COMBINED EFFECTS OF HEAVY METALS AND CYANOBACTERIAL CRUDE EXTRACTTO DAPHNIA MAGNA

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Abstract- A chronic test has been done to test chronic effects of combination of copper (Cu), Chromium (Cr) and cyanobacterial crude extract (CCE) to *Daphnia magna*. The result shows the combination of Cu, Cr and CCE impacted to survival rate, maturation and fecundity of *D.magna.* The combination of Cr +CCE caused the effects that more severe to survival rate and fecundity of *D.magna* than in the single Cr or CEE. Whereasthe combinationeffects of Cu + CCE or Cu +Cr+ CCE to *D. magna*were less toxic than in the single Cu. Maturation was significant been late in *D.magna*exposed to the combination of Cu, Cr and CCE.

I. INTRODUCTION

Heavy metals from mining, mechanical engineering, textile and plating are discharged in to the rivers in Vietnam. As a result, concentrations of the heavy metals such as copper (Cu), Chromium (Cr), lead (Pb), arsenic (As) in sediments of Saigon, To Lich and Kim Nguu Rivers have been exceeded the permitted threshold of both the Vietnam and Danish standards (Phuong *et al.*, 1998; Marcussen *et al.*, 2008). High concentration of heavy metals in water may have toxic effectson aquatic organisms. On the other hand, blooms of cyanobacteria in freshwater bodies have been recorded in Vietnam (Nguyen *et al.*, 2007; Christensen 2006; Dao *et al.*, 2010; Duong *et al.*, 2013). Many species of cyanobacteria can produce a secondary metabolites, namely cyanotoxins that can cause acute or chronic toxicity to animals and humans. The heavy metals and cyanotoxins in water bodies in Vietnam, therefore, may impact to aquatic organisms in which micro crustacean such as *D. magna* is known as the most common freshwater invertebrate model species to study ecotoxicity (OECD, 2004). Effects of heavy metals and cyanotoxins to *D. magna* have been examined by several studies (Khangarot *et al.*, 1987; Bodar *et al.*, 1989and Lürling, 2003; Smutná *et al.*, 2014; Dao *et al.*, 2010). However, combined effects of both heavy metals and cyanotoxins to *D. magna* are still unknown. In this study, we examined combined effects of copper (Cu), Chromium (Cr) and cyanobacterial crude extract (CCE) to survivor rate, maturation and fecundity of *D. magna*.

II. METHOD

2.1 Preparation of cyanobacterial crude extract (CCE)

Cyanobacterial crude extract was made from *Planktothrix*sp. isolated in a fish pond in Soctrang province (Vietnam) and cultured as single strain in our lab. The preparation is modified from Fastner et al. (1998). The biomass of cultures on GF/C filters (Whatman) were dried at 50°C over night to make dry mass. A certain weight of the dry mass was added to double distilled water and subjected to five freeze/thaw cycles (-70°C) and ultra-sonicated on ice (200 Hz, 1 minutes)to lyse cells. Cell debris was removed by centrifugation. Double distilled water was added to make final solution of 10g dry mass per litreand kept at -70°C.

2.2 Preparation of test solutions

A series concentrations of copper (Cu) combined with Crom (Cr) and CCE in Combo medium was prepared as test solution (see table 1). The concentration of Cu or Cr stocks are 1000 $mg.L^{-1}$ Cu or Cr in Nitric acid 2-3%(Merck). These stocks solution were added to Combo medium to obtain nominal exposure concentrations (see table 1). For instant, 20µL of the copper stock was added to 1L Combo medium to make the test solution of 20µg $Cu.L^{-1}$.

