Ecotoxicology and Environmental Safety 108 (2014) 217–223

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Comparison of laboratory batch and flow-through microcosm bioassays

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form
12 July 2014
Accepted 12 July 2014
Available online 2 August 2014

Keywords:
Microcosm
Cadmium
Flow-through assay
Ecotoxicity
Variability

ABSTRACT

Since 1997, we have been developing a protocol for ecotoxicological bioassays in 2-L laboratory microcosms and have applied it to the study of various pollutants and ecotoxicological risk assessment scenarios in the area of urban facilities and transport infrastructures. The effects on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) are assessed using biological responses such as growth, emergence (chironomids), reproduction (daphnids) and survival, with a duration of exposure of 3 weeks. This bioassay has mainly been used as a batch bioassay, i.e., the water was not renewed during the test. A flow-through microcosm bioassay has been developed recently, with the assumption that conditions for the biota should be improved, variability reduced, and the range of exposure patterns enlarged (e.g., the possibility of maintaining constant exposure in the water column). This paper compares the results obtained in batch and flow-through microcosm bioassays, using cadmium as a model toxicant. As expected, the stabilization of physico-chemical parameters, increased organism fitness and reduced variability were observed in the flow-through microcosm bioassay.

1. Introduction

Tests in laboratory microcosms are a good compromise in terms of cost and ecotoxicological pertinence when choosing between single-species and outdoor microcosm tests (Barry and Logan, 1998). They offer a large number of effect criteria and take into account several interactions between species (competition, predation, etc.), while presenting a sufficient level of standardization and replicability (Cairns and Cherry, 1993).

Two approaches to laboratory microcosms have been developed. Laboratory microcosms based on natural communities (Van Donk et al., 1995) can provide results closer to what can be observed in natural ecosystems, but are harder to replicate (Barry and Logan, 1998). As an alternative, laboratory microcosms can be entirely synthetic (or gnotobiotic, Taub, 1969), or assembled from a reconstructed environment and sediment so as to contain a definite number of species from laboratory cultures and breeding-stocks.

Regarding gnotobiotic microcosms, mention can be made of the Standardized Aquatic Microcosms (SAM) (Conquest and Taub, 1989), which are 3-L systems used to test the effects of toxic substances on a diversified community of laboratory organisms for 63 days. Clément and colleagues implemented another type of gnotobiotic laboratory microcosm. The laboratory aquatic microcosm bioassay (Clément and Cadier, 1998) enables evaluating the response of five species exposed simultaneously, under conditions in which they interact with each other and the environment, to toxic substances introduced via the water column or the sediment (Babut et al., 2002; Clément et al., 2004, 2013Triffault-Bouchet et al., 2005a, 2005b).

Despite its usefulness for ecotoxicological assessment, a recurrent problem of microcosm bioassays is the variability of the responses obtained, which affects detection capacity and the sensitivity of the toxicity endpoints (Caquet et al., 2001). This variability is inherent to microcosm bioassays, due to biological variability and the multiplication of sources of variability in complex systems with an increase in variation with time. Other problems encountered under batch conditions are: (i) the limitation of primary production due to nutrient consumption, and (ii) the possible accumulation of metabolites (ammonia or nitrites).

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http://dx.doi.org/10.1016/j.ecoenv.2014.07.015
0147-6513/

These problems can impair invertebrate development and could explain part of the variability observed.

Finally, batch bioassays in water-sediment systems may result in specific exposure conditions due to the rapid decrease of toxicant concentrations in the water column and the sorption of the toxicant onto the sediment during the bioassay, which may be appropriate and realistic or not, depending on the scenario studied. In some cases it can be interesting to keep toxicant concentrations in the water column as constant as possible. This can be achieved by a flow-through bioassay with continuous injection of toxicant at the water inlet (Lauth et al., 1996; Kottelat et al., 2010).

Following on from the example of flow-through single-species tests aimed at improving conditions for pelagic and benthic test organisms (Roman et al., 2007; Nebeker et al., 1986; Ankley et al., 1993), and maintaining constant toxicant concentrations (Bishop and Perry, 1981; Wang, 1991), we modified our original batch microcosm protocol in order to develop a flow-through bioassay. Our objective was to improve organism fitness, reduce variability and ensure constant exposure. Here, we compare the results obtained from a batch and a flow-through microcosm bioassay using cadmium as a model toxicant.

