

Effects of Heavy Metals Artificial Contamination on *Porcellio laevis* (Latreille, 1804) (Crustacea: Isopoda: Oniscidea)

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Abstract

This study aimed at determining the competition of cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) on their assimilation, on the food consumption and the growth of terrestrial isopod *Porcellio laevis*. Individuals were exposed to artificially contaminated litter of *Quercus* for 4 weeks and were weekly weighed. At the end of the experiment, the concentration of Cd, Pb, Zn and Cu in individuals were measured by atomic absorption spectrometry. Biological parameters such as growth, and bioaccumulation factor (BAF) were calculated and results from the various treatments were compared. Depending on metals, weight loss or gain were recorded for isopods during the four weeks of exposure. A weight loss was measured on individuals exposed to Cd-contaminated litter whereas a weight gain was highlighted for those exposed to the Zn-contaminated litter. BAF values revealed that *P. laevis* was macroconcentrator of Zn and Cu and deconcentrator of Cd and Pb.

Keywords Isopods · Microcosms · Heavy metals · Bioaccumulation

Due to the presence of contamination in the surrounding environments (*e.g.* water, soils, sediments, atmosphere), soils invertebrates are constantly exposed to various contaminants in the natural environment via soil and food (Ghannem et al. 2016; Van Gestel et al. 2018).

Among soil's invertebrates, isopods play an important role in the decomposition of litter material (Zimmer et al. 2003) and show promise as bioindicators used to assess environmental quality (Ghemari et al. 2018). Thus, they are

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characterized by their wide geographic distribution, they are easy to collect and they can be easily reared in a laboratory (Godet et al. 2011; Mazzei et al. 2013). Additionally, they have been recently described as tolerant to heavy metals and organic compounds and have been successfully used in biomonitoring programs (Agodi et al. 2015; Ghemari et al. 2018; 2019). The effects of heavy metal contamination levels on food consumption, growth (as an endpoint in toxicity tests) and metal accumulation in isopods have been well studied under laboratory conditions (Drobne and Hopkin 1994; Odendaal and Reinecke 1999; Van straalen et al. 2005; Godet et al. 2011). Porcellio laevis, a species characterized by its worldwide distribution, is well investigated in ecotoxicological approach (Ghemari et al. 2018). It is a potential species for a standard test organism as well as for monitoring heavy metals bioavailability in the soils (Odendaal and Reinecke 2003; Hussein et al. 2006; Mazzei et al. 2014; Ghemari et al. 2017). To date, in Tunisia, no study reports the effects of contaminated food on P. laevis under laboratory conditions. Thus, the aims of the current study were to evaluate the single effects of Cd, Pb, Zn and Cu contaminated litter (Quercus ilex), on (1) the growth of P. laevis individuals by weight measurements during four weeks and (2) the accumulation of these heavy metals by P. laevis individuals.

Materials and Methods

Porcellio laevis individuals were collected by hand from Wadi Joumin in northern Bizerte ($37^{\circ}0'37''N$, $09^{\circ}41'21''E$). They were transported to the laboratory in cylindrical plastic containers with soil. The containers were weekly moistened to ensure a constantly moist environment. Thus, the individuals were fed by disks of carrots and were then maintained at $20 \pm 2^{\circ}C$ and a 16:8 (Light:Dark) photoperiod until the exposure. For the experiments, we selected sexually mature adults 'males and non-gravid females' which body length varied between 10 and 15 mm.

Quercus ilex leaves were collected and dried during 24 h at 90°C at room temperature. Thus, dried leaves were weighed, remoistened, contaminated and then they were presented as food to the individuals. From CdCl₂, PbCl₂, ZnCl₂ and CuCl₂ stock solutions, different level concentrations were prepared with a concentration gradient for each metal as follow: 60, 80, 100 and 120 mg L^{-1} ; 1.5, 3,4.5 and 6 mg L^{-1} ; 800, 900, 1000 and 1100 mg L^{-1} and 50, 100, 150 and 200 mg L^{-1} in the form of CdCl₂, PbCl₂, ZnCl₂ and CuCl₂ respectively. For each metal, a stock solution of 10 mL (mg g^{-1}) was prepared and then sprayed onto the Quercus leaf disks (5 g dw) according to Köhler et al. (1996). Thereafter, they were acclimated for 3 days at room temperature at 25°C. Two replicates were prepared for each metal and each concentration with 10 individuals per replicate. Simultaneously, control was also prepared where leaves were sprayed by only bi-distilled water. An aliquot of 40 g dw of soil was added to each replicate. Individuals were placed individually in a box without food one day prior to the experiments in order to empty their gut. Once the experiments started, individuals were maintained at 20°C, with a 16:8 (Light:Dark) photoperiod during four weeks and were weekly weighed using a precision balance (RADWAG WTB).

