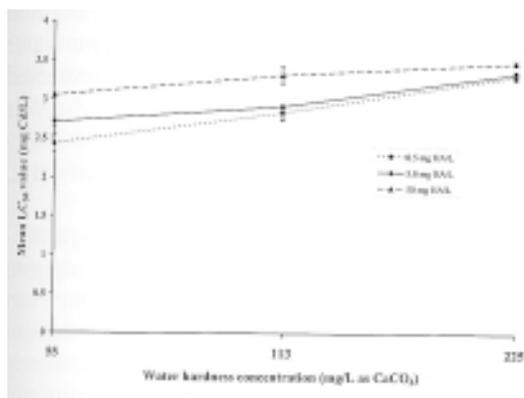

Fig 4. Mean 96-h LC_{50} values showing the combined effects of water hardness and humic acid concentrations to the toxicity of cadmium to *Puntius gonionotus*.
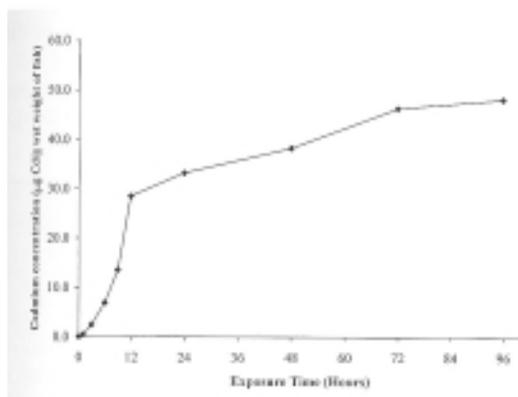

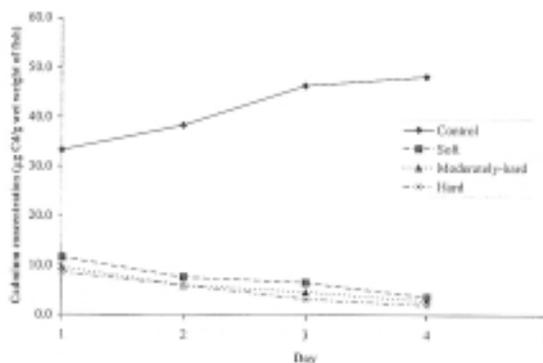**Fig 5.** Cadmium accumulation in *Puntius gonionotus* up to 96 hours.

Fig 6. Influence of water hardness concentration on cadmium accumulation.

cumulation at day 1 were 2.65, 2.72 and 5.40 μg/g wet weight of fish for soft, moderately-hard and hard water, respectively. However, the concentration of cadmium accumulation decreased as time increased.

Increasing concentration of humic acid also increased pHs in all three water hardnesses.

Effects of humic acid on cadmium accumulation

The results showed that there was a negative relationship between the exposure time and cadmium accumulation in all three humic acid concentrations (Fig 7).

Interactive effects of humic acid and water hardness on cadmium accumulation

Table 2 illustrates the interactive effects of humic acid and water hardness on cadmium accumulation in *P. gonionotus.* It appears that the accumulation of cadmium in each condition was different. There was no consistent relationship between the exposure time and cadmium accumulation.

Discussion

There was a negative relationship between water hardness concentration and cadmium toxicity. An increase in water hardness concentration significantly increased the LC_{50} values of cadmium. A reasonable

Fig 7. Influence of humic acid on cadmium accumulation.

explanation is that since fish skin and gills are in direct contact with water body and dissolved substances, they are potential uptake sites of dissolved substances. However, the skin is nearly impermeable to both water and dissolved substances, and so the uptake mechanism via this route seems to be of minor importance (Payan et al, 1981). Many researchers have accepted that cadmium uptake in freshwater fish occurs mainly via gills (Winner and Gause, 1986). There is also some evidence that indicates that after exposure to cadmium, fish gills were structurally damaged and hypoplasia occurred, including high mucus excretion (Part and Svanberg, 1981). This implies that the gills have a large effec-tively irrigated area serving as the main uptake site for cadmium. The water hardness ions, mainly calcium and magnesium ions, however, may have an influence on branchial permeability to cadmium uptake, probably by competing with cadmium ions for available binding sites. Additional mechanisms responsible for this action are also expected to be detoxification processes, such as physiological responses or the biological mechanisms to defend themselves, thereby reducing cadmium uptake and toxicity in fish. Hence, the water hardness ions, especially calcium ions, protected them from lethal effects. These results are similar to those found for brook trout (Carrol et al, 1979) and rainbow trout (Bradley et al, 1985; Davies et al, 1993).

The present study also shows that a high concentration of humic acid decreased the cadmium toxicity. There are possible mechanisms which can explain how humic acid may decrease cadmium toxicity in *P. gonionotus.* First, humic acid may modify the chemical speciation of cadmium and result in a decrease of cadmium bioavailability. Second, interference of humic acid with cell membrane permeability may cause a decrease in cadmium uptake. Third, it is certainly well established that many of the functional groups identified as com-
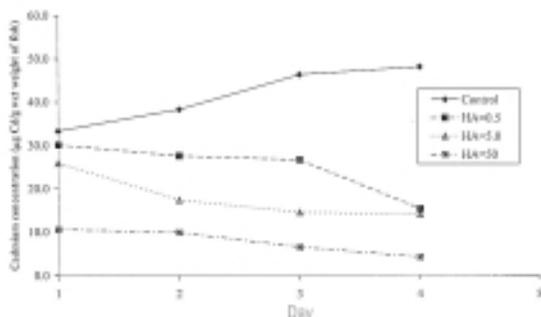
Table 2. Influence of humic acid concentration on the accumulation of cadmium. Cadmium accumulation was determined as mg Cd/g wet weight of *Puntius gonionotus* after exposed to the 96-h LC_{50} (2.3 mg Cd/L). The three hardness levels are S = soft water, MH = moderately - hard water, and H = hard water.

