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Toxicity of Ammonia to Benthic Amphipod *Grandidierella bonnieroides*: Potential as Confounding Factor in Sediment Bioasssy

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Abstract

Toxicity of ammonia was evaluated using amphipod Grandidierella bonnieroides to describe its role as confounding factor in sediment quality assessment. Ammonia is a toxic compound that is found naturally in seawater and sediment. High ammonia content in the pore water sediment can be potentially toxic to benthic biota, so that it will interfere with the results of sediment toxicity tests. Laboratory production amphipod was used in this ammonia toxicity test. Water-only toxicity tests was conducted to produce new toxicity data of ammonia, and is expressed as LC50, LOEC and NOEC for benthic amphipod G.bonnieroides. The study resulted the 96-h median lethal concentration (LC50) of ammonia for G. bonnioerides was 65.5 mg.L^{-1}*. While the value LOEC (low observed effect concentration) is 56 mg.L^{-1} and NOEC value (no observed effect concentration) was 32 mg.L^{-1} . This shows that ammonia has a relatively low toxicity to amphipod and ammonia does not act as a confounding factor in the sediment toxicity test, because the ammonia content in sediment does not pose any significant effect on amphipod survival. It can be concluded that the amphipod has a potential useful as test organism in sediment bioassay for assessing the quality of marine sediment. Moreover, the high dependence of total ammonia toxicity indicates that it is necessary to measure the total ammonia and pH of the medium when testing environmental samples.*

Keywords: *ammonia, amphipod, toxicity, confounding factor, bioasssay*

Abstrak

Toksisitas ammonia terhadap amphipod bentik Grandidierella bonnieroides : Potensi sebagai faktor pengganggu dalam bioassay sedimen

Toksisitas ammonia dievaluasi menggunakan amphipod Grandidierella bonnieroides untuk menggambarkan perannya sebagai faktor pengganggu dalam penilaian kualitas sedimen. Amonia merupakan senyawa beracun yang ditemukan secara alami dalam air laut dan sedimen. Kandungan amonia yang tinggi dalam air pori sedimen dapat berpotensi racun bagi biota bentik, sehingga akan mengganggu hasil uji toksisitas sedimen. Amphipod hasil produksi laboratorium digunakan dalam uji amonia. Uji toksisitas dilakukan untuk menghasilkan data toksisitas baru ammonia, dan dinyatakan sebagai LC50, LOEC dan NOEC untuk G.bonnieroides amphipod bentik. Studi ini menghasilkan nilai konsentrasi median letal 96-jam (LC50) ammonia untuk G. bonnioerides adalah 65.5 mg.L^{-1}. Sedangkan nilai LOEC (konsentrasi terendah yang menyebabkan dampak secara signifikan) adalah 56 mg.L^{-1} dan nilai NOEC (konsentrasi tertinggi yang tidah menyebabkan dampak secara signifikan) 32 mg.L^{-1}. Hal ini menunjukkan bahwa ammonia memiliki toksisitas relatif rendah untuk amphipod dan ammonia tidak bertindak sebagai pengganggu dalam untuk uji toksisitas sedimen, karena kandungan ammonia dalam sedimen tidak menimbulkan pengaruh yang nyata pada survival amphipod. Dapat disimpulkan bahwa amphipod memiliki potensi sebagai biota uji dalam sedimen bioassay untuk menilai kualitas sedimen laut. Selain itu, terdapat ketergantungan yang tinggi toksisitas amoniak terhadap pH, sehingga perlu untuk memantau total amonia dan pH saat melakukan pengujian sampel lingkungan.

