ECOTOX - BRASIL

J. Braz. Soc. Ecotoxicol., v. 7, n. 1, 2012, 49-54
doi: 10.5132/jbse.2012.01.008

JBSE

Biomonitoring of substrates containing sewage sludge: assessment of the feasibility in using the diplopod *Rhinocricus padbergi*

C.S. Fontanetti*, L.R. Nogarol, R.B. Souza, V. Bozzatto & D.G. Perez

Department of Biology, Institute of Biosciences, São Paulo State University (UNESP), Av. 24A, no1515, CEP 13506-900, Rio Claro, SP, Brazil.

(Received January 08, 2010; Accept February 22, 2011)

Abstract

Due to the constant release of potentially harmful substances in the environment, it is necessary the use of biomonitoring as a form to assess the impact of these substances on animals that inhabit this environment. Therefore, there is a better understanding of the possible effects that these exogenous substances may cause in the organisms present there. In the present study it was verified the feasibility of the use of the diplopod *Rhinocricus padbergi* as bioindicator of impacted soils by their exposition to substrates containing different concentrations of sewage sludge from different Sewage Treatment Stations (STSs). It was observed animals' behavior and the survival rate was analyzed by log-rank test (p=0,05). The analysis showed that the animals exposed to pure sewage sludge presented higher mortality index than the specimens exposed to different concentrations of the sludge mixed with soil. In general, the survival rate and the analysis of the behavior of the diplopod *R. padbergi* showed efficiency of this species in biomonitoring impacted soils.

Key words: millipede, sewage sludge, STS, toxicity.

Biomonitoramento de substratos contendo lodo de esgoto: avaliação da viabilidade do uso do diplópodo *Rhinocricus padbergi*

Resumo

Diante da constante liberação de substâncias potencialmente danosas no ambiente, faz-se necessário o uso do biomonitoramento como forma de avaliar o impacto destas substâncias sobre os animais que os habitam. Com isso, tem-se uma maior compreensão dos possíveis efeitos que estes agentes exógenos podem causar nos organismos ali presentes. No presente estudo, verificou-se a viabilidade do uso do diplópodo *Rhinocricus padbergi* como bioindicador de solos impactados, por meio de sua exposição a substratos contendo diferentes concentrações de lodo de esgoto oriundo de diferentes Estações de Tratamento de Esgoto (ETEs). Foi observado o comportamento dos animais e a taxa de sobrevivência foi analisada pelo teste log-rank (p=0,05). As análises evidenciaram que os animais expostos ao lodo de esgoto puro apresentaram maior índice de mortalidade do que os espécimens expostos a diferentes concentrações de lodo misturado com terra. De um modo geral, a taxa de sobrevivência e a análise do comportamento do diplópodo *R. padbergi* demonstraram eficiência desta espécie no biomonitoramento de solos impactados.

Palavras chave: milipede, lodo de esgoto, ETE, toxicidade.

*Corresponding author: Carmem Silvia Fontanetti, e-mail: fontanet@rc.unesp.br

INTRODUCTION

The interest to biomonitor terrestrial and aquatic ecosystems has considerably increased due to the constant release of potentially harmful substances in the environment. Despite the countless available analytical methods, collecting timely sufficient samples remains a major obstacle in the assessment of environmental damages. Furthermore, the determination of isolated substances by traditional chemical analysis does not detect the effects in the organisms and does not allow the obtainment of responses about what kind of chemical agent is being responsible for the toxicity and information about the possible interactions between the substances (additive, antagonistic or synergistic), as well as their bioavailability since only biological systems (organisms or parts of them) can detect the toxic effects of these substances (Magalhães & Ferrão Filho, 2008).

In this context, the biomonitoring appears as an alternative of ecotoxicological test, which consists in the systematic use of responses of living organisms to evaluate changes in the environment, generally caused by anthropogenic actions (Buss *et al*., 2003).

Ecotoxicological tests are carried out with indicator organisms, which, due to their characteristics of little ecological tolerance to certain chemical substances, present some alteration, be it physiological, morphological or behavioral, when exposed to certain pollutants. The expositions are made at different concentrations of the substances and chemical compounds for a certain period of time. The main advantage related to the use of these organisms is their capacity to interact with the environmental conditions throughout their lives. Therefore, the biological assessment can be used with efficiency in the detection of both intermittent acute toxic waves, in which the lethal concentration of the toxic agent is released in a single event and is rapidly absorbed, and continuous chronic release, when the agent is released in periodically repeated events, in sub-lethal concentrations over a long period of time (De Pauw & Vanhooren, 1983; Schvartsman, 1991).

