EVALUATION OF TOXICITY OF SEDIMENT FROM HOCHIMINH CITY,VIETNAM TO AMPHIPODS *Hyalella Azteca*

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ABSTRACT: Toxicity of sediment from Ho Chi Minh City (HCMC) on ***H.azteca*** *(14-days acute sediment toxicity test) was tested in this study. The amphipods* ***H. azteca*** *test showed that the 9 sediment samples from 5 canals of HCMC were toxic with mortality varying from 87.5% to 100%. The reference sediment Tri An showing no acute toxicity can be considered as being a non contaminated site. The order in mean survival in these canal samples was the following: TriAn(76.3%)>NL-TN(10%)>KD-KT(7.5%)>TL-BC(6.3%)>TH-LG (5%)>TH-BN (0%). All sediments of HCMC were classified as very toxic (effects greater than 50% difference from the control - according to Burton et al., 1996). The* ***H.azteca*** *sediment test is interesting for the assessment and classification of the sediment contamination in the HCM canals with the aim to assess and manage sediment quality, including dredged material in HCMC.*

1. INTRODUCTION

The objective in this research was to test the toxicity of sediment from HCMC on *H.azteca* (14-days acute sediment toxicity test). Contaminants from agricultural, municipal, and industrial source have been accumulating since some years in sediments of canals from HCMC, Vietnam. In HCMC, there are 5 main canals (Nhieu Loc - Thi Nghe NL-TN, Tan Hoa – Lo Gom TH-LG, Tham Luong - Ben Cat TL-BC, Kenh Doi - Kenh Te KĐ – KT and Tau Hu - Ben Nghe TH-BN. Waste water (domestic water, industrial waste water and agricultural water) of HCMC is discharged into these five canals and then to Sai Gon river. Sai Gon river is the main navigation and also the main water supply for the HCMC. The analysis of chemical contaminants in the sediments of the HCMC canals is realized since the 1980's and the levels of especially metallic contaminants is quite well known, however, the toxic effect on benthic, epibenthic and aquatic communities is unknown. The application of these toxicity assays to the sediment will enable to evaluate the potential toxicity of pollutants contained in the sediment from HCMC.

2. MATERIALS AND METHODS

The method for culturing *H. azteca,* the water toxicity test with pure chemicals, the toxicity test sediments from HCMC are described in EPS 1/RM/33 - Environment Canada - December 1997 and are recommended by the ecoxicology laboratory of CEMAGREF, Lyon, France.

2.1 Culture techniques

The organisms used for our experiments were obtained from the Ecotoxicology laboratory of CEMAGREF (CEMAGREF is a research center for engineering of agriculture and environment -3bis quai Chauveau, 69336 Lyon, France). The fist step in this research was to transport the amphipods *Hyalella azteca* from CEMAGREF to the ecotoxicology laboratory of the CEFINEA (HCMC, Vietnam) and to culture them appropriately in the conditions of our laboratory. About 100 adults amphipods were cultured in a culture chambre: glass beakers with a volume 5L contin about 4L culture media (prepared according the protocol of USEPA 1995). Cultures were maintained in a photoperiod of 16h light and 8h dark with neon tube. Light intensity adjacent to the water surface in the culture chamber was approximately 850-1000lux. Water to be used for cultures was aerated gently, the range of dissolved oxygen DO was 5-6mg/l. The organisms are fed 1ml Sera Micron (5g/L) daily and 10µg Sera Golgy three times per week. The following parameters were measured daily in the water: pH, conductivity,

dissolved oxygen and temperature. *Hyalella azteca* was chosen as a test organism in this research because this organisms has a high sensitivity, both test water and sediment tests can be conducted, they are more tolerant to salinity than other organisms (which is important for Vietnam: a range of the overlaying water salinity of the canal (max = 1.24%) is acceptable for *H. azteca*), the generation time is relatively short, ease of culture in the laboratory, tolerance to a wide range of sediment physicochemical characteristics, and direct contact of the organisms with the sediment.

