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Bioaccumulation of heavy metals in the bodies of workers of *Camponotus atriceps* and *Dorymyrmex brunneus* (Hymenoptera, Formicidae): an exploratory study

Bioacumulación de metales pesados en los cuerpos de obreras de *Camponotus atriceps* y *Dorymyrmex brunneus* (Hymenoptera, Formicidae): un estudio exploratorio

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Abstract: This study quantified the concentration of heavy metals (copper, zinc, cadmium, lead and nickel) in the biomass of two ant species, *Camponotus atriceps* and *Dorymyrmex brunneus*, which are dominant in forests and crop areas, respectively, in Sinop, Mato Grosso, Brazil. Ant sampling pitfall traps were used in both land use types, and metal concentrations in the biomass were obtained by means of atomic absorption spectrometry. The two species were selected because they were the most abundant in each land use type, considering the volume of biomass necessary for the chemical analysis. The results showed that with the exception of copper, heavy metals revealed higher concentrations in *D. brunneus* from crop areas than in *C. atriceps* from native forests. Such results are associated with the fact that the crop areas received high inputs of agrochemicals through the management of corn and soybean crops. Thus, ants are good indicators of contamination and environmental pollution in tropical regions, as the highest concentration of heavy metals was expected in the crop areas in relation to the adjacent forest area.

Key words: Agrochemicals. Ants. Bioindicators. Concentration. Environmental contamination.

Resumen: Este estudio cuantifica la concentración de metales pesados (cobre, zinc, cadmio, plomo y níquel) en la biomasa de dos especies de hormigas, *Camponotus atriceps* y *Dorymyrmex brunneus* de bosques y cultivos, respectivamente, localizadas en Sinop, Mato Grosso, Brasil. Para el muestreo de hormigas se utilizaron trampas en ambas áreas, y las concentraciones de metales presentes en la biomasa se obtuvieron por espectrometría de absorción atómica. Las dos especies se seleccionaron porque fueron las más abundantes en cada tipo de uso de la tierra, teniendo en cuenta el volumen requerido de biomasa necesaria para el análisis químico. Los resultados mostraron que, con la excepción del cobre, los metales pesados tuvieron más altas concentraciones en *D. brunneus* en áreas de cultivo que en *C. atriceps* procedentes de los bosques nativos. Los resultados se asocian con el hecho de que las áreas de cultivo recibieron alta entrada de insumos de agroquímicos a través del manejo de cultivos de maíz y soja. Por lo tanto, las hormigas son buenos indicadores de la contaminación y polución ambiental en las regiones tropicales y, como se esperaba, la mayor concentración de metales pesados se presentó en las zonas de cultivo en relación con la superficie forestal adyacente.

Palabras clave: Agroquímicos. Hormigas. Bioindicadores. Concentración. Contaminación ambiental.

Introduction

Agrochemicals are the main source of contamination in agricultural soils, and their distribution or degradation is related to the physicochemical properties and characteristics of the soil (Alves and Oliveira 2003; Carvalho and Pivoto 2011). The major agricultural chemicals used in agricultural fields are insecticides, herbicides, pesticides and fungicides, which contain heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn) (Gonçalves-Junior *et al.* 2000). The application of lime, sewage sludge, fertilizers and especially phosphates, among others, are examples of practices that may eventually raise the concentration of these metals in the soil (Alloway 1995; Kabata-Pendias and Pendias 2001; Mendes *et al.* 2006).

The potential risks of environmental contamination by inappropriate use of these products are dangerous for public health, through the contamination of the soil, water, air or agricultural products, and require an environmental monitoring (Guaratini *et al.* 2008). One way to monitor environmental quality is through surveys of different taxonomic groups, used as biological indicators or bio-indicators, because they provide information on the bioavailability of polluting agents, in other words, the form in which such pollutants are available in the environment and can be absorbed by a living being, as well as their contamination standard (Beeby 2001; Mcgeoch *et al.* 2002; Nicholson and Lam 2005).

Specifically, invertebrates can indicate the soil quality well (Crepaldi *et al.* 2014). Soil and leaf litter arthropods, particularly ants, are involved in important ecological processes that provide them as potential bio-indicators (Meloni 2012). Because ants have a wide geographical distribution, have a high abundance locally, act in various trophic levels, are easy to identify, use various ecological niches and can be classified into functional groups with biotic factors, they are ideal as bio-indicators (Osborn *et al.* 1999; Silva and Brandão 1999).