Diplopods are important invertebrates in the soil fauna since they colonize several soil layers, contributing, significantly, to its aeration and humification by revolving mineral and organic particles. These invertebrates live from one to ten or more years, depending on the species (Ruppert *et al*., 2005). Due to the habits of the group they have been considered good environmental indicators being used as test organisms in some soil analysis (Hopkin *et al*., 1985; Triebskorn *et al*., 1991; Köhler & Triebskorn, 1998; Nogarol & Fontanetti, 2010; Godoy & Fontanetti, 2010).

Due to the population growth and the food crisis in the world, the intensive use of the soil, monoculture and the use of pesticides and fertilizers have become common practices to increase the agricultural production. The use of these practices has caused loss of organic material of the soil, erosion and contamination of groundwater, besides damages to the microbiota and their biochemical process. In Brazil, cities that have increased the collection and the level of sewage treatment are facing difficulties to manage and dispose adequately the sludge generated in the Sewage Treatment Stations (STSs) (Epstein, 2003). Given these problems one of the alternatives for the final disposal of the sludge is its use in agriculture (Gomes *et al*., 2007), and its use in the remediation of the physical structure of degraded soils (Kocssis & De Maria, 2004). However, its use as source of nutrients and organic matter demands some caution, especially in relation to the metal content and health, since both metals and pathogenic agents such as helminthes eggs, spores of fungi and colonies of bacteria tend to co-precipitate with sewage and concentrate in the sludge (Andreoli *et al*., 1998).

In this context, the present study aimed to verify the efficiency of millipeds in biomonitoring assays through exposition of specimens of *Rhinocricus padbergi* to substrate containing sewage sludge in order to analyze their behavior and survival.

MATERIAL AND METHODS

Adult specimens of *R. padbergi* (mean length ± SD = 6,51cm ± 0, 95) were manually collected at São Paulo State University (UNESP) campus of Rio Claro city (22º24'36''S; 47º33'36''W), SP, Brazil and brought to laboratory where they were kept in soil from the collection site. Then they were acclimated for a period of 15 days at a temperature of 21±2ºC, respecting the environmental conditions of the collection site such as photoperiod (12/12h light-dark cycle), relative humidity (65%) and temperature. This species was chosen due to its abundance in the region and to the historical of studies developed with this species (Camargo-Mathias *et al*., 1998; 2004; Fantazzini *et al*., 1998; 2002; Camargo-Mathias & Fontanetti, 2000; Fontanetti & Camargo-Mathias, 2004a; 2004b; Fontanetti *et al*., 2004; 2006).

The sewage sludge samples were originating from different STSs of São Paulo State (AT, SG and PCJ). According to the Department of Water and Electric Energy of São Paulo State (Departamento de Águas e Energia Elétrica do Estado de São Paulo - DAEE) (2004 - 2007), the Basins of Alto Tietê (AT) and of Tietê River (PCJ - Piracicaba, Capivari and Jundiaí) have, respectively, a population of 19.2 million and 4.9 million people, both basing their economy in industrial activities. Now, the STS SG is located in the region of the São Paulo side of Grande River, which is inhabited by 685 thousand people with an economy geared principally in agriculture and livestock. Thus, the sludge generated in the STSs AT and PCJ contains domestic and industrial compounds while the sludge of STS SG is predominantly domestic.

Bioassays were mounted using glass terrariums of 0,02m³ volume; in each bioassay three Kg of soil were used.

During 13 weeks, animals' behavior was observed and death occurrence counted.

This study was conducted in two stages: the sewage sludge of first stage will be treated here as (a) and of the second as (b). In the first stage it was mounted three bioassays for each one of the sewage sludge coming from the four STSs: AT-1(a), AT-2(a), SG(a) and PCJ-1(a). The first bioassay contained soil from the animal collection site (control); the second, pure sewage sludge from the respective STS; and the third, the same sludge at 10% (sludge mixed with soil, 9 parts of soil for 1 part of sludge).