2.2 Sample collection

Samples stations were chosen in 5 main canals of HCMC: Nhieu Loc - Thi Nghe NL-TN, Tan Hoa – Lo Gom TH-LG, Tham Luong - Ben Cat TL-BC, Kenh Doi - Kenh Te KĐ – KT and Tau Hu - Ben Nghe TH-BN. For each canal, we monitored two stations: one upstream and one downstream. The reference sediment was obtained from the station Tri An (upriver of Dong Nai). The samples were sampled with Eckman Grab sampler. For each sampling station, about 5cm of the top layer of sediment were collected at a distance of 1.2m of the left and right bank. One liter of each was placed in to the 2L plastic bottles using an inox spoon, obtaining one mixed sample for each station. Each sample container was filled completely to exclude air. Sediments were transported in an ice box (dark, 1-4^{0}C) to CEFINEA and were stored at 4^{0}Cin fridge until testing (within 2weeks)

2.3. Analysis of chemical contaminants

The analysis of chemical contaminants in the sediments of the HCMC canals was realized by CEFINEA, 1999. Residues of heavy metals (Ni, Pb, Zn, Cu, Cr), PAHs and DDT exceeded the threshold effect levels (TEL) established for *H. azteca* in several samples. The water quality characteristics of the overlaying water of these sediment showed that the pH range observed was 6.1 to 8.2. High conductivity was observed in all samples (1143-1560 µS/cm) except for TL-BC2 and TH-BN2 with low values (346 and 424 µS/cm, respectively). Dissolved oxygen was relatively low in these samples (0.1-0.7mg/l).

2.4. Acute test ecotoxicology

After the establishment of the culture of the organisms, we first applied the 96h acute water toxicity test with some pure chemicals. This is necessary to assess, under standardized test conditions, the relative sensitivity of the culture of *H. azteca,* and the precision and reliability of data produced by our laboratory. We chose $CuCl_2$, $K_2Cr_2O_7$ and two pesticides methyl parathion and diazinon for this test. The endpoint for this test is the mortality of the organisms. A mean percent survival was calculated for each concentration and the LC_{50} for each chemical was calculated using a probits/log regression with confidence interval of 95% for each LC_{50}.

2.5. Test with collected sediments

The day before beginning the test, samples were homogenized using an inox spoon and sieved through 0.4mm sieve to remove large particulate material and indigenous macro-organisms present in sediments. At the start of the test, 75ml of sediment and 150ml of test water were transferred to the test beaker 250ml. Ten 2-9day old *H.Azteca* were randomly added to their respective test beakers. After 14-day of exposure, surviving amphipods were counted, isolated from the beakers with a Pasteur pipette and placed in 70% ethanol for counting and length measurement. For each collected sediment (including reference sediment Tri An) the percent survival of amphipods after 14-days was calculated (sum of 4 replicates). The growth of organisms was measured for each individual and the mean (±SD) percent of growth inhibition for each contaminated sediment was also calculated, and compared for significant differences to the reference sediment. Dissolved oxygen, pH, temperature and conductivity were measured in the overlying water each 2 days during the test.

3. RESULTS AND DISCUSSION

3.1 Culturing organisms

We have succeeded in establishing culturing conditions for *H. azteca* in CEFINEA's laboratory.

Several factors can have effects: light, temperature, water quality, system aeration, age of organisms, transport and feeding. A problem with one of these factors can provoke death of the culture. After some difficulties, we managed to culture the amphipods and to obtain sufficient neonates for the testing.

3.2 Toxicity of the tested chemicals to H. azteca

Without aeration the dissolved oxygen concentrations were in a range of 4.8 - 5.3mg/l. In general, pH was reduced after 96h test and conductivity was slightly increased. For the controls, survival is > 80% at the end of all the test. LC_{50}= 79.4µg/L was determined for $CuCl_2$, which is comparable to the effect concentrations obtained by other laboratories. The LC_{50} obtained for $K_2Cr_2O_7$ was 8.3µg/L. There LC_{50} obtained for diazinon and methylparathion were respectively 5.4µg/L and 1.1µg/L. *H.Azteca* seem to be just as sensitive or even more sensitive than other organisms like *D.magna.* The comparisons show that the amphipods of CEFINEA responded with a similar sensitivity to chemical toxicants. This demonstrated that our laboratory can perform the test consistently, and our data are comparable to those reported by other laboratories.

3.3 Testing of sediments from HCMC

This sediment toxicity test method with *H. azteca* is rapid, inexpensive and does not need special equipment. The method provides a direct measurement of the effect on organisms (e.g., mortality, growth...), reflecting an effect of all contaminants and interactions in sediment. Results of sediment toxicity on

H. azteca are shown in table 1. If survival rate was the only endpoint measured in the 14-days test, all samples of HCMC were classified as toxic. When both survival and growth rates were measured, only 1 sample (TH-LG2) reduced both rates compared to the control. No explanation could be found for this big

Difference in sensitivity between the responses of the two endpoints.