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Characteristics of each species analyzed must be taken into consideration, ants of the genus *Camponotus* Mayr, 1861 (Hymenoptera: Formicidae), are denominated as major structural pests (Wilson 2003). They feed on sugary and protein substances, have nocturnal habits (Fowler 1990) and are known for their opportunistic habit and high capacity for invasion (Marcolino 2000), which would make their survival in highly managed cultivated areas difficult. The representatives of *Dorymyrmex* Mayr, 1866 (Hymenoptera: Formicidae) forage in open areas, build nests in the ground, usually in arid and semi-arid regions, preferring open spaces with scarce vegetation cover (Cuezzo 2003), justifying its dominance in the cultivated areas in this study. In relation to their behavior, they have the ability to thrive in hot, dry areas and also nest in disturbed locations. Most workers are quite active and tolerate the heat better than most other species. Their diet is quite varied and they feed on pollen, nectar and vertebrate carcasses (Ward 2005).

Considering the importance of bio-indication and environmental monitoring in areas of agricultural cultivation and adjacent natural environments with intensive use of agrochemicals, this study evaluates the concentration of heavy metals (*sensu lato*) (Cu, Zn, Cd, Pb and Ni) in the bodies of workers of two species of predominant ants in crop areas (*Dorymyrmex brunneus* Forel, 1908) and adjacent forests (*Camponotus atriceps* (Smith, 1858)) in Southern Amazon, Sinop, Mato Grosso, Brazil. The aim was to evaluate the potential effects of agrochemicals on the bioaccumulation of these species.

Material and methods

Study area. The study was carried out in a private area (11°51'S 55°22'W) located in the municipality of Sinop, northern Mato Grosso, Brazil. The samples were taken in a crop area and an adjacent forest which is part of the permanent reserves of the property. The crop area has been planted several years with soybean and corn with use of agrochemicals. According to Souza *et al.* (2013) the region has two well defined seasons: rainy (October to April) and dry (May to September). The average annual rainfall is 1974.47 mm; average temperature of about 24.7 ºC. The municipality is situated in the Amazon rainforest, however, very close to El Cerrado, featuring a vegetation type known as semi-deciduous forest (Brasil 1979).

Sampling. The ants were sampled using pitfall traps (Adis 2002), in the dry period (September 2013) and rainy period (April/May 2014). The traps consisted of 500 ml plastic bottles containing 200 ml of water and drops of neutral detergent. These traps were baited with sardines in edible oil, and were placed in the field for 24 h to attract and trap the ants.

In forest and crop land three replicate sampling sites were selected with 50 m distance from each other. At each site three pitfall traps were installed, which were also in 50 m distance from each other. A total of 18 traps was installed. In the cultivated area maize was harvested before the sampling period, which could be seen by the presence of straw. Sampled material was transported to the Acervo Biológico da Amazônia Meridional (ABAM) da Universidade Federal de Mato Grosso, Campus Universitário de Sinop. Due to ant species collected in the areas being different, it was not possible to compare the data between species. The ants were screened and the most abundant species, *D. brunneus* (crop land) and *C. atriceps* (forest) were selected for chemical analysis. The ants were non countable, and only the workers were used in the study.

Chemical analysis. The replicate pitfall traps of each sampling site were combined to one sample for subsequent chemical analysis.The ants were dried at 60 °C in a drying oven for five days, weighed every 24 h, until constant weight was obtained. Subsequently, samples were grounded, separately, with the aid of a blender and melting pot for homogenization. An aliquot of 0.4 g of dry biomass of each species were weighed using a digital balance with ± 0.1 mg accuracy. This biomass was stored in the digester tube with six ml of digestion solution 3:1 nitric acid (HNO_3)/perchloric acid ($HClO_4$). The mixture was heated for 2 h at 200 °C. The digested sample was transferred to a 25 mL volumetric flask and the volume was completed with distilled water. The samples were analyzed using five replicates.

