Kasetsart J. (Nat. Sci.) 41 : 180 - 185 (2007)

Bioaccumulation of Cadmium in an Experimental Aquatic Ecosystem Involving Phytoplankton, Zooplankton, Catfish and Sediment

Suneerat Raungsomboon[1]* and Ladda Wongrat[2]

ABSTRACT

The accumulation of cadmium (Cd) was studied in an experimental aquatic ecosystem involving the phytoplankton, *Chlorella regularis*, the zooplankton, *Moina macrocopa*, and the catfish, (cross bridge *Clarias macrocepharus* X *Clarias gariepinus*), and sediment. All organisms were selected in this study because of their economically importance. The accumulation of cadmium was mainly on phytoplankton. The cadmium concentration in all organisms increased with increasing cadmium concentration in the ecosystem. When the input cadmium concentration was 3.5 mg l^{-1}, cadmium accumulated in the surface of sediment (0-1 cm.), phytoplankton, zooplankton, and catfish were 5.53±0.26, 586.18±23.37, 141.52±26.74 and 10.29±0.47 µg g^{-1} (dry wt.), respectively. Results showed that phytoplankton, zooplankton, and catfish exposed to cadmium represents a risk of secondary poisoning for their predators and such a risk could affect humans. The accumulation of cadmium in sediment may have an effect on benthic organisms.

Key words: aquatic ecosystem, cadmium, phytoplankton, zooplankton, catfish

INTRODUCTION

Contamination of heavy metals when released into aquatic environments caused toxic effects. Sensitive species may be impaired by sublethal effects or decimated by lethality, and this ecological alteration may initiate a trophic cascade or a release from competition that secondarily leads to responses in tolerant species (Fleeger *et al*., 2003.)

The presence of heavy metals in the environment is partially due to natural process, but is mostly the result of industrial waste (Mansour and Sidky, 2002). Contamination of aquatic ecosystems with heavy metal has increased worldwide. Cadmium (Cd) is a toxic heavy metal, which usually contaminates the aquatic ecosystem. It has many industrial purposes such as, cadmium batteries, anti-corrosive coating of metals, pigments, and as stabilizers for plastic (Stoeppler, 1991). The amount of cadmium used in the industry increases as the demand for its use increases. The presence of cadmium in different foods constitutes serious health hazards, depending on their relative levels. For example, a high amount of cadmium accumulated in the human body could injure the kidney, the liver, and cause symptoms of chronic toxicity, including impaired kidney function, tumors and hepatic dysfunction (Mansour and Sidky, 2002).

[1] Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
[2] Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: krsuneer@kmitl.ac.th

Received date : 18/04/05 Accepted date : 30/06/05

When cadmium contaminates the aquatic ecosystem, accumulations of cadmium in aquatic organisms are possible. Cadmium could enter the aquatic food chain through direct consumption of water or biota; and through non-dietary routes, such as absorption through epithelia. For fish, the gills, skin, and digestive tract are potential sites of absorption of cadmium in water.

Accumulation of cadmium in aquatic organisms varies with the intensity and duration of exposure to cadmium, and frequently studied, in part, because predictive criteria to estimate risk and establish permissible levels of contamination are based on species response to contaminants (Long *et al*., 1995). However, these accumulations are derived from laboratory tests that usually employ single species of organisms, which can not represent the simultaneous accumulation of all organisms in the ecosystem.

The aim of this study was to evaluate the accumulation of cadmium in an experimental aquatic ecosystem involving phytoplankton, zooplankton, and fish, which are important to fisheries. Cadmium concentrations at 0.35 and 3.50 mg l^{-1} were tested in this study. These concentrations represent the safety and LC_{50} value for catfish, which derived from preliminary test of this experiment.

