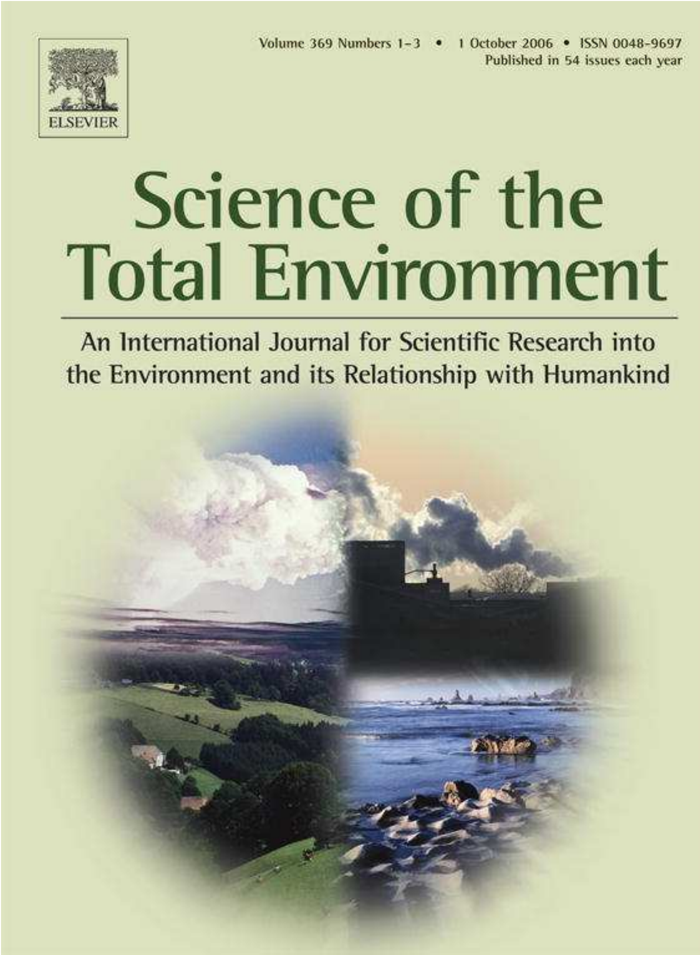
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Feeding behaviour of the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination

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Abstract

Soil decomposition is mainly dependent on the nature and characteristics of organic matter within the soil, the environmental conditions and the activity of microorganisms and soil fauna. Isopods play an important role in decomposition through litter fragmentation and stimulating and/or ingesting fungi and bacteria.

The aim of this study was to jointly evaluate the effect of different food types and the effect of heavy metal contamination of those foods through isopod feeding performance assays. These studies used the terrestrial isopod *Porcellionides pruinosus*. After feeding with different leaf types for the study on feeding performance, alder leaves were chosen for the contamination experiments. Feeding parameters like consumption, assimilation, egestion and growth ratios were calculated and compared among treatments and food type.

Lower quality food decreased isopods performance. Exotic food types were shown to be less preferred than alder or oak leaves. Contaminated food also resulted in a decrease in performance among the feeding parameters studies, although isopods can tolerate in certain cases high amounts of heavy metals. For this reason it is possible that in future this crustacean can be used as bioindicators of soil contamination or in the evaluation of contaminated sites or remediation processes.

Keywords: *Porcellionides pruinosus*; Leaf type; Heavy metals; Leaf contamination; Terrestrial isopods

1. Introduction

Decomposition within soil is a process mainly dependent on the nature and characteristics of the organic matter within the soil, the environmental conditions and the activity of microorganisms and soil fauna. Isopods play an important role in decomposition processes by the fragmentation of litter material and stimulating and/or ingesting fungi and bacteria that are very important in the cycling of nutrients. One major process is nitrogen mineralization; up to 30% of which is influenced by edaphic organisms. Isopods excrete ammonia (NH_3), thereby increasing the ammonium (NH_4) concentration in litter (Hoese, 1981), which is an important nitrogen supply

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for microbial growth. Changes in forest types can also result in changes in decomposition processes by altering the extent of fragmentation of litter material and subsequent microbial colonization. Changes in forest types can also induce changes in decomposition processes by altering decaying litter material in soil and therefore its fragmentation and microbial colonization. The adjustment in life traits like growth rate, offspring number, offspring size or the incubation period in invertebrates like isopods following modifications in their food source is related to changes in palatability, leaf toughness and nitrogen content (Rushton and Hassall, 1983; Sousa et al., 1998; Lardies et al., 2004). Another factor that can affect decomposition processes is the presence of contaminants in the terrestrial environment, which can result in changes in nitrogen availability and compartmentalization, by directly affecting detritivorous consumption (Van Wensem et al., 1997). Anthropogenic activities have increased the environmental burden of contaminants, and the consequent effects on the biosphere have become an important issue because of the potential increase of environment and human health problems. For the terrestrial ecosystem earthworms and isopods show promise as invertebrates for use as biomonitors (Hopkin et al., 1993; Cortet et al., 1999; Pokarzhevskii et al., 2000; Loureiro et al., 2002, 2005).