Kata kunci : *ammonia, amphipod, toksisitas, faktor pengganggu, bioassay*

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Introduction

It is important to identify the contaminant that is responsible for sediment toxicity in the sediment quality assessment studies. The biological fraction of contaminant available for benthic organisms was reported to be the most of concern and it has been highly correlated with the concentrations of contaminant in interstitial (pore) water (Swartz *et al.*, 1986; Burgess *et al.,* 1993; Whiteman *et al.,* 1996).These studies have confirmed that biological effects based on pore-water chemical concentrations are similar to concentration-response relationship resulted from water-only toxicity test with the same chemicals. Several studies revealed ammonia has been responsible for the toxicity of contaminant in sediment (Boardman *et al.,* 2004; Ingersoll *et al.*, 2015). High concentrations of ammonia in sediment pore water may have contributed to the observed toxicity. For these reason, misinterpretation of the sediment assessment studies result will be a critical to the development of successful remedial strategies.

Ammonia is a relatively toxic compound produced in natural waters and sediments by heterotrophic bacteria as a primary by-product of the decomposition of organic matter (Russo 1985) and become a potentially highly toxic occuring constituent of sediment pore waters for aquatic organisms (Burgess *et al.*, 1993; Whiteman *et al.*, 1996). Anthropogenic sources included waste water effluents and terrestrial runoff can significantly increase ammonia concentrations in coastal environments. Since ammonia is highly soluble and readyly released from sediment during resuspensions process, it is recycled via the pore water to the water column and epibenthic/benthic organisms can be exposed to high concentrations of ammonia in solid-phase and elutriate toxicity test (Alessandra *et al.,* 2003) so that ammonia may affect outcomes of contaminant toxicity test (Mehler *et al.*, 2010; Batley and Simpson 2009). It is, therefore, needed to determine the sensitivity limit to ammonia in the methods used in sediment toxicity assessment.

In aquatic environments, ammonia is in equilibrium between two chemical species: unionized ammonia (NH_3) and ammonium ion (NH_4^+) (Russo, 1985). Total ammonia is the sum of NH3 and NH4+, and it is total ammonia that is measured analytically in aqueous solution. The neutral, un-ionized form is highly toxic to fish and other aquatic life because of its ability to diffuse readily across phospholipid cell membranes; ammonium effects are less marked (US EPA, 2001a; Boardman *et al.*, 2004). Unionized ammonia is the most toxic form, although there is evidence that the ammonium ion also contributes to toxicity (USEPA, 1999). It has been recommended a water quality criteria for un-ionized ammonia value of 0.035 mg.L^{-1} for salt water acute effects (US EPA,1999), a guideline for ammonia in sediment pore waters, a guideline trigger value of 3.9 mg total NH3–N/Lbased on species sensitivity disribution (Batley and Simpson, 2009). The Australian and New Zealand currently revised guideline of 4 mg total NH3–N/L as conservative value after recommended previously as a guideline trigger value (Batley and Simpson, 2009).

The toxicity of ammonia to benthic organisms, especially amphipods, has been evaluated in 96-h water-only exposures (Kohn *et al.,* 1994; Schubauer-Berigan *et al.,* 1995; Alessandra *et al.*, 2003; Boardman *et al.*, 2004; Lee *et al.*, 2005a). Recent study using mussels demonstrated that the acute sensitivity to ammonia was not influenced by the presence of substrate in 4-d laboratory toxicity tests (Wang *et al.,* 2011). This indicated that results of the water-only toxicity test can be used to predict the toxicity of the sediment pore water toxicity. High levels of ammonia in sediment toxicity tests can potentially confound test results and a significant potential for ammonia induced interference has been reported in the 10-d test using estuarine amphipod (*Leptocheirus plumulosus*) but not for the 28-d tests due to rapid dissipation of ammonia via renewal of overlying water (Moore *et al.,* 1997). Toxicity of ammonia was found to be highly pH dependent (Van Sprang and Janssen 1997). Increased toxicity was observed at higher pH levels, whereas reduced toxicity was found lower pH levels.

