Environmental Pollution 238 (2018) 607–616

Contents lists available at ScienceDirect

Environmental Pollution

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

The use of time-averaged concentrations of metals to predict the toxicity of pulsed complex effluent exposures to a freshwater alga☆

Brad M. Angel [a, *], Kathryn Goodwyn [b], Dianne F. Jolley [b], Stuart L. Simpson [a]

[a] Centre for Environmental Contaminants Research, CSIRO Land and Water Locked Bag 2007, Kirrawee, NSW 2232, Australia
[b] Centre for Medical and Molecular Biosciences, School of Chemistry, University of Wollongong, Australia

ARTICLE INFO

Article history:
Received 12 February 2018
Received in revised form
26 March 2018
Accepted 26 March 2018

Keywords:
Dissolved metal
Labile metal
Complexation
Effluent
Regulation

ABSTRACT

Intermittent, fluctuating and pulsed contaminant discharges may result in organisms receiving highly variable toxicant exposures. This study investigated the toxicity of continuous and pulsed exposures of a complex, neutralised drainage water (NDW) and dissolved copper-spiked dilute NDW to the green alga, *Pseudokirchneriella subcapitata*. The effects of single pulses of between 1 and 48 h duration and continuous exposures (72 h) on algal growth rate inhibition were compared on a time-averaged concentration (TAC) basis. Algal growth rates generally recovered to control levels within 24–48 h of the pulse removal. Continuous exposures to NDW resulted in similar or marginally higher toxicity to the algae when compared to pulsed exposures of equivalent TAC (% NDW). The toxicity of the NDW was attributed mostly to the metals, with the major cations potentially causing effects that are both additive (direct toxicity) and antagonistic (lower bioavailability of trace metals). For dissolved copper in dilute NDW, the pulsed exposures caused slightly higher toxicity than continuous exposures of equivalent dissolved copper TAC, with much of the difference explained by differences in labile copper concentrations between treatments. The results indicate that water quality guideline values for toxicants derived from continuous chronic exposures may be relaxed for pulsed exposures by a factor related to the TAC with the intent to provide an adequately protective but not overly-conservative outcome. The study highlights the influence that natural water quality parameters such as water hardness and DOC can have metal speciation and toxicity, and indicates that these parameters are particularly important for site-specific water quality guideline value derivation where, on a TAC basis, pulsed exposures may be more toxic than continuous exposures typically used in guideline value derivation.

1. Introduction

Waterways may experience significant temporal fluctuations in concentrations of contaminants as a result of industrial, agricultural and urban-runoff inputs (Burton et al., 2000; Simpson et al., 2014). This may be particularly pronounced in close proximity to effluent discharge points, where concentrations of chemicals in receiving waters may rapidly decrease after the discharge has ended (Angel et al., 2010a; Beck and Birch, 2014; Simpson et al., 2014). Variations in rainfall duration, frequency and intensity also have a strong influence on contaminant concentration in waters receiving stormwater inputs (Burton et al., 2000). As a consequence, organisms within the receiving environment are frequently exposed to pulses, rather than constant concentrations of contaminants, and the assessment of the risk posed by the varying water quality (i.e. contaminants and associated non-chemical stressors such as variations in water pH, salinity, dissolved oxygen, suspended solid concentrations) should be considered (Gordon et al., 2012; Hoffman et al., 2000; Hogan et al., 2013). Pulses of dissolved metals may also occur in continuous exposure toxicity tests when the solubility limit is exceeded (Angel et al., 2016; Gilmore et al., 2016). Most whole effluent toxicity (WET)/direct toxicity assessment (DTA) test methods have been developed to assess continuous point source discharges, but frequently are applied in the same manner to episodic discharges (US EPA, 2002a; b).

There is generally a poor understanding of the magnitude, duration and frequency of pulsed exposure events or the biological effects resulting from such exposures. Few studies have

☆ This paper has been recommended for acceptance by Prof. W. Wen-Xiong.

* Corresponding author.
E-mail address: brad.angel@csiro.au (B.M. Angel).

https://doi.org/10.1016/j.envpol.2018.03.095
0269-7491/

investigated the effects of pulsed exposures of complex effluents that are typical of many trade waste and industrial discharges (i.e. often containing multiple potential chemical toxicants and other stressors), and there is little understanding of how factors that influence the bioavailability of contaminants may modify the outcomes when assessing potential impacts from episodic discharges that result in pulsed exposures to organisms. For example, variations in the ionic compositions (e.g. water conductivity, hardness) and dissolved organic matter will influence the bioavailability of many contaminants, particularly metals (Paquin et al., 2002; Apte et al., 2005). A number of studies have investigated the effects of pulsed exposures of a few chemicals added to synthetic media (Angel et al., 2017; Diamond et al., 2005; Hoang et al., 2007), and there is a need for more information on the effects of pulsed exposures in natural waters of chemical mixtures (e.g. effluents) and contaminants whose bioavailability is strongly influenced by water composition. The regulation of effluent discharges is likely to be improved by the consideration of these factors in assessment frameworks.

