RESPONSE OF FRESHWATER SNAIL (*Angulyagra polyzonata*) AS WATER BIOMARKER BY HEAVY METALS (*Cd, Cu, Zn, Pb*)

Dinh Tien Dzung*[1], Nguyen Thi Thanh Hoa[1],
Nguyen Truong Phu[1], Nguyen Thi Thu Ha[2]

Abstract

Metals toxicity testing of freshwater snail (*Angulyagra polyzonata*) was carried out in laboratory by artificially exposing to metals Cd, Cu, Zn, and Pb less than 48 hours test. The results showed that toxic levels of metals increases in the order: $Zn^{2+}<Pb^{2+}<Cd^{2+}<Cu^{2+}$. In particular, the highest toxicity level was observed by exposing to Cu^{2+} with effect concentrations (EC50) between 0.17 and 0.21 ppm; lethal concentration (LC50) was 2.937 ppm. Other metals (in toxicity order of Cd, Pb, and Zn) in the National Technical Regulation (NTR 08:2008/MONRE) did not affect seriously to snail. The maximum acceptable concentration of the metals for snail in exposure for 48 or 24 hours was Cd: 0018 - 0.021 ppm; Cu: 0.0008 - 0.0012 ppm; Zn: 0071 - 0.082 ppm and Pb: 0.134 - 0.276 ppm.

Keywords: EC, LC, MATC, heavy metals, *Angulyagra polyzonata*, toxicity testing

INTRODUCTION

Currently, along with the increase in the world population, economic development in the developing countries, the living quality is declining due to human activities (exploitation and use of natural resources, discharge of untreated waste physical...). According to the report on the national environments during the last years (2007 - 2015) water quality was severely affected by heavy metals arising from mining activities, industrial coating, metallurgy, transportation, production and processing of heavy metals in the rural regions. The current levels of heavy metals affecting aquatic ecosystems were accessed based on only environmental quality standards (NTR 08: 2008/MONRE for surface water quality). Meanwhile, different levels of metals affecting particular species and ecosystem in general are not the same and should be evaluated for determination of eco-safety concentration value (Capinera, 2008).

Asia freshwater snail (*Angulyagra polyzonata*) was chosen as the test organism because they are available, poor mobility, large size, easy to be observed and has an important role in the freshwater ecosystem. Snail's biological accumulation ability of heavy metals was shown in many previous studies (Dang Kim Chi *et al.*, 2006) because mollusks are benthic species consumed of organic sediments. This study aims to evaluate acute effects of heavy metal concentrations in the water on organism as a basis for determining maximum acceptable toxicant concentration (MATC).

MATERIALS AND METHODS

Materials

1000 ppm original standard solution of the chemicals: $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Zn(NO_3)_2$, $Pb(NO_3)_2$ respectively and individually were added with deionized water. Freshwater snails (*Angulyagra polyzonata*) were collected from the aquatic system (rivers, canals, ponds…) without waste sources and were used for identification according to invertebrate key (Dang Ngoc Thanh *et al.*, 1980). The individuals were evaluated at adult stage, strong, 10 – 15 mm length, 4 - 6g weight and were stabilized by clean water during 24 hours before the test.

Experiment design

Experiments were arranged based on artificial contamination water of heavy metal concentrations from lowest to highest. Each concentration, there were 5 individuals/500ml water (was repeated 3 times). A blank sample was similarly conducted by deionized water with 100% normally healthy individuals during the test. Based on some scientific data about the toxicity of metals (Rand, 2003; Le Huy Ba, 2006), chosen concentrations were as: Cd^{2+} (0, 0.1, 0.25, 0.5, 0.8, 1, 5, 10, 25, 50, 100 ppm); Pb^{2+} (0, 0.5, 1, 2, 3, 5, 7, 9 ppm); Cu^{2+} (0, 0.005, 0.025, 0.05, 0.01, 0.25, 0.5, 1, 5, 20, 30 ppm); Zn^{2+} (0, 0.1, 0.5, 1, 2, 5, 7, 9, 10 ppm).

The experiment was conducted in a laboratory with natural light, ventilation, average temperature (23 - 25^{o}C), dissolved oxygen DO (3 - 6mg/l), pH = 7.28 - 7.8, from April 2014 to June 2015. Observing the behavior of individuals after 10 minutes, 15 minutes, 30 minutes, 1 hour, 2h, 4h, 8h, 12h, 24h, 36h, and 48h. According to Alonso and Camargo (2010), responses of the organisms were identified as follow:

Normal responses: Moving, muscle contractions.

