Aquatic Toxicology 161 (2015) 138–145

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Copper sulphate reduces the metabolic activity of *Gammarus fossarum* in laboratory and field experiments

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 2 February 2015
Accepted 9 February 2015
Available online 14 February 2015

Keywords:
Amphipod
Feeding activity
Electron transport system (ETS)
Metal
Accumulation
Spring
Headwater

ABSTRACT

The specialised fauna of freshwater springs is affected by contamination of the water with xenobiotics from human activities in the surrounding landscape. We assessed the effects of exposure to toxins in laboratory and field experiments by using copper sulphate as a model substance and *Gammarus fossarum* Koch, 1836, as the model organism. This amphipod is a common representative of the European spring fauna and copper is a widespread contaminant, mainly from agricultural practice. The experiments were conducted in test chambers placed in flow channels and directly in a spring. The gammarids were fed with conditioned beech leaf discs, which had been exposed to a 0.8 mg Cu/L solution for 96 h. The feeding activity of the amphipods was quantified on the level of the organism; and the respiratory electron transport system (ETS) assay was conducted in order to determine changes on the cellular level in the test organisms. The results show that the feeding activity, when the leaf discs were contaminated with copper, was not significantly different from the control. The ETS activity of the gammarids, which had been feeding on the copper contaminated leaf discs was however significantly reduced. The results followed the same pattern for gammarids from both the laboratory and the spring. By conducting the experiments not only in a laboratory but also directly in a spring in the field, we took a crucial step towards a more realistic approach when examining environmental pollutants on an organism. Our findings demonstrate the importance of conducting experiments out in the field, in natural conditions, as well as in the laboratory.

1. Introduction

Pollution of our freshwaters is taking place rapidly, for example through pharmaceutical compounds, fertilisers and pesticides. Terrestrially applied pesticides are flushed into springs and rivers through runoff. This will take place more regularly when heavy rainfalls occur more frequently as a consequence of global change, which is causing an increase in extreme events such as floods in the temperate regions (IPCC, 2007). An increase of the mean winter precipitation in the northern and western part of Switzerland and an increase of heavy precipitation events are documented for Switzerland (Schmidli et al., 2002; Schmidli and Frei, 2005).

Copper salts are important ingredients in many fungicides and fertilisers used in agriculture (e.g. De Oliveira-Filho et al., 2004), for example in vineyards (e.g. Ruyters et al., 2013), and are amongst the most widespread contaminants (Debelius et al., 2009). Contamination of leaf litter with copper can happen, for example, in vineyards where fungicides containing copper are applied. Owing to its non-degradability, copper moves up food webs and is distributed in the entire biotic compartment of freshwaters (Lebrun et al., 2012). Copper concentrations in natural unimpacted waters are mainly influenced by the geology of the watershed of the area and are typically less than 4 μg/L (Schönborn and Risse-Buhl, 2013). The copper concentration of impacted waters can be considerably higher, reaching more than 10 mg/L (e.g. Sridhar et al., 2001). Although a certain amount of copper is essential for most organisms, it is extremely toxic for aquatic organisms beyond certain threshold levels (Prato et al., 2013). Copper poses a threat to many aquatic organisms when available in excess in water (De Martinez Gaspar Martins et al., 2011). A consequence of exposure to copper salts is the accumulation of these ions in the tissues of the exposed organisms. Bioaccumulation of copper has been observed in different aquatic species (e.g. Tattersfield, 1993; Reichmuth et al., 2010; Pinho et al., 2011).

Amphipods are frequently used as bioindicators in aquatic toxicity tests owing to their prolific breeding, high abundance in nature and sensitivity to anthropogenic compounds (e.g. Ladewig et al., 2006) such as metal ions in water bodies which they inhabit. Amphipods mainly take up ions via their gills since these are a large

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http://dx.doi.org/10.1016/j.aquatox.2015.02.005
0166-445X/

adsorptive organ system (Reichmuth et al., 2010) making them especially susceptible to water-borne pollutants (Rinderhagen et al., 2000).

The genus *Gammarus* is most commonly used in experiments in Europe (e.g. Brooks and Mills, 2003; Fialkowski et al., 2003; Dedourge-Geffard et al., 2009; Coulaud et al., 2011). Gammarids are also often more sensitive than *Daphnia magna* Straus, 1820 (Gerhardt, 2011) towards different types of pesticides, such as neurotoxic substances and especially pyrethroids. *Gammarus fossarum* Koch, 1836 (Crustacea; Amphipoda) is a relatively robust and abundantly occurring member of the macrozoobenthos of European springs. It inhabits springs and spring brooks in mountainous regions of central Europe (Janetzky, 1994; Pöckl et al., 2003). *G. fossarum* is a key species (e.g. Dangles et al., 2004) that mainly acts as an efficient shredder, but also feeds on fine particulate organic matter (FPOM) (Moog 1995). It plays a fundamental role in organic matter breakdown in springs and spring brooks, and hence, in the distribution of coarse particulate organic matter (CPOM) and FPOM (Wagner 1990; Simcic and Brancelj, 2006). *G. fossarum*, a typical inhabitant of running waters rich in oxygen (Lukancic et al., 2009) and low pH, is more sensitive than *Gammarus pulex* (L.) (e.g. Rinderhagen et al., 2000; Alonso et al., 2010) towards contamination of water and low oxygen. For these reasons *G. fossarum* can be deemed a suitable organism for assessing impacts of pollution and it is readily used in ecotoxicological assays (e.g. Westram et al., 2011; Gerhardt, 2011; Maltby et al., 2002).