The concentrations of Cu, Cd, Ni, Zn and Pb were determined, using an Atomic Absorption Spectrometer with Air/Acetylene Flame Atomization. For calibration analytical curves were made for the metal under study, using standards prepared from Standard Solutions of the respective metals, 1,000 ppm traceable to NIST. Data were carried out in triplicates, which generated the sample standard deviation. The statistical test was not applied due to the unification of the samples, so it is not possible to test the difference between the areas for the different species. The data were analyzed with descriptive statistics.

Results and discussion

With the exception of copper, the heavy metals showed higher concentrations in the *D. brunneus* from the crop area, than in the *C. atriceps* from native forest. For *D. brunneus* a higher concentration of Zn (41.76 ± 5.72 $\mu g. g^{-1}$), followed by Cu (14.33 ± 0.42 $\mu g.g^{-1}$), Pb (3.98 ± 0.33 $\mu g.g^{-1}$), Ni (0.87 ± 0.34 $\mu g.g^{-1}$) and Cd (0.62 ± 0.00 $\mu g.g^{-1}$) were observed. In the forest *C. atriceps* biomass showed higher concentration of Cu (37.93 ± 0.22 $\mu g.g^{-1}$), followed by Zn (29.09 ± 0.76 $\mu g.g^{-1}$), Pb (3.80 ± 0.27 $\mu g.g^{-1}$), Cd (0.61 ± 0.00 $\mu g.g^{-1}$) and Ni (0.49 ± 0.27 $\mu g.g^{-1}$). The Zn concentration was higher in the crop area, while Pb, Cd and Ni, although presenting higher values in crop area, were very close to values obtained in the forest area (Fig. 1).

Changes in the structure of the natural environment with the use of agrochemicals may be associated with increasing concentrations of Cd, Zn, Pb and Ni metals, found in *D. brunneus* biomass in the cultivated area, indicating not only environmental changes but also the plasticity of the species in tolerating such conditions. Ants are good bio-indicators of environmental contamination, however our study uses only the dominant ant species obtained in these two distinct habitats (crop area and native forest), aiming evidence the differences in the management of the areas, that present distinct ant communities, which allowed only the most abundant species to be used at each site.

The effect of heavy metals depends on the dosage and chemical bond. Many metals are essential for the growth of all organisms in low concentrations and may damage biological systems in high concentrations (Virga *et al.* 2007).

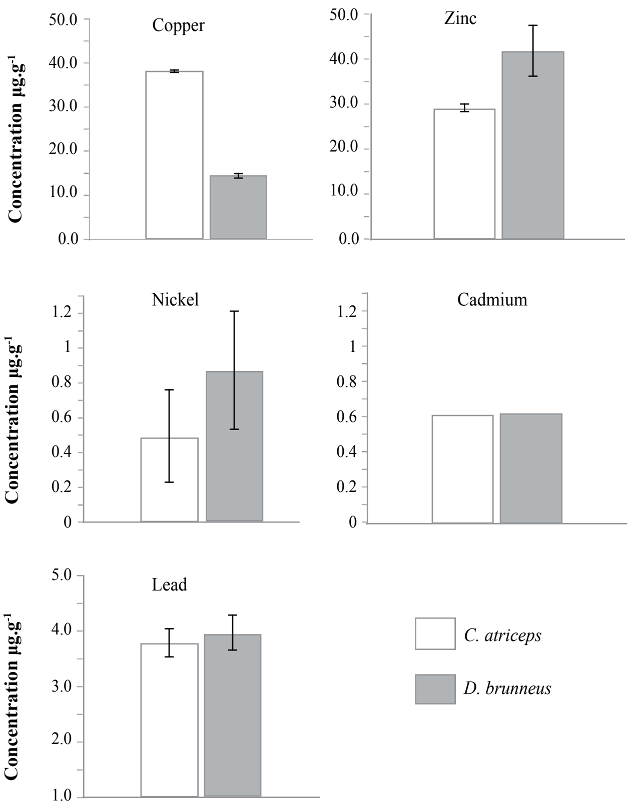

Figure 1. Metal concentrations (µg.g^{-1}) (Cu, Zn, Pb, Cd and Ni) in the *C. atriceps* biomass in forest area and *D. brunneus* in crop area in Southern Amazon, Sinop, MT, Brazil.