Affected (abnormal) responses: Immobile, not muscle contraction, closed operculum, death.

[1] Center of Environmental Analysis and Technology Transfer, Institute of Agriculture Environment
[2] Faculty of Environment, Vietnam National University of Agriculture
* Corresponding author: dinhtiendungnn1@gmail.com

Methods of evaluation

The percentage of tested organisms of normal and abnormal was recognized at the time of observation.

Acute toxicity 24 hours and 48 hours was expressed by the EC and the LC value. Where: EC50 (Effective concentration) is the concentration causing 50% impact on the organisms tested and LC50 (Lethal concentration) is 50% lethal concentrations organisms tested. EC50, LC50 is determined from the linear regression equation between l g (concentration) and the percentage of affected units converted at the probability (according to Rand, 2003 criticism of Finney, 1952). Similarly in EC10; EC90; LC10; LC90

Recommended safe level of metal is represented by the value MATC (Maximum Acceptable Concentration Tocicant - maximum acceptable concentrations of toxic substances).

$$MATC = \sqrt{(NOEC.LOEC)}$$

Where: NOEC (No Observed Effect Concentration) The concentration is not observed influence and LOEC (Lowest Observed Effect Concentration) is the lowest concentration of influence (Rand, 2003).

RESULTS AND DISCUSSION

Acute effects of heavy metals concentration on tested organisms

The responses of snail to tested concentrations showed significant change during the observation period. The number of abnormal snails increased when exposing time was prolonged in an individual concentration level of the metals. In particular, the common response was immobile, not muscle contraction, closed operculum.

The response of snails was powerfully changed when exposed to water contaminated with heavy metals (after 1- 8 hours of exposure, depending on the concentration tested), then abnormal expression of snail ascending to stabilize at around time between 24 to 48 hours.

Results presented in figure 2 showed that: In every metal tested, responses of organisms were not similar: For Cd, tested organisms manifestations: moving (I), clinging on the bottle wall (II), immobile (V) and closed operculum (VI). For Cu, organisms tested only three types of responses: wall clinging (II), not muscle contraction (III), closing (VI). For Zn and Pb, responses of organisms were more diverse, includes all the responses mentioned above, most common abnormal response was closed operculum (VI) in Pb testing while was mucus secretion (IV) in Zn testing.

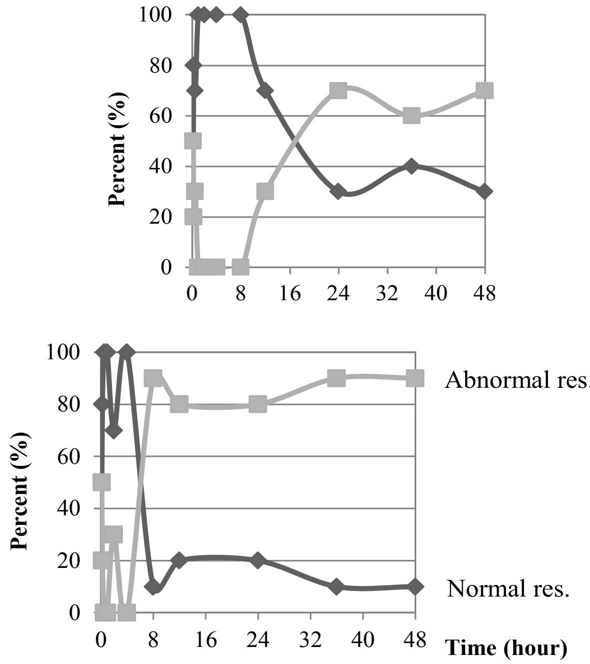

Figure 1. The change in response of tested organisms over time (Cd 0.1 ppm and 0.8 ppm)

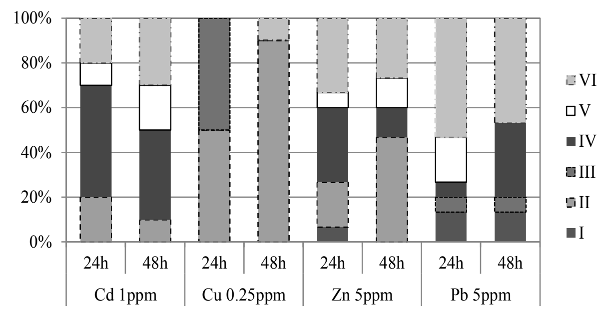

Figure 2. The Proportion of response types tested at 24 and 48 hours

At 24h, 48h in various concentrations of each metal, the proportion of abnormal (affected) response organisms increased gradually with increasing concentrations of metals. By probit method of Finney (1953) (according to Rand, 2003), this study has established the regression equation between the logarithm of the metal concentration with probit of affected organisms percentage to determine the EC50 level (Table 1).