In the second stage it was mounted new bioassays for the sewage sludge samples from five STSs (AT-1(b), AT-2(b), AT-3(b), SG(b) and PCJ-1(b)), in order to biomonitor the individuals at different concentrations of the sludge (1%, 10% and 50%).

In each sample, the differences between the survival curves of the control bioassay and the sewage sludge concentrations were analyzed by the log-rank test at a 5% significance level using the Prisma 3.0 program.

RESULTS

Once diplopods fulfilled part of their nourishment during the soil revolving, the behavioral analysis showed that, in general, the animals accepted the substrate being found buried in most of the time. An exception was observed for the sample of STS AT-1(a), in which it was verified that the individuals rejected the sludge.

The survival curves of the diplopods in the respective substrates are shown in figures 1, 2 and 3, and their significance in relation to the control group, according to the statistical test used, in Table 1. In the sludge samples whose tested concentrations were 1%, 10% and 50%, the survival of the diplopods was not altered by the increase in the concentration of the sludge, from 1% to 10%, but for the highest concentration, 50%. In the control and in the lowest concentrations, 1% and 10%, there was a higher survival rate (16.7% - 100%) with some variation, which seems to be related with the different concentrations: the lower the sludge concentration the higher the survival rate of the animals. Thus, only the survival in the highest exposure concentration differed significantly from the control group ($p<0.05$). Now, in the pure sewage sludge samples and in the 10% concentration, the survival of the animals was not affected by the lowest concentration, being that the survival rates ranged between 33.3% and 100%. However, the analysis of the pure sludge showed significance of the survival curves in two samples: AT-1(a) (Figure 1A) and SG (a) (Figure 2C).

DISCUSSION

The rejection of the animals to the substrate containing sludge from STS AT-1(a) may explain the fact there was a higher survival rate in the exposed group compared to the control (Figure 1A). Knowing that certain species of diplopods usually reject toxic foods (Hopkin *et al*., 1985), and thus they can die from starvation, it can be inferred that this behavior would indicate the presence of toxic substances in the sludge, which can be corroborated by the high mortality of the animals in sludge at 50% in AT-1(b) (Figure 1B) and AT-3(b) (Figure 1C).

The samples from STS PCJ-1 (PCJ-1(a) and PCJ-1(b)) seem to present low levels of toxicity (Figures 2A and 2B), since the survival of the animals exposed to sludge in all the concentrations did not differ significantly from the control group. Furthermore, among the samples analyzed in the second stage of this study, PCJ-1(b) was the only one in which there was no significance of the survival curve in the sludge at 50% (Table 1).

In a study carried out by Tsutyia (2001), it presented the variation amplitude in the composition of sewage sludge,