In terrestrial ecosystems some plants have also developed the ability to accumulate heavy metals in their tissues at high levels as a physiological adaptation. In mine sites, resident plants have displayed high levels of several trace metals like copper, lead, zinc, derived from previous mine extractions (Prasad and Freitas, 1999; Steinbörn and Breen, 1999; Cobb et al., 2000; Prasad and Freitas, 2003; Freitas et al., 2004). Also in natural environments like serpentine areas (e.g. in the north-east of Portugal) the local flora have developed some resistance to high heavy metal levels in soil (Freitas et al., 2004).

Isopods are also able to accumulate heavy metal and organic compounds from food and soil and are renowned for their ability to tolerate high contamination levels in the environment (e.g. copper), by physiological adaptations. One example is the compartmentalization of metal elements in granules in the hepatopancreas cells and their capacity of detoxification by excreting harmful compounds (Wieser and Klima, 1969; Donker et al., 1990; Van Brummelen and Van Straalen, 1996; Stroomberg et al., 1999; Sousa et al., 2000; Loureiro et al., 2002). The isopod *Porcellionides pruinosus* Brandt 1833 is a cosmopolitan and synanthropic isopod species playing an important role in the decomposition of agriculture and cattle waste material. Some previous studies with *P. pruinosus* have suggested again that these animals can be used in one of the steps of bioremediation processes, helping to promote the degradation of pesticides (Loureiro et al., 2002). They can also be used as biomonitoring organisms either in contaminated or even remediated areas (Takeda, 1980; Hoese, 1989; Warburg, 1993; Vink et al., 1995; Vink and Van Straalen, 1999). Their sensitivity to several chemicals and, on the other hand, their tolerance to metal elements might seem contradictory but both behaviours are balanced by physiological processes and by lethal and sublethal thresholds, above which animals will be affected. One good example is copper accumulation and its compartmentalization. Copper ingestion will reach a certain level where it can become toxic and, when this happens, there are two main possibilities: egestion or/and compartmentalization. These physiological processes will create an equilibrium between what can be ingested, accumulated and excreted or egested so that animals are not affected by contaminants exposure.

The aim of this study was to evaluate isopods feeding performance with different food types and the effect of heavy metal contamination via food to the terrestrial isopod *P. pruinosus*, by assessing its feeding performance.

2. Materials and methods

2.1. Test animals, leaf material and test chemicals

The specimens of *P. pruinosus* used in the feeding experiments were collected from a laboratory culture maintained for 4 years in the Department of Biology, University of Aveiro. Animals in culture were fed *ad libidum* with alder leaves (*Alnus glutinosa*) and maintained at 25°±2 °C, with a 16:8 (light: dark) photoperiod. From the lab cultures sub-adults were selected (4–15 mg) and only males and non-gravid females were used. All isopods were checked for pregnancy at the beginning of the test although there is no possibility to know if females will develop or not a future pregnancy. This is explained by the fact that females can accumulate sperm and use it to get pregnant in several occasions. In order to not include data from pregnant females in our study females that were found pregnant during the experiments were discarded. Our cultures are not synchronized but all isopods were within a weight range, so they were assumed to belong to the same age stage.

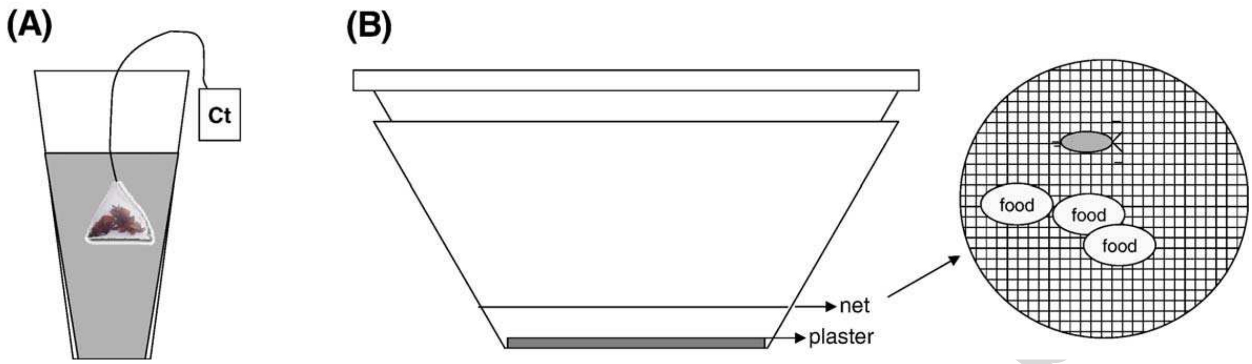

Fig. 1. Scheme for leaf contamination (A) and experimental test boxes (B).

Animals were fed with alder, oak (*Quercus robur*), and eucalyptus (*Eucalyptus globulus*) leaves and pine needles (*Pinus* sp.) to test feeding performance. The selection criteria for these species were their physico-chemical characteristics (Sousa et al., 1998) and their status of autochthonous (alder and oak) and introduced (this pine species and eucalyptus) species.