Table 1: Test solution and test substances

Test solutions	Tested substances		Concentration
1	Cu+CCE	0	20 ($\mu g.L^{-1}$) +100 ($mg.L^{-1}$)
2	Cr+CCE	0	100 ($\mu g.L^{-1}$) +100 ($mg.L^{-1}$)
3	Cu+Cr+CCE	0	20 ($\mu g.L^{-1}$) +100 ($\mu g.L^{-1}$) +100 ($mg.L^{-1}$)

2.3Experimental organisms

D. magna was obtained from MicroBioTests Inc. Belgium and has been maintained in the laboratory of Institute for Environment and Resource, HoChiMinh city, Vietnam for several years under controlled conditions: temperature= 22 ± 1°C, light intensity = 1000 Lux (Lux-meter, Testo, Germany), photoperiod = 14:10 hours light:dark in ISO medium. The animal was fed by *Scenedesmus* sp. for every two days. The

medium was weekly refreshed. The ISO medium consists of $NaHCO_3$ (67.75 $mg.L^{-1}$),$CaCl_2.2H_2O$ (294 $mg.L^{-1}$), $MgSO_4.7H_2O$ (123.25 $mg.L^{-1}$), KCL (5.75 $mg.L^{-1}$). The pH was adjusted to 7.0 ± 0.2.

2.4 Experimental design

Fifteen adults of*D. magna* were individually placed in separate 200-mL plastic cups containing 100 mL of ISO medium, *Scenedesmus* sp. and YTC (yeast, cerrophyl and trout chow digestion).Tennew-borns (less than 24h old)were randomly selected and put in 250mL plastic beaker containing test solutionsincluding combination of Cr + CCE, Cu + CCE, Cu+Cr+CCEand food (*Scenedesmus*2mgC.L^{-1} and YTC). Control included the new-borns in ISO medium and food. Each concentration and the control consists of three beakers as three replicates. Test solutions and food were refreshed every two days and culture conditions were kelp as described above. The survival, maturation (time at which the instar has the first eggs deposited into the brood chamber) and fecundity of each exposure and control experiment were recorded within 21 days.

Kruskal-Wallis test (Sigma Plot, version 12) was used to determine the significant difference between *Daphnia*'s maturation of control and exposures.

III. RESULT AND DISCUSSION

3.1 Survival

After 21 days of exposure, survival of D. magna was decreased inthe threecombinations Cr + CCE, Cu + CCE, Cu+Cr+CCE. The number of *D. magna*in the exposure to combinations Cr + CCE and Cu + CCE were stable within the first 8 days but quickly decreased afterwards, with 33% and 53% survival at terminal experiment. The combination of Cu+Cr+CCE,however, showna weaker and later effects on survival of *D. magna*in comparison with the combination of Cr + CCE and Cu + CCE. Survival rate of *D. magna*in Cu+Cr+CCE was quickly reduced from 90% on 16th day to 63% at terminal experiment. The control experiment had no deaths (Fig. 1b).

Single Cr and CCE had a weak and a moderate effects on survival of *D. magna*, with survival rates of 93% and 43% respectively (Fig. 1a, Tran*et al.* 2014) but their combination had high toxicity to *D. magna*with only 33% survived after 21 days of exposure. The combination of Cr + CCE increased toxicity to *D. magna* in comparison with the singles.On the other hand, the combination of Cu + CCE, Cu+Cr+CCE decreased toxicity of Cu to *D. magna*. More than 90% *D. magna* was died after 21 days of exposure to single Cu (Tran et al. 2014) but this numberwas less than 50% and 40 % in the combination of Cu + CCE and Cu+Cr+CCE.Concentrations of Cu, Cr and CCE were the same in the single and combination treatment experiments.

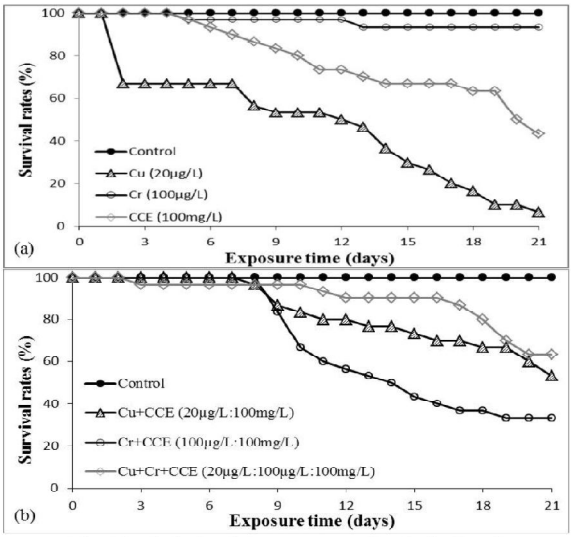


Fig. 1 Survival rate of *D. magna* exposed to single (a) and combined (b) Cu, Cr and CCE