2. Materials and methods

2.1. Microcosm bioassay protocols

2.1.1. Microcosm batch assay

Batch and flow-through bioassays were conducted sequentially and not concurrently. We performed the batch bioassay in June and the flow-through bioassay in October. Both bioassays lasted 21 days.

Our microcosms consisted of cylindrical polypropylene (PMP) beakers (diameter 13 cm, height 18.5 cm) containing 2 L of synthetic water column and 100 g of artificial sediment. The characteristics of the freshwater used were: pH:7.7, hardness: 60 mg $CaCO_3$ L^{-1}, conductivity: 290 μS cm^{-1}, moderate nutrient contents (100 μg L^{-1} phosphorous, 1308 μg L^{-1} nitrogen) and oligo-elements and vitamins of M4 medium (Elendt and Bias, 1990). In order to minimize Cd complexation and increase Cd bioavailability (Huebert et al., 1993; Guilhermino et al., 1997), we avoided EDTA, initially present in the M4 medium. The sediment was composed of 88.35 g siliceous sand (Fontainebleau sand, Sigma Aldrich), 10 g pristine fine-grained lacustrian sediment (Lake of Aiguebelette, Savoie, France), 1.5 g α-cellulose (Sigma-Aldrich), and 0.15 g TetraMin® fish food (Tetrawerke, Melle, Germany). Seven days before introducing the organisms (Day-7), we introduced each sediment ingredient in the 2-L glass beakers, and mixed them with a spoon before gently adding 2 L of medium so as to limit sediment resuspension. Continuous aeration of the water column was started a few hours afterwards, using a Pasteur glass pipette connected to a Rena 100 aquarium pump. Aeration ensured that the dissolved oxygen content remained close to saturation level throughout the test. The temperature was kept at 20 ± 2 °C. We left the beakers for 7 days (Day-7 to Day 0) in the dark to ensure equilibrium between water and sediment.

On Day 0, we illuminated the beakers for 16 h d^{-1} (2200 lux, CV: 5 percent) and introduced into the water column of each microcosm 4×10^7 algal cells of an exponentially growing culture of the green alga *Pseudokirchneriella subcapitata*, two three-frond colonies of the duckweed *Lemna minor*, 10 neonates of the cladoceran *Daphnia magna* (age ≤ 24 h), 10 young amphipods *Hyalella azteca* (age 7–14 d), and ten first-instar *Chironomus riparius* larvae (age ≤ 2 d after hatching). Algae and duckweeds were grown as recommended by French standard methods (AFNOR, 1993, 1996). Cladocerans, amphipods, and chironomids were bred in aerated groundwater available on site, with TetraMin® added as food for the chironomids, the microalgae *P. subcapitata* for cladocerans, and the microalgae *P. subcapitata* +TetraMin® for amphipods.

We tested four Cd concentrations (10–20–40–80 μg L^{-1}) and introduced Cd on Day-7, 7 days before adding the organisms. Four replicates were used for the control (no Cd) and for each of the treatments with added-Cd.

We monitored abiotic parameters for 28 days, and biotic parameters for 21 days (Supplementary Table 1). We assessed water quality as a function of general parameters such as temperature, oxygen content, electrical conductivity, pH, and anion and cation contents. To monitor total Cd concentrations, 10 mL of unfiltered water was taken from the water column of each microcosm. These samples were acidified to pH 2.0 with nitric acid, and stored at 4 °C until they were analyzed by graphite furnace atomic absorption spectrometry (AAS) at 1500 °C (Hitachi, model Z-8200, Tokyo, Japan, detection limit: 0.1 μg L^{-1}). We analyzed final Cd contents of sediment were analyzed by AAS using 10-g samples dried at 60 °C, following the mineralization of 100-mg sub-samples with 2 mL ultra-pure HNO_3 + 6 mL ultra-pure HCl in a microwave oven (CEM/Express 180 °C, 30 mn). Algal density (cells mL^{-1}) of the water column was measured using a particle counter (Beckman Coulter, model Z1) set to a threshold of 3.2 μm (counting all particles > 3.2 μm). We assessed duckweed growth through frond number and final dry weight. Final total frond area was determined by image analysis of photographs with Gimp and Creatools software (Creatis). The daphnids introduced into the systems initially were counted twice a week; neonates were collected at the same time, counted and discarded. The body length of mothers (distance from the eye to the base of the spine) was measured using a micrometric binocular (precision: 0.01 mm) once a week, after checking that the manipulation of daphnids under the binocular had no effect on their development. We collected and counted the living amphipods at the end of the bioassays, and their fresh weight was measured at a precision to within 0.1 mg, using a Mettler Toledo weighing scale, after gathering all the individuals of one microcosm. Emerging chironomids were collected daily, males and females counted, and numbers of larvae and nymphs measured at the end of the test.