Exposure Time (days)	Humic acid concentration (mg/L)											
	0			0.5			5.0			50		
	S	MH	H	S	MH	H	S	MH	H	S	MH	H
1	11.55	9.56	8.54	2.65	2.72	5.40	3.71	3.15	5.19	6.72	2.08	4.22
2	7.58	5.97	5.91	2.95	1.67	5.10	2.57	2.60	3.44	4.58	2.63	3.98
3	6.53	4.66	3.21	5.18	2.57	2,91	2.23	2.86	3.06	2.76	3.97	2.70
4	3.59	2.77	1.91	3.23	2.31	1.71	3.28	2.13	1.26	3.53	2.28	1.51

ponents of humic acid contain suitable atoms, particularly nitrogen and oxygen, which are capable of acting as ligands for cadmium (Stackhouse and Benson, 1988). Presumably, the mechanism by which humic acid decreases cadmium toxicity may be because of the success of humic acid in forming complexes with cadmium ions through the removal of a proton from either carboxylic acid or hydroxyl functional groups followed by a bond formation with cadmium ions. The formation of cadmium-humic complexes results in the precipitation of the complexes and reduces the free cadmium ions available for the fish. Such interactions of humic acid and cadmium ions may thereby decrease cadmium toxicity to *P. gonionotus.*

The results on the effect of humic acid on cadmium toxicity in *P. gonionotus* agree with those from the clam, *A. anatina* (Hamsten et al, 1996), rainbow trout (*O. mykiss*) (Hollis et al, 1996), and barnacle (*S. balanoides*) (Stackhouse and Benson, 1988). However, they disagreed with those of Winner (1984), Winner and Gause (1986) and Penttinen et al (1995) who reported that humic acid increased the cadmium toxicity in *D. magna*. Hence, the influence of humic acid on cadmium toxicity may vary with the tested species, possibly depending on the specific detoxification mechanism or immune response of the tested species.

Comparisons of toxicity effects among combinations of humic acid and water hardness were also performed. Interestingly, it was apparent that the combined effects of soft water with 50 mg/L of humic acid, moderately-hard water with 50 mg/L of humic acid, and hard water with 5.0 and 50 mg/L of humic acid resulted in a statistically significant increase in the LC_{50} values of cadmium when compared to that of cadmium alone. It is possible that these resulted from competition between Ca^{2+} and Cd^{2+} for the binding sites of humic acid (Part and Svanberg, 1981; Zitko and Carson, 1976; Wicklund and Runn, 1988; Markich and Jeffree, 1994). In soft and moderately-hard water, Ca^{2+} might not compete with Cd^{2+}. At 50 mg/L of humic acid, Cd^{2+} was effective in forming complexes with humic acid. In hard water, the high concentrations of Ca^{2+} might increase the soluble concentration of Cd^{2+} by replacing Cd^{2+} from its complexes with humic acid. Hence, the high concentration of humic acid at 5.0 and 50 mg/L resulted in a significantly increase in the LC_{50} values of cadmium.

The accumulation of cadmium within 96 hours was evaluated in this study. The results from the sensitivity test of *P. gonionotus* to cadmium showed that the rate of cadmium accumulation significantly increased during the first 12-hour exposure period, then the rate was slower until 96 hours of exposure. This indicates that the rate of cadmium accumulation is approaching the maximum limit.

There was a negative relationship between water hardness concentrations and cadmium accumulation. An increase in water hardness concentration resulted in a reduction of cadmium accumulation throughout the exposure time. Similarly, an increase of humic acid also resulted in a reduction of cadmium accumulation throughout the exposure time. Both experimental results can be explained as previously described. The Ca^{2+} ion may compete significantly for Cd^{2+} binding sites in fish. Hence, the reduction of cadmium accumulation resulted in a decrease of cadmium toxicity in *P. gonionotus*, and these data corresponded with the toxicological data.

Conversely, when humic acid and water hardness, were combined, the cadmium accumulation did not decrease throughout the exposure time. It sometimes decreased or increased during the exposure time, but still decreased from the control on the fourth day of exposure. However the accumulation of cadmium appeared to depend on the selected time interval examined and the humic acid concentration in exposure water. These data may indicate the mechanisms responsible for cadmium accumulation in *P. gonionotus.* There are several possible explanations for describing the pattern in the whole-body cadmium accumulation during the exposure period. First, it is possible that the fraction of the total accumulated cadmium is concentrated in some target organs that can significantly alter the cadmium concentration. Second, it is possible that much of the accumulated cadmium is in some non-toxic form, like being complexed with a metabolically inactive protein, such as metallothionein (Winner and Gause, 1986). There is a considerable support for a protective role of metallothionein in cadmium poisoning. Torres et al (1997) reported that cadmium tolerance in a diatom *Phaeodactylum tricornutum* was due to the ability of this diatom to synthesize class III metallothionein, which can bind to cadmium ions by thiolate coordination, thus yielding intracellular cadmium-metallothionein complexes that contribute to the detoxification process. Similarly, Roesijadi et al (1997) found that the exposure of *Crassostrea virginica* to cadmium influenced the metallothionein concentration. Furthermore, the expression of the metallothionein gene was also associated with the ability to resist cadmium toxicity. Therefore, this protein provides an effective attenuation of cadmium

toxicity. Thirdly, the disparity may be due to the direct change in the physiological response with respect to the ions in the exposure water (Torres et al, 1997). This process may prevent an initial adsorption on the gill surface by reducing either the ambient free cadmium ion concentration, or the number of surface binding sites on the gill, thus resulting in the lower toxicity of cadmium.

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