INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–190/DJZ/2009/11–6–783–786
http://www.fspublishers.org

Full Length Article

Bioaccumulation of Copper and Lead in Asian Clam Tissues from Bung Boraphet Reservoir, Thailand

TINNAPAN NETPAE AND CHITCHOL PHALARAKSH[1]†
Environmental Science Program and Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Faculty of Science, Chiang Mai University, Thailand 50200
†*Department of Biology and Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Faculty of Science, Chiang Mai University, Thailand 50200*
[1]Corresponding author's e-mail: chitchol@chiangmai.ac.th

ABSTRACT

Bung Boraphet is the largest fresh water reservoir in Thailand. The accumulations of copper (Cu) and lead (Pb) in water, sediment and tissues of Asian clam (*Corbicula* sp.) with in Bung Boraphet were carried out to indicate possible polluting effect of soil erosion and the dissolution of water soluble salts from Nan river. Samples were collected from 12 study sites with in Bung Boraphet between February to December 2008. Cu and Pb in water were found at low levels but the heavy metals were detected at high levels in the sediment and tissues of *Corbicula* sp. Accumulation rate of Cu and Pb in tissues *Corbicula fluminea* was to determine under laboratory condition. Pb was accumulated in tissue more than Cu. The maximum 168 h Pb accumulation in tissues was found as 129.5 µg g^{-1} dry weight, whereas the Cu accumulation was maximum at 120 h as 26.83 µg g^{-1} dry wt.

Key Word: Bung boraphet; *Corbicula* sp.; Lead; Copper

INTRODUCTION

Bung Boraphet is located between latitude 15°40' N, 15°45' N and longitude 100°10' E, 100°23' E (about 20 m above sea level) Nakhon Sawan Province, Thailand. The reservoir covers approximately 212.38 km^2. It has been used for water supply, flood control, waste disposal and fisheries. Heavy metals in Bung Boraphet come from natural as well as artificial sources. Natural heavy metals introduced into the reservoir come primarily from such sources as soil erosion the dissolution of water soluble salts from Nan rivers. The concentration of zinc (Zn), lead (Pb) and cadmium (Cd) in reservoir sediments were found at normal levels. However, Pb was the only metal that showed an increasing trend. Copper (Cu) found at 23.50 µg $^{-1}$, which was slightly higher than the normal level for earth sediment (Petpiroon *et al*., 1996). Asian clam (*Corbicula* sp.) are also present in Bung Boraphet by frequency of occurrence (%OC) was 17.24% (Duangsawasdi, 2003). These clams have been shown to act as good bioaccumulators of aquatic pollution (Belanger *et al.*, 1990).

The objectives of this research were to determine the concentration of Cu and Pb in sediments, water and tissues of *Corbicula* sp. in Bung Boraphet and accumulation patterns of Cu and Pb in the tissues of *C. fluminea* under laboratory conditions.

MATERIALS AND METHODS

Determination of Cu and Pb in water, sediments and *Corbicula* sp. from bung boraphet. Water, sediment and *Corbicula* sp. samples were collected every 2 months from 12 study sites around bung boraphet (Fig. 1) between february to december 2008. Water samples were taken approximately 30 cm beneath the water surface. Water samples were transferred directly to bottles after sedimentation and acidified to a pH < 2 by adding concentrated nitric acid. Sediment samples were collected by a specially modified Smith-McIntyre grab made from high grade stainless steel, while offshore samples were collected by core sampler. *Corbicula* sp. samples were collected from the field and taken to the laboratory. An approximate clam size ranged between 15 and 25 mm in length and we collected at least 10 clams from an area approximately 500 m^2 in size from each location.

Clams were dried at 40-50°C. Soft tissues of 5 to 10 individuals were removed from each shell, prior to homogenizing with a pestle and mortar. Sediment samples were dried at 100°C in hot air oven for 24 h. Dried sediment samples were crushed with an agate mortar. The homogenized *Corbicula* sp. tissues were transferred to acid-washed 250 mL conical flasks. The *Corbicula* sp. tissues were added in 5 mL HNO_3, 10 mL H_2SO_4 and glass bead. The mixtures were evaporated on a hot plate until appearing

To cite this paper: Netpae, T. and C. Phalaraksh, 2009. Bioaccumulation of Copper and Lead in Asian Clam Tissues from Bung Boraphet Reservoir, Thailand. *Int. J. Agric. Biol.*, 11: 783–786

dense white fumes of SO_3 just appear. For sediment digestions, 4 mL conc. HNO_3, 16 mL HCl and glass bead were used. The sediment mixtures were evaporated on a hot plate until a volume of approximately 5 mL remained. Digested samples were allowed to cool to approximately 55°C, followed by further addition of concentrated HNO_3 20% (25 mL). Flasks were returned to the hot plates and digested again until approximately 3 mL volume remained. The digests of *Corbicula* sp. tissues and sediment were diluted to 25 mL with deionized water and transferred to separate glass bottles prior to analyses.