These metals may be absorbed by species and accumulated from water, sediment, soil or diet. The bioaccumulation is affected by the bioavailability of the substance which can be influenced by metal species and physicochemical processes. The greater the bioavailability, the greater the potential toxicity or bioaccumulation of the substance (Pedrozo 2003).

Heavy metals can usually be found in various organs and body tissues (Hopkin 1989). The internal distribution of metals in the tissues can vary between populations living in polluted areas and non-contaminated areas, as well as by the storage capacity, which may be increased in polluted environments (Hopkin 1982; Donker 1992; Martins 2000). In invertebrates the bioaccumulation of metals in tissues and the tolerance is based on the absorption, restriction to immobilization and its elimination (Grzes 2010).

Among the metals analyzed in this study, greater variations were found for Zn and Cu with respect to Cd, Ni and Pb. Some of these metals, including Cu and Zn, which are harmful to the environment in high concentrations, are necessary as micronutrients for plants (Martins *et al.* 2011). Plants need fertile soils with essential elements such as Fe, Mn, Zn, B, P, and Cu; deficiencies of one essential element and a surplus of others can affect productivity (Malavolta 1976). However, Cd even in low concentrations is not essential for plants. Therefore, its bioaccumulation in ants may indicate an environmental contamination (Floss 2006). According to Di Martos (2014) Cd occurs in phosphate rocks which are used as a source of phosphorus, an important element which limits agricultural productivity must be added by fertilizer.

According to Grześ (2010) metal concentrations in ants coming from polluted and contaminated areas are higher than in ants from non-polluted areas. But the differences between sites are species dependent. In the present study the ant species differ between both areas, therefore, it can be said that the higher concentration of heavy metals in the *D. brunneus* biomass in the crop area in relation to the *C. atriceps* in the forest area, is associated with the management and cultural care that are employed aside from fertilizers, pesticides such as insecticides, herbicides, fungicides which in most cases have metals in their formulations.

The Cu concentrations, only, exhibited higher concentration in the forest area than in the crop area. According to Nummelin *et al.* (2007), ants correspond to the weakest indicator in detecting differences in pollution by Cu. Di Martos (2014) points out that some elements such as Cu, Fe and Zn, are essential for plant development, so the occurrence of higher concentrations of Cu in the forest area can be justified by the ecological structure, as it is a conserved and unexplored area when compared to crop areas where Cu deficiency can occur due to excessive consumption of this micronutrient by the constant cultivars.

These results allow us to infer that the concentration of some metals in the *D. brunneus* biomass of apparently greater intensity in the crop area, can be explained by the fact that ants forage near their nests, having direct contact with residues of fertilizers and pesticides during their activities, unlike *C. atriceps* in the forest foraging in an area in which this contact is not direct, evidencing the different management conditions of these areas. Del Toro *et al.* (2010) analyzing the distribution of As, Cd, Cu and Pb in soils, seeds and ants correlated their concentration significantly with distance from the contaminated area. Thus, it was shown that *Pogonomyrmex rugosus* Emery, 1895 (Hymenoptera: Formicidae), accumulate heavy metals from seeds that they consume making them ideal bioindicators of contamination in above mentioned ecosystem.

Grześ (2009) in a study on tolerance to Zn pollution in *Myrmica rubra* (Linnaeus, 1758) (Hymenoptera: Formicidae) concluded that larval mortality was independent of local pollution, and among the soldier ants mortality decreased with local pollution, indicating possible tolerance to the metal Zn. Furthermore, according to the same author, the impact from metals may be relatively stronger for smaller animals compared with larger animals. Therefore, it is expected that small animals accumulate higher metal concentrations compared to larger animals.

Conclusion

Higher levels of Zn, Cd, Pb and Ni were observed in the *D. brunneus* tissue in the crop area. This species may be tolerant to contamination and can be used as a bioindicator of environmental contamination. In contrast, *C. atriceps* was dominant in the forest area with greater concentration of Cu in their tissue. Some metals, such as Cu, are considered as essential micronutrients for plants. Their higher concentration in the forest area in relation to the cultivated area is justified because of agricultural practices in which the soil may be more damaged or over-used, and the transfer of metals to the ant biomass be impaired. Our results showed that ants are good bio-indicators of environmental conditions, however, studies using the same ant species under distinct contamination conditions and in different habitats should be done, to improve protocols to use ants as environmental bio-indicators.

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