

## Acute toxicity of cadmium to amphipod *Grandidierella bonnieroides*: implications as test organism for sediment bioassay

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**Abstract.** *Grandidierella bonnieroides* was exposed to cadmium to investigate their potential as test species for sediment toxicity assays in Indonesia. The species was found to be abundant in tidal area of Kramat Kebo estuary. Cadmium is a widespread environmental pollutant and also commonly used as reference toxicant in toxicity testing. Three batch of amphipod were exposed to series of cadmium concentrations in static toxicity testing for 96 h. Seven-day old amphipod productions laboratory have been used in this cadmium toxicity test. The study revealed that sensitivity vary within species, the median lethal concentrations (LC50) values were in the range of 0.35 mg/L and 0.88 mg/L. Amphipods provide response that made them suitable test species for toxicological testing including sediment bioassay.

**Key Words:** heavy metal, benthic organism, adverse effect, estuarine, environmental assessment.

**Introduction.** The problem of contaminated sediment particularly heavy metals has increased obvious attention, especially in Indonesia (Puspitasari & Hindarti 2009; Arifin 2011; Lestari & Budiyanto 2013). Elevated metal concentrations in sediment can exert toxic effects, therefore can affect benthic organisms living in, or coming into contact with, the sediments. In recent years, toxicity testing has been involved in the assessment of contaminated sediments as they provided importance information on their impact on biota which may include human sources of food (Morales-Caselles et al 2008; Carr et al 1996; Louma & Carter 1993; Chapman & Long 1983). At present, the potential toxicity of field-contaminated sediments can be assessed using bioassays approach (Anderson et al 2007), including benthic organisms that are exposed to field-collected sediments and its toxic effects determination. There are number of requirements for a test organism in marine sediment toxicity test: the species should be native and numerical abundance in intertidal coastal, wide-range salinity tolerance, sensitive to common sediment contaminants, occupied microhabitat to make sure a consistent exposure to sediment contaminants, less sensitive to natural sediment types, including grain size and organic matter, less sensitive to ammonia, short life cycles, widely distributed, easy to collect, handle and maintenance in the laboratory, ecologically relevance and available continuously throughout the year (Chapman 1988; DeWitt et al 1989; Smith & Logan 1993; Ingersoll 1995). Cadmium is a widespread environmental pollutant as well as a reference toxicant. Sources of cadmium in aquatic environment include waste from Pb-mines, various chemical industries (Landis & Yu 2005). Chemical forms of cadmium may result in different toxicity and bioconcentration factors, which have adverse effects on aquatic organisms (Callahan et al 1979). Cadmium bound-particulate matter and – organic material in fresh water and seawater may not be bioavailable and hence may not be harmful to aquatic organisms (Callahan et al 1979; Kramer 1988). The toxicity of Cd to amphipods has been extensively tested (Lee et al 2005a; Kohn et al 1994; DeWitt et

al 1989). A pattern of increased tolerance to toxicity with increasing size or age has been reported in the amphipods *Leptocheirus plumulosus* (McGee et al 1998).

Amphipods are dominant taxa living in the marine and estuarine soft bottom. They are an important prey for many fish, birds and larger invertebrate species. Most of them are detritus feeders and ingest sediment; consequently they can be directly exposed to variety of contaminants from sediments. Amphipods have been shown to be sensitive to contaminated sediments (Mann et al 2009). Amphipods have also been successfully used in toxicological studies due to their sensitivity to a wide variety of contaminants, easy collection and handling in the laboratory, but also because their protection ensures the protection of the whole benthic community. Amphipods from the genus *Grandidierella* such as *G. japonica* are one of the standard species for use in the sediment quality assessment and have been widely used in environmental quality studies. *G. japonica* collected from Korean waters was reported to be sensitive to various contaminant and contaminated sediment tested (Lee et al 2005a,b). However, very few works (Hindarti et al 2010, 2015) have paid attention to evaluate the use of *G. bonnieroides* as test organism in toxicity testing.

In this study, the Indonesian benthic amphipod *G. bonnieroides* that locally found in Banten waters was evaluated as test organism in the sediment bioassay. The amphipod was chosen to test cadmium as reference toxicant to determine its median lethal concentrations (LC50) values as indicator of the amphipod sensitivity in relation to established test organisms. Similar work has been done with amphipod from the temperate regions by Lee et al (2005a), Anderson et al (2004), and Nipper et al (1989). Thus, this paper serves to introduce tropical species from related genera as test organisms for toxicity bioassays carried out in the Southeast Asian region, especially in Indonesia.

