Ecotoxicology and Environmental Safety 129 (2016) 257–263

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Contribution of aqueous and dietary uptakes to lead (Pb) bioaccumulation in *Gammarus pulex*: From multipathway modeling to *in situ* validation

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ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form
22 March 2016
Accepted 25 March 2016
Available online 6 April 2016

Keywords:
Biodynamic model
Amphipod
Biokinetics
Multi-exposure
Trophic route
Field data

ABSTRACT

Although dynamic approaches are nowadays used increasingly to describe metal bioaccumulation in aquatic organisms, the validation of such laboratory-derived modeling is rarely assessed under environmental conditions. Furthermore, information on bioaccumulation kinetics of Pb and the significance of its uptake by dietary route is scarce in freshwater species. This study aims at modeling aqueous and dietary uptakes of Pb in the litter-degrader *Gammarus pulex* and assessing the predictive quality of multipathway modeling from *in situ* bioaccumulation data. In microcosms, *G. pulex* were exposed to environmentally realistic concentrations of Pb (from 0.1 to 10 μg/L) in the presence of Pb-contaminated poplar leaves, which were enclosed or not in a net to distinguish aqueous and dietary uptakes. Results show that water and food both constitute contamination sources for gammarids. Establishing biodynamic parameters involved in Pb aqueous and dietary uptake and elimination rates enabled to construct a multipathway model to describe Pb bioaccumulation in gammarids. This laboratory-derived model successfully predicted bioaccumulation measured in native populations of *G. pulex* collected *in situ* when local litter was used as dietary exposure source. This study demonstrates not only the suitable applicability of biodynamic parameters for predicting Pb bioaccumulation but also the necessity of taking dietary uptake into account for a better interpretation of the gammarids' contamination in natural conditions.

1. Introduction

Lead (Pb) is one of the priority substances singled out by the European Water Framework Directive for the protection of aquatic ecosystems (Directive, 2008/105/EC). Indeed, this metal is a common contaminant in freshwaters and its occurrence is often related to human activities, *i.e.* mining, coal burning, industries, traffic… (Urien et al., 2015). Although chemical analyses enable the determination of total concentrations of dissolved or particulate Pb in water, they do not give information about its bioavailability, *i.e.* the metallic fraction internalized and potentially toxic for aquatic organisms. In this regard, metal determination in biota is more relevant than metal determination in water insofar as the bioaccumulation process integrates both the different exposure routes for organisms (*i.e.* water and food), and the effects of the water's geochemistry on metal bioavailability (Bourgeault et al., 2011; Rainbow, 2007).

The amphipods of the genus *Gammarus* are widely distributed in Europe and known as ecosystem engineers with important roles in litter decomposition, nutrient remobilization and metal cycling in litter of freshwaters (Schaller et al., 2011a, 2011b). Furthermore, gammarids are strong candidates to monitor bioavailable metals in freshwaters because of their efficient ability to accumulate metals (Besse et al., 2013; Lebrun et al., 2015). However, a reliable interpretation of bioaccumulation data depends on the knowledge of the organism's behavior against contamination: which exposure route is more important? what is the organisms' ability to regulate contaminants according to the exposure route? (Ponton and Hare, 2010; Rainbow, 2007). In gammarids, bioaccumulation studies have long focused only on the aqueous uptake of freely dissolved metals because water is assumed to be the main exposure route through direct contact (Lebrun et al., 2011; Pellet et al., 2009; Urien et al., 2015; Xu and Pascoe, 1994). But the ingestion of

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http://dx.doi.org/10.1016/j.ecoenv.2016.03.033
0147-6513/

contaminated food can also be an important pathway of contamination, as reported in *Gammarus pulex* fed with Cd-contaminated leaves in controlled conditions (Pellet et al., 2014). Characterizing the relative contribution of metal uptakes from both water and food is thus of great interest for the effective use of gammarids as biomonitors, especially for Pb, a metal known for its high affinity for organic matter, including food sources for animals, in natural conditions (Gaillardet et al., 2004; Schaller et al., 2011b)

In last decades, conceptual models have been proposed to describe bioaccumulation in aquatic organisms (Luoma and Rainbow, 2005). These models are usually calibrated in the laboratory by the establishment of kinetic parameters describing physiological mechanisms involved in metal uptake from both water and food, and metal elimination. Such biodynamic approaches have been shown to be well suited to several freshwater species including gammarids (Croteau and Luoma, 2007; Goulet et al., 2007; Komjarova and Blust, 2009; Lebrun et al., 2012, 2011). However, information on Pb bioaccumulation kinetics and the importance of its dietary uptake is very scarce in the literature, mostly as a result of the lack of suitable tracer for Pb to use in the laboratory (Croteau et al., 2013; Urien et al., 2015).

Although dynamic approaches are nowadays increasingly used, the validation of such laboratory-derived models is still rarely assessed *in situ*, especially for freshwaters. Yet, biodynamic modeling has been reported as a powerful tool to predict field bioaccumulation of Ag, Cd, Cu, Ni or Se in various lake, estuarine and marine species, providing that both exposure routes (water and food) are considered (Luoma and Rainbow, 2005; Ponton and Hare, 2010). To date, the use of biodynamic modeling to predict field Pb bioaccumulation is poorly documented (Urien et al., 2015). Thus, the acquisition of environmental datasets including Pb concentrations determined in native gammarids, ambient water and local food source remains a crucial step for validating multipathway modeling with *in situ* data.