In the contaminants exposure experiments, animals were exposed to copper (II) sulphate pentahydrated (Merck), lead (II) acetate (Merck), zinc sulphate heptahydrated (Merck) and cadmium chloride (Aldrich) via food (alder leaves).

2.2. Experimental setup

All leaves were cut into disks (Ø 10 mm) with a cork borer (nickel-plated) and dried (48 h at 60 °C). Pine needles were fragmented into 1 cm pieces and also dried. All dry leaf material was weighed, remoistened with distil water and given to isopods (25–30 mg dw/replica).

For the alder leaves contamination experiment, leaf disks (25–30 mg dw) were enclosed in a net bag and submerged in the contaminated solution for 4 days at room temperature. Leaf disks were submerged to 10, 20, 40, 80 and 100 mg/L for all contaminant solutions except copper, for which the 10 mg/L solution was not used. Control leaf disks were submerged in distilled water. Extra leaf disks were also contaminated for subsequent chemical analysis. In the beginning of the experiments leaf disks were removed from the net bags, air dried and placed in the test boxes.

In all experiments two plastic boxes (Ø 80 mm; 45 mm high), one placed within the other were used as test chambers (Fig. 1). The upper box had a net bottom to allow faeces collection in the lower box, and avoid coprophagy. The lower box had a plaster bottom that was used to maintain moisture. All individuals used in the tests were placed individually in a box without food one day prior to the start of the test to allow them to empty their gut. The same procedure was followed at the end of the test period. Isopods were kept individually isolated during the duration of the experiment. Ten and five replicates were used per food type and concentration, respectively. The test system was maintained at 25±2 °C, with a 16:8 (light: dark) photoperiod regime.

Isopods were daily checked for mortality during the full duration of the tests, i.e. 28 days in the feeding performance experiment, and 14 days in the contamination experiments; every other day faeces were collected to individual eppendorff vials and plaster was remoistened. At the end of the test period animals (after emptying their gut) were weighed and leaf disks and faeces were dried for 48 h, at 60 °C, and weighed.

2.3. Chemical analysis

In the food contamination experiment, extra leaf disks were analysed to verify their contamination status/efficiency.

Leaf materials were stored at −20 °C, after each experiment; for the chemical analysis procedure extra leaf disks were acid digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), according to the USEPA 3050B protocol (USEPA, 1996). All samples were analysed for heavy metal content by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy).

2.4. Parameters calculation and statistical analysis

Isopod consumption, assimilation and egestion ratios and assimilation and growth efficiencies were calculated as follows:

$$C_R = (W_{Li} - W_{Lf}) / W_{isop}$$

$$A_R = [(W_{Li} - W_{Lf}) - F] / W_{isop}$$

$$AE = [(W_{Li} - W_{Lf}) - F] / (W_{Li} - W_{Lf}) * 100$$

$$E_R = F / W_{isop}$$

$$GE = [(W_{isop} - W_{isop\ f}) / W_{isop}] * 100$$

where, dw—dry weight; W_{Li}—initial leaf weight (mg dw); W_{Lf}—final leaf weight (mg fw); W_{isop}—initial isopod weight (mg dw); $W_{isop\ f}$—final isopod weight (mg fw); C_R—Consumption ratio (mg leaf/mg isopod); A_R—assimilation ratio (mg leaf/mg isopod); F—faeces (mg); AE—assimilation efficiency (%); GE—growth efficiency (%); E_R—Egestion ratio (mg faeces/mg isopod).

One-way analysis of variance (ANOVA) using the SigmaStat statistical package (SPSS, 1995), were used to test for statistical differences between food type/treatment. Whenever significant differences between food type/treatments were found the post-hoc multiple comparison Dunnett's method was performed (Zar, 1996). Whenever data were not normally distributed and data transformation did not correct for normality, a Kruskal–Wallis ANOVA on Ranks was performed (Zar, 1996), followed by the Tukey test, Dunnett's or Dunn's method when significant differences were found.

For the feeding inhibition tests EC_{50} values were calculated, when possible, using a sigmoidal decay function, using the software package SigmaPlot (Systat Software Inc., 2002).

Table 2
Measured concentration (mean values) of metal elements in alder leaves (*Alnus glutinosus*) soaked in solution of copper sulphate, lead acetate, zinc sulphate and cadmium chloride.— not possible to determine

Chemical compound	Solution concentration (mg/L)	Measured concentration (μg/mg leaf dw)	(SE)
Copper sulphate	Control (distil water)	0	–
	20	6.31	1.36
	40	7.80	0.63
	80	10.50	0.18
	100	13.71	0.55
Lead acetate	Control (distil water)	0	–
	10	2.69	1.21
	20	5.29	1.07
	40	5.05	0.88
	80	31.78	2.79
	100	42.07	0.85
Zinc sulphate	Control (distil water)	0.40	0.06
	10	3.81	0.17
	20	5.51	0.74
	40	7.60	0.47
	80	8.77	0.40
	100	9.43	0.83
Cadmium chloride	Control (distil water)	0	–
	10	7.93	–
	20	11.83	–
	40	16.05	–
	80	19.85	–
	100	21.61	–

3. Results

3.1. Feeding performance experiment

Isopods fed on alder and oak leaves were demonstrated to have higher consumption and assimilation ratios when compared with the other leaf material (Table 1). Pine needles and eucalyptus leaves were less preferred by the isopods, showing the lowest values for consumption, assimilation ratios and assimilation efficiency (Table 1).