## Material and Method

**Test organism.** *G. bonnieroides* 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), Tangerang, Banten Province. Methods of amphipods transportation and handling in laboratory was carried out as described Hindarti et al (2015). Amphipod used in this test was seven-days larvae (neonates) of amphipod obtained from laboratory larvae production.

**Acute toxicity tests.** Cadmium acute toxicity tests were conducted using three different amphipod laboratory productions. Stock solution of cadmium was prepared by dissolving CdCl<sub>2</sub> (E-MERCK) in the treated natural seawater at 25°C and salinity 30 ppt. All of the tests used the same stock cadmium solution. Five concentrations and three or four replicates, 20 individuals for each treatments were performed for all of the tests. The amphipods were exposed to cadmium for 96 hours. 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 endpoint was survival and acceptability of test results was fixed a percentage of amphipod survival ≥90% in all negative control, and only if the LC50 with the reference toxicant fell within the acceptable range (ASTM 2006).

**Data analysis.** LC50s with 95% confidence limits were calculated by the Trimmed Spearman-Kärber statistical method (Hamilton et al 1977). The assessments of no-observed effects concentration (NOEC) and low-observed effects concentration (LOEC) were made by analysis of variance (ANOVA) and Steels Many-One Rank test on arcsine square-root transformed data using TOXSTAT software (West & Gulley 1995).

