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The use of *Porcellio laevis* (Crustacea, Isopoda) as organism for the avoidance test in response to a metal contaminated litter

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Abstract

Terrestrial isopods have become tools for the ecotoxicological assessment of metal-contaminated litter and soil. This study aimed to determine the EC₅₀ of *Porcellio laevis* individuals after their exposure to a contaminated *Quercus* litter by CdCl₂ and ZnCl₂. The net response was also calculated. All these results showed the potential of *P. laevis* which might be suggested as bio-indicator in litter quality assessment.

1. Introduction

Metals are naturally available in the environment, but since the industrial revolution, its amount has significantly increased (Hopkin, 1989). Metal pollution could be related to various anthropogenic sources such as mining, smelting and agricultural activities (Godet *et al.*, 2011). Isopods as soil organisms and as part of terrestrial ecosystems show promise as invertebrates used as bioindicators to assess environmental quality (Nannoni *et al.*, 2014). Among them, the species *Porcellio laevis* Latreille 1804 which have shown its ability to accumulate heavy metals under both laboratory exposure (Mazzei *et al.*, 2014) and in heavily contaminated areas (Ghemari *et al.*, 2017). Among toxicity tests conducted under laboratory conditions, avoidance tests seem to be a useful complement to evaluate the toxicity of a substance and can be considered as a first evaluation (Römbke, 2003). It was first performed on earthworms *Eisenia fetida* and *Lumbricus terrestris* (Stephenson *et al.*, 1997) and then for different organisms such as collembolans, enchytraeids, and isopods that might show a behavioral response (Amorim *et al.*, 2008). The present study aimed to determine the EC₅₀ and the net response of woodlice after their exposure to heavy metals under laboratory conditions.

2. Material and Methods

P. laevis individuals were collected from Wadi Joumin in Bizerte, Northern Tunisia (37° 0' 37" N and 009° 41' 21" E). *Quercus* leaves were prepared and contaminated with two salt solutions of Cadmium chloride (CdCl₂) and Zinc chloride (ZnCl₂) according to Köhler *et al.* (1996) and were presented as food for *P. laevis* individuals. For each metal, four concentrations (C₁, C₂, C₃, and C₄) were prepared with three replicates for each concentration and with 10 individuals per replicate. A control was also prepared in which the leaves were sprayed only with bi-distilled water. An aliquot of 40 g of uncontaminated soil was added to each replicate. The avoidance test consists on maintaining *P. laevis* individuals in rectangular plastic containers, divided into two equal compartments (A and B) by a removable plastic wall. The control leaf was placed in compartment A and the same amount was placed in compartment B. Animals were kept during 48 h defined as the test period. After this period, the plastic wall was reintroduced aiming to count individuals in each compartment. Dead specimens were linked to the contamination so were counted as they belong to compartment B. Concerning animals found in the wall which separates both of the compartments, they were considered regarding their head direction (A or B). The EC₅₀ was calculated using a Probit regression conducted by *Xlstat* software. Results of the avoidance tests were represented in terms of average net response (NR) expressed as a percentage and calculated as follow $NR = ((C-T) / N) * 100$ according to Amorim *et al.* (2008).

Where C = individuals observed in the control litter; T = individuals observed in the tested litter; N = total number of individuals per replicate.

A positive (+) net response indicates avoidance and a negative net response (–) indicates a non-response (or attraction) to the metals.

3. Results and Discussion

The EC₅₀ was estimated to 71.7 (95% CL, 2.5 - 494.3 mg L⁻¹) for CdCl₂ while it was estimated to 2503.4 (95% CL, 433.4 - 2844.7 mg L⁻¹) for ZnCl₂.

Conducting avoidance test on *Enchytraeus albidus*, the EC₅₀ was estimated to 362 and 92 mg Kg⁻¹ for Cd and Zn respectively (Amorim *et al.*, 2008).

Furthermore, Loureiro *et al.* (2005), have calculated the EC₅₀ regarding different types of contaminants between two models: *Porcellionides pruinosus* and *Eisenia andrei*. For Copper, the estimated EC₅₀ was equal to 802.26 and 181.10 mg Kg⁻¹ for *P. pruinosus* and *E. andrei*, respectively. The net response was calculated and results were expressed in average values ± standard error in Table 1. The lowest CdCl₂ nominal concentration (60 mg L⁻¹) showed the highest positive net response with 53.33 ± 13.33 while by

Table 1: Results (average values ± standard error) of the net response of *P. laevis* individuals exposed to contaminated *Quercus* litter by CdCl₂ and ZnCl₂

	CdCl ₂	ZnCl ₂
C₁	53.33 ± 13.33	-53.33 ± 13.33
C₂	16.66 ± 14.52	-3.33 ± 44.84
C₃	33.33 ± 6.66	40 ± 11.54
C₄	-13.33 ± 43.71	60 ± 11.54

CdCl₂ nominal concentrations (C₁=60, C₂=80, C₃=100 and C₄=120 mg L⁻¹); ZnCl₂ nominal concentrations (C₁=800, C₂=900, C₃=1000 and C₄=1100 mg L⁻¹).

increasing concentration, the net response decreased to a negative value with -13.33 ± 43.71. The inverse was observed in ZnCl₂; a negative response was obtained at the first nominal concentration (800 mg L⁻¹), then a positive is obtained at the last one (1100 mg L⁻¹) with 60 ± 11.54. A positive (+) net response indicates avoidance while a negative net response (-) induced a non-response (or attraction) to the saline soil (Owojori & Reinecke, 2009). In accordance with the Draft Guideline for the Earthworm Avoidance Test (ISO, 2006), the habitat function of soils was considered to be limited if an average (>80%) of worms were found in the control soil as shown for *Eisenia foetida* (Amorim *et al.*, 2008). Thus, considering the two studied metals, significant differences were observed in term of distribution of *P. laevis* specimens in the two compartments of the containers. In the present study, we obtained an avoidance response only with CdCl₂ suggesting that it might depend on the type of contaminant. As reported by Lukkari & Haimi (2005), a behavioral test was conducted on three different species where they were exposed to a mixture contamination but they don't exhibit the same response. It seemed that species did not react by the same way regarding contamination and developed their characteristics like chemoreceptive (Stephenson *et al.*, 1998), physiologic, morphologic (Edwards & Bohlen, 1996), ecologic and notably behavioral characteristics (Lukkari & Haimi, 2005).

4. Conclusion and Recommendations

Through low exposure, this study has shown the effect of contaminated litter by heavy metals on *P. laevis* individuals. The determinations of EC₅₀ for CdCl₂ and ZnCl₂ will be useful to highlight this result by increasing nominal concentrations to obtain a clear gradient of dose-response.

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