MATERIALS AND METHODS

Preparation of phytoplankton

Chlorella regularis was axenic-cultured in Chlorella Medium (without EDTA) (Becker, 1994) under continuous illumination of 300 µE. $m^{-2}.s^{-1}$ at 25°C. Ten-day-cultured cells (late exponential phase) were harvested and used in the cadmium accumulation experiments. Upon harvesting, *C. regularis* was washed 3 times with sterile, deionized water and separated by centrifugation at 3,500 rpm for 5 minutes at 4°C. Cadmium contaminated in *C. regularis* was analyzed for use in the cadmium accumulation experiments.

Preparation of zooplankton

Moina macrocopa was cultured in sterile deionized under 12:12 h light and dark period at 25°C and fed with non-contaminated *C. regularis*. The adult *M. macrocopa* was carefully harvested (filter through 20 µm nylon net) for use in the cadmium accumulation experiments.

Preparation of catfish

Catfishes (hybrid catfish: *Clarias macrocepharus* x *Clarias gariepinus*) weighing 7.48 ± 0.10 g (X±S.E.) were cultured in 50 l indoor aquaria with unchlorinated and aerated water 25° C under 12:12 h light and dark period. They were fed with non-contaminated *M. macrocopa*.

Acclimation of tested organism: Organisms were transferred to 50 l acrylic container in the laboratory and maintained at 25° C. They were acclimated in the laboratory for 96 h before testing.

Preparation of sediment

Prior to this study the sediment collected from fish pond at Department of Fisheries Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang was cleaned by soaking with 10% HNO_3 for 2 nights and washed by sterile deionized water. These were done 3 times. The pH of sediment was adjusted to 6 using the sterile deionized water at pH 4, and then sediment was dried in the open air.

Preliminary test

To study the toxicity of cadmium to catfish, 96-h acute toxicity tests were conducted on catfish weighting 7.34 ± 0.45 using standard techniques developed by Lassus *et al.* (1984). The test of LC_{50} was performed at cadmium concentration 0.1-5.0 mg l^{-1}, for each test, three replicates of 30 catfish were exposed to each treatment. Each group of catfish was monitored daily for mortality. Preliminary tests were conducted to establish a mortality range at 1-100%. LC_{50} values were computed using probit analysis (Forget *et al.*, 1998). The results show that the 96 h LC_{50} and safety values for catfish was 3.5 and 0.35 mg l^{-1} cadmium. Since these cadmium concentrations were LC_{50} and safety values, these concentrations were selected for further experiment.

Accumulation of cadmium in an experimental aquatic ecosystem

To study the accumulation of cadmium, modified techniques from Scheifler *et al*. (2002) were used. Fifteen grams wet weight of *C. regularis*, and *M. macrocopa*, five fish (average weight 7.48 ± 1.10 g) and 1.5 l of cadmium solution 0.35 and 3.5 mg l^{-1} were placed in the indoor acrylic tanks containing the 10 cm height of non cadmium contaminated sediment. Each tank was divided into three sections using 0.2 µm membrane filters, each section was placed with one type of organism in order to prevent any consumption. The tanks were left for 72 h, then the residual cadmium concentrations in water were measured. Each organism was separated and dried at 100°C for 24 h, cadmium concentrations were determined. Because all organisms were not fed during the experiment, to prevent the mortality from undernourished the accumulated cadmium was determined after 72 h. The experiments were carried out in triplicate.

Analysis of cadmium samples

The stock standard solution of cadmium was obtained from Merck in concentration of 1,000 ± 0.2 mg l^{-1}. An atomic absorption spectrophotometer (GBC Avanta, Australia) was used for the analysis. The detection limit was 1 µg l^{-1}, using graphite furnace methods.

The analyzed samples were prepared according to methods prescribed by the Association of Official Analytical Chemists (AOAC, 2000). In the *C. regularis* and *M. macrocopa* analyses, whole body parts were examined. The analyses involved fish muscle samples only. In the sediment analyses, each depth was divided and examined. Prior to the study, cadmium concentrations in all organisms and sediment were determined.