Chemical analyses were performed according to Ghemari et al. (2018; 2019). An aliquot of 300 mg dry weight (dw) leaf litter was digested by adding 5 mL of HNO₃ (70%) within a Hot block digester for 1h15 at 95°C. After cooling, 5 mL of (H₂O₂) was added and the mixture was heated for 3 h at the same temperature. Heavy metals concentrations in isopods were performed on a mixture of 5 males for each replicate and for each concentration modality. The selected individuals were dried in an oven (Binder, Tuttlingen, Germany) at 105°C overnight until they reach a constant dry weight (dw) according to the NF ISO 11465 procedure. An aliquot of 100 mg of individuals was placed in a tube in which 5 mL of HNO₃ was added. The mixture was heated at 95°C for 1h15 using a Hot Block digester.

Leaves and isopods digests were filtered over an acetate Millipore membrane (0.45 μm porosity, Minisart) and

adjusted to 25 mL with bi-distilled water. Solutions were then stored at 4°C prior to analysis. Analytical determinations were conducted using an atomic absorption spectrometry by flame (FAAS-6800, Shimadzu) or by graphite furnace (GFA-EX7-6800, Shimadzu) depending on Cd, Pb, Zn, and Cu concentrations.

Reagent blanks were used to control quality measurements. Cd, Pb, Zn and Cu concentrations in blanks were below the detection limits. To ensure the analytical procedures, Virginia Tobacco leaves (CTA-VTL-2) and ERM_ CE278 (mussel-tissue) from the European Institute for Reference Materials and Measurements (IRMM) were used as certified materials for leaves and isopods, respectively. The heavy metals concentrations measured in these two certified samples did not differ more than 10% of the certified concentrations.

Bioaccumulation factor (BAF) defined as the ratio between heavy metal concentrations in the organism body (C org) and those in the surrounding environment soil or leaf litter (Cm) provides an estimation regarding the heavy metal accumulated by isopods (Ghemari et al. 2017, 2019). The BAF was used to classify the species as macroconcentrator (BAF>2), microconcentrator (1 < BAF < 2) or deconcentrator (BAF<1) according to Dallinger's (1993) classification.

Prior to analyses, all data were checked for normality. The statistical design was applied at the significance level $\alpha = 0.05$. To compare weight variations between the used nominal concentrations, the Kruskal–Wallis test was conducted. A Mann–Whitney test was used to compare weight variations between replicates. Thus, a comparison of weight variations over the 4 weeks of exposure was conducted using the Friedman test. The one-way ANOVA test was used to compare metal concentrations in woodlice and BAF values between the nominal concentrations. All statistical tests and analyses were conducted using XLSTAT 2012.6.04 software, used as a Microsoft Excel plug-in.

Results and Discussion

Weight variations of the individuals during the four weeks of exposure are summarized in Table 1. During the first week of exposure, a weight loss was observed for individuals exposed to Cd contaminated litter whatever the concentration (e.g. at 100 mg L^{-1} , it varied from 108.30 ± 15.52 to 105.10 ± 17.04 between the first and the second week of exposure; Table 1). Thus, a weight gain was highlighted for individuals exposed to Pb, Zn and Cu between the weeks particularly the first and the last week (e.g. at 800 mg L^{-1} ZnCl₂, it varied from 114.07 ± 27.27 to 125.66 ± 33.56 ; Table 1). In addition, the comparison of weight variation in individuals between the first and last week of exposure using Kruskal–Wallis test showed significant differences