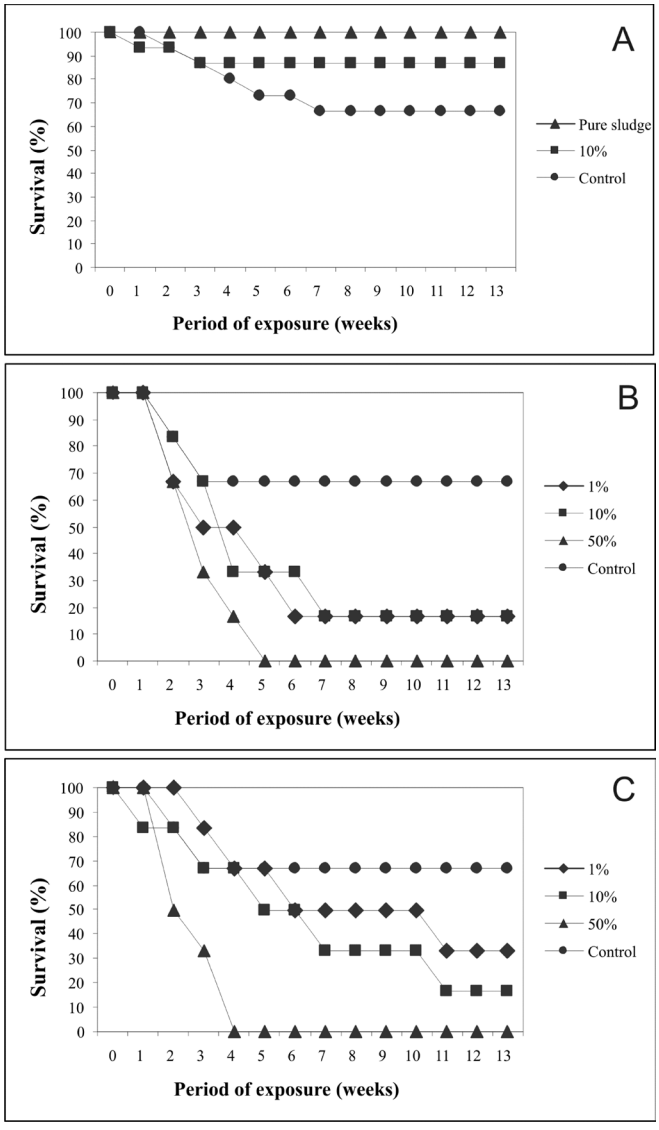

Figure 1 – Percentage of survival of the diplopod *R. padbergi* exposed to different samples and concentrations of sewage sludge for 13 weeks. STS AT-1(a) (A), STS AT-1(b) (B), STS AT-3(b) (C).

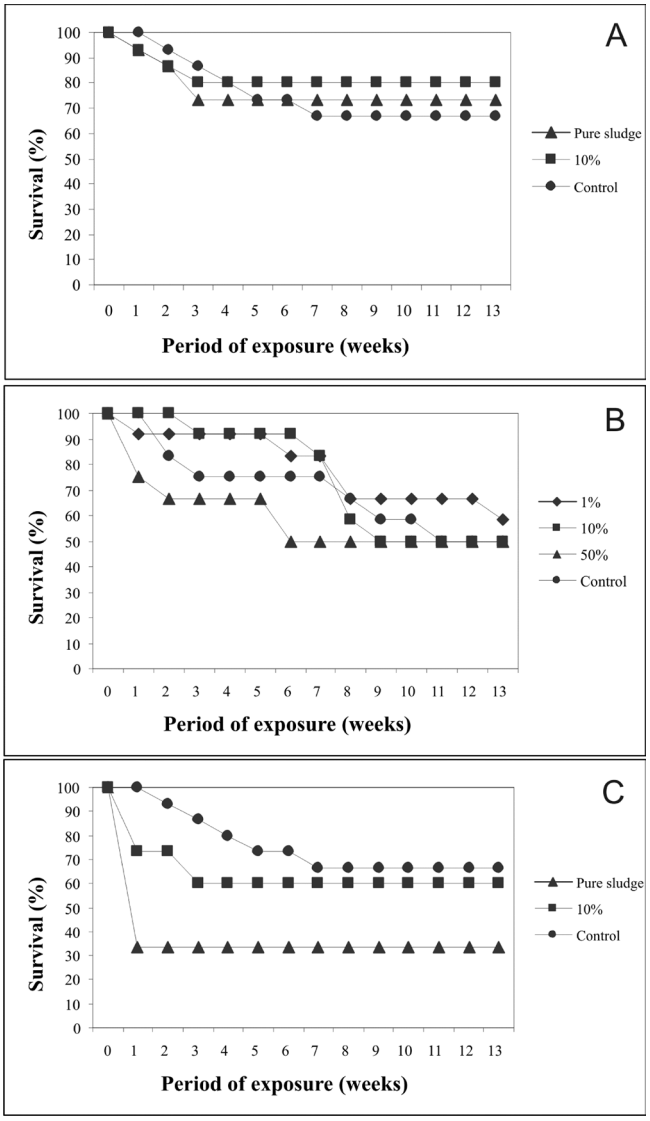

Figure 2 – Percentage of survival of the diplopod *R. padbergi* exposed to different samples and concentrations of sewage sludge for 13 weeks. STS PCJ-1(a) (A), STS PCJ-1(b) (B), STS SG(a) (C).