There were no significant differences in isopod growth efficiency among food types although alder exposure exhibited a 10 times higher value. In some cases isopods displayed negative growth efficiencies, with a loss of weight during the 28 days of exposure. This caused a high variability in our data and therefore no statistically differences could be found.

Table 1
Feeding parameters calculated for *Porcellionides pruinosus* fed on different leaf types

Leaf type	Consumption ratio (mg/mg body fw)	Assimilation ratio (mg/mg body fw)	Assimilation efficiency (%)	Growth efficiency (%)	Egestion ratio (mg/mg body fw)
Alder	2.11^{a}	1.83^{a}	86.77^{a}	12.85	0.31
	(0.12)	(0.13)	(3.48)	(4.20)	(0.08)
Oak	$1.95^{a,b}$	1.34^{b}	68.33^{b}	1.82	0.61
	(0.15)	(0.14)	(3.64)	(1.77)	(0.07)
Pine	1.11^{b}	0.39^{c}	40.51^{c}	1.67	0.71
	(0.22)	(0.07)	(4.91)	(6.20)	(0.16)
Eucalyptus	1.12^{b}	0.49^{c}	45.34^{c}	1.73	0.63
	(0.15)	(0.06)	(5.43)	(4.40)	(0.14)

Data are expressed as mean values and SE (between brackets) and superscript letters indicate a significantly difference among parameters ($p<0.05$).

The egestion ratio was not significantly different among food type (Kruskal–Wallis One Way Analysis of Variance on Ranks, $df=3$, $H=6.721$, $P>0.05$), although isopods fed with alder leaves displayed a mean egestion ratio two times lower those provide with the other food sources.

Mortality was observed in isopods exposed to all food types especially after 14 days of exposure. Among isopods provided with alder, oak and eucalyptus, 20% mortality was observed, while among isopods provided with pine needles there was a mortality of 10%.

3.2. Contaminated food experiments

The contamination methodology for the alder leaves showed to be adequate for this kind of studies due to small variation between replicates. Measured concentrations of metals within leaf material are presented in Table 2.

The control groups for all chemical experiments were compared and there were no differences between them regarding consumption ratio (one-way ANOVA, $F_{3,16}=3.098$, $P>0.05$), assimilation ratio (one-way ANOVA, $F_{3,16}=1.983$, $P>0.05$), egestion ratio (one-way ANOVA, $F_{4,20}=2.249$, $P>0.05$), assimilation efficiency (one-way ANOVA, $F_{3,16}=1.356$, $P>0.05$) and growth efficiency (Kruskal–Wallis One Way Analysis of Variance on Ranks, $df=3$, $H=3.343$, $P>0.05$).

During the 14 days test period no mortality was observed among the control groups. Dead animals were only observed at the lead exposure experiment, with a mortality rate of 20% at 2.69 μg Pb/mg leaf dw and 5.05 μg Pb/mg leaf dw (corresponding to one organism each).

Among animals in the copper exposure there was a significant decrease in food consumption (Kruskal–Wallis One Way Analysis of Variance on Ranks, $df=4$, $H=10.501$, $P<0.05$) and egestion ratios (one-way

