Chemosphere 151 (2016) 220–224

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Short communication

The sediment-contact test using the ostracod *Heterocypris incongruens*: Effect of fine sediments and determination of toxicity thresholds

M. Carmen Casado-Martinez [a, *], Karen F. Burga-Pérez [b], Rebecca Bebon [a], Jean-François Férard [b], Etienne L.M. Vermeirssen [c], Inge Werner [c]

[a] Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Station 2 (GR B0 391), 1015 Lausanne, Switzerland
[b] Université de Lorraine (UdL), Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) - UMR 7360 CNRS, Campus Bridoux, Bât. IBiSE, 8, rue du Général Delestraint, 57070 Metz, France
[c] Swiss Centre for Applied Ecotoxicology Eawag/EPFL, Überlandstrasse 133, 8600 Dübendorf, Switzerland

HIGHLIGHTS

- The presence of fine sediment has no effect on ostracod survival.
- Increasing the presence of fine sediment reduces ostracod growth.
- Toxicity thresholds defined on the basis of sensitivity to sediment properties.

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
21 January 2016
Accepted 30 January 2016
Available online 15 March 2016

Handling Editor: Jim Lazorchak

Keywords:
Sediment toxicity
Ostracodtoxkit®
Clay content
Toxicity threshold
Grain size

ABSTRACT

The toxicity test using freshwater ostracods of the species *Heterocypris incongruens* is a sub-chronic static test that exposes individuals to whole sediments over a period of 6 d, the endpoints being mortality and growth. We tested the hypothesis that endpoints of the sediment bioassay using *Heterocypris incongruens* are affected by the presence of fine sediment particles by testing control sediment supplied with the commercial test kit with increasing proportions of kaolin clay as a proxy for fines. While mortality was not affected, the results showed that increasing the presence of clay reduced ostracod growth. Based on the variability in growth, a sublethal toxicity threshold of 35% is proposed to distinguish effects due to sediment properties from those due to toxicity. The relevance of this threshold was verified using data from toxicity tests of ambient sediment samples with low levels of contamination.

1. Introduction

The toxicity test using freshwater ostracods of the species *Heterocypris incongruens* is a sub-chronic static test that exposes individuals to whole sediments over a period of 6 d, the endpoints being mortality and growth. This bioassay can be purchased as a kit (Ostracodtoxkit F™, MicroBioTests Inc.) and it has a number of advantages over other conventional bioassays using benthic invertebrates in that it does not require stock culturing of organisms, is miniaturised into a microscale test that only requires limited bench space, sample volume and materials, and is user-friendly. Since its establishment in the early 2000s, it has been widely applied for sediment quality assessment either for toxicity screening or as part of a toxicity test battery (Chial et al., 2003; Garcia-Lorenzo et al., 2009; Törökne and Toro, 2010; Mwanamoki et al., 2014) and for testing other complex matrices, including soils (Oleszczuk, 2008; Plaza et al., 2010; Khanal et al., 2015). In 2012 a standard protocol for testing freshwater sediments with the ostracod *H. incongruens* was released (ISO, 2012).

As for other analytical and testing methods, the assessment of ruggedness (robustness) for sediment bioassays should be part of

* Corrresponding author.
E-mail address: carmen.casado@centreecotox.ch (M.C. Casado-Martinez).

http://dx.doi.org/10.1016/j.chemosphere.2016.01.126
0045-6535/

the development phase (ASTM, 1992; Dillon, 1994). Because responses of benthic organisms may be influenced by a variety of sediment characteristics, test ruggedness addresses the sensitivity to sediment characteristics in addition to the sensitivity of the test method to deviations from normal test conditions and protocols (Dillon, 1994). The experimental determination of ruggedness is meant to provide a matrix of conditions for which the sediment bioassay is or is not appropriate. Chial and Persoone (2002a; 2002b) studied the sensitivity of the ostracod test endpoints to deviations in the test protocol, including incubation conditions, and an interlaboratory comparison was carried out for its standardization (Janssen and Persoone, 2010). The sensitivity of *H. incongruens* to unionized ammonia and pH changes has recently been determined (Watanabe et al., 2013). Test sensitivity to fine sediment (the term is used here as the load of silts and clays, which have diameters smaller than 0.0625 mm), an important factor in sediment toxicity assays (Dillon, 1994), has not yet been determined.

According to the ISO guideline 14371 (2012), the ostracod bioassay is based on the comparison of mortality and growth of freshly hatched ostracods in control and test sediments after a 6 d exposure. The controls are used to determine compliance with acceptability criteria (mortality $\leq$ 20% and a mean ostracod length increase of at least 1.5-fold over the test duration) and to quantify growth inhibition as a % control response if the % mortality is low (<30%). A commercial (not toxic and not calcareous) river sand or marine sand, which is water-washed and sieved to eliminate dirt and debris and then air-dried, can be used as control sediment provided it is a medium-coarse sand, with a particle size < 2 mm (ISO, 2012). However, the grain size of natural sediments can vary greatly, ranging from sand, through silty-sand, to sandy–silt, to clays. If fine sediments have an effect on test endpoints, the interpretation of results solely on the basis of comparison with responses in a medium-coarse control sediment may overestimate or underestimate toxicity.

