Science of the Total Environment 521–522 (2015) 173–182

Contents lists available at ScienceDirect

Science of the Total Environment

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

Mesocosm validation of the marine No Effect Concentration of dissolved copper derived from a species sensitivity distribution

E.M. Foekema [a,*], N.H.B.M. Kaag [a], K.J.M. Kramer [b], K. Long [c]

[a] IMARES Wageningen UR, PO Box 57, 1780 AB Den Helder, The Netherlands
[b] Mermayde, P.O. Box 109, 1860 AC Bergen, The Netherlands
[c] Regulatory Compliance Limited, Bilston Glen, Midlothian, EH20 9LZ, UK

HIGHLIGHTS

- Ecological impact of dissolved copper was investigated in outdoor marine mesocosms.
- Six, triplicated, exposure concentrations were actively maintained for 82 days.
- Development on the plankton and benthic community was followed.
- Bivalve reproduction formed the most sensitive endpoint.
- NOEC was comparable with PNEC from SSD based on single species lab studies.

ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 18 March 2015
Accepted 20 March 2015
Available online 30 March 2015

Editor: Mark Hanson

Keywords:
Marine mesocosm
Copper
Ecosystem
Species sensitivity distribution

ABSTRACT

The Predicted No Effect Concentration (PNEC) for dissolved copper based on the species sensitivity distribution (SSD) of 24 marine single species tests was validated in marine mesocosms. To achieve this, the impact of actively maintained concentrations of dissolved copper on a marine benthic and planktonic community was studied in 18 outdoor 4.6 m^3 mesocosms. Five treatment levels, ranging from 2.9 to 31 μg dissolved Cu/L, were created in triplicate and maintained for 82 days. Clear effects were observed on gastropod and bivalve molluscs, phytoplankton, zooplankton, sponges and sessile algae. The most sensitive biological endpoints; reproduction success of the bivalve *Cerastoderma edule*, copepod population development and periphyton growth were significantly affected at concentrations of 9.9 μg Cu/L and higher. The No Observed Effect Concentration (NOEC) derived from this study was 5.7 μg dissolved Cu/L. Taking into account the DOC concentration of the mesocosm water this NOEC is comparable to the PNEC derived from the SSD.

1. Introduction

Copper is an essential element and enables multiple metabolic functions in all life forms when present at low levels. At elevated concentrations copper has been shown to trigger a number of adverse physiological, histological and behavioural responses, and it is in the dissolved cupric ion (Cu^{2+}) form that copper is the most bioavailable, although labile forms of copper are also suggested as contributing to observed toxicity (Brooks et al., 2007, 2008).

The bioavailability of metals in the aquatic environment can be influenced by many factors, but is especially affected by pH (Hyne et al., 2005; De Schamphelaere and Janssen, 2004), and dissolved organic carbon (DOC) concentrations (DePalma et al., 2011a). The influence of these factors can be very metal specific (e.g. Sánchez-Marin et al., 2010), but for dissolved copper in the marine environment the DOC concentration is the most significant environmental factor controlling the toxicity for pelagic organisms. When incorporated into a complex with DOC, the bioavailability of dissolved copper is strongly reduced. This has been demonstrated for a wide range of marine species, covering macroalgae (Brooks et al., 2008), rotifers (Arnold et al., 2010b), echinoderms (e.g. Lorenzo et al., 2002; Arnold et al., 2010a), bivalves (e.g. Zamuda and Sunda, 1982; Arnold et al., 2006, 2010a; Brooks et al., 2007) and fish (Gheorghiu et al., 2010). DOC concentrations in natural marine and estuarine waters show large variations, as illustrated by the analysis of 72 water samples from coastal marine and estuarine sites in the USA and Canada, with DOC levels ranging between 0.8 and 21 mg C/L (DePalma et al., 2011b). Therefore, the comparability of copper toxicity data from experiments performed with seawater from a different origin is strongly improved after normalisation of the effect concentration on DOC (Arnold et al., 2010a). By following this approach a Predicted No Effect Concentration (PNEC) for dissolved copper was calculated from

* Corresponding author.
E-mail address: edwin.foekema@wur.nl (E.M. Foekema).

http://dx.doi.org/10.1016/j.scitotenv.2015.03.086

a species sensitivity distribution (SSD; see Posthuma et al., 2002) by Van Sprang and co-workers (Van Sprang et al, 2008). This calculation was based on No Observed Effect Concentrations (NOECs) from 24 high quality marine single species laboratory tests covering 8 taxonomic groups, normalised for a DOC concentration of 2 mg/L, which can be considered as typical for coastal marine areas. The PNEC was 5.2 μg dissolved Cu/L and was calculated as the median Hazard Coefficient 5-50 (HC5-50) being the lower 50th percentile of the 95% protection level. It thus predicts that 95% of the species in a marine environment with 2 mg/L DOC will not be affected by a dissolved copper concentration that does not exceed 5.2 μg/L.

The aim of the study presented in this paper was to evaluate the robustness of this PNEC in a marine mesocosm study, mimicking a marine, soft sediment, near shore European ecosystem that is chronically (>80 days) exposed to a concentration series of dissolved copper ranging from 1 to 31 Cu μg/L.