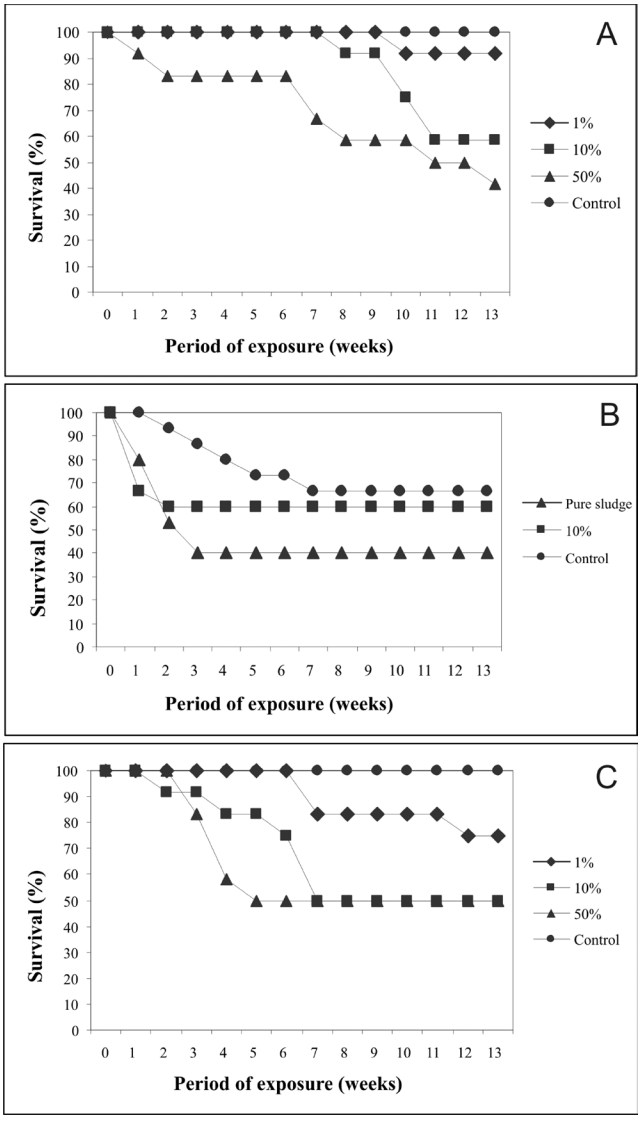

Figure 3 - Percentage of survival of the diplopod *R. padbergi* exposed to different samples and concentrations of sewage sludge for 13 weeks. STS SG(b) (A), STS AT-2(a) (B), AT-2(b) (C).

Table 1 – Significance of the survival curves in the different samples and concentrations of the sewage sludge in comparison to the control group. * = significative at 5%; ns= not significative at 5%.

Samples	Significance		
	1%	*10%*	*50%*
AT-1 (b)	ns	ns	*
AT-2 (b)	ns	*	*
AT-3 (b)	ns	ns	*
PCJ-1 (b)	ns	ns	ns
SG (b)	ns	ns	*
	10%	Pure Sludge	
AT-1 (a)	ns	*	
AT-2 (a)	ns	ns	
PCJ-1 (a)	ns	ns	
SG (a)	ns	*	

obtained from several STSs of São Paulo State. The results showed there are no great differences regarding the presence of chemical elements in the sewage sludge of the different locations; however, considering the quantitative aspects, it can be noted great variability, which can be credited to the origin of the sludge. Although physical-chemical analyses were not carried out in this study, it is known that different treatment systems and their respective stages generate sludge with variable characteristics (Andreoli *et al*., 1998). Sewage sludge, depending on the place of origin, present peculiar composition, which can reflect several particularities of the local population, such as diet habits, sanitation conditions, industrialization, among others (CETESB, 1999).

According to the origin of the residues, the sludge can be classified in domestic and industrial and the concentration of metals differ between the two types of sludge. The STS SG receives sewage affluents predominantly domestic, being the industrial contribution almost insignificant. Therefore, it

could be presumed its low toxicity due to the domestic origin. However, in the sludge samples from SG(a) and SG(b) the mortality of the specimens was high in the pure sludge and in the sludge at 50% (Figures 2C and 3A), indicating toxicity of the sludge in higher concentrations. According to Bettiol and Camargo (2006), even those sludges of exclusively domestic origin contain concentration of metals higher than those found in the soil, which could lead to problems of soil contamination.