EC50 (24 hours) of Cadmium (Cd^{2+}), Copper (Cu^{2+}), Zinc (Zn^{2+}), Lead (Pb^{2+}) were 1.2299, 0.2064, 2.8199, 4.6664 ppm. EC50 (48 hours) were 0.8603, 0.1748, 3.8000, 3.5873 ppm, respectively. The impact of metal ranked according metal toxicity levels was: At 24 hours: $Pb^{2+}<Zn^{2+}<Cd^{2+}<Cu^{2+}$; At 48 hours: $Zn^{2+}<Pb^{2+}<Cd^{2+}<Cu^{2+}$. The freshwater snail was sensitive to Zn and Pb, tolerant to Cu.

Compared with surface water quality standards (NTR 08: 2008/MONRE) or water quality standards protect aquatic life (NTR 38: 2011/MONRE), the biological impact was negligible in concentrations for permission of the metal, except for Cu. For Cu exists as Cu^{2+}, at a concentration of 0.2 ppm, it began to appear abnormal response in some organisms, then at 24 - 48 hours, more than 50% of the organisms were identified abnormal response.

Also according to the study results, the concentrations causing adverse effects on most organisms (EC99) ranked by decreasing toxicity of the metal were: Cu^{2+}: 3.8 to 8.7 ppm; Cd^{2+}: 5.2 to 9.7 ppm; Pb^{2+}: 8.5 to 11.2 ppm; Zn^{2+}: 13.9 to 22.4 ppm.

Table 1. Affective Concentrations (EC) of the metal to snails at 24 and 48 hours

Time (h)	Metals	Equation	R	Effective concentration				NTR 08: 2008	
				EC10	EC50	EC90	EC99	A2	B1
24	Cd^{2+}	*Y=3.4392X+4.6908*	0.92	0.3863	*1.2300*	2.8979	9.7357	0.005	0.01
	Cu^{2+}	*Y=1.9008X+6.3026*	0.89	*0.0254*	*0.2064*	*0.9730*	*8.7165*	0.2	0.5
	Zn^{2+}	*Y=4.4504X+2.9963*	0.78	1.1521	*2.8199*	5.4682	13.9492	1.0	1.5
	Pb^{2+}	*Y=11.694X-2.8231*	0.98	3.3193	*4.6664*	6.0040	8.5748	0.02	0.05
48	Cd^{2+}	*Y=3.9393X+5.2574*	0.94	0.3130	*0.8603*	1.8180	5.2367	0.005	0.01
	Cu^{2+}	*Y=2.3097+6.7497*	*0.88*	*0.0311*	*0.1748*	*0.6261*	*3.8044*	0.2	0.5
	Zn^{2+}	*Y=4.0092X+2.6755*	0.77	*1.4070*	*3.8001*	7.9261	22.4140	1.0	1.5
	Pb^{2+}	*Y=6.2471X+1.5343*	0.96	1.8960	*3.5873*	5.7500	11.2047	0.02	0.05

Where: Y: probit of percentage of affected organisms; X: logarithm of the metal concentration; NTR 08: 2008: National Technical Regulation on Surface Water Quality; A2: The quality of surface water for domestic purposes; B1: The quality of surface water for irrigation and similar activities.

Lethal effects of heavy metal concentration on tested organisms

The high concentration of metals in the aquatic environment adversely affected the life of snails *Angulyagra polyzonata* especially when the metal was accumulated. In the metals mentioned above, Cu^{2+} had high toxicity values, so in this study Cu^{2+} was chosen to determine potential death on snails.

Accordingly, the started lethal concentration of Cu^{2+} was 0.1 ppm. With increasing concentration to 1 ppm (10 times), 2.5 ppm (25 times higher), and the snail lethal rates observed after 48 hours remained at 10%. Only at the level of 5 ppm (increased 50 times), mortality percentage observed was 20%. In the highest concentration (20 ppm), mortality percentage was 100% of organisms tested. Based on observations of the mortality rate, LC50 (Lethal Concentration) of Cu was calculated:

Probit (Mortality rate) = 1.8677 log (Concentration) +4126 (r = 0.77, n = 45)

Lethal concentration of 50% tested organisms: LC50 (48 hours) of Cu^{2+} is 2.937 ppm; Likewise, lethal concentrations of 10%, 90% and 99% tested organisms: 0.606; 14.232; 51.937 ppm, respectively. Thus, although the toxicity of Cu are relatively high compared with the rest of the test metals, but the Cu concentration causing destruction with *Angulyagra polyzonata*was determined higher 100 times than its was in the NTR 08: 2008 B1 column.