This study aims at characterizing different uptake routes of Pb by *Gammarus pulex* and modeling the physiological processes involved in its bioaccumulation. In aquatic microcosms, *G. pulex* were exposed to environmentally realistic concentrations of Pb (from 0.1 to 10 μg/L) in the presence of Pb-contaminated poplar leaves, which were enclosed or not in a net to distinguish aqueous and dietary uptakes. Then, gammarids were transferred into uncontaminated microcosms to assess their ability to eliminate Pb according to the exposure route. Finally, the predictive quality of multipathway modeling was assessed from *in situ* bioaccumulation data.

2. Materials and methods

2.1. Collection and maintenance of gammarids

Adult *G. pulex* were selected according to their size using a series of sieves (± 1 cm in length) in a stream (Ru de l'Etang, Doue, France) in April 2013. This sampling site located at the head of a watershed is not impacted by urban or industrial activities and displays a good physicochemical quality (Lebrun et al., 2011; Urien et al., 2015). In the laboratory, gammarids were acclimated for 7 d in aquaria containing aerated Volvic® mineral water (Ca^{2+} 11.5, Na^{+} 11.6, Mg^{2+} 8.0, K^{+} 6.2, Cl^{-} 13.5 and SO_4^{2-} 8.1 mg/L; pH 7). Aquaria were maintained at 14 °C under a 12:12-h light:dark photoperiod. Amphipods were fed *ad libitum* with poplar leaves (*Populus nigra*) until 24 h before exposure experiments. The poplar leaves, which constitute the main local food supplies for gammarids at the sampling site, were previously collected in autumn (October 2012), dried and then stored for the following experiments.

2.2. Experimental design

After weighing batches of 2 dried poplar leaves, they were rehydrated in Volvic® mineral water for 24 h. Then, each batch of leaves was enclosed in a nylon net (150 μm-pore; 6 × 6 cm) to constitute leaf bags and immersed in a 1.5 L beaker contaminated with $Pb(NO_3)_2$ (> 99% purity) at final concentrations of 0.5, 1, 5 or 10 μg/L Pb. The aqueous solutions were stirred and renewed daily for a week to ensure that the metallic exchanges between the media, encaging systems of leaves (nylon bag) and leaves were at equilibrium.

Aquatic microcosms were comprised of beakers containing 500 mL of Volvic mineral water spiked with Pb at 0.5, 1, 5, and 10 μ/L. Before exposing gammarids, microcosms were previously equilibrated for 24 h and renewed with contaminated water to minimize the further binding of Pb to the inside wall of the beakers. Then, 50 gammarids were exposed for 6 d in each equilibrated microcosms in the presence of a leaf bag. For each exposure concentration, the leaf bags were either opened or closed in independent microcosms. Thus, opening the bags gave the organisms access to the free leaves and they were thus exposed simultaneously by aqueous and dietary routes. On the contrary, closed leaf bags meant that the organisms were only exposed by the aqueous route. Experiments were performed in triplicates, *i.e.* three microcosms for each exposure condition, all maintained at 14 °C under a 12:12-h light:dark photoperiod. Exposure media were renewed every day to ensure constant exposure, and 0.45 μm-filtered water was sampled before and after renewing exposure media to measure dissolved Pb.

During the exposure phase, pools of 5 gammarids were sampled after 1, 2, 3 and 6 days for each exposure condition. Gammarids were rinsed with 2 and 0.5 mM EDTA, then twice with ultrapure waters for 1 min, so as to remove the metal potentially adsorbed on the organisms (Lebrun et al., 2011). Then, they were frozen at − 80 °C for further analysis of internalized Pb. At the end of the exposure period, the amount of free leaves ingested by the animals was determined in each replicate by weighing the remaining leaves once dried. The encaged leaves were frozen at − 80 °C for Pb quantification in vegetal material.

To assess elimination rates, gammarids initially exposed to Pb were transferred into uncontaminated microcosms for 8 d and fed *ad libitum* with clean poplar leaves. During this depuration phase, pools of 5 gammarids were sampled after 1, 2, 4 and 8 days in each condition and treated as previously described for metal determination in their bodies.

2.3. Metal analysis in samples

Frozen amphipod pools as well as encaged leaves were lyophilized in 50 mL polypropylene tubes (SCP Science), weighed and digested by adding nitric acid and hydrogen peroxide (Urien et al., 2015). Digested samples were analysed by using a Zeeman graphite furnace atomic absorption spectrophotometer (SpectrAA 220Z, Varian) to determine Pb concentrations in animals and in poplar leaves. This analysis was performed by standard additions to avoid matrix effects. Reference materials successfully validated the digestion method of animals and vegetal material, *i.e.* mussel tissue ERM-CE278 and aquatic plant BCR 60 with recovery means of 92% and 115% respectively.

Water samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS, X series 2, Thermo Fisher Scientific). The detection limit for Pb was 0.01 μg/L and recovery of the standard (NIST 1640a) was of 96%.