Determination of Cu and Pb accumulation pattern in *C. fluminea.* *C. fluminea* were collected from bung boraphet and kept in water tanks at 25-30°C water temperature. The specimens were transported to the laboratory where they were detoxified by maintaining them for 20 days in an aquarium containing continuously aerated well water. During acclimation and detoxification periods, *C. fluminea* were fed with micro algae (*Chlorella* sp.) dissolved in water at a concentration of 10^5 algal cell mL^{-1} per unit (Marie *et al.*, 2006). Selected living *C. fluminea* were sorted by length (2.0-2.5 cm) and distributed over the experimental treatments. An experimental treatment consisted of 60 clams placed in an aquarium (10 L), containing 6 L of Cu and Pb solution. Water temperature was kept at 29 ± 1°C. Turbidity was 0 ± 1 FTU at 7 ± 0.2 pH under 12 h light (680 ± 5 lux) and 12 h dark period. The water was aerated and always air-saturated with oxygen. The *C. fluminea* were treated with Cu and Pb concentration ranging from 0.1 to 6 mg L^{-1} and 0.1 to 10 mg L^{-1}, respectively. The Cu and Pb stock solutions were prepared using copper (II) sulfate ($CuSO_4$) and lead nitrate ($PbNO_3$). Concentrations of Cu and Pb in water, sediment and clam tissues were analyzed by Atomic Absorption Spectrophotometry (Perkin Elmer-3110).

Statistical analysis. Effects of seasonal changes of heavy metal toxicity were evaluated with a one way analysis of variance (one - way ANOVA) and Post Hoc, Duncan test ($p<0.05$). Accumulation rate data were analyzed by two way analysis of variance (two way ANOVA) and significant differences between groups were determined by Scheffe's multiple range test.

RESULTS AND DISCUSSION

Cu and Pb in water, sediment and *C. fluminea* from bung boraphet. Cu and Pb were generally higher in sediment than clams and water, respectively (Table I & II). The highest concentration of Cu in water of bung boraphet was observed in April (< 2 µg L^{-1}). The concentrations of Cu were less than 2 µg L^{-1} in february, june, august and October, while the Cu concentration was less than 1 µg L^{-1} in December. The highest concentration of Pb (< 5 µg L^{-1}) in water was detected in April, while the concentration of less than 5 µg L^{-1} in August. The Pb concentrations of less than 4 µg L^{-1} were observed in february, june, october and

Fig. 1. Sampling sites for water, sediments and aquatic organisms on bung boraphet

Fig. 2 Time curve of Cu concentration in tissue tread with *C. fluminea* by 0 mg Cu l^{-1}, 0.1 mg Cu l^{-1}, 0.5 mg Cu l^{-1}, 1 mg u l^{-1}, 2 mg Cu l^{-1}, 3 mg Cu l^{-1} and 4 mg Cu l^{-1}

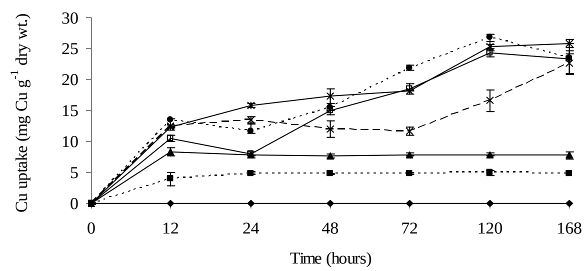
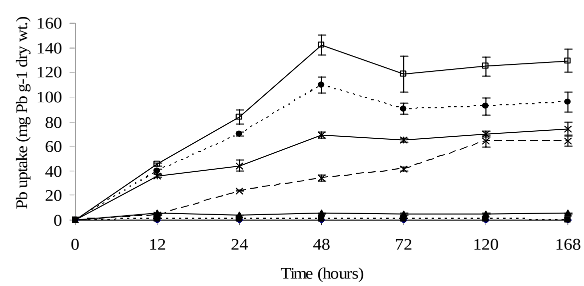


