



Revista Brasileira de Ciência do Solo

ISSN: 0100-0683

revista@sbcs.org.br

Sociedade Brasileira de Ciência do Solo  
Brasil

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Revista Brasileira de Ciência do Solo, vol. 35, núm. 3, junio, 2011, pp. 743-750

Sociedade Brasileira de Ciência do Solo

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## Nota

# NEOTROPICAL WOODLICE (ISOPODA) COLONIZING LEAF-LITTER OF PIONEER PLANTS IN A COAL RESIDUE DISPOSAL ENVIRONMENT<sup>(1)</sup>

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## ABSTRACT

The irregular disposal of coal combustion residues has adverse impacts on terrestrial ecosystems. Pioneer plants and soil invertebrates play an important role in the recovery of these areas. The goal of this study was to investigate the colonization patterns of terrestrial isopods (Oniscidea) in leaf litter of three spontaneous pioneer plants (grass - Poaceae, shrub - Euphorbiaceae, tree - Anacardiaceae) at sites used for fly ash or boiler slag disposal. The experiment consisted of eight blocks (four per disposal site) of 12 litter bags each (four per plant species) that were randomly removed after 6, 35, 70 or 140 days of field exposure. Three isopod species were found in the litter bags: *Atlantoscia floridana* (van Name, 1940) (Philosciidae; n = 116), *Benthana taeniata* Araujo & Buckup, 1994 (Philosciidae; n = 817) and *Balloniscus sellowii* (Brandt, 1833) (Balloniscidae; n = 48). The isopods colonized the three leaf-litter species equally during the exposure period. However, the pattern of leaf-litter colonization by these species suggests a conflict of objectives between high quality food and shelter availability. The occurrence of *A. floridana* and the abundance and fecundity of *B. taeniata* were influenced by the residue type, indicating that the isopods have different degrees of tolerance to the characteristics of the studied sites. Considering that terrestrial isopods are abundant detritivores and stimulate the humus-forming processes, it is suggested that they could have an indirect influence on the soil restoration of this area.

**Index terms:** detritivores, coal residues, litter bags.

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<sup>(1)</sup> Part of the Master's Dissertation of the first author, Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul – UFRGS. Received for publication in March 2010 and approved in February 2011.

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**RESUMO:** *TATUZINHOS NEOTROPICAIS (ISOPODA) COLONIZANDO SERAPILHEIRA PIONEIRA EM UM AMBIENTE COM DEPÓSITOS DE RESÍDUOS CARBONÍFEROS*

*Depósitos irregulares de resíduos da combustão do carvão têm impactos adversos nos ecossistemas terrestres. Plantas pioneiras e invertebrados do solo têm importante papel na recuperação dessas áreas. O objetivo deste estudo foi investigar os padrões de colonização de isópodos terrestres (Oniscidea) em serapilheira de três plantas pioneiras (uma gramínea – Poacea; um arbusto – Euphorbiaceae; e uma árvore – Anacardiaceae), as quais ocorrem espontaneamente em depósitos de cinzas leves e de escória. O experimento consistiu em oito blocos (quatro por depósito,) cada um tendo 12 bolsas de serapilheira (quatro por espécie de planta), que foram removidas ao acaso em 6, 35, 70 ou 140 dias de exposição em campo. Três espécies de isópodos foram encontradas colonizando as bolsas de serapilheira: Atlantoscia floridana (van Name, 1940) (Philosciidae; n = 116), Benthana taeniata Araujo & Buckup, 1994 (Philosciidae; n = 817) e Balloniscus sellowii (Brandt, 1833) (Balloniscidae; n = 48). Os isópodos terrestres colonizaram igualmente as três espécies de serapilheira ao longo do tempo de exposição das bolsas. Entretanto, o padrão de colonização da serapilheira por esses isópodos sugere um “dilema” entre alimento de boa qualidade e disponibilidade de abrigo. A ocorrência de A. floridana e a abundância e fecundidade de B. taeniata foram influenciadas pelo tipo de resíduo, sugerindo que as espécies de isópodos apresentam diferentes graus de tolerância aos ambientes estudados. Considerando que os isópodos terrestres são detritívoros abundantes e estimulam os processos de formação do húmus, sugere-se que as espécies encontradas possam estar indiretamente influenciando a restauração do solo nessa área.*