RESULTS

The accumulations of cadmium in aquatic organisms and the sediment are shown in Table 1. Cadmium accumulation in all organisms and sediment of prior to the study was non-detected. After 72 h of cadmium exposure, the concentrations of cadmium accumulated in all organisms increased when the input cadmium solutions were increased from 0.35 to 3.5 mg l^{-1}. The highest cadmium accumulation was found in *C. regularis*, 81.60 ± 14.78 µg cadmium g^{-1} dry

Table 1 The cadmium accumulated in an experimental aquatic ecosystem. Data are expressed as means ± S.E (n=9).

Cadmium accumulation	Initial cadmium (mg l^{-1}) in water	
	0.35	3.5
Residual cadmium in water (mg l^{-1})	0.01 ± 0.01	0.05 ± 0.02
C. regularis (µg g^{-1} dry wt.)	81.60 ± 14.78	586.18 ± 23.37
M. macrocopa (µg g^{-1} dry wt.)	44.02 ± 2.63	141.52 ± 26.74
catfish (µg g^{-1} wet wt.)	1.54 ± 0.05	1.98 ± 0.15
(µg g^{-1} dry wt.)	8.00 ± 0.18	10.29 ± 0.47
sediment (0-1 cm.) (µg g^{-1} dry wt.)	1.27 ± 0.10	5.53 ± 0.26

weight in cadmium 0.35 mg l^{-1} and 586.18 ± 23.37 μg cadmium g^{-1} dry weight in cadmium 3.50 mg l^{-1}. The accumulations of cadmium in *C. regularis* were 1.8 (initial cadmium 0.35 mg l^{-1}) and 4.2 (initial cadmium 3.50 mg l^{-1}) times higher than the accumulations in *M. macrocopa*, 64.3 (initial cadmium 0.35 mg l^{-1}) and 106.0 (initial cadmium 3.50 mg l^{-1}) times higher than in the sediment, and 10.2 (initial cadmium 0.35 mg l^{-1}) and 57.0 (initial cadmium 3.50 mg l^{-1}) times higher than in the catfish.

After organisms were exposed to 0.35 and 3.50 mg l^{-1} cadmium, the residual cadmium in solution was only 0.01±0.01 and 0.05±0.02 mg l^{-1}. *C. regularis* exposed to cadmium 3.50 mg l^{-1} contained 586.18 ± 23.37 μg cadmium g^{-1} dry weight, which was 7.2 times higher than that exposed to cadmium 0.35 mg l^{-1}. *M. macrocopa* exposed to cadmium 3.50 mg l^{-1} contained 141.52 ± 26.74 μg cadmium g^{-1} dry weight which was 3.5 times higher than that exposed to cadmium 0.35 mg l^{-1}. Cadmium accumulated in the catfish muscle that exposed to cadmium 3.50 mg l^{-1} was 1.98 ± 0.15 μg cadmium g^{-1} wet weight, which was 1.3 times higher than that exposed to cadmium 0.35 mg l^{-1}.

The accumulation of cadmium in sediment at various depths was shown in Table 2. When cadmium concentration in water was increased, cadmium accumulation in the sediment was increased. The accumulations in the surface of sediment were 1.27±0.10 and 5.54±0.2 μg cadmium g^{-1} (dry wt.) after exposed to cadmium 0.35 and 3.5 mg l^{-1} (Table 2). Cadmium accumulated in the sediment was decreased when the sediment depth was increased.

Cadmium accumulated in the sediment at 8-10 cm was 0.21±0.03 and 0.04±0.03 μg cadmium g^{-1} dry weight when exposed to 0.35 and 3.5 mg l^{-1} cadmium respectively. Cadmium accumulated in the surface of sediment (0-1 cm) was 146 times higher than in the bottom (8-10 cm), when the initial cadmium concentration was 3.50 mg l^{-1}.