Toxicity testing applications in the management of ecological risk of heavy metals

Although the study was conducted only on one type of aquatic life, as well as only conducted on 4 metals in the environment, but the basis of toxicity studies in experimental biology, we can set the world term safety of students living marrow of the concentration of the toxin. Accordingly, the Maximum Acceptable Concentration Toxicant - MATC is defined to indicate the maximum value does not cause adverse effects to organisms (including death). Then, this value was converted to the factor of safety (Applied Factor - AF) to thereby allow the construction value of the metal (according to Rand, 2003: AF usually takes the value of 10). The Maximum Allow Value (MAV) = MATC/AF.

With the above calculation results, calculated MAVs for *Angulyagra polyzonata* (excepted Cu) were higher than the NTR for surface water irrigation (Cd - 2 times; Pb - 5 times; Zn - 20 times). Meanwhile issues of concern in this study are the concentration of Cu allows for lower real current NTR. This is why using Cu and Cu compounds to kill organisms complex, but the problem also needs to pay attention to the source of Cu containing waste going into the environment.

Table 2. The maximum allow value of the metals for organisms in acute testing

Time	Metals	NOEC	LOEC	Mean of MATC	Cal. MAV	NTR 08: 2008	
24 hours	Cd^{2+}	0.1	0.25	0.207 ± 0.017	0.021	0.005	0.01
	Cu^{2+}	0.005	0.01	*0.008 ± 0.001*	*0.001*	0.2	0.5
	Zn^{2+}	0.5	1	0.710 ± 0.060	0.071	1.0	1.5
	Pb^{2+}	2	3	2.761 ± 0.258	0.276	0.02	0.05
48 hours	Cd^{2+}	0.1	0.25	0.181 ± 0.015	0.018	0.005	0.01
	Cu^{2+}	0.01	0.25	*0.012 ± 0.001*	*0.001*	0.2	0.5
	Zn^{2+}	0.5	1	0.822 ± 0.068	0.082	1.0	1.5
	Pb^{2+}	1	2	1.343 ± 0.119	0.134	0.02	0.05

CONCLUSION

Metal toxicity testing of freshwater snail (*Angulyagra polyzonata*) was carried out in laboratory by artificially exposing to metals Cd, Cu, Zn, and Pb less than 48 hours test. The results showed that the metal sensitivity of snail ascending in order: $Zn^{2+}<Pb^{2+}<Cd^{2+}<Cu^{2+}$.

In particular, Cu was the most interesting metal with affective concentrations (EC50) were 0.21 ppm and 0.17 ppm, respectively at 24 and 48 hours of exposure. Cd, Pb and Zn were lower toxicity level metals with EC50 in 48 hours was 0.86; 3:58 and 3.8 ppm, respectively. Similarly, lethal concentrations (LC50) of Cu was 2.937 ppm (higher nearly 5 times the NTR 08:2008 B1). According to toxicity testing methods, research also indicated the maximum allowed concentration of copper in NTR 08:2008 was not ecological safe for freshwater snail (*Angulyagra polyzonata*).

REFERENCES

Álvaro Alonso, Julio A. Camargo, 2011. "Toxic Effects of Fluoride Ion on Survival, Reproduction and Behaviour of the Aquatic Snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)". *Water, Air, "http://link.springer.com/journal/11270" Soil Pollution* Volume 219 (1), pp 81-90.

C. A. Crichton, A. U. Conrad, D. J. Baird, 2004. Assessing Stream Grazer Response to Stress: A Post-Exposure *Bulletin of Environmental Contamination and Toxicology*, Volume 72, (3), pp 564-570.

Ian C. Campbell, 2012. Biodiversity of the Mekong Delta, The Mekong Delta System. *Springer Environmental Science and Engineering 2012*, pp 293-313.

John L. Capinera, 2008. Bioassay. *Encyclopedia of Entomology*, p 475.

Gary M. Rand, 2003. *Fundamental of Aquatic Toxicology: Effects, Environmental fate and Risk Assessment 2nd edition*, Taylor and Francis Group.