Amphipods have been used in the assessment of sediment quality studies due to their sensitivity, abundant, ease of handling and widely distribution (Angel *et al.,* 2010, US EPA 2001a). Among the amphipods, *Grandidierella japonica* was commonly used as standard test organism in other countries (Lee *et al*., 2005, USEPA, 2001b). However, available information was limited on the sensitivity of benthic amphipod *Grandidierella bonnieroides* to sediment particle size (Hindarti *et al.,* 2015). Then this study was aimed essentially to evaluate the effect of ammonia on mortality of benthic amphipod *Grandidierella bonnieroides* to elucidate the role the environmental factor in sediment assessment studies. Also, it is required to conclude whether or not the amphipods is suitable as test organism for sediment bioassay in terms of their sensitivity to ammonia contamination.

Materials and Methods

Benthic indigenous amphipod *Grandidierella bonnieroides* Stephenson 1948 was collected from the upper layer of sediment from the low intertidal zone located in Kramat Kebo estuary (5°98'33" N, 106°70'33" E), Banten, Indonesia, using a stainless-steel spoon. Sediment containing amphipods were transferred to plastic container containing a 2- to 3-cm layer of sediment and seawater. They were transported to the laboratory in low temperature by given ice pack into container to prevent overheating. In the laboratory, amphipods were sieved out of the sediment and sorted based on size, > 3,5 mm and $\leq$ 3,5 mm lengths (Hindarti *et al.*, 2015). Amphipod used in this test was obtained from larvae production of gravid females. Seven days larvae (neonates) of amphipod or approximately 3-5 mm length are ready for used for bioassay. To determine the 7 days old is by the time they release from the pouch.

Acute toxicity test of ammonia was conducted based on procedures of ASTM (2006) with slight modification to tropical temperature. UV-sterilized and filtered natural seawater at salinity 30 ppt was used as a dilution water in the toxicity tests and a negative control factor. Stock solution of total ammonia was prepared by dissolving (NH4)2SO4 (E-MERCK) in the treated natural seawater at 25°C and salinity 30 ppt. Five nominal ammonia concentrations were prepared for acute toxicity test, the values were 18, 32, 56, 100, and 180 mg.L^{-1} total ammonia(NH_4^++NH_3), and were exposed to the amphipods for 96-h. Amphipods were also exposed to cadmium as a reference toxicant and it was conducted concurrently to the test.

The tests was conducted in four replicates and 20 individuals of amphipod were allocated to each treatments. Observation of dead individual and water quality of the test media were made in the daily basis. No test water renewal was made for all of the tests. The pH was measured with a Model 290 Orion meter, dissolve oxygen and temperature were measured with a YSI model 58 DO meter.

The acceptability of test results was fixed a percentage of amphipod survival $\geq$ 90% in all negative control, and only if the LC50 with the reference toxicant fell within the accepable range (ASTM, 2006). All calculations originate from nominal ammonia concentrations and expressed as total ammonia nitrogen (i.e. mg N/L). Median lethal concentrations (LC50s) with 95% confidence limits were calculated by the Trimmed Spearman-Karber statistical method (Hamilton *et al.*, 1977) and were based on total ammonia and unionized ammonia. The fraction of unionized ammonia was calculated using ammonia calculator (SVL Analytical, 2010) at differing values of pH and temperature, and known concentration of total ammonia. After tests were completed, survival data was analyzed to determine the LC50,NOEC, and LOEC by analysis of variance (ANOVA) and Dunnett's one-tailed t-test on arcsine square-root transformed data using TOXSTAT software.