DISCUSSION

Normally, sediment has long been recognized as a potential route for trapping a variety of contaminants, especially for metal ions (Cheggour *et al*., 2001). In this study, *C. regularis* showed the highest sorption ability. This probably due to *C. regularis* suspends in the water volume, therefore, it has higher surface areas to expose to cadmium solution while only the surface of sediment could expose to the water.

C. regularis could accumulate cadmium in their cells because, its exterior contains proteins and different carbohydrates, which the metal ions could react (Volesky, 1990). The uptake of heavy metals by phytoplankton occurs in two stages: short-term uptake involves physical sorption on the cell surface: and long-term uptake involves intracellular accumulation (Khummongkol *et al*.,

Table 2 The accumulation of cadmium at various sediment depth. Data are expressed as means ± S.E (n=9).

Depth (cm.)	Cadmium (μg g^{-1} dry wt) Initial cadmium 0.35 mg l^{-1}	Initial cadmium 3.5 mg l^{-1}
0-1	1.27±0.10	5.54±0.26
1-2	1.09±0.09	0.83±0.13
2-4	0.46±0.04	0.57±0.27
4-6	0.23±0.10	0.42±0.37
6-8	0.22±0.05	0.05±0.73
8-10	0.21±0.03	0.04±0.03

1982). It was reported that the marine microalgae: *Chlorella* sp. exposed to cadmium solution 50 μM for 12 days accumulated cadmium both on cell surface and more than 70% in cell (Matsunaga *et al*., 1999). In this study it was indicated that *C. regularis* is able to accumulate high amount of cadmium. Thus in the natural water bodies, when cadmium is input, cadmium would be mostly accumulated in phytoplankton cell, due to the highest sorption ability compared to other organisms in this study. Because phytoplankton is the primary producer of aquatic ecosystem, therefore, the sorption of cadmium therefore, could be transferred to higher trophic levels, for example zooplankton, mollusks, and herbivorous fish.

M. macrocopa accumulated relatively high amount of cadmium. The accumulation of cadmium in *M. macrocopa* in this experiment occurred through the adsorption mechanism on their carapaces. The carapace of *M. macrocopa* is chitin, which could bind to metal ions (Benguella and Benaissa, 2002). The surface bound contaminants not only have direct effects on the adsorbing organism, but also have effects on their consumers through dietary exposure (Robinson *et al*., 2003). It was reported that the surface-bound fraction of *M. macrocopa* contaminated with cadmium could significantly affect food uptake and feeding behavior in consumers, such as carnivorous fish, even at very low concentrations (Robinson *et al*., 2003). Thus cadmium could be transferred to higher trophic level via consumption of secondary consumer.

Catfish accumulated cadmium in muscle via direct exposure to cadmium in water, because cadmium diffused across the body membrane, bound to the gill surface, diffused to blood vessel, and dispersed through the body.

The permissible limit recommendation for the concentration of cadmium in fish muscle is not greater than 0.2 mg kg^{-1} fresh weight (FAO, 1983), while in water is not greater than 0.001 mg l^{-1} (Sindermann, 1996). In this study the cadmium concentrations in both fish muscle and water were higher than the permissible limits. Cadmium concentrations in fish muscle were 200 and 800 folds higher than residual cadmium concentration found in water. This might be a warning that although the cadmium concentration in water is lower than the permissible limit, the concentration of cadmium in fish muscle may exceed the permissible limit.

Accumulated cadmium in the sediment could affect fish buried in the mud or the benthic organisms at the bottom surface (biofilter organisms). The enrichment of cadmium was found in mollusks cultured in farms near industry areas. Mollusks consume the first order of the food chain and can accumulate a high amount of heavy metals without exhibiting marked visible physiological effects (Cheggour *et al*., 2001).