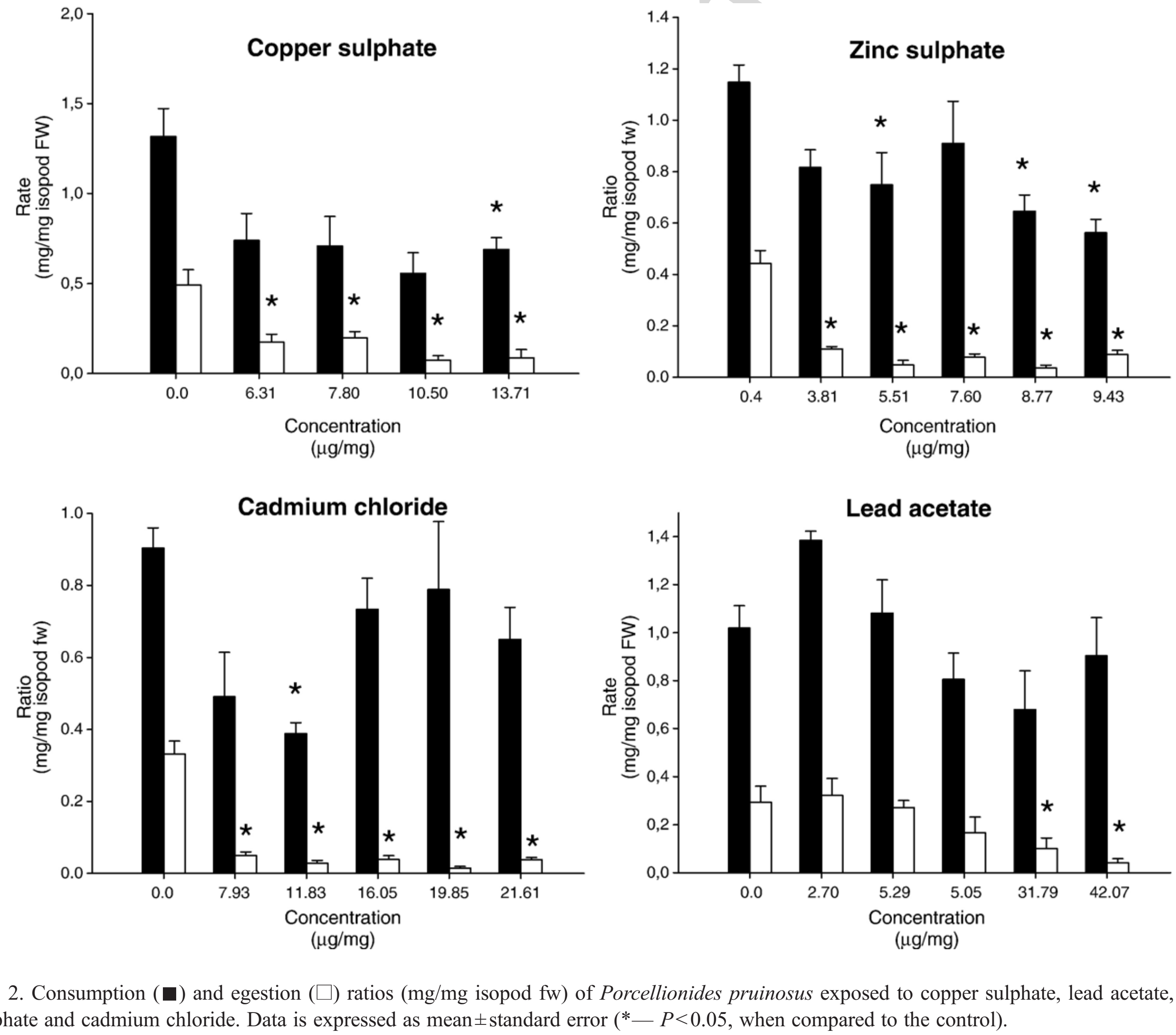

Fig. 2. Consumption (■) and egestion (□) ratios (mg/mg isopod fw) of *Porcellionides pruinosus* exposed to copper sulphate, lead acetate, zinc sulphate and cadmium chloride. Data is expressed as mean ± standard error (*— $P<0.05$, when compared to the control).

ANOVA, $F_{4,20}=12.252$, $P<0.05$) by isopods (Fig. 2). There were also differences between all treatments and the control (Dunnett's method, $P<0.05$). Isopods growth efficiency was not affected by the 14 day exposure (one-way ANOVA, $F_{4,20}=1.183$, $P>0.05$). Food assimilation efficiency increased significantly with the increase of toxicant exposure (one-way ANOVA, $F_{4,20}=2.999$, $P<0.05$) in the two highest concentrations (Fig. 3). Isopods from the control group showed assimilation efficiencies of 63.25±2.67% and 86.77±7.35% (mean±SE) at the highest concentration. For this exposure EC_{50} values were calculated for consumption and egestion ratios using a sigmoidal logistic curve (3 parameters); the values are shown in Table 3.

Among animals in the lead exposure, the consumption ratio showed a significant decrease when its concentration increased, mainly on the two highest concentrations (one way ANOVA, $F_{5,22}=3.300$, $P<0.05$). Food assimilation efficiency increased significantly in the highest concentration (one way ANOVA, $F_{5,27}=4.689$, $P<0.05$; Dunnett's Method, $P<0.05$) from 71.92±4.22% in the control to 97.18±2.10% in the 42.07 μg Pb/mg leaf dw concentration (mean±SE). Although presenting negative values in all concentrations and control, growth efficiency showed a significant decrease in the two highest concentrations (one way ANOVA, $F_{5,27}=4.296$, $P<0.05$; Dunnett's Method, $P<0.05$). Isopods egestion ratio also showed a significant decrease in the two highest concentrations (one way ANOVA, $F_{5,27}=5.208$, $P<0.05$; Dunnett's Method, $P<0.05$), being 8 times lower in the highest concentration relative to the control group. It was possible to calculate an EC_{50} value (Table 3) only for the egestion ratio, using a sigmoidal logistic curve (3 parameter).