*Termos de indexação: detritívoros, resíduos do carvão, bolsas de serapilheira.*

## INTRODUCTION

Coal has been the fastest-growing major fuel source in the world (BP, 2010). However, its extraction and combustion cause serious environmental impacts. Coal combustion in thermal power plants produces large amounts of waste that end up as backfill and landfill material, even though they could be used in the construction industry (Scheetz & Earle, 1998; Rohde et al., 2006). Coal and its combustion residues contain polycyclic aromatic hydrocarbons and trace elements (Querol et al., 1995; Teixeira et al., 1999) that can cause genotoxic effects in the surrounding environment (Silva et al., 2000).

One of the major adverse impacts of these residues in terrestrial ecosystems is the decline in plant establishment and growth (Carlson & Adriano, 1993). This decline is caused by changes in soil properties, both physical (such as compacted layers, reduced bulk density) and chemical (such as nutrient depletion, high toxicity and low pH-value) that create unfavorable conditions and reduce the soil microbial and animal activity (Tordoff et al., 2000; Gupta et al., 2002). Despite these challenges, pioneer plants with heavy metal tolerance or resistance are able to colonize such environments spontaneously (Whiting et al., 2004). Once these plants are established, a positive feedback loop may occur between the pioneer plants and the substrate (Wilson & Agnew, 1992), as their presence improves the microclimate through shading and

stabilization of coal residues and increases availability of food and shelter for microorganisms and animals by litter production (Carlson & Adriano, 1993). In this way, pioneer plants may pave the way for subsequent species in a facilitation model (Connel & Slatyer, 1977).

Soil invertebrates play a crucial role in the early succession of the restoration process in polluted or damaged ecosystems (Frouz et al., 2006, 2007). Terrestrial isopods (Isopoda, Oniscidea) are commonly found in metal-polluted environments (Jones & Hopkin, 1996, 1998; Grelle et al., 2000; Tajovský, 2001). As abundant litter detritivores in terrestrial ecosystems they participate in the leaf-litter processing (Quadros & Araujo, 2008), by breaking up the organic matter and returning nutrients to soil through their feces, which boosts microbial activity (Hassall et al., 1987). To neutralize the toxic effects of the ingested contaminated food, isopods are capable of immobilizing high levels of heavy metals in their hepatopancreas (Raessler et al., 2005). This capacity, however, may affect their life history traits by changing resource allocation strategies (Donker, 1992). These changes are related to increased mortality, slower growth rates, reduced body size and other effects on reproduction (Donker, 1992; Donker et al., 1994; Jones & Hopkin, 1996, 1998). However, there is strong evidence for the occurrence of heavy metal adaptation in natural populations of isopods (Posthuma & van Straalen, 2002).

The Southern region of Brazil is very rich in coal reserves which have been exploited, extracted and invested in energy generation in thermal power plants for decades (Pires & Querol, 2004). Consequently, this region has a number of sites exposed to long-term coal ash pollution (Teixeira et al., 1999). A recent research at one of these sites investigated the leaf decomposition of three abundant pioneer plants and the diversity of the associated soil invertebrate macrofauna and identified the terrestrial isopods as the most abundant soil macrofauna group in the leaf-litter (Podgaiski & Rodrigues, 2010). In view thereof, this study focused on this terrestrial isopod community and investigated (1) the colonization patterns of different isopod species during the leaf decomposition of three pioneer plants and (2) the isopod abundance and reproductive traits at different coal residue deposits (fly ash and boiler slag).

## MATERIALS AND METHODS

### Study area

The study was conducted in the city of São Jerônimo in the State of Rio Grande do Sul, Brazil (29° 57' 55.6" S; 51° 44' 14.9" W), in an area along a riparian forest of the Jacuí river. The regional climate is temperate (Cfa by the Köppen-Geiger climate classification; Peel et al., 2007) with hot summers and no dry season.