Table 1-Sediment toxicity results for H. azteca

Samples	Survival (%)	Length (mm)	Inhibition (%)
NL-TN1	12.5	3.35 ± 0.35	1.47
NL-TN2	7.5	3.26 ± 0.15	4.12
TH-LG1	2.5	3.50 ± 0	0
TH-LG2	7.5	3.07 ± 0.15	9.71
TH-BN1	Not tested	Not tested	Not tested
TH-BN2	0	-	-
TRI AN	77.5	3.40 ± 0.19	0
TL-BC1	10	3.06 ± 0.42	5.75
TL-BC2	2.5	3.00 ± 0	7.69
KD-KT1	10	3.10 ± 0.16	4.62
KD-KT2	5	3.30 ± 0.14	0
TRI AN	75	3.25 ± 0.29	0

*Notes: Starting body length of amphipods was 1.20mm (2-9 days old)

The amphipods *H.azteca* test identified that the 9 sediment samples from HCMC were toxic with mortality varying from 87.5% to 100%. The reference sediment Tri An showing no toxicity can be considered as being a non contaminated site. In order to compare the test response to the reference sample Tri An, the sediments of HCMC were grouped into two categories of effect levels according to Burton et al., 1996. Following this classification, all sediments from HCMC are in the second category (effects greater than 50% difference from the control), and can therefore be classified as very toxic.

3.4. Relation between chemical characteristics and toxicity.

The order in mean survival in these canal samples was the following: Tri An (76.3%)>NL-TN (10%)>KD-KT (7.5%)>TL-BC (6.3%)>TH-LG (5%)>TH-BN (0%) - Sources of pollution: Nhieu Loc - Thi Nghe canal shows the lowest toxicity among these 5 canal. This canal is mainly polluted by domestic waste water and by solid waste but not by industrial waste. This shows that the toxicity

observed in the other canals is probably mainly related to industrial pollution. They are no clear-cut explication for the toxicity observed in the other 4 canals. They are contaminated by both industrial (from many different industries) and domestic waste water. The sampling station TH-BN2 (in which survival was 0%) is located close to the biggest boat factory of Vietnam, which could explain the observed toxicity.

Classification of chemical characteristics and toxicity.

The survival of amphipods was not clearly correlated to PAH contamination. The toxicity *to H. azteca* probably depends on each PAH compound and not on total PAH. The survival of amphipods showed a slight correlation with heavy metals contamination of the sediment. We found no clear correlation between the sediment DDT concentration and the survival.

Chemical Classification: We used the threshold effect level (TEL) for *H. azteca* defined by Ingerssol et al. (1996) as parameter for ranking. The chemical concentration in the sediment of each station was compared to these TEL values (it is noted as "1 point" if a concentration in the sediment is higher than the TEL value and "0 point" if the concentration is Lower than the TEL value). The TEL values of heavy metals, PAHs and metabolites for *H.azteca* 28-days test are reported by Ingerssol et al., 1996. The TEL values of metabolites of DDT are reported by Environment Canada, 1999.

Biological classification was noted by % survival of *H. azteca* in the toxicity tests with the collected sediment. Reference sediment Tri An is also classified in table 2.

No correlation could be established between the observed toxicity and the individual chemical pollutants. But when comparing the total charge (by taking into consideration the number of pollutants exceeding the threshold effect levels for *H. azteca*) with the observed toxicity, a correlation could be observed, with the most polluted sites being the most toxic ones.

4. CONCLUSION

The water-only test and the sediment test with *Hyalella azteca* seem to be well adapted to the needs of CEFINEA laboratory. The *H.Azteca* sediment test is interesting for the assessment and classification of the sediment contamination in the HCM canals. With the aim to assess and manage sediment quality, including dredged material in HCMC. Our data suggests that dredged-sediment material can pose a hazard to terrestrial and aquatic ecosystems if they are used as fertilizes for the agriculture.