Le Huy Ba, 2006. *Environmental toxicology vol1 and vol2. Vietnam National University, Ho Chi Minh City publishing house in Vietnamese*

Dang Kim Chi, Hoang Thu Huong, Vu Thi Hong Nhung, 2006. Investigating possibility of Chromium (Cr) and Cadmium (Cd) accumulation in bodies of mollusca (mussels and snail) to development of biological indicators for industrial wastewater quality. *Scientific conference report, 20th Hanoi University of Science and Technology*, p 94-97 (in Vietnamese)

Dang Ngoc Thanh, Tran Thai Bai, Pham Văn Mien, 1980. Classification of Freshwater Invertebrate in North Vietnam, Science and Technics Publishing House Vietnamese (in Vietnamese)

Date received: 18/09/2016
Date reviewed: 17/10/2016
Reviewer: Associate Prof. Dr. Pham Quang Ha
Date approved for publication: 20/12/2016

PREPARATION OF A SOIL REFERENCE SAMPLE AND INITIAL RESULTS ON LABORATORY QUALITY IMPROVEMENT

Bui Bich Luong*[1], Le Thi My Hao[1],
Bui Hai An[1], Do Duy Phai[1], Paul Milham[2]

Abstract

The mineral constituents of soils were analysed for their agricultural potential by the Soils and Fertilizers Research Institute (SFRI). Until recently the laboratories had no reference materials to validate the tests. This deficit was overcome by creating a reference soil sample. The first step was to collect about 100 kg of surface soil from an unfertilized area adjacent to a rice paddy in the Red River Delta. The soil was air dried, and roots and pebbles were removed before grinding, mixed thoroughly and divided into two 50 kg portions. One portion was sent to Australia where it was circulated as a proficiency sample to 39 agricultural laboratories as part of a certification procedure run by the Australasian Soil and Plant Analysis Council (ASPAC). The inter-laboratory means and coefficients of variation for a wide range of tests were returned to SFRI where they are being used in method validation and quality control. The retained portion was divided into 100 jars each of ~500 g and the nitrogen content (semi-micro Kjeldahl) was used to assess the homogeneity of the material within jars (4 replicates) and between jars using the within-jar means. The mean N content for all jars was 0.067% and the CV was ± 6.22%, and the corresponding ASPAC statistics were 0.066% and ± 12.29%. Other parameters were analyzed and compared with the ASPAC inter-laboratory proficiency results as a part of method validation. For example, there respectively means for SFRI and for ASPAC (in parentheses) were: for the pH of 1 g/5 mL suspension in water 7.96 (8.04); for organic carbon, 0.46% (0.44%); for total-P as P_2O_5, 0.09% (0.08%); and for available K, 4.24 mg/100 g as K_2O (4.7 mg/100 g). However, the agreement for other parameters, such as cation exchange capacity, was poor: 13.2 cmol/kg (10 cmol/kg) and the causes are being investigated. Finally, it is planned to develop additional reference materials to complement the Hanoi reference soil. These materials will strengthen the quality of testing at SFRI and provide objective measures of that quality into the future.

Keywords: ASPAC, homogeneity, reference sample, SFRI

INTRODUCTION

The Soils and Fertilizers Research Institute (SFRI) is the peak body for soil classification and agronomic testing in Vietnam. The SFRI consequently operates laboratories in a number of locations, with the central facility being located in Hanoi. The organization has developed a comprehensive suite of test methods that are officially recognized (SFRI, 1998). Precision and accuracy are important requirements of chemical tests and can be achieved by skilled analysts using validated test methods and replication to reduce random errors. The validation of test methods includes measures of precision and accuracy, and these measures rely on reference materials, i.e. on homogeneous materials of known composition.

The International Organization of Legal Metrology defines reference samples as materials or substances for which one or more characteristic(s) is homogenous and the material is made to standardize equipment, evaluate a method or to define the composition of materials. The standard values of reference samples are ideally created following a technical protocol with perfect accuracy and reproducibility. Under some conditions the composition is certified and approved by official agencies.

Agronomic properties of soils are operationally defined and as such are unsuitable for certification. Nonetheless, reference materials of a suitable standard can be prepared and used to validate operationally defined test methods. This is the process we describe here for a reference soil sample. To our knowledge this is the first example of a reference soil sample being developed and used in Vietnam.

MATERIALS AND METHODS

Materials

For effective long-term use, the key requirements for a soil reference sample are homogeneity and a large quantity (about 200 kg). We selected an alluvial soil from the Red River delta that had been fallowed for a long time. This soil was taken from Tho An commune, Dan Phuong district, Hanoi capital.

[1] Soils and Fertilizers Research Institute, Vietnam

[2] Institute for the Environment and School of Science and Health, Western Sydney University, LB 1797, Penrith, New South Wales 2751, Australia.

* Corresponding author: buibichluong2009@gmail.com