The study area was used for the disposal of coal combustion residues for more than 30 years, but waste disposal has recently been interrupted. Two sites are found in the area, of which one is a deposit of predominantly fly ash and the other of boiler slag (for details see Podgaiski & Rodrigues, 2010). Fly ash and boiler slag are aluminosilicate minerals, with the predominant components SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> that differ in both physical and chemical properties (Rohde et al., 2006). Fly ash is a fine-grained powder, composed of spherical glassy and hollow particles captured by air pollution control equipment in thermal power plants. Boiler slag is a specific type of bottom ash, which is a molten, vitreous and grained material, composed of angular particles with high C contents from unburnt coal in the wet ash removed from wet-bottom furnaces (ECOPA, 2010). The elemental concentrations vary according to parent coal composition and combustion technology. Rohde et al. (2006) studied leaching from fly ash and boiler slag from the thermal power plant of São Jerônimo and found Sn, Ni and Mo as the most abundant elements, and in lower proportion Cr, As, Hg, Al, Pb, Mn, V, Cd, Ba, and Zn. Boiler slag had higher concentrations of Sn, Mo, Cr, Al, Pb, and Mn than fly ash, which, in turn, had higher concentrations of Ni, Hg, Cd, and Zn. The pH was lower at the boiler-slag than the fly-ash site (Podgaiski & Rodrigues, 2010).

At the ash deposits, three species of pioneer plants are commonly found and were chosen for the experiments: *Ricinus communis* L. (Euphorbiaceae) and *Cynodon dactylon* (L.) Persoon (Poaceae), which are exotic and invasive species in Brazil and *Schinus terebinthifolius* Raddi (Anacardiaceae), a native, very abundant tree in the south of Brazil.

### Experimental design and laboratory procedures

Ninety-six nylon litter bags (30 x 20 cm), made of coarse mesh (1.0 x 0.2 cm) were filled with 20.3 ± 0.2 g of recently fallen, air-dried leaves of *C. dactylon*, *R. communis* and *S. terebinthifolius* (32 litter bags per plant species). In June 2007, the litter bags were placed on bare ground in eight blocks: four at the site of fly ash disposal and four at the site of boiler slag disposal. Each block consisted of 12 litter bags (four litter bags per plant species), at least 2 m away from each other (see details in Podgaiski & Rodrigues, 2010). There were four successive samplings, 6, 35, 70, and 140 days after exposure, when one litter bag of each plant species was randomly removed from each block.

The isopods from the litter bags were manually collected, identified at the species level and counted. The reproductive traits measured were the size of ovigerous females (cephalothorax width; Araujo & Bond-Buckup, 2004) and their fecundity (marsupial content). After the inspection of the litter bags to sort out the animals, the remaining leaf-litter in each bag was dried and weighed.

Keeping in mind that isopods prefer leaf-litter with a low C:N ratio (Zimmer, 2002), the C and N content of green leaves of each plant leaf-litter species was determined using the methods of moisture combustion/Walkley-Black (Walkley & Black, 1934) and Kjeldahl (Kjeldahl, 1883), respectively (detection limit 0.01 %). The C:N ratio was lowest for *R. communis* (8.0), followed by *C. dactylon* (26.7) and *S. terebinthifolius* (34.2) (Podgaiski & Rodrigues, 2010).

### Statistical analysis

Isopod abundance in litter bags was standardized and expressed as individuals g<sup>-1</sup> d w litter (dry weight). The values of isopod abundance (total and for each isopod species) of each plant leaf-litter species and sampling dates were compared with repeated measures analysis of variance (ANOVA) in blocks for each site. A posteriori Tukey test was performed to verify specific differences among significant treatments.

The relationship between the size of ovigerous females (mm) and fecundity was assessed between sites using analysis of covariance (ANCOVA). The size of the ovigerous females at both sites was compared with ANOVA. Residue analyses were carried out to check all models used in this study. The statistical analyses were conducted using SYSTAT 11.0 for Windows and R- 2.11.0 for Windows.