In previous studies, we have observed that the time-averaged concentration (TAC, where the TAC is equivalent to the net exposure the organisms receive) of pulsed dissolved copper exposures was a good predictor of toxicity to the marine amphipod, *Melita plumulosa* (Angel et al., 2010b), the marine alga, *Phaeodactylum tricornutum* (Angel et al., 2015a), and the freshwater algae, *Psedokirchneriella subcapitata and Chlorella* sp (Angel et al., 2017). The designs of many other studies have not always enabled a direct comparison of the observed organism toxicity with the TAC of the major toxicants, however much of the literature indicates that over a range of pulse duration-magnitude exposures, the toxicity to organisms is of a similar or lesser magnitude for exposures to pulses that result in a similar or lower TAC of the major toxicants (Diamond et al., 2005; Hoang et al., 2007; Naddy et al., 2000). Beyond the different study methodologies, the comparison of toxicity and toxicant exposure on a TAC-basis may be complicated by many factors, including changes in the organism's life-stage sensitivity over the time-scale of longer toxicant exposure durations, acclimatisation of organisms following multiple exposures, and variations in recovery between pulses before test endpoints are assessed (Chen et al., 2012; Diamond et al., 2006; Hoang and Klaine, 2007; Hosmer et al., 1998; Naddy et al., 2000). When similar toxicity is observed for different types of exposure with equivalent TACs, the toxicant exposure duration and concentration may be varied in an inversely proportional manner and result in similar toxicity to an organism.

The current study investigated the toxicity of a freshwater effluent that contained known concentrations of metals and major ions that were expected to cause toxicity to aquatic organisms. The test organism studied was a freshwater microalga (*P. subcapitata*), selected as microalgae are among the most sensitive organisms to contaminants (Golding et al., 2015; Stauber and Davies, 2000). The position of microalgae at the bottom of the food chain implies that their toxicity during exposure and rate of biomass recovery post exposure affects higher trophic organisms directly through toxicant entry into the food chain and indirectly when decreased biomass leads to starvation of grazing invertebrates. Two hypotheses were evaluated: (i) that exposures (continuous and pulsed) with an equivalent TAC (% of effluent water) result in equivalent toxic effects, and (ii) that the algal population recovers rapidly from pulsed exposures of $\leq$48 h in duration. Two series of waters were studied: (i) a neutralised drainage water (NDW) from a dairy farm area impacted by acidity due to oxidised acid-sulfate soils; and (ii) a less-contaminated natural water spiked with dissolved copper (CuW), for comparison to previous studies conducted in synthetic freshwater, and for assessing the influence of metal bioavailability. The algal growth rate inhibition (toxicity) resulting from continuous and pulsed exposures was compared on a TAC basis for all tests, and algal cell densities of treatments were compared to controls to measure algal toxicity and recovery during and after exposure, respectively.

Particularly for metals, the toxicity of waters is most strongly related to the concentrations of non- or weakly complexed metals (e.g. 'free' aqua ions and weak inorganic metal-ligand complexes) (Campbell, 1995; Paquin et al., 2002). An appreciable portion of filterable metals in many natural waters may be strongly complexed by organic ligands or in colloidal forms that are non-labile and exhibit low bioavailability and hence toxicity (Apte et al., 2005; Bowles et al., 2006; Eriksen et al., 2001). Only a few studies have compared organism toxicity from continuous and pulsed toxicant exposures in natural waters where metal complexation may significantly influence the observed toxicity. In the present study, we used measurements of metal lability to provide information on metal bioavailability, where greater lability is predicted to result in greater metal bioavailability. The contribution of major cations to the observed toxicity was also evaluated.

2. Methods

2.1. Test waters

Two natural waters were collected and filtered through an acid-washed 0.45 μm cartridge filter (Sartobran P sterile midicap, Sartorius Stedium Biotech, Germany) and refrigerated in acid-washed 5-L high-density polyethylene containers in the dark below 4 °C until use. Approximately 20 L of an acidic, metal-rich, water was collected from a drainage channel of a farm at Mobilong, South Australia (Simpson et al., 2014). At this location the soils are impacted by acidity due to oxidised acid-sulfate soils that result in the farm drainage waters containing a range of metals and metalloids at concentrations that exceed Australian and New Zealand freshwater water quality guideline values (WQGVs) (ANZECC/ARMCANZ, 2000), and potentially a number of farm-derived chemicals (trace concentrations of some herbicides below WQGVs). Approximately 60 L of river water was collected from the Murray River, a few kilometres upstream of where the farm drainage waters are discharged to the river system on an intermittent basis. The farm drainage water pH was 3.2, and the Murray River pH was 7.8 and had an alkalinity of 95 mg $CaCO_3$/L which resulted in quite rapid neutralisation of the acidic drainage water inputs (Mosley et al., 2015).

The neutralised drainage water (NDW, pH 7.8) was prepared by adding small volumes of 1 M NaOH (Merck) to the Mobilong water without resulting in significant dilution of the drainage water. The NDW was allowed to stand at room temperature for 24 h before it was filtered (0.45 μm) to remove precipitates, after which it was used within 2 h to prepare test treatments. Metal and major cation concentrations at various pH values are shown in Table S1 of the Supporting Information (SI).

Two series of water treatments were used for ecotoxicity tests: NDW series and CuW series. The NDW treatments were prepared by diluting the NDW in Murray River water, where NDW-100, NDW-50, NDW-30, NDW-10, NDW-2 correspond to 100% (undiluted), 50%, 30%, 10% and 2% NDW dilutions, respectively. The NDW dilutions were selected following preliminary toxicity testing that covered a full range of toxic responses (i.e. 0–100% toxicity). A feature of the NDW series was the relatively high concentrations of Al, Co, Ni, Mn and Zn (Table S1 and S2), but not copper (<1.5 μg/L) compared to Australian and New Zealand water quality guideline values for 95% species protection (ANZECC/ARMCANZ, 2000).

The CuW treatments were prepared by spiking dissolved copper