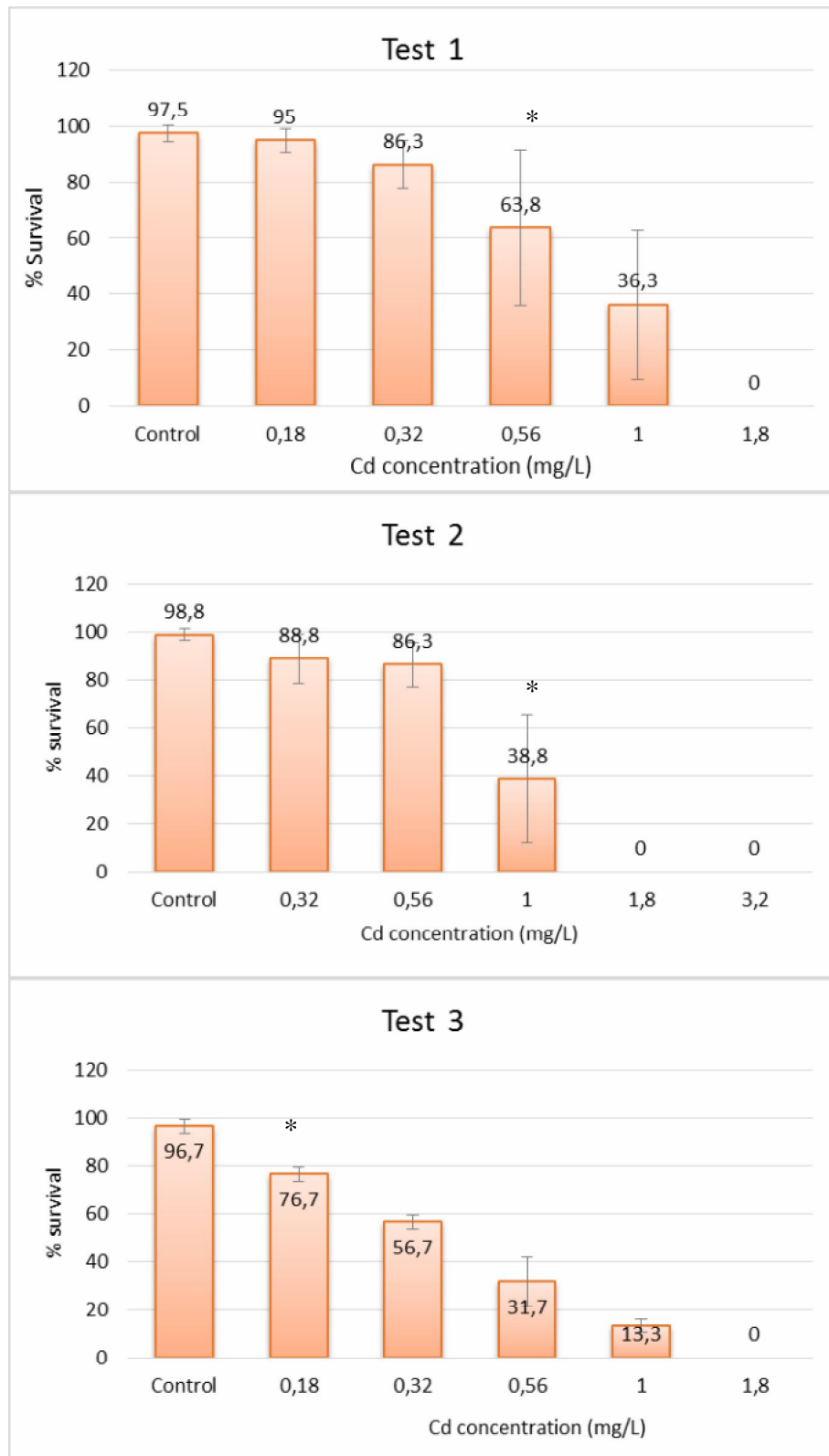
## Results and Discussion

**Water quality measurements.** Water quality conditions measured during all of tests are presented in Table 1. All conditions appeared suitable for survival of the amphipod, with the exception of dissolved oxygen (DO) of the second test. In the second cadmium toxicity test, concentration of DO dropped at 96 h of exposure >3 mg/L. Mean DO concentration was > 4 mg/L for most of the cadmium concentration tested and during the experiment. The DO concentration met the acceptable level of DO for static acute toxicity test (Parish 1985), that should remain  $\geq 40\%$  saturation ( $\pm 4.6$  mg/L DO).

Table 1  
Ranges in water quality condition during the 96 h cadmium toxicity tests

Test	Cd conc (mg/L)	Temperature (°C)	pH	Salinity (ppt)	DO (mg/L)
1	Control	24.0 – 25.6	7.10 – 7.84	31	4.13 – 4.98
	0.18	23.5 – 25.7	7.40 – 7.88	31	4.01 – 4.78
	0.32	24.2 – 26.1	7.83 – 7.92	31	4.24 – 5.83
	0.56	23.8 – 26.8	7.43 – 7.99	31	3.95 – 4.64
	1.0	24.5 – 27.1	7.52 – 8.06	31	4.11 – 4.79
	1.8	25.1 – 26.5	7.53 – 8.00	31	4.08 – 4.38
2	Control	23.0 - 26.2	7.44 - 7.97	30	3.72 - 5.52
	0.32	23.7 - 26.4	6.01 - 7.98	30	4.02 - 4.83
	0.56	24.0 - 26.3	7.86 - 7.98	30	3.64 - 4.70
	1	24.3 - 27.2	7.74 - 7.98	30	3.41 - 4.96
	1.8	24.4 - 27.0	7.83 - 7.98	30	3.92 - 4.85
	3.2	24.6 - 26.8	7.70 - 7.98	30	3.58 - 5.12
3	Control	23.4-25.8	6.32 - 7.64	31	4.73 - 6.34
	0.18	22.7-24.9	7.15 - 8.00	31	4.40 - 5.40
	0.32	22.3-25.0	7.60 - 7.99	31	4.54 - 5.83
	0.56	22.4-25.5	7.55 - 7.95	31	4.09 - 4.99
	1.0	23.7-25.2	7.56 - 7.95	31	4.06 - 4.66
	1.8	22.8-26.2	7.50 - 7.96	31	4.18 - 4.91

**Cadmium toxicity.** A wide range of cadmium concentration was tested to determine the effect of cadmium on the survival of amphipod, *G. bonnieroides*. All controls resulted in low mortalities, fewer than 5%, which indicated the acceptability of the experiment and are presented in Figure 1. Survival of amphipod decreased markedly at higher concentrations of cadmium. The first significant ( $p < 0.05$ ) effect of cadmium (LOEC) was in the range of 0.18–1.0 mg/L. The LC50 value of Cd for *G. bonnieroides* in the present study is presented in Table 2. The LC50 values generated from this toxicity tests varied with batch of amphipods collected from wild, these were 0.70 mg/L, 0.88 mg/L and 0.35 mg/L. The different sensitivities of amphipods to aqueous Cd may be related to individual variation within a population. The variation may be related to season (Kater et al 2000), the size of animals (McGee et al 1998), behavior (Peeters et al 2009), sexes (Magurran & Garcia 2000), and morphology (Wainwright & Richard 1995). In theoretical studies conspecific individuals are treated as ecologically equivalent or identical (Bolnick et al 2003). However, different studies reported variation among individuals in, for example, foraging ability or vulnerability to predation (Bridcut & Giller 1995). When individuals are exposed to concentrations below lethal concentrations, the performance of organisms can be affected. For instance, the energy budget of organisms may change due to direct expenditures to resist contaminants (Kooijman & Bedaux 1996; Kooijman 2000). Individual variation may also be related to other factors including feeding state (Sih et al 2004), and light conditions (Peeters et al 2009), health status (Burton et al 2011), metabolic rate (Calosi et al 2013). Behavior is the overall outcome of an organism response to physiological and environmental factors (Dell’Omo 2002). Taylor et al (1993) observed changes in feeding behavior of *Gammarus* when exposed to copper.