## RESULTS

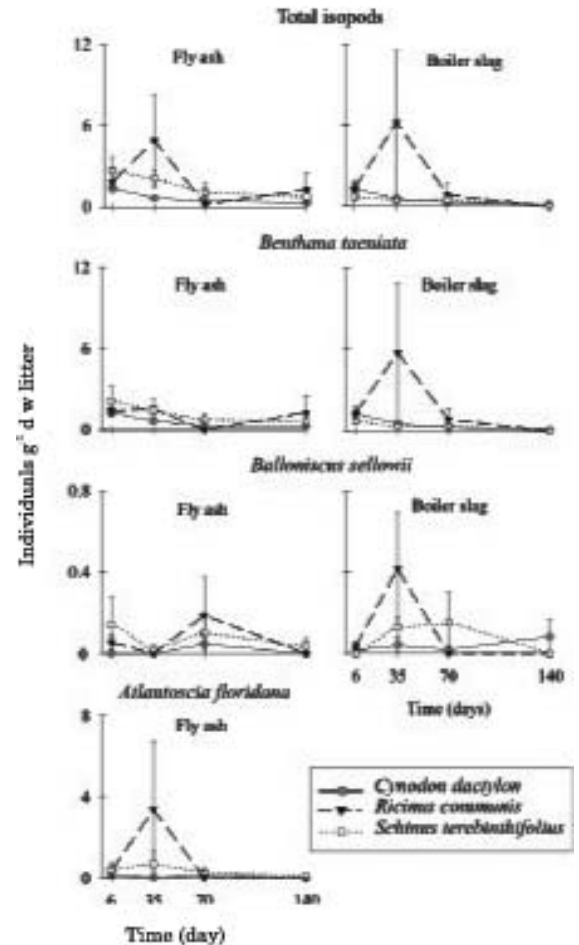
Three native neotropical isopod species were found, totaling 981 individuals (Table 1): *Benthana taeniata* Araujo & Buckup, (1994) (Philosciidae), *Atlantoscia floridana* (van Name, 1940) (Philosciidae) and *Balloniscus sellowii* (Brandt, 1833) (Balloniscidae). The species behave differently with regard to the two disposal sites. *Atlantoscia floridana* occurred exclusively at the fly ash site (116 individuals), the abundance of *B. taeniata* was much higher than of the others (817 individuals), with 63 % of individuals at the fly ash site. *Balloniscus sellowii*, on the other hand, was rarer than the others (48 individuals) and showed no difference between the two sites (Table 1).

According to results of repeated measures ANOVA, the total abundance of isopods as well as the abundance of each isopod species was not significantly different among leaf-litter species at both sites (Figure 1). There was no interaction between treatments (leaf-litter species) and time (sampling dates). However, a tendency towards a higher abundance of all isopod species in *R. communis* leaf-litter could be observed after 35 days of leaf exposure (Figure 1). The time of leaf exposure only had a significant effect on the abundance of *B. taeniata* at the fly ash disposal site ( $F_{3,18} = 3.70$ ;  $p < 0.031$ ). In this case, *B. taeniata* was more abundant after 6 than after 70 days of leaf-litter decomposition ( $p = 0.04$ ).

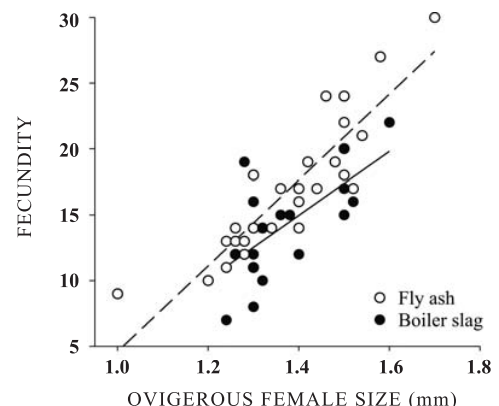
*Benthana taeniata* was the only species with a sufficient number of ovigerous females for the analysis of reproductive traits (53 individuals: 34 at the fly ash and 19 at the boiler slag site). ANCOVA indicates that fecundity in relation to female size ( $F_{1,50} = 107.36$ ;  $P < 0.001$ ) was lower in females from the boiler slag disposal site than in females from the fly ash disposal site ( $F_{1,50} = 10.79$ ;  $p = 0.002$ ; Figure 2). Nevertheless, the size of ovigerous females was not significantly different between sites ( $F_{1,51} = 0.02$ ;  $p = 0.88$ ).

