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Relative Sensitivity of Hyporheic Copepods to Chemicals

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Abstract The sensitivity of harpacticoid copepods was tested against selected pollutants. Acute toxicity tests were carried out for five hyporheic species exposed to pesticides, ammonia, and metals. The stygoxene *Bryocamptus zschokkei*, *B. minutus*, *B. pygmaeus* and *Attheyella crassa*; and the stygophilous *B. echinatus* were sampled and cultured during 8 months in controlled conditions. A first test protocol is presented. The acute endpoints among species fell within one order of magnitude. The sensitivity among various species evaluated in this study is consistent and the choice of species for further sediment/groundwater assessment is not specific to a chemical class. These potential test organisms would be more suitable to protect meiofaunal communities.

Keywords Copepods · Freshwater · Hyporheic · Groundwater

Sediment toxicity assessment is commonly performed by selecting macroinvertebrate species, predominantly selected among amphipod crustaceans, oligochaetes, and chironomid insect larvae (USEPA 2000). On the contrary, only poor information is available on the effects of pollutants on the meiofaunal species living in the interstitial habitat, of many porous sand-and gravel-bed, beneath and lateral to a stream–river, the so-called hyporheic habitat (Williams and Fulthorpe 2003). As a matter of fact, very few studies of the hyporheic contamination have been made, although this important ecotone, linking surface and subsurface freshwater is increasingly becoming contaminated with a variety of chemicals (Hancock 2002; Hancock and Boulton 2005), such as chlorobenzenes, pesticides, hydrocarbons, and heavy metals. In agriculturally dominated areas, groundwater may be enriched in nutrients, such as nitrates, and this condition may lead to eutrophication of surface waters. On the reverse, contaminated surface water may dramatically lowers groundwater quality, with alteration of hyporheic and groundwater communities (Notenboom et al. 1994; Mösslacher et al. 2001), and deterioration of drinking water. In order to preliminarily assess the effects of some chemicals on species strictly linked to the interstitial habitat, the sensitivity of harpacticoid copepods is tested herein against selected pollutants. Harpacticoid copepods are abundant and ubiquitous in alluvial sediments and possess many attributes that make them good candidates as toxicity test organisms, and they have been already used for evaluating the acute effects of marine contaminants for more than 20 years (Bengtsson 1978). They are small, have relatively short life cycles, are easily cultured under laboratory conditions, and have easily recognized larval stages, allowing the comparative sensitivity along the different phases of their post-embryonic development to be assessed (Verriopoulos and Moratou-Apostolopoulou 1982; Green et al. 1996). Harpacticoids have holobenthic life-cycle and pass through six naupliar stages followed by

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six copepodite stages, copepodite VI being the adult (Huys et al. 1996).

According to Burton et al. (2002) harpacticoid copepods have been shown to be sensitive to environmental variables and more susceptible to high metal concentrations than epibenthic fauna. The sensitivity of harpacticoids, such as *Bryocamptus zschokkei* and *B. praegeri* suggests that these taxa might have a role as indicators of metal pollution and as potential laboratory test organisms. The high sensitivity of harpacticoids has also been highlighted in marine systems (Van Damme et al. 1984). Brown et al. (2005) have applied toxicity test methods, established for marine copepods, to test sensitivity of the freshwater harpacticoid species *B. zschokkei*. The objective of this study was to develop a protocol for evaluating and determine the sensitivity of five harpacticoid species to pesticides, ammonia, and metals.