3.2 Maturation

Maturation of *D. magna*in the three combinationwas significantly later than in the control (Fig. 2). The maturation of *D. magna*in the control was reached around the age of 5 days, meanwhile *D. magna*in exposure treatments reached their maturity around the age of 6 days. Maturation of *D. magna*was similar among the three combinations. The maturationof *D. magna* in the combination of Cu + CCE was earlier than in single Cu but later than in single CCE (Tran*et al.* 2014). Therefore, effect of the combination of Cu + CCE was weaker than effect of single Cu but stronger than effect of single CCE to maturation of *D. magna*.From this result, we give two hypothesis that either CCE may help to reduce toxicity of Cu to *D. magna*in the combination of Cu + CCE or nutritional compounds in CCE may help to improve tolerance of *D. magna* .

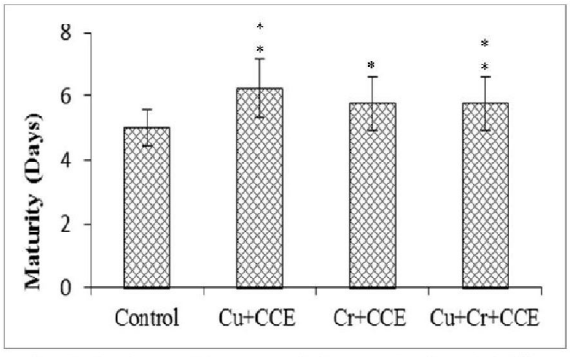


Fig. 2 Maturity age of *D. magna* in the three combinations. Asterisks indicated statistically significant differences from control (*, $p < 0.01$; **, $p < 0.001$, Kruskal-Wallis test)

3.3 Fecundity

The highest fecundity was observedin the control with 454 cumulative neonates born after 21 days of culture. Low fecundities were found in the combination of Cr+CCE, Cu+Cr+CCE and Cu+CCE with only13, 54 and86 neonates born over 21

days,respectively. The interesting thing is that reproduction was stimulated in *D. magna*exposed to either single Cr or single CCE (Tran*et al.* 2014)with but the reproduction was inhibited in *D. magna* exposed tothe combination of Cr+CCE. Abortion observed in *D. magna*exposed to the three combinations (fig. 4) was the reason for the low fecundity.

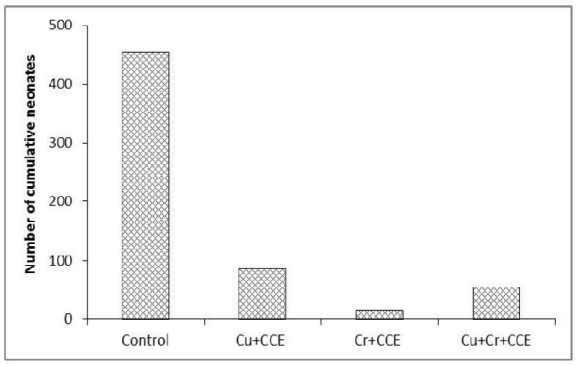


Fig. 3 Fecundity of *D. lumholtzi* when exposed to the three combinations

CONCLUSION

D. magna exposed tothe combinationof Cr + CCE, Cu + CCE, Cu+Cr+CCE was decreased survival rate, fecundity and late maturation. The combinationof Cr + CCEcausedsignificant effects to survival rate, fecundity of *D. magna* and the effects was moresevere in the combinationof Cr + CCE than in the single Cr, CEE. Toxicity of Cu to *D. magna* was decreased in the combinationof Cu + CCE, Cu+Cr+CCE.



Fig. 4 Abortion in *D. magna* exposed to the metals and CCE. Arrow indicates dead embryo

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