ĐÁNH GIÁ ĐỘ ĐỘC BÙN LẮNG KÊNH RẠCH THÀNH PHỐ HỒ CHÍ MINH TRÊN BỌ NƯỚC *Hyalella Azteca*

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TÓM TẮT: *Nghiên cứu này áp dụng thử nghiệm độc học cấp tính 14-ngày trên sinh vật* ***H.azteca*** *để đánh giá độ độc của bùn lắng kênh rạch tp.HCM. Thử nghiệm độc tính 9 mẫu bùn lắng của 5 kênh tp.HCM với bọ bơi nghiêng* ***H.azteca*** *cho kết quả % chết của sinh vật từ 87.5 đến 100%. Mẫu bùn kiểm chứng Trị An không độc với sinh vật thử nghiệm chứng tỏ ở hồ Trị An, bùn chưa bị ô nhiễm. Giá trị trung bình % sống sót của sinh vật với các mẫu bùn lắng sắp xếp như sau: Tri An (76.3%)>NL-TN(10%)>KD-KT(7.5%)>TL-BC(6.3%)>TH-LG(5%)>TH-BN(0%). Các mẫu bùn lắng đều được xếp vào loại rất độc (ảnh hưởng trên 50% so với mẫu kiểm chứng – phân loại theo Burton và đồng nghiệp, 1996). Thử nghiệm độc học với sinh vật* ***H.azteca*** *của bùn cho phép chúng ta đánh giá và*

phân loại mức độ độc của các loại bùn lắng giúp cho việc quản lý chất lượng bùn lắng và kể cá bùn nạo vét của kênh rạch tp.HCM.

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Table 2 - Classification of chemical characteristics and toxicity.

Samples	NL-TN1	NL-TH2	TH-LG1	TH-LG2	TL-BC1	TL-BC2	KD-KT1	KD-KT2	TH-BN2	Tri An	TEL–HA28	PEL–HA28
PAHs*(ppb)*	-	1402	-	927	1221	1083	729	588	1168	90,71	260	3400
PYR *(ppb)*	NA	234,8	NA	311,6	112,9	116,3	154,6	149,3	367,0	20,72	44	490
BAA *(ppb)*	NA	122,6	NA	85	115,8	134,8	68	54,8	109,2	6,94	16	280
CRY *(ppb)*	NA	312,8	NA	177,3	213,5	242,4	159,7	105,9	233,6	18,72	27	410
BBF *(ppb)*	NA	254,3	NA	113,3	246,9	235,6	106,8	82,0	140,3	22,28	27	160
BKF *(ppb)*	NA	115,7	NA	44,8	214,3	114,5	38,4	33,8	55,6	4,47	27	320
BAP *(ppb)*	NA	118,4	NA	87,9	68,2	103,6	59,7	69,8	115,9	5,75	32	320
DBA *(ppb)*	NA	16,3	NA	9,7	23,7	22,6	25,8	7,6	10,2	3,56	10	28
BGP *(ppb)*	NA	226,8	NA	97,3	225,8	113,4	115,7	81,9	139	8,27	16	250
Cr *(mg/kg)*	35,49	46,77	100,54	32,36	14,5	32,90	87,78	53,85	70,35	29,44	36	120
Cu *(mg/kg)*	54,64	84,21	275,24	18,47	26,18	53,75	69,08	112,06	87,43	26,73	28	100
Ni *(mg/kg)*	24,54	40,54	94,54	29,42	trace	30,74	60,13	54,05	30,64	25,65	20	33
Pb *(mg/kg)*	56,78	86,92	479,16	27,41	53,53	47,89	76,68	118,8	93,21	25,56	37	82
Zn *(mg/kg)*	371,7	531,7	1210,9	132,04	16,65	322,87	18,77	129,55	260	46,55	98	540
p,p'-DDE *(ppb)*	NA	50,23	NA	9,2	5,11	7,76	9,27	3,73	19,33	NA	1,42	6,75
p,p'-DDD *(ppb)*	NA	35,50	NA	13,61	1,36	9,56	10,38	10,46	23,19	NA	3,54	8,51
p,p'-DDT *(ppb)*	NA	3,3	NA	1,55	1,36	1,33	1,07	trace	4,9	NA	1,99	4,77
Total points	NA	16	NA	11	10	14	12	11	16	1		
Chemical.Class	NC	1	NC	4	5	2	3	4	1	6		
Biological.Class	6	4	2	4	5	2	5	3	1	7		

NA: Not analyzed - NC: Not classified

TEL-HA28: Threshold Effect Level for *H.Azteca* 28-days test (Ingersoll et al, 1996)

PEL-HA28: Probable Effect Level for *H.Azteca* 28-days test (Ingersoll et al, 1996)