Materials and Methods

Copepods were sampled in the Presciano spring system, located on the southeastern part of the Gran Sasso Massif, at 330 m a.s.l., coordinates 42°16′05″ N, 13°46′56″ E, near Capestrano town (L'Aquila, Italy). Quantitative samples were taken by pumping 20 L of water with a Bou-Rouch pump at 50 cm below the bottom, and filtering through a 60 μm mesh net. Specimens were transported to the lab in the same spring water, refrigerated at 10°C (pH: 7.7, conductivity: 436 μS/cm, total hardness: 190 $CaCO_3$, NH_4^+: <0.03, NO_3^-: 3.9, PO_4^-: 0.01, organic N: 0.8, Cl^-: 4.3, SO_4^-: 15, Cu^{+2}: <0.005 and Zn^{+2}: 0.01, all expressed in mg/L). In the lab, copepods were cultured during 8 months in a commercial spring water Santa Croce (pH: 7.84, conductivity: 306 μS/cm, hardness: 56 $CaCO_3$, $NH_4{}^+$: <0.03, NO_3^-: 1, Na^+: 1.23, K^+: 0.16, SO_4^-: 1.3, SiO_2: 1.7, all expressed in mg/L), at temperature of 10°C and 24 h dark. Fed with natural dehydrated organic matter (mean concentration of POM: 1 ± 0.5 mg/L) collected at the same place and reared in coarse-fine sand sediment (0.06–2 mm). Five species were selected, namely: *Bryocamptus zschokkei*, *B. minutus*, *B. echinatus*, *B. pygmaeus* and *Attheyella crassa*. At this time, mono-specific cultures were started for each species.

Acute toxicity tests were carried out with aldicarb, methidathion, α-endosulfan, copper added as nitrate pentahydrate, chromium as potassium dichromate, all of these were purchased from Riedel-de – Germany, and ammonia added as nitrate (Fluka – Germany). All chemicals were analytical reagent-grade. Experiments were performed during 96 h, using the same environmental conditions as described for the cultures, in the absence of food. Adult males and non-ovigerous females were evaluated. Copepods were exposed (two replicates each of ten animals) in 5 cm diameter polystyrene Petri dish, containing 10 mL of the appropriate test solution. Acetone was used as pesticide carrier, at a final concentration lower than 500 μL/L. Appropriate controls were designed. Every 24 h, each replicate was observed for the presence of dead animals (no movement after gentle stimulation), and test water was renewed to ensure 10 mL as final volume.

All endpoint estimates were based on nominal values. Acute toxicity data (mortality) were analyzed by Probit method whenever the data structure was suitable. If insufficient numbers of partial kills were present in a study, the data were alternatively analyzed by the Trimmed Spearman–Karber method. Validated software and programs used for statistical analyses were USEPA (1994a, b), Probit V1.5 and Trimmed Spearman–Karber V1.5. LC50's values were compared for each chemical and species by one way ANOVA in conjunction with Tukey's test using Toxstat V 3.5.

Results and Discussion

Acute toxicity data are showed in Table 1. LC50-96 h values and their 95% confidence limits are reported for all assayed species. The different acute responses among the studied species fell below one order of magnitude (Table 2). Inter-specific differences were recorded; *B. zschokkei* (stygoxene) and *B. echinatus* (stygophylous) were the most sensitive species to chromium and α-endosulfan to exposures, illustrating similar sensitivity among these different ecological categories. Acute toxicity for methidathion and copper has been evaluated only for *A. crassa*, LC50s values and ±95% confidence limits for these chemicals were: 0.064 (0.049–0.096) and 1.44 (1.13–2.22) mg/L, respectively. Burton et al (2002), using *B. zschokkei* as test species, have reported a LC50-96 h for copper of 290 μg/L and a NOEC value, measured as offspring production, of 50 μg/L, giving an acute to chronic ratio close to six. This high sensitivity to copper, in relation to *A. crassa*, could be explained due to hardness value of the dilution water, since in this case stream water having less than 1 mg/L of Ca^{+2} was used. On the other hand, for aldicarb, Notenboom et al. (1994) have reported a LC50-96 h value of 2.9 μg/L for *Parastenocaris germanica*, a stygobiont species.