\* Significant at  $p < 0.05$ .

Figure 1. Percent survival of amphipod at 96 h exposure to cadmium.

De Lange et al (2006) observed that sediments contaminated with PAH were actively avoided by *Gammarus*. Burton et al (2011) revealed that individual differences in the energy cost of self-maintenance (resting metabolic rate, RMR) may influence fitness because self-maintenance is considered as a life-history component along with growth

and reproduction. Furthermore, treating individual differences as biologically meaningful can lead to a better understanding of the physiological responses themselves to environmental changes.

Table 2

Summary of cadmium toxicity data for 96-h water-only test conducted with *Grandidierella bonnieroides*

Test	NOEC	LOEC	LC50	96h-LC50 95% CI	
				LL	UL
1	0.32	0.56	0.70	0.63	0.78
2	0.56	1.0	0.88	0.79	0.97
3	< 0.18	0.18	0.35	0.30	0.42

The LC50 values of this study were within the range of the LC50s value reported for most of the amphipods tested (Table 3), which varied in the range from 0.36 mg/L for *Leptocheirus plumolatus* and 2.9 mg/L for *Ampelisca abdita*, with the exception of the third test that having slight lower value of LC50 than that for *L. plumulosus*. *G. bonnieroides* also demonstrated higher sensitivity to cadmium than other tube-dwelling species (*A. abdita* and *G. japonica*) that have been used as standard test species. Sensitivities also vary within the same organisms depending on life-stage. The early life-stage of *G. bonnieroides* used in this study indicates their sensitivity to Cd Robinson et al (1988) found juveniles of *R. abronius* significantly more sensitive to cadmium (CdCl<sub>2</sub>) than the adults. On the other hand, Ciarelli et al (1997) found no significant differences in sensitivity between *Corophium volutator* of different size classes.

Table 3

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

Species	96-h LC50 (mg/L)	Reference
<i>Grandidierella bonnieroides</i>	0.70	Present study
	0.88	
	0.35	
<i>Ampelisca abdita</i>	2.9	USEPA (2001)
<i>Grandidierella japonica</i>	1.17	USEPA (2001)
	1.47	Lee et al (2005a)
<i>Eohaustorius estuarius</i>	2.4	ASTM (2006)
<i>Leptocheirus plumulosus</i>	0.36	USEPA (2001)
	0.53	Kohn et al (1994)
<i>Reposynius abronius</i>	0.76	DeWitt et al (1989)
	0.75; 1.1	ASTM (2006)
<i>Monocorophium achersicum</i>	0.7-1.4	Lee et al (2005a)

Published literature has confirmed that amphipods are more sensitive to polluted sediments than other benthic organisms (Gesteira & Dauvin 2000; Dauvin & Ruellet 2007, 2009). Since amphipods are dominant members of all major marine and freshwater assemblage systems, they are very good candidates for ambient reference indicators. Standardized sediment toxicity tests have been developed for burrowing amphipods, including *R. abronius* (Swartz et al 1985), *L. plumulosus* (Schlekat et al 1992), and tube-dwelling species, including *A. abdita* and *G. japonica* (Kohn et al 1994), and *Corophium volutator* (Ciarelli 1994; Ciarelli et al 1997; Conradi & Depledge 1998). *Grandidierella* sp. is the Indonesian amphipods that have been examined for sediment toxicity testing (Puspitasari & Hindarti 2010).

The results of the present study have provided information about a tube-dwelling amphipod, *G. bonnieroides*, as a potential test species in the sediment toxicity assessment. *G. bonnieroides* was chosen for many reasons. It is readily available and easy to handle. Also, it shows a high tolerance to different sediment textures (Hindarti et al 2015), ammonia (Hindarti et al 2015b), salinities (Hindarti et al 2016 -paper in preparation), yet is sensitivity to the reference toxicants.

In the evaluation of potential information value of numerous amphipod groups, one that should be considered is ecological factors. The sensitivity of a benthic organism is related to feeding behavior (Simpson & King 2005) and living strategy (King et al 2006), in which all of these affect the route of exposure to contaminant. Thomas (1993) revealed epifaunal nestler and fouler amphipod demonstrate different habitat needs and dispersive abilities than infaunal burrowers, tube-builder, and other cryptofaunal and commensal groups. These features must be taken into account when considering amphipods for monitoring and biodiversity programmes.

**Conclusions.** This species fulfills most of the criteria required for suitable sediment toxicity tests. It may be an alternative or complementary test species for ecotoxicological studies in Indonesian ecosystems. However additional experiments should be carried to give a broad picture of sensitivity by examining the species sensitivity to other contaminant such as toxic organic pollutant.

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