Sewage sludge is a complex material, resulting from the sewage primary and secondary treatment processes in the STSs. The interaction between the chemical compounds present in the sludge with environmental factors (temperature, sediment, humidity, among others) creates countless combinations of substances and reactions, which may cause effects on the biota. One reason that makes the study of the toxic effects of sewage a challenge is the variation of the treatment systems of sewage and the characteristics of the receptor environment (in this case the soil). The size of the treatment station, the volume and the quantity of sludge produced by the station can strongly influence the toxicity of sewage over the fauna where it will be applied (Akaishi, 2007).

Besides pollutants such as microorganisms and metals, synthetic chemical substances from several sources may be present in the solution or may be sorbed in the solids of the sewage sludge. The occurrence of organic pollutants in the sewage sludge has grown due to the increase in the production of synthetic substances of several natures by the chemical industry. In Brazil, studies about the environmental impacts of the constituents of sewage sludge were directed to pathogenic organisms, metals and nitrates (Andrade & Mattiazzo, 2000; Boeira *et al*., 2002; Rocha *et al*., 2003; Vieira & Cardoso, 2003). Some of these substances may have caused the reduction in the survival rates of the animals exposed to sludge at different concentrations.

In relation to the samples from STS AT-2, it was verified that while AT-2(a) did not present significative differences in the survival of the animals exposed to sludge at 10% (Figure 3B), AT-2(b) had high rates of mortality in the sludge of the same concentration (Figure 3C), being that in all the samples analyzed in the present study, this was the most toxic in the concentration 10%. Although both samples are from the same STS, the difference found, probably, is related to the period of collection of the sludge.

According to USEPA (1985), the composition and the quality of the liquid effluents, mainly from Treatment Stations that receive domestic and industrial discharges, may vary along the time due to alterations in the quantity and quality of the effluents and also, due to variations in the efficiency of the treatment system; these variations are reflected in the toxicity of the discharge as a whole.

Analyzing the toxicity potential of the mentioned samples, it is observed that the higher the percentage of sludge, the sharper will drop in the survival rate, tendency observed in the sludge samples at 50%.

The data obtained in the present study suggest the presence of toxic substances to the diplopod *R. padbergi* in certain samples of sewage sludge used. It was verified that for the same STS, samples of sludge collected in different periods presented varied degrees of toxicity, probably because depending on the time that the sewage is treated, the sludge generated can present specific contaminants (USEPA, 1985).

The diplopod *R. padbergi* showed sensitive to higher sludge concentrations. Since the effects of the pollutants present in the sewage sludge in long term on the edaphic fauna are still unknown, it is important to emphasize that further studies should be carried out on the impacts of this residue in other receptor organisms. The study also indicates the need for caution in the application of sewage sludge on soil remediation, owing to the peculiarities of the sludge produced in different STSs.

Acknowledgements: This research has been supported by FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) Grant n. 08/50309-0, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FUNDUNESP (Fundação para o Desenvolvimento da UNESP). The authors thank Dr. Gisela de Aragão Umbuzeiro, Engineer Samira Issa and Marcus da Matta for providing the sewage sludge, biologist Juliana Aparecida Preto de Godoy for supporting the orientation of the study, Prof. Dr. José Silvio Govone for helping with the statistical analysis and Prof. Dr. Odair Correa Bueno for letting us use the software for statistical analysis.

REFERENCES

AKAISHI, F.M., 2007, *Avaliação dos efeitos tóxicos de esgoto doméstico tratado e não-tratado em bivalve marinho Mytilus edulis*. Tese de Doutorado, Programa em Biologia Celular e Molecular, Universidade Federal do Paraná, Curitiba, 121p.

ANDRADE, C.A. & MATTIAZZO, M.E., 2000, Nitratos e metais pesados no solo e nas árvores após aplicação de biossólidos (lodo de esgoto) em plantações florestais de *Eucalyptus grandis*. *Sci. For.,* Piracicaba, 58: 59-72.

ANDREOLI, C. V., DE LARA, A. I., FERREIRA, A.C., BONNET, B. R. P. & PEGORINI, E.S., 1998, A Gestão dos biossólidos gerados em Estações de Tratamento de Esgoto Doméstico. *Engenharia e Construção*, Curitiba, 24:18-22.

BETTIOL, W. & CAMARGO, O.A., 2006, A Disposição de lodo de Esgoto em Solo Agrícola. *In*: *Lodo de Esgoto: Impactos Ambientais na Agricultura*. Jaguariúna: Embrapa Meio Ambiente, 349p.