Results and Discussion

A common practice during bioassay, most experiments use a reference toxicant as a positive control in order to have a compatibility of all across experiment conditions and to determine the level of sensitivity of biota used as test biota. Cadmium (Cd) was used in the present study as a reference toxicant due to its highly toxicity and stability along the duration of the test (Gorbi *et al.*, 2012). The LC50 value calculated from this data was 0.70 mg.L^{-1}, the NOEC and LOEC value were 0.32 mg.L^{-1} and 0.56 mg.L^{-1}, respectively. The toxicity of Cd to amphipods has been extensively tested (Lee *et al.*, 2005b; Kohn *et al.*, 1994; DeWitt *et al.*, 1989). The LC50 value of Cd for *G. bonnieroides* in the present study toxicity was within the range of the LC50s value for most of the amphipods tested (Table 1), which varied in the range from 0.36 mg.L^{-1} for *Leptocheirus plumolatus* and 2.9 mg.L^{-1} for *Ampelisca abdita*. Therefore, the amphipod of *G. bonnieroides*was as sensitive as the standard test-species used in other countries and proves to be a suitable species to use in estuarine acute toxicity testing showing high potential to identify both overlaying water contamination and sediment degradation.

A wide range of ammonia concentrations was tested to cover the effects on mortality of amphipods. The water-only test of ammonia with *G. bonnieroides* had acceptable control survival and exhibited a conccentration-dependent increase in the toxicity of ammonia (Figure 1). Throughout the experiment period, control showed 97.5 $\pm$ 5% of amphipod survival. The first significant effect onmortality (LOEC) was found at an exposure to 56 mg.L^{-1} total ammonia and the concentration of ammonia that have no a significant effect on mortality was 32 mg.L^{-1} (Figure 1). The effects increased markedly at higher exposure levels and the median lethal concentration (LC50-96h) value of ammonia calculable from this data was 65.49 mg.L^{-1}

Table 1. The 96-h LC50 values of cadmium for various marine and estuarine amphipods

Species	96-h LC50 (mg.L-1)	Reference
Ampelisca abdita	2.9	USEPA (2001a)
Grandidierella japonica	1.17 1.47	USEPA (2001b) Lee *et al.,* (2005a)
Eohaustorius estuarius	2.4	ASTM (2006)
Leptocheirus plumolatus	0.36	USEPA (2001a)
Repoxynius abronius	0.53 0.76 0.75, 1.1	Kohn *et al.,* (1994) DeWitt *et al.,* (1989) ASTM (2006)
Monocorophium achersicum	0.7-1.4	Lee *et al.,* (2005b)

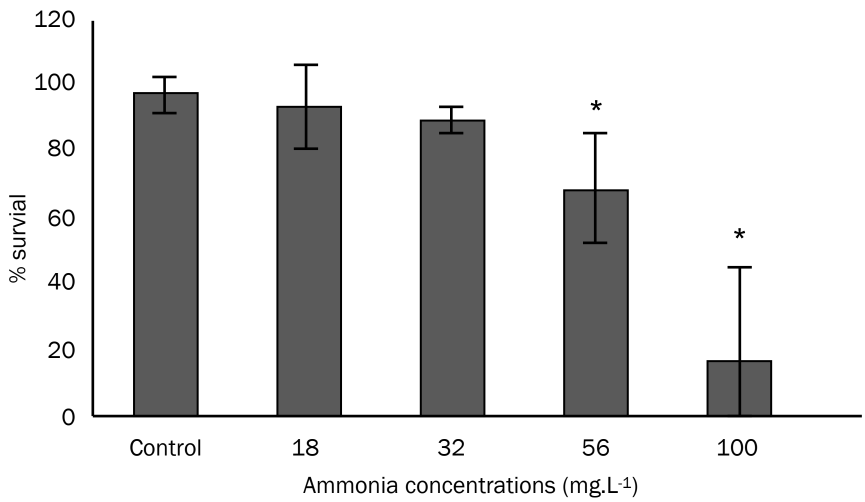

Figure 1. Survival of amphipods at 96-h of ammonia exposure. *statistical significant at p<0.05