One way of dealing with sediment matrix issues is by use of an uncontaminated reference sediment with similar properties to the test sediments. However, this approach may not be practicable in monitoring programs, either because the samples being tested vary in grain size composition, or due to difficulties finding appropriate uncontaminated reference sediments. An alternative to using reference sediments is to determine the natural variability of the test endpoints by testing natural sediments with a broad range of physico-chemical properties and low levels of contamination (Höss et al., 2010; Durand, 2012). The main advantage of this method over the reference sediment approach is that it avoids additional testing.

In the present study, we tested the hypothesis that endpoints of the sediment bioassay using *H. incongruens* are affected by the presence of fine sediments. Tests were performed with control sediment supplied with the commercial test kit amended with increasing proportions of kaolin clay as a proxy for fine sediment load. The results were used to derive toxicity thresholds that distinguish the effect of grain size from that of toxicity, and the relevance of these thresholds was verified using data from toxicity tests of ambient sediment samples with low levels of contamination.

2. Materials and methods

2.1. Test with ostracods

The sediment toxicity test using *H. incongruens* was performed following the corresponding ISO guideline 14371 with no modifications (ISO, 2012). Tests with artificial sediments were performed using a commercial Ostracodtoxkit F™ (MicroBioTests Inc.) which provides all materials and allows the test to be completed in accordance with the guideline. Tests with ambient sediment samples were carried out using commercially available cysts of *H. incongruens* (MicroBiotest Inc.), but they were fed *Pseudokirchneriella subcapitata* from a pre-culture in the exponential growth phase. The clean sand provided with the commercial kit was also used for all trials.

2.2. Test with artificial sediments

The medium-coarse sand (>0.2 mm) provided with the commercial kit (this sediment is provided dried and has virtually no fine sediment and an organic matter content of <1%) was mixed with a spatula with dry pure kaolin ($Al_2Si_2O_5(OH)_4$; 0.1–4 μm; Fluka, Sigma Aldrich) until complete homogenization to form the following mixtures (expressed as % clay): 0, 2.5, 5, 10, 15, 20, 40 and 80. Each sediment was tested in triplicate and two separate trials were performed.

2.3. Tests with natural sediments

Eight ambient sediment samples were tested in six replicates and three separate trials. These sediments are characterized by low levels of contamination and toxicity. Further information on the sediment sampling and characterization can be found elsewhere (Durand, 2012; Burga-Pérez, 2012). The clay content in these sediments ranged between 0.04 and 19%, while the proportion of fine sediment (including silt and clay) ranged between 6 and 91%. Organic matter content (measured as loss on ignition) ranged between 2 and 15%.

2.4. Data analysis

Statistical analyses were performed with GraphPad Prism®. For comparison of means a Kruskall–Wallis test was carried out because the requirements for a parametric test, normal distribution and equal variances, were not met. Mann–Whitney test was used for post-hoc comparisons.

Toxicity criteria were derived based on the minimal detectable difference and the maximal tolerable inhibition following Höss et al. (2010). Briefly, the variability of the test endpoints among replicates was expressed as the coefficient of variation (*CV*i) and the minimal detectable difference (MDD), also referred to as minimum significant difference (Denton et al., 2003). The variability of endpoints when expressed in relative values (% effect with respect to controls) associated with differences in the sediment matrix was estimated using the coefficient of variation (CV_s) and the maximal tolerable inhibition (MTI). This approach was applied for all tests including those for which requirements for a parametric test were not formally met. Thus this paper presents indicative threshold values as they are calculated using parametric statistics as an approximate index of test precision and variability.

A *CV*i for each test sediment was calculated as *CV*i = SD/Mean × 100 where Mean and SD are the mean and standard deviation of test endpoints (mortality and growth) from replicates of each test sediment sample. The mean, maximum and minimum *CV*i were calculated separately for the set of controls (CVi_c) and sediments (CVi_s). Then the MDD, which is the smallest difference between the mean responses in a test sediment and a control that is statistically significant given the observed test variability and the number of replicates, was calculated as

$$\%\ MDD_s = 100t\ \sqrt{[(SD^2{}_c / n_c) + (SD^2{}_s / n_s)]} / Mean_c \quad (1)$$

where t is the tabulated value of the Student's t distribution (alpha = 0.05, one-sided, df = $n_c + n_s - 2$), $SD^2{}_c$, $SD^2{}_s$ and $Mean_c$ are,