BOEIRA, R.C., LIGO, M.A.V. & DYNIA, J.F., 2002, Mineralização de nitrogênio em solo tropical tratado com lodos de esgoto. *Pesq. agropec. bras.,* Brasília, 37: 1639-1647. doi: 10.1590/S0100-204X2002001100016

BUSS, D. F., BAPTISTA, D. F. & NESSIMIAN, J. L., 2003, Bases conceituais para aplicação de biomonitoramento em programas de avaliação da qualidade da água de rios. *Cad. Saúde Pública*, Rio de Janeiro, 19: 465-473.

CAMARGO-MATHIAS, M. I. & FONTANETTI, C. S., 2000, Ultrastructural features of the fat body and oenocytes of *Rhinocricus padbergi* Verhoeff (Diplopoda, Spirobolida). *Biocell*, Mendoza, 24: 1-12.

CAMARGO-MATHIAS, M. I., FONTANETTI, C. S. & MICÓ-BALAGUER, E., 1998, Histochemical studies of *Rhinocricus*

padbergi Verhoeff ovaries (Diplopoda, Spirobolida, Rhinocricidae). *Cytobios*, 94:169-184.

CAMARGO-MATHIAS, M. I., FANTAZZINI, E. R. & FONTANETTI, C. S., 2004, Ultrastructural features of the midgut of *Rhinocricus padbergi* (Diplopoda: Spirobolida). *Braz. J. morphol. Sci.*, 21:65-71.

CETESB – Companhia de Tecnologia de Saneamento Ambiental. Aplicação de lodos de sistemas de tratamento biológico em áreas agrícolas – Critérios para projeto e operação (Manual Técnico – P4230), 1999, 32p.

DAEE - Departamento de Águas e Energia Elétrica do Estado de São Paulo, 2006, *Plano Estadual de Recursos Hidricos*: 2004/2007 Resumo. 92p., DAEE, São Paulo.

DE PAUW, N. & VANHOOREN, G., 1983, Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*, 100: 153-168. doi: 10.1007/BF00027428

EPSTEIN, E., 2003, *Land application of sewage sludge and biosolids*, 1ª ed., Lewis Publishers: Londres, Nova York, Washington. 220p.

FANTAZZINI, E.R., FONTANETTI, C.S. & CAMARGO-MATHIAS, M.I., 1998, Anatomy of the digestive tube and histochemistry of the foregut and salivary glands of "*Rhinocricus padbergi"*: Verhoeff, (Diplopoda: Spirobolida: Rhinocricidae). *Arthropoda Selecta,* 7:257-264.

FANTAZZINI, E.R., FONTANETTI, C.S. & CAMARGO-MATHIAS, M.I., 2002, Midgut of the millipede "*Rhinocricus padbergi"* Verhoeff, 1938 (Diplopoda: Spirobolida): histology and histochemistry. *Arthropoda Selecta*, 11: 135-142.

FONTANETTI, C. S. & CAMARGO-MATHIAS, M. I., 2004a, External morphology of the antennae of *Rhinocricus padbergi* Verhoeff, 1938 (Diplopoda: Spirobolida). *Braz. J. morphol. Sci.,* 21:73-79.

FONTANETTI, C. S. & CAMARGO-MATHIAS, M. I., 2004b, Presence of calcium in oocytes of the diplopod *Rhinocricus padbergi* Verhoeff (Spirobolida, Rhinocricidae). *Acta Histochem. Cytochem.*, 37: 301-306. doi:10.1267/ahc.37.301

FONTANETTI, C.S., CAMARGO-MATHIAS, M. I. & TIRITAN, B. M. S., 2004, The fat body in *Rhinocricus padbergi* (Diplopoda, Spirobolida). *Iheringia*, Ser. Zoologia, 94: 351-355. doi: 10.1590/S0073-47212004000400002

FONTANETTI, C. S., TIRITAN, B. & CAMARGO-MATHIAS, M. I., 2006, Mineralized bodies in the fat body of *Rhinocricus padbergi* (Diplopoda). *Braz. J. morphol. Sci.* 23: 487-493.