Heavy metals	Nominal concentrations	Week 1	Week 2	Week 3	Week 4
Cd	Control (bi-distilled water)	94.69 ± 20.30	91.00 ± 20.06	90.53 ± 20.84	91.41±19.85
	60	109.13 ± 26.76	108.68 ± 26.91	101.83 ± 29.73	101.00 ± 29.28
	80	97.52 ± 26.99	95.16 ± 28.48	92.22 ± 29.53	91.83 ± 30.43
	100	108.30 ± 15.52	105.10 ± 17.04	102.00 ± 15.55	100.83 ± 17.23
	120	99.00 ± 24.05	97.76 ± 28.27	94.15 ± 25.12	91.78 ± 25.05
Pb	Control (bi-distilled water)	95.05 ± 22.73	96.77 ± 23.09	96.44 ± 21.92	99.47 ± 20.97
	1.5	100.20 ± 22.21	102.07 ± 22.32	107.20 ± 29.52	119.28 ± 31.18
	3	109.00 ± 29.73	111.00 ± 30.72	113.94 ± 30.11	118.80 ± 35.60
	4.5	102.11 ± 24.48	101.61 ± 24.30	102.55 ± 24.79	107.27 ± 23.94
	6	96.52 ± 27.43	100.15 ± 27.94	101.38 ± 28.90	105.50 ± 27.57
Zn	Control (bi-distilled water)	96.66 ± 17.87	99.20 ± 21.33	99.07 ± 20.65	98.14 ± 21.23
	800	114.07 ± 27.27	113.15 ± 26.61	108.25 ± 33.65	125.66 ± 33.56
	900	110.84 ± 25.45	111.68 ± 24.41	111.88 ± 24.65	116.06 ± 26.42
	1000	104.05 ± 25.13	106.47 ± 25.35	106.27 ± 26.60	111.33 ± 29.41
	1100	106.50 ± 35.94	101.45 ± 29.41	102.60 ± 28.75	106.44 ± 26.83
Cu	Control (bi-distilled water)	99.11 ± 21.74	98.93 ± 22.74	97.42 ± 22.28	98.57 ± 22.93
	50	87.87 ± 23.03	90.25 ± 23.15	90.46 ± 23.92	95.53 ± 24.63
	100	92.25 ± 24.89	93.05 ± 24.63	92.31 ± 21.92	97.94 ± 25.96
	150	100.30 ± 34.27	102.40 ± 36.20	104.40 ± 35.54	110.38 ± 37.42
	200	98.83 ± 23.62	99.41 ± 24.72	107.45 ± 26.61	109.66 ± 28.85

Table 1 Fresh weight variations (mean±standard deviation in mg) resulting from the exposure of *Porcellio laevis* during four weeks to Cd, Pb,Zn and Cu contaminated *Quercus* litter

n = 400 individuals

for Cd (p = 0.001), Zn (p = 0.019) and Cu (p = 0.010). In contrast, using Mann-Whitney no significant differences were revealed regarding the comparison of weight variations of individuals between replicates whatever the metal (p > 0.05). The comparison of weight variations during the four weeks of exposure using Friedman test revealed a non-significant difference for individuals exposed to Cd contamination (p > 0.05), whereas significant differences were statistically observed for those exposed to Pb (p < 0.0001), Zn and Cu (p = 0.003). Many studies conducted in-situ and ex-situ have shown negative effects of Cd exposure on the weight or size of woodlice (Jones and Hopkin 1998; Odendaal and Reinecke 1999; Witzel 2000; Godet et al. 2011). Godet et al. (2011) have reported a significant decrease in Porcellio scaber growth was shown when they were exposed to different Cd, Pb and Zn contaminated litters. Although Zn is an essential metal, a significant weight loss for P. laevis individuals was observed after exposure to spiked food with 1000 mg kg^{-1} zinc sulfate solution for 14 days (Odendaal and Reinecke 2004). The weight loss induced by metals exposure may be related to the resistance of individuals to such amount of metals, which suggest that they may expend more energy, thus affecting their growth (Donker et al. 1993).

Metal concentrations in individuals were measured at the end of the exposure and significantly increase for the four studied metals (Table 2). For instance, Cd concentration increased from 0.62 ± 0.16 to 1.63 ± 0.44 mg kg⁻¹ dw in the control treatment and the CdCl₂ nominal concentration (100 mg L^{-1}), respectively (Table 2). For Zn, it increased from 301.39 ± 25.28 to 404.88 ± 50.89 mg kg⁻¹ dw in the control treatment and the ZnCl₂ nominal concentration (1100 mg L^{-1}), respectively (Table 2). Considering metals concentrations in the leaves, they ranged from (i) 1.57 ± 0.18 to 4.22 ± 0.27 mg kg⁻¹ dw for Cd; (ii) 2.26 ± 0.03 to 8 ± 0.64 mg kg⁻¹ dw for Pb; (iii) 19.16 ± 0.91 to 24.85 ± 0.56 mg kg⁻¹ dw for Zn and (iv) 11.49 ± 1.84 to 19.18 ± 0.54 mg kg⁻¹ dw for Cu (Table 2). Metal concentrations in P. laevis individuals indicates that although the species may have the ability to distinguish and to avoid contaminated Quercus leaves, they still consume such amount of contaminated leaves presented as food during the exposure, thus explaining their great tolerance to heavy metals (Agodi et al. 2015; Ghemari et al. (2019); Thus, among the hypotheses that could be added, the avoidance behavior which is common in terrestrial isopods (Loureiro et al. 2005). Thus, Zidar et al. (2009) showed that exposure to Cd and Zn artificially contaminated food-induced low assimilation of both metals in P. scaber, whereas, at a high concentration in the food (>13) 00 Zn mg kg⁻¹ > 180 Cd mg kg⁻¹), this exposure reduced only the Cd's assimilation (and not Zn).

