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W. Niyommaneerat, F. Nakajima, T. Tobino, K. Yamamoto. Toxicity of urban road dust on reproduction of a benthic ostracod, *Heterocypris incongruens*. Novatech 2016 - 9ème Conférence internationale sur les techniques et stratégies pour la gestion durable de l'Eau dans la Ville / 9th International Conference on planning and technologies for sustainable management of Water in the City, Jun 2016, Lyon, France. <hal-03321991>

HAL Id: hal-03321991

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Submitted on 18 Aug 2021

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Toxicity of urban road dust on reproduction of a benthic ostracod, *Heterocypris incongruens*

Toxicité des poussières de route sur la reproduction d'un ostracode benthique, *Heterocypris incongruens*

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RÉSUMÉ

Les poussières de route en zone urbaine contiennent toute une série de polluants et peuvent détériorer la qualité de l'environnement dans les plans d'eaux recevant le ruissellement urbain. Les poussières de route peuvent s'être accumulées dans les sédiments sur une longue période, aussi la toxicité chronique devrait-elle être évaluée. Cependant, l'évaluation de la toxicité de la poussière des routes urbaines est limitée, en particulier dans l'effet chronique sur les organismes benthiques. Cette étude a pour objet d'évaluer la toxicité des poussières de route sur la reproduction d'un ostracode benthique, l'*Heterocypris incongruens*. La toxicité des poussières routières a été testée au moyen d'une méthode récemment mise au point pour tester la toxicité chronique et avec la méthode ISO14371. Les critères d'évaluation observés dans cette étude ont été la mortalité, l'inhibition de la croissance, la production d'œuf et le taux d'éclosion des œufs produits. Les concentrations à effet non observé sur la mortalité à 6 jours et l'inhibition de la croissance des poussières routières étaient de 12,5% et 6,25%, respectivement. Des différences statistiquement significatives ont été constatées le premier jour sur le taux de couvaison et d'éclosion dans les échantillons de poussières de route avec la méthode de test non-toxique et ISO. L'échantillon de poussière de route a montré un effet toxique sur la reproduction de l'ostracode dans la faible concentration et n'a montré ni une toxicité fatale à 6 jours, ni une inhibition de la croissance.

ABSTRACT

Urban road dust contains a variety of pollutants and might deteriorate the environment quality in the receiving water bodies of urban runoff. Urban road dust may be accumulated in the sediment for the periods of time, thus chronic toxicity should be evaluated. However, toxicity assessment of urban road dust is limited especially in the chronic effect on benthic organisms. This study aimed to assess the toxic effect of road dust on reproduction of a benthic ostracod, *Heterocypris incongruens*. Road dust toxicity was tested by a newly developed chronic toxicity test method along with ISO14371 method. Endpoints observed in this study were mortality, growth inhibition, egg production and hatching ratio of the eggs produced. Non observed effect concentrations in 6-day mortality and growth inhibition of the road dust were 12.5% and 6.25%, respectively. Statistically significant differences were found in first day of brooding and hatching ratio in the road dust samples which non-toxic in ISO test method. The road dust sample showed toxic effect on ostracod reproduction in the low concentration which did not exhibit 6-day lethal toxicity and growth inhibition.

KEYWORDS

Chronic toxicity test, ostracod, reproduction, urban road dust

1 INTRODUCTION

Sediment contamination poses ecological risks in the receiving water body. Urban road dust is a potential source of sediment contamination and may provide any adverse effect to benthic organisms and deteriorate environmental quality of receiving water bodies of urban road runoff. Previous studies reported that urban road dust contained high concentrations of many potentially toxic substances such as hydrophobic organic compounds, heavy metals, polycyclic aromatic hydrocarbons and perfluorinated surfactants (Fang et al., 2004; Murakami and Takada, 2008; Atiemo et al., 2011). However, ecotoxicity assessment of urban road dust (not the runoff water) has been rarely conducted.

To test the freshwater sediment, the ostracod toxicity test has been standardized as ISO14371 (ISO, 2012) which has great advantages in the small size of the test scale and in the commercial availability of cysts without keeping the species in each laboratory. The toxicity test has already been applied to various solid samples such as sediment (Torokne and Toro, 2010), soils (Chial et al., 2003; Santorufo et al., 2012) and road dust (Watanabe et al., 2011, 2013; Khanal et al., 2014, 2015).

From the ecological point of view, chronic toxicity should be also evaluated; but the 6-day ISO14371 method seems too short to observe the chronic effect of the ostracod species, *Heterocypris incongruens* (*H. incongruens*). Hence, the objective of this study was to assess the toxic effect of urban road dust on reproduction of a benthic ostracod. For this objective, we developed a test method to investigate the chronic or long term effect using the freshwater benthic ostracod *H. incongruens*. Toxicity of urban road dust on the ostracod reproduction was assessed by 14-day exposure to the dust in parallel with the standard 6-day test. From various endpoints including mortality, growth inhibition, egg production and hatching, the toxicity of urban road dust was examined.