**Table 1. Abundance of terrestrial isopods in bags with leaf-litter of three pioneer plant species, cyn: *Cynodon dactylon*, ric: *Ricinus communis* and sch: *Schinus terebinthifolius* at two coal ash disposal sites. N = 96 litter bags**

Site Terrestrial isopods	Fly ash disposal			Boiler slag disposal			Total
	cyn	ric	sch	cyn	ric	sch	
<i>Atlantoscia floridana</i>	12	29	75	0	0	0	116
<i>Benthana taeniata</i>	130	100	286	122	92	87	817
<i>Balloniscus sellowii</i>	2	4	18	6	4	14	48
Total	144	133	379	128	96	101	981



**Figure 1. Mean  $\pm$  standard error of abundance of terrestrial isopods in different plant species in litter bags in four samplings (after 6, 35, 70, and 140 days) at two coal ash disposal sites.**



**Figure 2. Relationship between *Benthana taeniata* fecundity (marsupial content) and body size (cephalothorax width), in females from two coal residue deposits (fly ash and boiler slag). Each point represents one ovigerous female ( $n = 53$ ;  $R^2 = 0.70$ ).**



## DISCUSSION

The terrestrial isopods found in the coal ash deposits are native to the Neotropical region. Among them, *A. floridana* is the most common and abundant species in Southern Brazil. Populations can reach up to 1040 ind m<sup>-2</sup> (Araujo & Bond-Buckup, 2005), with an average biomass of 1 kg ha<sup>-1</sup> (Quadros & Araujo, 2008). *Balloniscus sellowii* is also commonly found in southern Brazil (Araujo et al., 1996; Lopes et al., 2005). *Benthana taeniata* occurs exclusively in this region (Araujo et al., 1996) and no data on its biology and ecology are available. In São Jerônimo, the fragment of riparian vegetation that remains between the disposal sites and the adjacent river probably serves as a source for these colonizing populations. Interestingly, synantropic isopods such as *Armadillidum* spp., *Porcellio* spp. and *Porcellionides* spp., which are very abundant in urban areas in Brazil (Araujo et al., 1996), were not observed in the area of the thermal power plant and at the disposal sites.

### Isopods at coal ash disposal sites

The isopod species seems to have different levels of tolerance to the conditions at the sites created by coal ash disposal. The boiler slag site is clearly less suitable for the isopods, especially for *A. floridana*. The low pH and high amount of contaminants such as Se, Mo and Mn (Rohde et al., 2006) present in the boiler slags (Podgaiski & Rodrigues, 2010) probably contributed to the low abundances of *B. taeniata* and the absence of *A. floridana* at the boiler slag site, possibly because these characteristics increase mortality, slow down growth rates and/or stimulate isopod emigration. On the other hand, *B. sellowii* seems to be more tolerant to this habitat, since it is commonly found in other anthropic habitats, such as monocultures of exotic plants (*Eucaliptus* spp. and *Pinus* spp.) and urban parks (Araujo et al., 1996). Moreover, *B. sellowii* shows an increased fecundity (Quadros et al., 2008), which is a key reproductive trait related to a species' colonization ability (Quadros et al., 2009).

The fecundity of *B. taeniata* females was differently affected at the two sites. Previous studies evidenced changes in life history traits of woodlice inhabiting contaminated environments (Donker et al., 1994; Jones & Hopkin., 1996; van Brummelen et al., 1996) due to changes in resource allocation (Donker, 1992). The lower fecundity of *B. taeniata* females at the most polluted site may be an example of such a trade-off, but further research is needed since there are no other studies concerning the ecology and reproduction of *B. taeniata*, especially at non-polluted sites.