Fig. 3 Time curve of Pb concentration in tissue after tread with *C. fluminea* by 0 mg Pb l^{-1}, 0.1 mg Pb l^{-1}, 0.5 mg Pb l^{-1}, 1 mg Pb l^{-1}, 3 mg Pb l^{-1}, 5 mg Pb l^{-1} and 10 mg Pb l^{-1}



december. Cu and Pb were satisfactorily determined at concentrations accepted as surface water quality standards of Thailand (Pollution Control Department, 2000). The Cu and Pb concentrations in the sediments were between 28.73-41.15 mg kg^{-1} and 16.44-26.89 mg kg^{-1}, respectively. Concentrations of the metals in the bung boraphet sediments were compared to the recommended lowest levels for Canadian sediment quality guidelines for the protection of

Table I. Concentrations of Cu and Pb in water and sediments collected from bung boraphet

	Cu in water (µg L^{-1})				Pb in water (µg L^{-1})			
	$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max
February	$1.29^{a,b}$	1.14	ND	3.57	3.11^{a}	1.06	2.12	4.86
April	2.07^{b}	1.10	ND	3.29	5.79^{d}	0.26	5.36	6.08
June	1.77^{b}	0.17	1.58	2.27	3.42^{a}	0.21	3.05	3.71
August	$1.29^{a,b}$	0.11	1.1	1.49	4.77^{c}	0.4	4.32	5.51
October	1.10^{a}	0.10	0.9	1.26	3.95^{b}	0.18	3.66	4.23
December	0.94^{a}	0.09	0.79	1.06	3.5^{a}	0.31	3.01	4.12
	Cu in sediment (mg kg^{-1} dry wt.)				Pb in sediment (mg kg^{-1} dry wt.)			
	$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max
February	28.73^{a}	4.68	21.02	36.15	26.41^{c}	1.421	29.63	24.47
April	$30.21^{a,b}$	4.67	24.12	34.79	26.89^{c}	2.53	28.89	20.65
June	41.15^{c}	11.72	27.57	64.18	17.94^{a}	3.45	24.83	9.54
August	$31.38^{a,b}$	4.64	24.33	38.75	16.44^{a}	4.36	24.58	11.93
October	$32.16^{a,b}$	4.63	25.52	41.44	19.29^{a}	2.46	22.45	14.46
December	34.89^{b}	5.31	26.02	43.06	22.04^{b}	4.73	29.28	17.73

$\bar{X}$ = Average, SD. = Standard deviation, Min = Minimum, Max = Maximum, ND = not determined

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$)

Table II. Concentrations of Cu and Pb in the tissue of *Corbicula* sp. from bung boraphet

	Study site*	Cu in tissue of *Corbicula* sp. (mg kg^{-1} dry wt.)				Pb in tissue of *Corbicula* sp. (mg kg^{-1} dry wt.)			
		$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max
February	1, 3, 4, 6, 8, 12	13.43^{a}	0.76	12.15	14.25	20.60^{b}	2.13	18.35	23.04
April	1, 2, 3, 4, 6, 11, 12	13.34^{a}	1.10	11.21	14.35	19.53^{a}	1.37	18.30	22.50
June	1, 2, 3, 4, 11, 12	12.99^{a}	0.93	11.38	13.93	$18.04^{a,b}$	1.12	16.37	19.45
August	1, 3, 4, 6, 10, 11	12.28^{a}	1.37	10.13	13.71	18.54^{a}	2.02	16.83	22.43
October	1, 2, 3, 4, 7, 12	12.51^{a}	2.14	9.84	14.94	$18.70^{a,b}$	0.68	17.94	19.55
December	1, 3, 4, 12	13.06^{a}	0.76	12.35	13.78	18.07^{a}	1.01	16.87	19.30

* Study site 5 and 9 cannot collected *Corbicula* sp.

$\bar{X}$ = Average Average, SD. = Standard deviation, Min = Minimum, Max = Maximum

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$)

aquatic life (Canadian Council of Ministers of the Environment, 1999). However the concentrations of Cu in sediments during June exceeded the permissible limits.

Sediments are the major depository of metals in some cases, holding more than 99% of total quantity of a metal present in an aquatic system. The observed high concentrations of Cu and Pb in the present work are consistent with the findings of Petpiroon *et al*. (1996). These high concentrations of Cu and Pb in the sediment can also cause metal pollution in the reservoir. The concentrations of Cu and Pb in tissues of *Corbicula* sp. were determined in 10 study sites (Table II). The average concentrations of Cu ranged between 12.28 and 13.43 µg g^{-1} based on dry weight. Comparison between seasons, using one-way ANOVA test showed that there was no significant difference ($p<0.05$) for Cu concentration in tissues of clams during one year. The average concentrations of Pb in *Corbicula* sp. tissues (dry weight) ranged between 18.04 and 20.60 µg g^{-1} in august and february, respectively.