As the hyporheic environment is a dynamic transition zone between surface water and true groundwater, several species, with different degree of adaptation to groundwater may be found in it. More in detail, according to the degree of adaptation to groundwater life, the copepods living in the hyporheic habitats may be classified as stygobionts, stygophilous and stygoxenes. Stygobionts are strictly linked to the groundwater environment during their entire

Table 1 Median lethal concentrations (96-h; ±95% confidence limits) for adults of five harpacticoid species exposed to chromium, aldicarb, endosulfan and ammonia. Expressed in mg/L

Chemical	*B. pygmaeus*	*B. minutus*	*B. zschokkei*	*B. echinatus*	*A. crassa*
Chromium Cr^{+6}	3.48[a] (3.11–3.91)	3.56[a] (3.17–3.99)	1.85[b] (1.71–2)	1.26[b] (1.13–1.4)	3.82[a] (3.49–4.19)
Aldicarb	2.42[c] (2.05–2.86)	2.5[c] (2.19–2.78)	2.47[c] (1.69–3.6)	2.71[c] (2.42–3.04)	3.17[c] (1.72–5.84)
α-Endosulfan	0.2[d] (0.19–0.21)	0.2[d] (0.19–0.21)	0.07[e] (0.06–0.09)	0.095[e] (0.048–0.12)	0.247[d] (0.19–0.43)
Ammonia	18.22[f] (15.37–21.61)	18.22[f] (15.37–21.61)	18.63[f] (16.63–20.87)	14.61[f] (12.76–16.73)	17.8[f] (15.89–19.83)

Different lower case letters show within-row comparisons for a chemical significant at $p < 0.05$ (ANOV–Tukey's test)

Table 2 Relative sensitivity between each pair of species, taking the higher values divided for the lower value for each assayed chemical. Bold number indicated major sensitivity, which is calculated as the quotient between the highest and the lowest LC50 values

	B. pygmaeus	*B. minutus*	*B. zschokkei*	*B. echinatus*	*A. crassa*
Chromium	2.8	2.8	1.5	**1**	3.0
Aldicarb	**1**	**1.03**	**1.02**	1.1	1.3
α-Endosulfan	2.9	2.9	**1**	1.4	3.5
Ammonia	1.2	1.2	1.3	**1**	1.2

life cycle, and frequently show adaptations to the biotic and abiotic conditions of subterranean waters; they can reach the hyporheic zone during the recharge phase of the aquifers (Galassi 2001). Stygophilous, as *B. echinatus*, can live and reproduce in subterranean habitats, as well as in some epigean marginal habitats, such as springs, edaphic habitats, near-surface sediments of running waters, and lentic water bodies; moreover, they may or may not possess incipient troglomorphic features; stygophylous may stably live in the hyporheic environment, and some of them are, for this reason, defined hyporheobionts. The stygoxene concept is defined as the accidental or occasional presence of species in subterranean waters, as *B. zschokkei*, *B. minutus*, *B. pygmaeus* and *A. crassa*. They are very frequently found in the hyporheic habitats, especially in the downwelling sectors of the stream bed. *Attheyella crassa*, even if was the most resistant species to the assayed compounds, possesses several features considered as good prerequisites to be suitable as test organism, such as: (1) wide geographical distribution, across Eurasia and circum-Mediterranean areas, (2) gonochorism, (3) widespread distribution in several freshwater habitats, with high densities during all year, (4) easy to be reared in the laboratory, (5) relatively large body size. Recently, Turesson et al. (2007) pointed out that *A. crassa* is an ecologically relevant test species for freshwater ecosystems and particularly for the cold, oligotrophic and often acidic lakes of northern Europe. Regardless of the relatively long generation time of this species, their results clearly show that sediment-associated toxicity related to development and sexual reproduction can be assessed within 2–3 weeks exposure with the developed bioassay.