### Isopods in pioneer leaf-litter during decomposition

Terrestrial isopods prefer to feed from leaf litter of decayed, dicotyledonous (Rushton & Hassall, 1983;

Hassall et al., 1987) plants with low C:N ratio (Zimmer, 2002). Considering that they may be attracted to a patch that offers more palatable food (Tuck & Hassall, 2005), a higher abundance was expected in *R. communis* bags (based on its lower C:N ratio) and in leaf-litter from the earliest days of decomposition (most decayed leave). Likewise, a low abundance in *C. dactylon* bags was expected, as it is a monocotyledonous species. However, our predictions were not supported as the isopods used the three plant species at all times of leaf decomposition. Another possibility is that woodlice were attracted to the litter bags for sheltering and protection from direct light, high temperatures (Hassall & Tuck, 2007) and potential predators. For animals that use the litter both as food and as habitat, there is a trade-off between these two resources, because the decomposition process that increases the palatability of the litter (Hassall et al., 1987) at the same time saps the structural integrity that makes it suitable as shelter (Hooper et al., 2000). The nitrogen-rich leaf-litter of *R. communis* is an example of such a trade-off. It may constitute a valuable food source; however, it decomposes much faster than the others (Podgaiski & Rodrigues, 2010). In the case of *C. dactylon* and *S. terebinthifolius*, if the litter was not suitable for feeding in the beginning of the experiment, the isopods may have been attracted to the litter bags mainly for sheltering and, during the decay process and after considerable microbial degradation (Wolters, 2000), they may have fed from the litter.

The isopod populations studied here inhabit a contaminated and highly modified riparian ecosystem in the south of Brazil. Considering that these animals are abundant detritivores, they may be influencing soil restoration processes in this area by the acceleration of humus-forming processes that efficiently contribute to nutrient availability for the establishment of the plant community (Frouz et al., 2008; Quadros & Araujo, 2008). On the other hand, as bioaccumulator organisms that are predated by a wide range of animals, both invertebrates and vertebrates (Sunderland & Sutton, 1980), they are likely to take part in the process of biomagnification of heavy metals through the food chain. Although some studies have been conducted with woodlice and other invertebrates in polluted environments in Europe and other regions of the world (Grelle et al., 2000; Tajovský, 2001; Frouz et al., 2006, 2007; Majer et al., 2007), no such studies with neotropical invertebrates are available yet in Brazil (Quadros, 2010). Studies focused on plants and invertebrate detritivores, ecosystem processes (such as litter decomposition) and effects of contaminants on invertebrate biology would be very important for the understanding of the ecology of polluted environments. This knowledge would be essential to achieve goals of restoration and diversity conservation.

## CONCLUSIONS

1. Three native neotropical isopod species were found colonizing leaf-litter of spontaneous pioneer plants in the coal residue disposal environment: *Benthana taeniata*, *Atlantoscia floridana* (Philosciidae) and *Balloniscus sellowii* (Balloniscidae).

2. Woodlice colonized the three leaf-litter species *Cynodon dactylon* (grass), *Ricinus communis* (shrub) and *Schinus terebinthifolius* (tree) equally during the exposure period.

3. The woodlice species seems to present different degrees of tolerance to the environments studied (fly ash and boiler slag disposals). The abundance of *Atlantoscia floridana* and the abundance and fecundity of *Benthana taeniata* were lower in the boiler slag deposit.

## ACKNOWLEDGEMENTS

The authors are indebted to André F. B. Lima, André Castillo, André L. Casara, Tamires B. da Silva and Verônica G. Sydow for their help with the fieldwork and/or laboratory procedures; to Adriano S. Melo and Gislene Ganade for advice on statistical analysis; to Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for the scholarships awarded to Luciana R. Podgaiski and Aline F. Quadros; to the National Council of Scientific and Technological Development (CNPq) for a scholarship in research productivity of Paula B. Araujo; and to the company Companhia de Geração Térmica de Energia Elétrica (CGTEE) for access to the study site.

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