2 MATERIAL AND METHODS

2.1 Test organism

Freshwater benthic ostracod *Heterocypris incongruens* was purchased as a dormant eggs (cysts) from MicroBioTests Inc.

2.2 Test samples

Urban road dust (RD) (<2000 μm) was collected from road side in Tokyo in 2012 (Khanal et al., 2014). The road dust was diluted with clean sediment into different concentrations (3.125%, 6.25%, 12.5%, 25% and 50% (v/v)). The clean reference sediment (RF) was obtained as a part of Ostracodtookit F (MicroBioTests Inc.).

2.3 Ostracod toxicity test

6-day ostracod toxicity test (ISO14371) (ISO, 2012) and chronic ostracod toxicity test were conducted in parallel. The chronic toxicity test was newly designed and was composed of three consecutive parts as 14-day sediment exposure phase, reproduction phase and hatching test

2.3.1 14-day sediment exposure phase

Road dust exposure was conducted following the same procedures of the 6-day ostracod toxicity test (ISO14371) but for a longer period of time (14 days). The hatching of the ostracod cysts was initiated 52 h prior to the toxicity test. The test was started by inoculating 10 ostracod neonates to each well of six-well microplate containing a mixture of sediment (1 mL) and standard freshwater (SFW) (2 mL) (held for 24 h at 25°C in the dark prior to the test) after that adding 2 mL of algal suspension of green algae *Scenedesmus acutus* NIES-94. Laboratory-cultured green algae *S. acutus* were used for feeding ostracod in the toxicity test which was adjusted by SFW to 1.5×10^7 cells/mL as described earlier (Watanabe et al., 2011). Particularly on the day 7 of this sediment exposure phase, each well was fed with TetraMin suspension (ground and sieved through 250 μm mesh screen). Test with clean reference sediment was performed concurrently with urban road dust samples. The pH, conductivity and salinity of the overlying water were measured at the start of the test, on day 7 and day 14 of the sediment exposure phase. The endpoint measurements in this phase are 14-day mortality and growth inhibition by body length measurement.

2.3.2 Reproduction phase

Reproduction was observed continually from the 14-day sediment exposure phase until all the ostracods died. The reproduction phase was conducted without sediment by transferring the surviving

ostracods individually into another well (one individual per one well) containing 2 mL of SFW and 1 mL of 1 mg/L of TetraMin suspension. Every other day of reproduction phase, mortality and reproduction of ostracod were recorded by counting the number of surviving ostracods and of eggs produced in each well. Then, the surviving ostracod was again transferred into a new well in the same procedure on the 14th day. The pH, conductivity and salinity in the overlying water were also measured before and after transferring the ostracod. Endpoints of the reproduction phase were life span, total number of eggs produced, first day of brooding, mean day of egg production, lifetime egg production and egg-laying ratio.

2.3.3 Hatching Test

The produced eggs were collected and further observed their hatching for 14 days to determine hatching ratio. The endpoints of this test were hatching ratio and F2 generation rate. The hatching ratio was defined as the number of hatched eggs within 14 days divided by the total number of egg produced. We also calculated F2 generation rate based on hatching ratio and lifetime egg production.

Lifetime egg production = (total number of egg produced)/(total number of ostracod tested)

Hatching ratio (%) = (number of hatched eggs in 14 days)/(total number of egg produced) × 100

F2 generation rate = (number of hatched eggs in 14 days)/(total number of ostracod tested)

2.4 Statistical analysis

All data and results were reported with standard deviation. For each variable, Dunnett test was used to determine the significant difference ($p < 0.05$) between urban road dust samples and control using Minitab 17 software. Chi-square analysis was applied to hatching ratio.

3 RESULTS AND DISCUSSION

3.1 Mortality, growth inhibition and life span of ostracods

Mortality and life span of ostracod exposed to the road dust are shown in **Figure 1**. 25%RD and 50%RD showed a significant difference from RF (control) in 6-day as well as mean life span of ostracods. There was a statistically significant difference ($p < 0.05$) in 14d-mortality between the control and 12.5%RD, 25%RD, 50%RD sample. Mean life span of ostracods considerably decreased in high concentrations of urban road dust.