As preliminary approach to evaluate potential relationship between sensitivity of *Attheyella* sp. and species of the genus *Bryocamptus*, correlation between LC50s was calculated and results showed in Fig. 1. In relation to the assayed chemicals, inter-specific and inter-generic responses seemed to be homogeneous. However, the interrelations among different freshwater copepods should be further developed, increasing the data set presently available. Anyway, it should be stressed that this taxonomic group, highly dominant in hyporheic and true groundwater habitats, has a great potential for being used as test species group in a battery of test organisms, in order to obtain a realistic evaluation of sediment toxicity assessment. As a matter of fact, traditional approaches,

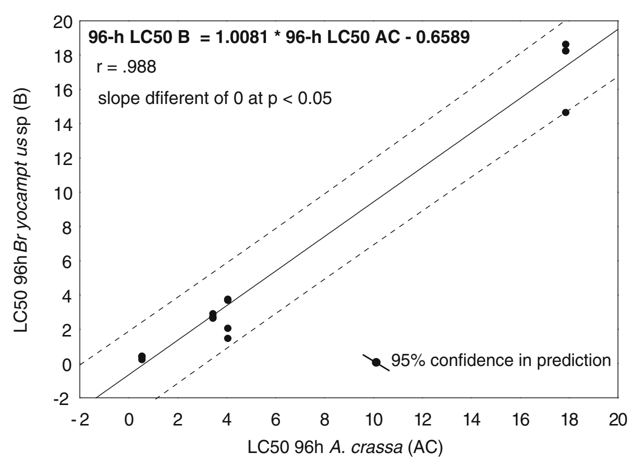

Fig. 1 96-hr LC50 values, correlation between the genus *Bryocamptus* sp (B) and *Attheyella crassa* (AC). Regression analysis significative at $p < 0.05$

Table 3 Hazardous concentration values for total ammonia and aldicarb, calculated using Wagner and Løkke (W&L) and Aldenberg and Slob (A&S) models

Number of species	8	5	3	4	3
Ammonia mg/L	C + MI[a]	C[b]	MI[c]	MI + C1[d]	C × 3[e]
HC5 (W&L)	0.04	1.14	1.78	0.008	0.63
HC5 (A&S)	0.03	1.11	1.49	0.005	0.59
Aldicarb (μg/L)	C + MI	C	MI	MI + C1	C × 3
HC5 (W&L)	0.018	0.17	0.0003	0.003	0.1
HC5 (A&S)	0.015	0.16	0.0002	0.0018	0.099

HC5: hazardous concentration 5%

[a] Copepods plus macroinvertebrates, [b] Only copepods, [c] Only macroinvertebrates, [d] Macroinvertebrates plus one copepod species, [e] Only three copepod species out of five assayed species

based on the selection of taxa not directly linked to the interstitial environments, cannot be predictive of the ecological risk for the hyporheic fauna.

An application of these findings for a sediment risk assessment with total ammonia or aldicarb was performed. Extrapolation methods proposed by OECD (1995) were used to assess hazardous concentrations (HC). These methods use variability in sensitivity among different test species in order to calculate the concentration that is expected to be low risk for most of the species in aquatic ecosystems. We used, for total ammonia, acute toxicity data for *Hyalella azteca* (200 mg/L), *Chironomus tentans* (370 mg/L) and *Lumbricus variegatus* (390 mg/L). LC50 values for aldicarb were 3.99, 23 and 28 mg/L for *Hyalella azteca, Chironomus riparius* and *Chironomus thummi*. Data were taken from US EPA ECOTOX database. A conservative acute/chronic value of ten was used for NOEC extrapolation. HC values are indicated in Table 3.

We included in Table 3 hazardous concentration 5% (HC5) estimated with a minimum number of three species, as it is used in sediment studies, although the applied models require, as minimum data set, five test species. For total ammonia, only HC5 estimation using three macroinvertebrate LC50 values failed to protect copepod species. On the contrary, all HC5 for aldicarb may be protective for both macroinvertebrates and copepods; however, HC5, estimated by using MI and MI + C1, overprotects both groups for one or two order of magnitude, regarding the final aldicarb concentration threshold.

A whole sediment toxicity assessment should consider the ecotoxicological evaluation of the hyporheic zone and/or define which organism is more suitable to define a battery of toxicity tests.

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