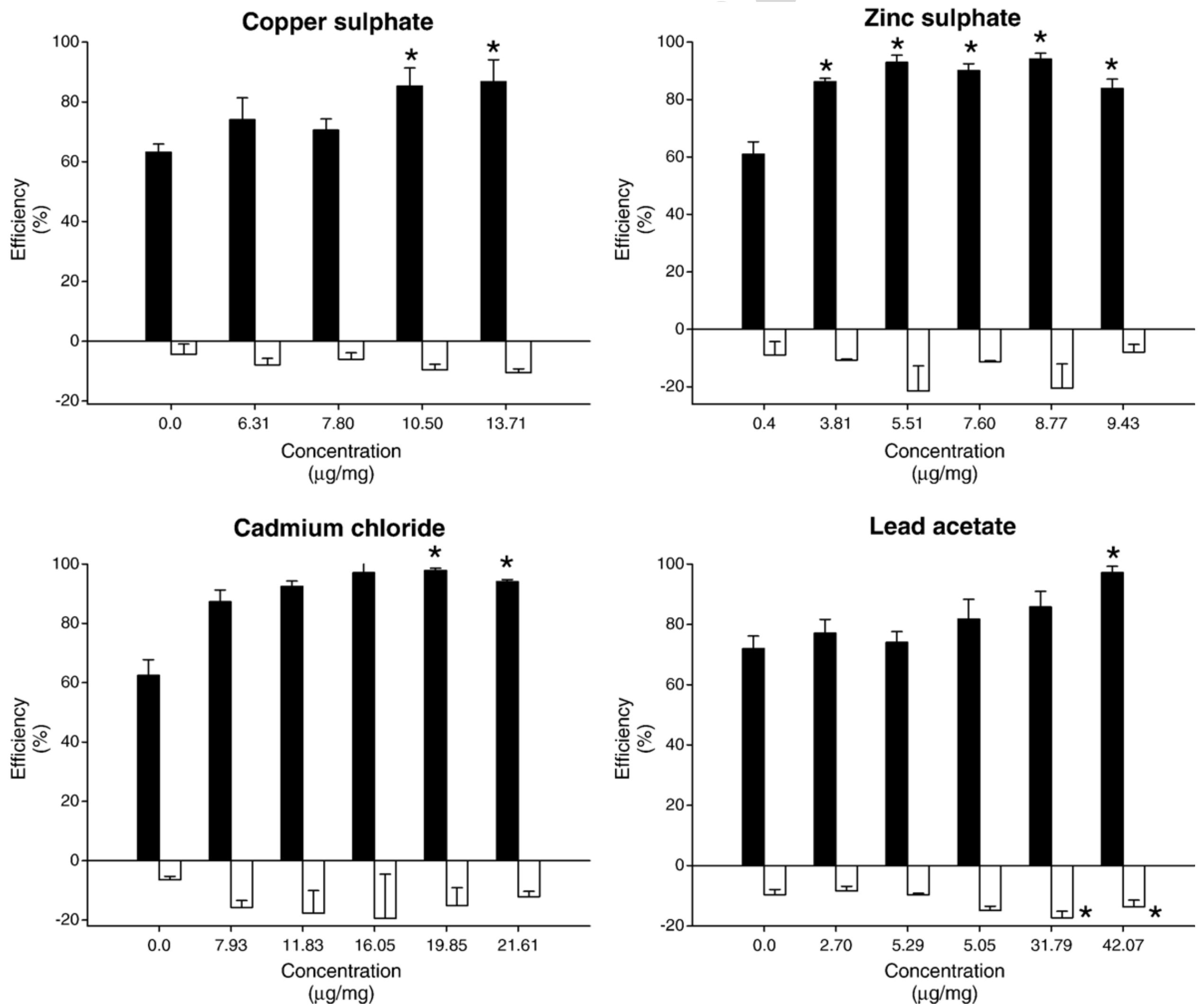

Fig. 3. Assimilation (■) and growth (□) efficiencies (%) of *Porcellionides pruinosus* exposed to copper sulphate, lead acetate, zinc sulphate and cadmium chloride. Data is expressed as mean±standard error (*— $P<0.05$, when compared to the control).

Table 3
EC_{50} values (with SE values between brackets) for feeding parameters for *Porcellionides pruinosus* exposed to copper sulphate, lead acetate, zinc sulphate and cadmium chloride

Chemical compound	Consumption ratio (µg/mg leaf dw)	r^2	Assimilation efficiency (%)	r^2	Egestion ratio (µg/mg leaf dw)	r^2
Copper sulphate	10.38 (8.67)	0.421	– –	–	4.83 (1.18)	0.633
Lead acetate	– –	–	– –	–	14.05 (7.31)	0.425
Zinc sulphate	11.10 (7.61)	0.315	3.65 –	0.701	3.52 –	0.869
Cadmium chloride	– –	–	– –	–	0.37 (1.44)	0.909

(r^2 values express the fit of data to the sigmoidal logistic function).