CONCLUSIONS

The highest accumulation of cadmium was in the primary producer (phytoplankton) followed by primary consumer (zooplankton) and secondary consumer (fish). Since cadmium concentration in fish muscle was much higher than the residual cadmium concentration found in water, which was lower than standard limit. Therefore, cadmium concentration in water might not represent the safety of aquatic organisms served as human food. In addition both *C. regularis* and *M. macrocopa* exposed to cadmium could have effects on their consumers through dietary exposure including fish, and also human. Thus it should be concerned about heavy metal contamination when collecting organisms from natural water sources for both feeding fish and human food.

ACKNOWLEDGEMENTS

The present work was financially supported by The Thailand Research Fund (TRF).

LITERATURE CITED

AOAC. 2000. **Official Methods of Analysis of the Association of Analytical Chemists**. (Vol. I; 17 th ed.). Association of Official Analytical Chemists, Washington, DC.

Becker, E.W. 1992. **Microalgae: Biotechnology and Microbiology.** Cambridge University Press. 361 p.

Benguella, B. and H. Benaissa. 2002. Effects of competing cations on cadmium biosorption by chitin. **Colloids and Surfaces A: Physiochemical and Engineering Aspects** 201: 143-150.

Robinson, K., D.J. Baird and F.J. Wrona. 2003. Surface metal adsorption on zooplankton carapaces: implications for exposure and effects in consumer organisms. **Environ. Pollut.** 122: 159-167.

Cheggour, M., A. Chafik, W.J., Lagnston, G.R., Burt, S. Benbrahim and H. Texier. 2001. Metals in sediments and the edible cockle *Cerastoderma edule* from two Moroccan Atlantic lagoons: Moulay Bou Selham and Sidi Moussa. **Environ. Pollut.** 115: 149-160.

FAO. 1983. **Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products.** FAO fishery circular No. 464, pp. 5-100.

Fleeger, J.W., K.R. Carman and R.M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. **Sci. Total Environ.** 317: 207-233.

Forget, J., J.F. Pabillon, M.R. Menasria and G. Bocquene. 1998. Mortality and LC_{50} values for several stages of the marine copepod *Tigriopus brevicornis* exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos, and malathion. **Ecotoxicol. Environ. Saf.** 40: 239-244.

Khummongkol, D., G.S. Canterford and C. Fryer. 1982. Accumulation of heavy metals in unicellular algae. **Biotechnol. Bioeng.** 24: 2643-2660.

Long, E.R., Macdonald, D., S.L. Smith and F.D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. **Environ. Manage.** 19: 81-97.

Lussus, P., C. Lebaut, L. Le Dean, M. Bardouil, P. Truquet and G. Boc-quene. 1984. **Ecotoxicological Testing for the Marine Environment.** Institute of Marine Science Research, pp. 131-142.

Mansour, A.A. and M.M. Sidky. 2002. Ecotoxicological studies. 3. heavy metals contaminating water and fish from Fayoum Governorate, Egypt. **Food Chem.** 78: 15-22.

Matsunaga, T., H. Takeyama, T. Nakao and A. Yamazawa. 1999. Screening of marine microalgae for bioremediation of cadmium-polluted seawater. **J. Biotechnol.** 70: 33-38.

Robinson, K., D.J. Baird and F.J. Wrona. 2003. Surface metal adsorption on zooplankton carapaces: implications for exposure and effects in consumer organisms. **Environ. Pollut.** 122: 159-167.

Scheifler, R., A. Gomot-de Vaufleury, M.L. Toussaint and P.M. Badot. 2002. Transfer and effects of cadmium in an experimental food chain involving the snail *Helix aspersa* and the predatory carabid beetle *Chrysocarabus splendens*. **Chemosphere.** 48: 571-579.

Sindermann, D.J. 1996. **Ocean Pollution Effects on Living Resources and Humans**. CRC Press, London. 275 p.

Stoeppler, M. 1991. **Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance**. VCH Publishers, Germany. pp. 803-851.

Volesky, B. 1990. **Biosorption of Heavy Metals**. CRC Press, Inc., Boston. 397 p.