as total ammonia ($NH4^+$ + NH3). Toxicity test using amphipods generally conducted in order to clarify the role of ammonia as confounding factor in sediment quality assessment studies. The 96-h LC50 of total ammonia for *G. bonnieroides* in the present study showed that G. *bonnieroides* was relatively tolerable to ammonia in water-only exposure when compare to *Paracentrosus lividus* (5.7 mg.L^{-1}) (Alessandra *et al*., 2003), *Ampelisca abdita* (50 mg.L^{-1}) (US EPA, 1994) and *Leptocheirus plumulosus* (44-89 mg.L^{-1}) (Moore *et al*., 1997), but less tolerance when compare to *Repoxynius abronius* (79 mg.L^{-1}) (US EPA, 1994), *Eohaustorius estuaries* (126 mg.L^{-1}) (Kohn *et al*., 1994) and *G. japonica* (141 mg.L^{-1}, 148 mg.L^{-1}) (Lee *et al*., 2005a; US EPA, 1994) which were used as standard test organism in sediment bioassay in the USA and other countries. Ammonia concentrations in sediment porewater are commonly thousands of times higher than in overlying water; therefore, ammonia can exert severe toxicity in some organically polluted sediments (Ferretti *et al.,* 2000). The effect of contaminant in sediment pore water can be predicted by water-only toxicity data for the same contaminant both for freshwater and seawater species (Newton and Bartsch, 2007; Whiteman *et al.*, 1996; Burgess *et al*, 1993).Hence, the bioavailability of ammonia to benthic macroinvertabrates can indeed be accurately related to sediment pore water contentrations. If the pore water ammonia concentrationsis in the range of the water-only LC50 for the species of concern, the ammonia could be presumed as possibly being responsible for observed toxicity. Therefore, relatively high tolerance of *G. bonnieroides*to ammonia can be useful as test species for sediment bioassay.

The relative ammonia toxicity (expressed as a total ammonia) for *G. bonnieroides* increased as pH increases.The first significantly (P<0.05)increase in pH value was found in the 48 h of exposure and afterwards (Figure 2). At the end of the test (96-h) the pH value was significantly increased (P<0.05) compared to the control at the beginning of the test, and then increases in mortality of the amphipods.

This is due to differences in the relative toxicity of unionized ammonia (NH3) versus ammonium ion ($NH4^{+}$) as explained by Schubauer-Berigan *et al.* (1995) that at low pH values the ionized ammonium ion predominates, and as pH increases the equilibrium shiifts toward the more toxic unionized ammonia. Schubauer-Berigan *et al.* (1995) found that over the pH range of 6.5 to 8.6 the sensitivity of the benthic macroinvertebrates to total ammonia increased by 4–40 order of magnitudes.

Un-ionized ammonia is the form of ammonia that is toxic to aquatic life due to its characteristic that can most readily reach entry to aquatic organism. The un-ionized ammonia calculated as a function of pH, temperature and total ammonia