Among isopods in the zinc exposure there was a significant decrease in the consumption ratio (half value at 9.43 µg Zn/mg leaf dw) (one way ANOVA, $F_{5,24}=4.454$, $P<0.05$, Dunnett's method, $P<0.05$) but no differences in the assimilation ratio were obtained (one way ANOVA, $F_{5,24}=1.496$, $P>0.05$). This led to a significant increase in the assimilation efficiency, reaching 83.87±3.24% (mean±SE) in the highest concentration when compared to the control group (60.96±4.36%) (Fig. 3). Isopods showed a significant decrease in their egestion ratio in all zinc concentrations (one way ANOVA, $F_{5,24}=33.16$ $P<0.05$; Dunnett's method, $P<0.05$). In this experiment it was possible to calculate EC_{50} values for the consumption and egestion ratios and assimilation efficiency (Table 3) using a sigmoidal logistic curve, 3 parameters (consumption ratio) and 4 parameters (egestion ratio and assimilation efficiency).

Isopods exposed to cadmium chloride showed a significant decrease in their consumption ratio when exposed to 11.83 µg Cd/mg leaf dw. The assimilation efficiency of the animals exposed to 19.85 and 16.05 µg Cd/mg leaf dw increased significantly (Kruskal–Wallis One Way Analysis of Variance on Ranks, $df=5$, $H=18.009$, $P<0.05$; Dunn's method, $P<0.05$) (Fig. 3). The egestion ratio showed a significant decrease of approximately 10 times at the highest concentration (one way ANOVA, $F_{5,23}=36.73$ $P<0.05$; Dunnett's method, $P<0.05$) (Fig. 2).

4. Discussion

4.1. Feeding performance experiment

Alder leaves were the preferred food source for *P. pruinosus*. The same was observed for *Trichoniscus pusillus* fed with differently acidified leaves of alder and *Betula pendula* (Kautz et al., 2000) However, in contrast, *Porcellio dilatatus* in a study comparing palatability of leaves from several exotic and native tree species (Sousa et al., 1998), displayed higher consumption of pine compared to alder. In the latter study, isopods displayed better growth performances (in terms of biomass gained) when fed with alder than when fed with the other food types (including pine). In the *Trichoniscus pusillus* studies (Kautz et al., 2000), isopods fed with alder leaves showed a significantly higher reproductive success and a continuous reproduction throughout 3 years and also a higher survival rate.

As alder leaves were also used as food in isopods cultures for 4 years, isopods preference by this leaf type could be seen as a reflection of habituation. But, in the study of Sousa et al. (1998) isopods used in tests were also from a laboratory culture fed with alder leaves and their preference was re-directed to pine needles.

In this study high food quality resulted in higher assimilation efficiencies, in contrast to the studies of Sousa et al. (1998), but in agreement with the studies of Van Straalen and Verweij (1991). The increase of assimilation efficiency might be explained by a slower passage of leaf material through the isopods' guts, which consequently decreased faecal production. The longer the leaf material stays in the gut the higher the assimilation of food and it might be related with food characteristics, like leaf hardness and nutrients availability.

Consumption and assimilation ratios were higher for alder leaves and lower for pine and eucalyptus. This might be related to physico-chemical properties of the leaves: alder leaves have lower leaf resistance, lower concentrations of polyphenolic compounds and higher nitrogen content when compared to other leaf material (Sousa et al., 1998; Zimmer et al., 2005). These results are not in concordance with the study by Sousa et al. (1998), where a higher consumption and digestibility of pine was observed, followed by alder, acacia and eucalyptus, and finally oak as the lowest consumed item (for an 8 days exposure). In another study, consumption and digestibility of oak and eucalyptus by *Eluma caelatum* and *Porcellio dispar* were compared but no differences were observed (Zimmer et al., 2005), whereas here although consumption ratios did not show any differences, the assimilation ratio for isopods was significantly higher in oak when compared with eucalyptus and pine.

A low body mass gain or even a decrease in body mass was observed in most animals. A possible explanation for this might be the fact that while still within the stock cultures, isopods were fed *ad libitum* and might have become hyperphagous. When they were placed in test conditions that differed from cultures they probably lost weight. This was also observed by Sousa et al. (1998). Also, the low density of one animal per test box is likely to alter conspecific interaction, which may affect isopod feeding. Isopods are well known for their aggregation behaviour (Takeda, 1980; Loureiro et al., 2005) and at low densities might encounter difficulties in life-trait performances. For example, aggregation behaviour in isopods reduces water loss and oxygen consumption.

4.2. Contaminated food experiments

The contamination procedure used yielded a range of metal concentrations in leaves that were similar to those used in other studies or that can be found in the environment (Drobne and Hopkin, 1995; Prasad and Freitas, 2003; Zidar et al., 2004). As an example, in industrially polluted areas one can find leaf litter with high levels of copper up to and over 15 μg Cu/mg leaf dw (Zidar et al., 2004), and in some previous studies where isopods were exposed to zinc in maple leaves, concentrations used where similar to those used in this study, ranging from 1 to 10 μg Zn/mg leaf dw (Drobne and Hopkin, 1995). As stated above, there are also some plant species that are able to accumulate high amounts of metals, like *Thlaspi caliminare* and *Canna* sp., in which bioaccumulated concentrations of 39.6 μg Zn/mg leaf dw and 32 μg Pb/mg leaf dw, respectively, have been measured, or the case of *Arabidopsis halleris* exposed to a mixture of zinc and cadmium that accumulated 37.35 μg Cd/mg dw in roots and 5.72 μg Cd/mg dw in shoots (Küpper et al., 2000).