Table 1 show growth inhibition of the road dust samples. 6-day growth inhibition also increased with increasing the road dust concentration. We also found a significant difference ($p < 0.05$) in 6-day growth inhibition between the control and 12.5%RD sample although no significant different was found for 3.125% and 6.25% road dust samples. 14d-growth inhibition also showed significantly difference among samples ($p < 0.05$). As expected, the toxicity (mortality and growth inhibition) on Day 14 was greater than on Day 6, except for mortality by 3.125%RD and the growth inhibition by 12.5%RD.

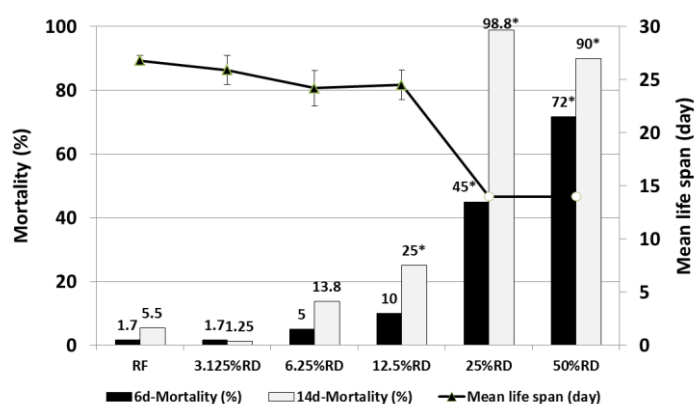


Figure 1. 6-day and 14-day mortality and life span of ostracod exposed to urban road dust.

Table 1. 6-day and 14-day growth inhibition (mean ± standard deviation) of urban road dust.

	RF	3.125%RD	6.25%RD	12.5%RD	25%RD	50%RD
6-day growth inhibition (%)	-	0.9 ± 4.1	1.2 ± 7.3	15.1 ± 10.7*	N/a**	N/a**
14-day growth inhibition (%)	-	5.5 ± 4.3*	15.9 ± 6.0*	7.2 ± 2.9*	N/a**	N/a**

* $p < 0.05$ in Dunnett's test. ** N/a: Not available due to the high mortality (>30%, the criteria in ISO 14371)

3.2 Reproduction and hatching

After 14 days of exposure to the road dust, reproduction of the ostracod was observed in order to evaluate the long-term effect of the road dust. Life history characteristics of ostracod under control condition and different concentration of road dust sample are shown in **Table 2**. Life span of egg-laying individuals was slightly longer in the road dust exposure cases than in control condition. The results fall in the range which is not considerably different from the study of Havel and Talbott (1995). They reported the average life time of this species was 25 days (14 - 37 days) and its reproduction started from 19th day (13th - 25th day). Mean day of egg production was slightly delayed in urban road dust sample than control but no statistically significant difference was found.

Table 2. Life history characteristics of ostracod under control condition and after the exposure to the different concentrations of road dust.

Parameters	RF (Control)		3.125%RD		6.25%RD		12.5%RD		25%RD	50%RD
	All individuals	Egg-laying individuals	All individuals	Egg-laying individuals	All individuals	Egg-laying individuals	All individuals	Egg-laying individuals	All individuals	All individuals
n (number of ostracod)	220	141	80	43	80	36	80	36	80	80
Egg-laying ratio (%)	64.1±16.5		54.0±22.6		45.0±25.6		45.0±29.8		0	0
Life span (day) [range]	26.8±2.3	31.0 ± 3.0 [20-48]	25.9 ± 3.9	31.6 ± 3.3 [20-48]	24.2±4.1	31.0±2.5 [22-46]	24.5±4.0	32.5±1.8 [26-42]	<14	<14
Days to first brood (day) [range]	-	23.0±2.2 [18-32]	-	22.5±1.8 [20-28]	-	24.2±2.2 [20-30]	-	25.2±1.6* [20-28]	-	-
Mean day of egg production (day) [range]	-	25.5±2.3 [22-30]	-	26.0±2.5 [24-31]	-	26.6±1.4 [24-28]	-	27.6±1.2 [26-30]	-	-
Total number of eggs produced (egg)	4275		1793		1264		974		0	0
Lifetime egg production (egg/ind.) [range]	19.4±8.90	30.3±24.6 [1-109]	22.4±13.4	41.7±37.5 [1-154]	15.8±10.8	35.1±29.9 [1-122]	12.2±9.80	27.1±21.4 [3-82]	0	0
Hatching ratio (%)	10.4		12.8**		4.4**		16.8**		-	-
F2 generation rate	2.02		2.87		0.70		2.05		0	0

mean ± standard deviation; values in parenthesis indicate the observed range in min-max.

All individuals = calculated for all the individuals used in the test; Egg-laying individuals = calculated only for the individuals which laid eggs; Days to first brood = first day of laying egg; mean day of egg production = average day of egg productions.