Accumulation pattern of Cu and Pb in *C. fluminea.* Cu and Pb accumulation in the tissue of clams had a significant ($p<0.05$) relationship with the heavy metal concentration and exposure time (Fig. 2 & 3). However decrease in Cu and Pb accumulations during 12 and 48 h period were not significant. The accumulation of heavy metals in the clam tissue increased significantly ($p<0.05$) but the relationship between exposure time and heavy metal concentration was not linear. Cu and Pb concentrations in the clams exposed for 168 h increased with increasing metals exposure concentration. The maximum Pb accumulation in clam tissues was found at 168 h exposure period at the concentration of 129.5 µg g^{-1} dry wt., whereas the Cu accumulation was maximum at 120 h under metal concentration of 26.83 µg g^{-1} dry wt., suggesting significant accumulation of Cu and Pb at these concentrations.

During 168 h metal exposure the *C. fluminea* showed Cu and Pb regulation but Pb accumulation in body tissue was significantly higher than Cu that exerted more toxicity, especially the juveniles (Doherty, 1990). Long-term exposure of the clams to Cu at 0.008-0.017 mg L^{-1} results in impairment of growth resulting in dwarf populations (Belanger *et al.*, 1990). Clarke *et al.* (1979) reported that the amount of Pb absorption by *C. fluminea* was high at xposure concentration range of between 4.1-69.5 mg L^{-1}. However, the contaminations of Cu and Pb in surface water should not exceed 0.1 mg L^{-1} and 0.05 mg L^{-1}, respectively sustainability of the aquatic health (Pollution Control Department, 2007). The results suggested that *C. fluminea* can act as suitable indicator of Cu and Pb contamination in the aquatic habitat.

CONCLUSION

Concentration of Cu and Pb in water of bung boraphet were found at low levels but the heavy metals accumulations in clams were high that correlated with the toxicities of these metal in water and sediments predicting that *C. fluminea* is a good bioaccumulator for Cu and Pb contaminations in aquatic habitats.

Acknowledgement. Financial support from Commission on Higher Education of Thailand, Center for Environmental Health, Toxicology and Management of Chemical (ETM) are gratefully acknowledged.

REFERENCES

Belanger, S.E., J.L. Farris, D.S. Cherry and J. Cairns, 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. *Canadian J. Fish. Aquat. Sci.*, 47: 904–914

Canadian Council of Ministers of the Environment, 1999. *Protocol for the Derivation of Canadian Sediment Quality Guidelines for the Protection of Aquatic Life*. Canadian Council of Ministers of the Environment, Winnipeg, Manitoba, Canada

Clarke, J.H., A.N. Clarke, D.J. Wilson and J.J. Friauf, 1979. On the use of *Corbicula fluminea* as indicators of heavy metal contamination. In: *Proc. First Int. Corbicula Symposium*, pp: 153-163

Doherty, F.G., 1990. The Asiatic clam, *Corbicula* spp. as a biological monitor in freshwater environments. *Environ. Monitor. Assess.*, 15: 143–181

Duangsawasdi, S., 2003. Abundance and diversity of benthic fauna in bung boraphet, nakhon sawan province. *Thai Fish. Gaz.*, 6: 503–520

Marie, V., P. Gonzalez, M. Baudrimont, J.P. Bourdineaud and A. Boudou, 2006. Metallothionein response to cadmium and zinc exposures compared in two freshwater bivalves, *Dreissena polymorpha* and *Corbicula fluminea*. *Biometals*, 19: 399–407

Petpiroon, P., K. Loichuen and S. Petpiroon, 1996. The distribution of heavy metal in the sediment of bung boraphet swamp. *Thai Fish. Gaz.*, 48: 148–155

Pollution Control Department, 2000. *Water Quality Standards and Criteria in Thailand*, 4th edition, Ministry of Science, Technology and Environment, Bangkok, Thailand

Pollution Control Department, 2007. *Thailand State of Pollution Report 2006*. Bankok, Thailand: Report. Ministry of Science, Technology and Environment, Bangkok, Thailand

(Received 01 June 2009; Accepted 09 September 2009)