2.1.2. Microcosm flow-through assay

For the flow-through bioassay, we used the same beakers as for the batch assay, with the same quantity of synthetic water and artificial sediment introduced in the same way. The water was stored in 30-L PEHD containers and transported continuously to the beakers using a peristaltic pump (IPC, Ismatec®) at a flow rate of 1 L day^{-1}. These conditions resulted in the total renewal of the microcosm water every two days. The water entered the beakers through a 10-mL pipet cone fastened at the center of a transparent plastic lid covering the beaker, and immersed in the water. Four lateral holes were drilled in the cone, allowing the homogeneous distribution of the water which was removed through an elbow tube fitted with a 300-μm nylon mesh to retain the organisms but not the microorganisms (microalgae, bacteria, etc.). The outlet water was discharged into the sewage network unless used for analyses. Aeration was not necessary, as water renewal started on Day-6 ensured that oxygen content was kept above 80 percent. The temperature was maintained at 19 ± 1 °C. Illumination differed slightly (3100 lux 16 h d^{-1}, CV: 4 percent).

We prepared and inoculated the beakers on Day 0, as in the batch bioassay, with organisms from the same breedings and cultures. We also added 4×10^7 algal cells on Day 2 in order to compensate for the loss of cells in the outlet during the lag phase.

We tested four Cd concentrations (1.25–2.5–5–10 μg L^{-1}) and Cd was introduced continuously from Day-6, 6 days before adding the organisms. As in the batch bioassay, four replicates were used for the control and for each of the added-Cd treatments.

The monitoring of abiotic and biotic factors during the course of the test was carried out as for the batch bioassay (Supplementary Table 1). We measured the Cd on the sediment at the end of the test in the 0–5 mm and the 5–10 mm layers.

2.2. Data analysis

R Development Core Team (2010) was used to plot all the figures and perform the statistical analyses.

2.2.1. Expression of toxicity values

Cd concentrations decreased in the water column and increased in the sediment, so we calculated different values of EC50s based on nominal Cd concentrations (e.g., EC50), or measured Cd concentrations in a compartment at a given time (e.g., $EC_0$50, measured on Day 0), or mean Cd concentrations in a given time interval (e.g., EC_m50). In some cases, we calculated ECx (concentrations leading to *x* percent effect) using a three-parameter logistic model. In these cases the normality and homogeneity of residue distributions were checked.

2.2.2. Comparison of batch and flow-through assay data

To compare the two treatments (e.g., control data of batch and flow-through bioassays), we used a Fisher test for the binary data (e.g., survival) and a Student *t*-test (parametric test) or a Mann–Whitney–Wilcoxon *U*-test (non parametric test) for continuous data (e.g., growth and reproduction).

When comparing more than two treatments at a given time (e.g., results at different Cd concentrations), we used a Cochran–Armitage test for the binary data and the monotonous effect of the treatment on the parameter tested, and a Fisher test with Bonferroni–Holm correction if the effect was not monotonous. An Anova was performed for continuous data following a normal distribution. When the Anova showed an effect and the residues were normally distributed, Dunnett test was carried out to determine which treatments were different from the control. A Williams test was performed for the monotonous data. In the case of non-normally distributed residues and data, we used a Kruskal–Wallis test rather than an Anova. If a significant global effect was shown, a Jonckeere test was carried out for monotonous responses, and a Dunn test for non-monotonous responses.