Table 2 Concentration of metals measured in *Porcellio laevis* and in the *Quercus* leaves (in mg kg⁻¹ dw), and bioaccumulation factor (BAF) of Cd, Pb, Zn and Cu

Nominal concen-	Metal concentration	BAF	
trations (mg L^{-1})	In Porcellio laevis In Quercus leaves		
Cd			
Control	0.62 ± 0.16	1.57 ± 0.18	0.52
60	1.48 ± 0.10	2.10 ± 0.14	0.70
80	1.40 ± 0.30	2.85 ± 0.13	0.49
100	1.63 ± 0.44	3.46 ± 0.30	0.47
120	1.32 ± 0.09	4.22 ± 0.27	0.31
Pb			
Control	1.61 ± 1.23	2.26 ± 0.03	0.71
1.5	1.39 ± 0.32	3.11 ± 0.97	0.45
3	2.37 ± 0.41	4.87 ± 0.40	0.49
4.5	2.66 ± 1.34	6.49 ± 1.37	0.41
6	3.23 ± 0.05	8.00 ± 0.64	0.40
Zn			
Control	301.39 ± 25.28	19.16 ± 0.91	15.73
800	328.31 ± 21.55	19.65 ± 1.22	16.71
900	336.92 ± 30.54	20.34 ± 0.59	16.56
1000	397.48 ± 5.48	21.54 ± 0.62	16.00
1100	404.89 ± 50.89	24.85 ± 0.56	16.26
Cu			
Control	223.37 ± 12.47	11.49 ± 1.84	19.44
50	235.78 ± 1.82	13.65 ± 0.23	17.27
100	248.49 ± 16.14	15.40 ± 0.04	16.14
150	255.17 ± 3.35	17.90 ± 0.35	14.26
200	271.12 ± 24.69	19.18 ± 0.54	14.14

n = 400 individuals

Table 2 summarized the calculated BAF values for the different studied metals. The BAF of Cd ranged from 0.31 to 0.70 whereas, for Pb, it was in the range 0.40 up to 0.71. For Zn, BAF values ranged from 15.73 to 16.71 and were between 14.14 and 19.44 for Cu (Table 2). The comparison of BAF values between the nominal concentrations revealed a highest significant difference for Cd, Zn and Cu (p < 0.001)and for Pb in a lesser extent (p = 0.006). In view of these results, P. laevis individuals may be characterized as deconcentrator of Cd and Pb (BAF value were below 1) and as macroconcentrator of Zn and Cu since BAF values were higher than 1 (from 15.7 to 19.44; Table 2). Those results are in accordance with those of Mazzei et al. (2013) which demonstrated that BAF values are less than 1 for Pb and globally in 1-12 range for Cd. The differences observed in Cd and Zn BAF values might be explained by the tolerance of P. laevis individuals to those metals and by their excretions mechanisms (Ghemari et al. 2017, 2019). According to our results, the studied metals exhibited varying enrichment levels: Cu > Zn > Cd > Pb. Those findings corroborated well with those of Ghemari et al. (2017) and Mazzei et al. (2014) where isopods species accumulated lower levels of Pb than Cd. Many authors suggested that the strategy of metals' accumulation and their excretion depends on the species (Godet et al. 2011; Mazzei et al. 2013, 2014; Ghannem et al. 2018a, b; Ghemari et al. 2019). Thus, isopods tend to absorb metals with a concentration range using their hepatopancreas as the main storage organ (Hopkin 1989). Once they reach a certain metals' concentration, a defecation phenomenon is observed through detoxification within the hepatopancreas (Hames and Hopkin 1989).

This work underling the usefulness of the microcosm bioassay approach using *P. laevis* and confirming their influence on physiological parameters such as growth and their ability to cope with heavy metals levels within the *Quercus* litter. More experiments should be conducted scrutinized aiming to its possible use as a bioindicator of heavy metals' pollution.

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