As shown in other studies (Drobne and Hopkin, 1995; Zidar et al., 2003, 2004) 14 days might be enough to perform feeding inhibition experiments. Increasing the duration of exposure can lead to high mortality rates because isopods rapidly attain their critical body burden for the metals. Zidar et al. (2004) reported that dead animals exposed to copper had body-copper burdens that did not exceed that of surviving isopods. Also, in a study that examined feeding performance of *P. scaber* and *Oniscus asellus*, exposed to 10 μg Zn/mg leaf dw, the isopods stopped eating after 4 days and between the 7th and the 14th day, respectively (Drobne and Hopkin, 1995).

In this study assimilation efficiencies among isopods provided with contaminated food were opposite to those observed in the feeding performance experiments. At the highest level of contamination, isopods displayed assimilation efficiencies between 83.87% and 97.18%, even though isopods from control groups displayed assimilation efficiencies between 60.95% and 71.92%. Since isopods consumed less food in highly contaminated leaves, food must have stayed longer in the gut so that more nutrients could be assimilated, with a consequent decrease in fecal production. Increased assimilation efficiency was also observed in a 5-week exposure of *P. scaber* to 10 μg Zn/mg leaf dw; assimilation efficiency in these animals reached a value of 100% (Drobne and Hopkin, 1995).

Since isopods consumed less food in highly contaminated leaves, their egestion ratio was obviously decreased. Also it could have happened that food have stayed longer in the gut so that more nutrients could be assimilated, decreasing their egestion ratio.

Although different species cope with environmental contaminants using different behavioural and physiological mechanisms, and despite the fact that there are no feeding inhibition studies on the species *P. pruinosus*, a comparison between endpoints and species sensitivity can be made. In the copper exposure *P. scaber* showed an EC_{50} value for consumption ratio of 1.34 μg Cu/mg leaf dw, almost 10 times lower than the one obtained in this study (Zidar et al., 2003). This may indicate that *P. pruinosus* is able to ingest higher amounts of copper in food, despite the relatively low EC_{50} value of 4.83 μg Cu/mg leaf dw for the egestion ratio. Also, *P. scaber* exposed to lead oxide in food showed a LOEC value of 12.80 μg Pb/mg leaf dw for adult survival, number of juveniles produced and survival of young (Beyer and Anderson, 1985), which is very similar to the one obtained in this study for the egestion ratio (Table 3). In the same study *P. scaber* exposed to zinc oxide showed LOEC values for the number of juveniles and life span of 1st generation of 1.60 μg Zn/mg leaf dw and 6.40 μg Zn/mg leaf dw, respectively, which are similar to the EC_{50} values found in this study for the assimilation efficiency and egestion ratio parameters. The EC_{50} for zinc chloride based on feeding ratio of *P. scaber* was

2.60 μg Zn/mg leaf dw (Zidar et al., 2003), which is also very similar to the values for assimilation efficiency and egestion ratio, but lower than the EC_{50} found for consumption ratio. Finally, for cadmium exposure Zidar et al. (2003) also found an EC_{50} value of 0.35 μg Cd/mg leaf dw based on the feeding ratio; very similar to the one found in our study for egestion ratio.

The egestion ratio, or faeces production, was the most sensitive parameter used in this study. Egestion is considered an ecologically relevant parameter because faecal production occurs as a direct consequence of litter fragmentation, included in the primary step in the leaf decomposition process. It is also well known that faeces acquire more fungi and bacteria than decay leaves, thereby accelerating decomposition. Egestion can be compared to other ecologically relevant parameters used in other studies such as survival and number of juveniles.

In 14 days similar results were obtained when compared to those using higher exposure periods as described above. Hence, a 14 day exposure period might be enough to observe chemical effects in isopods.

5. Conclusions

Changes in forest type can induce changes in decomposition processes which can ultimately increase the retention period of litter in soil. This will in turn slow down the nutrient cycle in soils and increase the probability of occurrence of forest fires, which has became a crucial issue in Mediterranean countries.

This study confirms that contamination in terrestrial ecosystems decreases decomposition processes by inhibiting the feeding performance of terrestrial isopods and consequently the microbial community associated to litter material.

The species *P. pruinosus* is usually found near places used by man, like cattle and agriculture pills where high amounts of xenobiotics are present. Although it has been already shown and stated that these organisms are affected by chemical products, it is also true that they can survive in places where low levels of chemicals are observed. For example, (Heikens et al., 2001) sampled several soil invertebrates from a site contaminated with Cd, Cu, Pb and Zn and analysed them for accumulated metals. Among all the invertebrates sampled (including, Lumbricidae and Coleopterans), isopods were the organisms that were able to accumulate higher levels of metal elements. Therefore they might be useful as bioindicators of metal contamination or presence in soils and leaf litter during the evaluation of contamination or even after remediation processes.

For future work, more studies on chemical bioaccumulation by isopods should be implemented and their use as bioindicators tested.

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