Laboratory experiments guarantee reproducibility of the results by exactly defining the test conditions. They provide numerous replicates and are suitable for a variety of experiments on different biological levels, for example the population level. Furthermore, they are useful tools when assessing contamination effects on certain species. Quite a few studies on laboratory experiments concerning the effects of copper exposure on freshwater species have been published (e.g. Sroda and Cossu-Leguille, 2011; Reichmuth et al., 2010). However, laboratory conditions are, depending on the experimental design, far from natural (e.g. petri dishes, static), usually very standardised and optimised so that species might react differently under natural conditions. Laboratory experiments conducted in flow channels provide a certain degree of reproducibility and control and are much more realistic than other laboratory experiments. Experiments in artificial flow channels are more suitable than other laboratory set-ups, especially for stream invertebrates. Mesocosms have been used in artificial indoor streams (e.g. Böttger et al., 2013) and in a few natural streams (e.g. Coulaud et al., 2011). Furthermore, flow-through microcosms were used to analyse the impact of elevated temperature on the emergence of the mayfly *Baetis bicaudatus* Dodds (Harper and Peckarsky, 2006). However, the best approach to natural conditions is gained with field experiments, which are generally not easy to establish and usually lack reproducibility but are crucial for understanding, for example, the real effects of pollutants on certain species in the field.

In this study, we conducted feeding tests and the respiratory electron transport system (ETS) assay in order to determine the effects of copper-contaminated leaf discs on *G. fossarum* both in the laboratory in artificial flow channels and in the field, using test chambers. The feeding activity is a suitable non-lethal endpoint (Pestana et al., 2007) and gives insight into the metabolic activity of the organisms, on the level of the organism. It has been used widely in the last decade in many different experiments (e.g. Bundschuh et al., 2009; Dedourge-Geffard et al., 2009). The ETS assay was conducted to quantify the effects of the copper on the cellular level of the organisms. The ETS is an enzyme system found in the inner mitochondrial membranes of eukaryotes which controls the oxygen consumption (G.-Toth, 1999) and the results reflect the maximum oxygen consumption when all enzymes are functioning optimally (Kenner and Ahmed, 1975).

Our aims were to find out how *G. fossarum* reacts to a copper-contaminated food source, and to see if laboratory results differed from those in the field.

2. Materials and methods

2.1. Sampling site

Specimens were collected from one natural rheocrene in the Röserental near Liestal, in Switzerland (see von Fumetti et al., 2007). They were pipetted with a turkey baster into white trays and counted out for use in the spring and laboratory. Individuals of both sexes were collected and their average wet weight was 11.1 ± 5.2 mg. Organisms were used regardless of their sex, as has been done in other studies (e.g. Cold and Forbes 2004; Bundschuh et al., 2009; Alonso et al., 2010). Selected specimens showed no sign of being parasitised and their movement was normal. Specimens for use in the laboratory were transported in closed plastic boxes with spring water and leaves from the spring. They were then kept in the transport containers at 10 °C for 46 h, for technical reasons not 48 h, owing to transport time, for acclimatisation to laboratory conditions before being used in an experiment.

The temperature of the spring water was 10.4 ± 0.1 °C. The pH was 7.1 ± 0.2 and the electrical conductivity of the spring water was 571.2 ± 17.7 µS/cm. The oxygen concentration was 7.3 ± 1.5 mg/L and the air saturation was 68.0 ± 13.9%. The water properties were all measured using portable metres (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

The nutrients phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_4^+) of the spring water were measured with inductively coupled plasma optical emission spectroscopy (ICP-OES) (SPECTRO MS, Spectro Analytical Instruments GmBh, Kleve, Germany). The nitrate concentration of the spring water was 18.0 mg/L, the nitrite <0.05 mg/L, the ammonia concentration <0.1 mg/L and the phosphate <0.5 mg/L.

The copper concentration of the natural spring water measured with ICP-OES was found to be smaller than 0.001 mg Cu/L. The spring water has a ionic composition as follows: potassium (Na^+) 2.5 mg/L, calcium (Ca^{2+}) 106.5 mg/L, magnesium (Mg^{2+}) 7.9 mg/L, chloride (Cl^-) 12.7 mg/L and sulphate ($SO4^{2-}$) 33.9 mg/L. The carbonate hardness is 24.7 mg/L. The spring water exhibits a total hardness of 29.9 mg/L.

2.2. Conditioning of the leaf discs

The spring from which the gammarids were obtained is surrounded mainly by beech trees. These leaves form the most important food source for its inhabitants including *G. fossarum*. Therefore, we collected beech leaves (*Fagus sylvatica* L.) from the litter layer near the spring after abscission in autumn 2013. The collected leaves were first washed, dried in an oven at 40 °C and then stored as described by Bloor (2010). Leaf discs (diameter 1 cm) were cut out of the collected leaves using a cork borer. Ten leaf discs were always weighed together and then placed together in numbered stainless steel herb infusers (ø 9 cm). The infusers were submerged into aerated spring water with fine particulate organic matter from the spring for conditioning. They were conditioned at a water temperature of 17 ± 0.5 °C for four weeks.

2.3. Choice of copper concentration for leaf disc exposure

We conducted pre-tests with beech leaf discs using the following nominal copper concentrations (all in mg Cu/L): 0.4, 0.8, 1.6, 3.2 and 6.4, in order to decide to which copper concentration to expose the leaf discs in our experiments. The range of 0.4–6.4 mg Cu/L was chosen loosely on previously conducted LC_{50}-tests (see Schmidlin