GODOY, J. A. P.; FONTANETTI, C.S., 2010, Diplopods as bioindicators of soils: analysis of midgut of individuals maintained in substract containing sewage sludge**.** *Water, Air and Soil Pollution*, 210: 389-398. doi: 10.1007/s11270-009-0261-z

GOMES, S. B. V., NASCIMENTO, C. W. A. & BIONDI, C. M., 2007, Produtividade e composição mineral de plantas de milho em solo adubado com lodo de esgoto. *R. Bras. Eng. Agríc. Ambiental*, 11 :459-465. doi: 10.1590/S1415-43662007000500002

HOPKIN, S. P., WATSON, K., MATIN, M. H. & MOULD, M. L., 1985, The assimilation of heavy metals by *Lithobius variegates* and *Glomeris marginata* (Chilopoda; Diplopoda). *Bijdr. Dierk.*, 55: 88-94.

KOCSSIS, M. A.; DE MARIA, I. C. O efeito do lodo de esgoto na recuperação da estrutura física de solos degradados. In: Congresso Brasileiro de Geógrafos, 2004, Goiânia. Setenta *Anos da ABG:* as transformações do espaço e a geografia no século XXI; caderno de resumos. Goiânia: Associação Brasileira de Geógrafos, 2004. v.1, p.262.

KÖHLER, H. R. & TRIEBSKORN, R., 1998, Assessment of the cytotoxic impact of heavy metals on soil invertebrates using a protocol integrating qualitative and quantitative components. *Biomarkers*, 3: 109-127. doi:10.1080/135475098231273.

MAGALHÃES, D. P. & FERRÃO FILHO, A. S., 2008, A ecotoxicologia como ferramenta no biomonitoramento de ecossistemas aquáticos. *Oecol. Bras.*, 12: 355-381.

NOGAROL, L.R.; FONTANETTI, C.S., 2010, Acute And Subchronic Exposure Of Diplopods To Substrate Containing Sewage Mud: Tissular Responses Of The Midgut. *Micron* (Oxford. 1993), 41: 239-246. doi: 10.1016/j.micron.2009.10.009

ROCHA, R.E.M., PIMENTEL, M.S., ZAGO, V.C.P., RUMJANEK, N.G. & DE-POLLI, H., 2003, Avaliação de biossólido de águas servidas domiciliares como adubo em couve. *Pesq. agropec. bras.*, Brasília, 38: 1435-1441. doi: 10.1590/S0100-204X2003001200010

RUPPERT, E. E., BARNES, R. D. & FOX, R. S., 2005, *Zoologia dos Invertebrados:* Uma Abordagem Funcional-evolutiva. Editora Roca LTDA: São Paulo, 7ª ed, 1146p.

SCHVARTSMAN, S., 1991, *Intoxicações agudas*. Sarvier: São Paulo, 4ª ed., 355p.

TRIEBSKORN, R., KÖHLER, H. R., ZANH, T., VOGT, G., LUDWING, M., RUMPF, S., KRATZMANN, M., ALBERTI, G. & STORCH, V., 1991, Invertebrate cells as targets for hazardous substances. *Z. Angew. Zool.*, 78: 277-287.

TSUTIYA, M.T., 2001, Características de biossólidos gerados em estações de tratamento de esgotos, pp. 89-131. *In:* TSUTIYA, M.T., COMPARINI, J.B., SOBRINHO, A.P., HESPANHOL, I., CARVALHO, P.C.T. & MELFI, A.J. (eds.), *Biossólidos na agricultura*. São Paulo: Sabesp.

USEPA - United States Environmental Protection Agency, 1985, *Technical support document for water quality based toxics control*. 74p., EPA, Washington D.C.

VELTMAN, K., HUIJBREGTS, M. A. J., VIJVER, M. G., PEIJNENBURG, W. J. G. M., HOBBELEN, P. H. F., KOOLHAAS, J. E., VAN GESTEL, C. A. M., VAN VLIET, P. C. J. & HENDRIKS, A. J., 2007, Metal accumulation in the earthworm *Lumbricus rubellus.* Model predictions compared to field data. *Environmental Pollution*, 146: 428-436. doi: 10.1016/j.envpol.2006.06.033

VIEIRA, R.F. & CARDOSO, A.A., 2003, Variações nos teores de nitrogênio mineral em solo suplementado com lodo de esgoto. *Pesq. agropec. bras.*, Brasília, 38: 867-874. doi: 10.1590/S0100-204X2003000700011