2. Materials and methods

2.1. Mesocosms

In total 18 mesocosms were used, each consisting of a circular glass-fibre tank located on land, partly buried in the ground, with a volume of 4.6 m^3 (diameter 190 cm, depth 180 cm). The mesocosms were installed with about 20 cm of natural sandy sediment that was collected from the coastal North Sea and a 150 cm deep water column of natural seawater collected from the Oosterschelde, a relatively pristine tidal bay in direct connection with the North Sea, often used as a reference site in marine ecotoxicological studies in the Netherlands (e.g. Kuiper et al., 2007; Foekema et al., 2008, 2012). Concentrations of selected metals measured in the batch of water used for this study were 0.2 μg Cd/L, 1.26 μg Ni/L, 0.5 μg Pb/L, 59 μg Zn/L and 1.1 μg Cu/L.

The water level was not manipulated to simulate a tidal cycle. The water column of each mesocosm was continuously mixed by aeration at about 10 cm above the sediment. Each mesocosm was covered with a transparent lid as a defence against rainfall, birds and litter. Evaporation losses were replenished with demineralised water so that salinity was maintained at 32 ± 1 throughout the study. Prior to the first application of dissolved copper, the water was circulated through the hydraulically connected mesocosms for 33 days to ensure a similar development of the plankton community and water characteristics in all mesocosms. On day 0, before dosing commenced, the continuous circulation was stopped and the mesocosms were isolated.

2.2. Test substance and dosing

Copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$, Sigma-Aldrich, purity 99.995%) dissolved in 0.45 μm-filtered seawater was used to achieve and maintain the concentration of dissolved copper in the mesocosm water at the appropriate level. For each mesocosm the stock solution was prepared at the appropriate concentration in 10 l aliquots of water in a polyethylene tank, which was then added to the mesocosm with continuous flow of 1 to 8 mL/min starting on April 29, 2009 (Day 0). Within a week the concentration of dissolved copper in the water column of each mesocosm was built-up until the intended (nominal) copper concentration was reached. These concentrations were, in addition to the controls, 2.6, 5.2, 9.0, 15 and 27 μg Cu/L. From then on, a constant concentration of dissolved copper was actively maintained by adjusting the dosing rate for each individual mesocosm, based on the analysis of dissolved copper (three times per week). All treatments and untreated controls were triplicated in a randomised block design. Filtered seawater without added copper was added to the control mesocosms in amounts comparable to the treated mesocosms. As somehow similar volumes of water were removed from the mesocosms during each sampling event (see below) the additions of the dosing solutions did not result in substantial increase of water levels.

The mesocosms were installed on March 24, 2009, exposure started on April 29, 2009 and lasted until the final sampling on July 22, 2009. The total duration of the exposure period was 82 days. The start of the exposure period is defined as Day 0 and the pre-exposure period is indicated by negative day numbers throughout this publication.

2.3. Flora and fauna

Phyto- and zooplankton and small benthic invertebrate species were introduced with the water and sediment during installation. A selection of macroinvertebrates was introduced in known numbers during the first days of the establishment phase (Table 1). The introduced species are commonly present in shallow soft sediment coastal ecosystems of the North Sea and representatives from various taxonomic classes: sponges, crustaceans, molluscs and annelids. All introduced fauna were collected from relatively pristine field locations and were introduced in the mesocosms following a random table. Small parts of larger specimens of the sponge *Halichondria panicea* were suspended about 20 cm below the water surface.

2.4. Biota sampling and analyses

Phytoplankton was sampled by submerging a bottle in the mesocosm ca. 30 cm below water surface. Phytoplankton biomass was measured in these samples as chlorophyll-a concentration twice a week by means of a 1Hz-kuvetten Fluorimeter (BBE-Moldaenke). Subsamples of 100 mL were collected on days −2, 12, 26, 54 and 82, preserved with lugol and stored to be analysed by visual microscopic determination and counting of the various taxa.

On a weekly basis five water samples of about 1.5 L each were collected per mesocosm for determination of the zooplankton community by means of a core sampler covering the entire water column depth. The zooplankton from these samples was collected using a 55 μm plankton net and the composite sample was preserved in a formaldehyde solution. Visual microscopic analyses were performed on the samples collected on days −9, 12, 26, 54 and 82.

The development of periphyton (sessile algae) was monitored on three glass microscope slides (76 × 26 mm) that were placed in each mesocosm in vertical position facing south at ca. 10 cm below the water surface, out of reach of gastropods. At 28 days intervals the development of the periphyton on the slides was determined by measuring the chlorophyll-a fluorescence using a microtiter plate reader (BioteK FLx800).

The development of the sponges was monitored by determining the wet weight of the individual sponges with 28 day intervals.

In order to avoid disturbance of the system periwinkles, cockles, and lugworms were only sampled at the end of the study, after the water was pumped off. Survival and growth (biomass and where appropriate shell length) of the introduced individuals were determined. The density of small macroinvertebrates, including mudshrimps and juveniles of introduced species, was also determined only at the end of the study. For this, two rings (30 cm diameter each) were pressed in the sediment surface before the water was fully pumped off. The top 5 cm sediment layer within each ring was collected and sieved (500 μm). All macroinvertebrates that were recovered were stored in formaldehyde for taxonomic identification and counting.

2.5. Physico-chemical measurements

Water temperature and dissolved oxygen concentration (Hach LDO101), salinity (Hach CDC401), and pH (Hach PHC101) were determined twice a week by submerging electrodes at half water depth.

Sampling for the determination of the concentration of nutrients, Mg, Ca (bi weekly) and DOC (weekly) was performed by immersing