* $p < 0.05$ in Dunnett test, ** $p < 0.05$ in Chi-square test

We recorded the number of eggs produced by each individual ostracod until all the ostracod died in order to calculate lifetime egg production. The number of eggs produced in total were 4,275 for reference sediment (from 220 individuals) as accounted for 1,555 eggs from 80 individuals, while 1,793, 1,264 and 974 eggs were produced from 80 individuals in the cases of 3.125%, 6.25% and 12.5% diluted road dust samples, respectively. Egg-laying ratio (%) decreased as increasing concentrations of urban road dust but no statistically significant difference was found. These samples (12.5%RD and 6.25%RD) also showed lower lifetime egg production than control although the statistical difference was not significant due to the considerable individual variability (**Figure 2**). In addition, 12.5%RD and 6.25%RD showed slightly delayed first brood than control condition. Statistically significant difference for days to first brood was found in 12.5%RD sample ($p < 0.05$). This suggests that those diluted road dust samples have an impact on growth rate of the ostracod population.

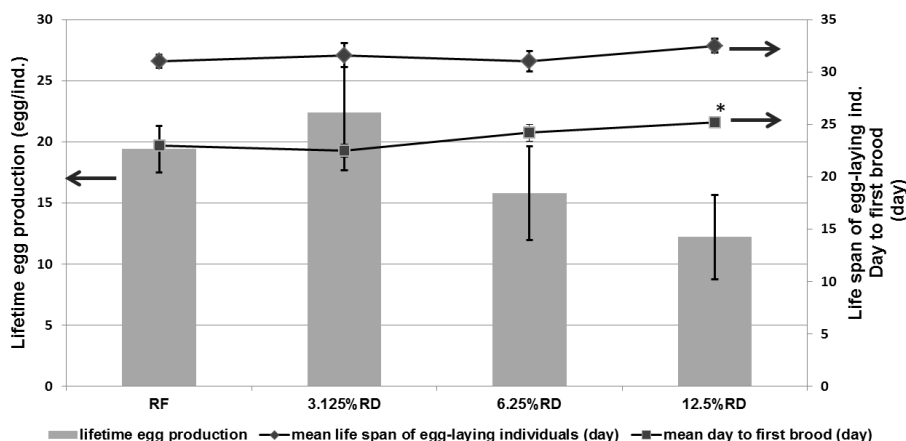


Figure 2. Lifetime egg production, Mean life span for egg-laying individuals and days to first brood under different road dust concentrations. Error bar means standard error. * $p < 0.05$ in Dunnett' test

Hatching ratio increased by 3.125%RD but became lowest by 6.25%RD (**Table 2**) with significant differences ($p < 0.05$, Chi-square test). F2 generation rate was calculated based on hatching ratio and lifetime egg production. 3.125%RD caused a slight increase of both lifetime egg production and hatching ratio, and resulted in the highest F2 generation rate among the tested conditions. In contrast, 6.25%RD brought lower fecundity and the lowest hatching ratio so that the F2 generation rate became lowest. This suggests that the diluted urban road dust had an impact on growth rate of the ostracod population (below 1 indicating the possibility of extinction).

These results presumably showed that urban road dust might inhibit the reproduction of the benthic ostracod even in the low proportion in the sediment which showed no toxicity in 6-day mortality and growth inhibition. Since the results cannot be generalized only by one road dust sample, future research of the known toxicants and more environmental samples would be required. Nevertheless, this new test method seems effective, but the laboriousness should be improved in egg-counting and hatching tests.

4 CONCLUSIONS

Urban road dust has been known to be toxic to benthic ostracod in 6-day toxicity test but there is no information available on reproduction toxicity. Our concern is to confirm if lower concentrations of urban road dust have any detectable effect in the chronic or long term toxicity test. In the preliminary test of 6-day toxicity test, the lowest observed effect concentration of the road dust was 12.5%(v/v). 25%RD and 50%RD showed a significant difference from control in 6-day and 14-day mortality and mean life span. 14d-growth inhibition also showed statistically significant difference among samples ($p < 0.05$). 12.5%RD and 6.25%RD showed lower lifetime egg production and slightly delayed first brood than control. Egg-laying ratio (%) decreased as increasing concentrations of urban road dust but no statistically significant difference was found, first day of brooding of 12.5%RD and hatching ratio in all the road dust samples. 6.25%RD brought lower lifetime egg production and the lowest hatching ratio so that the F2 generation rate became lowest (below 1). In conclusion, the current study presents the new bioassay to evaluate the reproduction effect on benthic ostracod *H. incongruens* even in the urban road dust which was not toxic in the standard 6-day toxicity test (ISO 14371). This result would draw attention to the management of such diffused pollutants and to the further identification of responsible stressors in road dust.

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