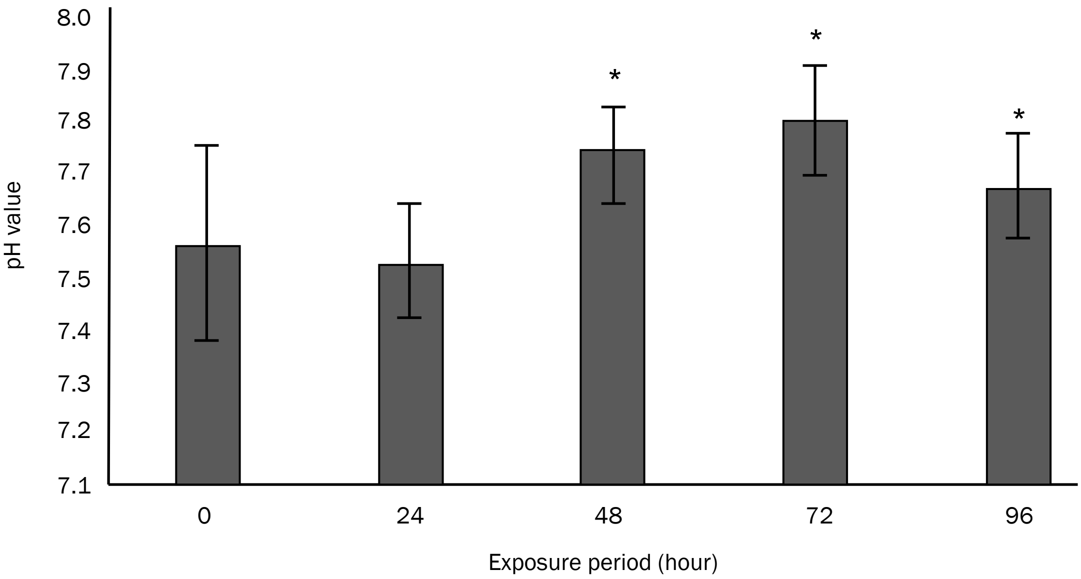

Figure 2. Changes in pH value during the test. * statistical significant at $p<0.05$

Table 2. Summary of of toxicity data for 96-h water-only test conducted with *G. bonnieroides*. Ammonia concentrations are indicated as nominal total ammonia (mg N/L)

Chemical	NOEC	LOEC	LC50	96h-LC50 95% CI	
				LL	UL
Ammonia (mg N/L)	32	56	65.5	59.46	72.15
Unionized ammonia ($mg.L^{-1}$)	1.039	2.760	1.70	1.574	1.843

CI – Confidence Interval; LL – Lower limit; UL – Upper limit

Table 3. Summary of toxicity data for 96-h water-only ammonia toxicity test conducted with *G. bonnieroides* Stephenson 48. Ammonia concentrations are indicated as total ammonia (nominal).

Ammonia conc. ($mg.L^{-1}$)	pH	Temperature (^{o}C)	Unionized conc. ($mg.L^{-1}$)	Mortality (%)
Control	7.67 (7.33-7.88)	25.06 (24.1-26.2)	0	2.5 (0-10)
18	7.74 (7.61-7.88)	25.36 (24.4-26.6)	0.535	6.3 (0-25)
32	7.72 (7.5-7.87)	25.76 (24.7-26.7)	1.039	10 (5-15)
56	7.70 (7.59-7.83)	25.86 (24.7-27.1)	1.421	31.3 (15-45)
100	7.62 (7.42-7.73	25.7 (24.4-27.2)	2.542	83.8 (40-100)
180	7.50 (7.32-7.62)	26.18 (25.2-27.1)	3.61	100

concentration was presented in Table 3. The LC50 value of unionized ammonia for *G. bonnieroides* was 1.70 mg NH3/L (Table 2) and was lower than the value of 3.5 $mg.L^{-1}$ found for*G. japonica*(Lee *et al.*, 2005), was higher than the value of 0.43 $mg.L^{-1}$ found for *Allorchestes compressa* (Adams and Strauber, 2007). The LC50 value of un-ionized for *G. bonnieroides* was higher than the water quality criteria for un-ionized ammonium set by US EPA (1999), indicating the amphipod used in this study was tolerant to un-ionized ammonia exposure. The sensitivity of *G.* bonnieroides to un-ionized exposure was comparable to other standard tests species used in other countries indicating the benefecial used of the species in the sediment bioassay.

Conclusion

Survival of *G. bonnieroides* significantly responded to ammonia concentrations and the toxicity of ammonia was relaitively low to the amphipods. The sensitivity of *G. bonnieroides* was comparable to other standard amphipod test species, and Itindicates that the interference of ammonia on the sediment toxicity results is relatively less important. Therefor, this study suggest that the amphipod has a potential useful as test organism for sediment bioassay in the marine sediment assessment. Moreover, the high dependence of total ammonia toxicity indicates that it is necessary to measure total ammonia concentrations and pH of the medium accurately when testing environmental samples.

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