A brief report on the bioaccumulation of small terrestrial mammals: a suggestion for a new line of research in Brazil

Breve relato sobre bioacumulação em pequenos mamíferos terrestres: uma sugestão para nova linha de pesquisa no Brasil

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Abstract: Small mammals are potential elements to evaluate mining impacts because they occupy key positions in food webs and can be sampled using relatively inexpensive, easy and quick methods. They have been used as environmental indicators for various purposes, such as ecological succession gradients and pollution, among others. However, little is mentioned about bioaccumulation in South America. Our objective is to present data from a heavy metal accumulation test using the Brazilian gracile opossum *Gracilinanus microtarsus* (J. A. Wagner, 1842) as an experimental model. We concluded that the contents of heavy metals found in the animals' tissues showed differences when compared to other individuals of the same species from an area without the influence of heavy metals. As a result, we encourage the scientific community to carry out more studies in this little mentioned line in the literature in South America and incipient in Brazil.

Keywords: Bioaccumulation of heavy metals. Didelphimorphia. Ecology. *Gracilinanus microtarsus*.

Resumo: Pequenos mamíferos são elementos potenciais para avaliar os impactos da mineração, porque ocupam posições-chave nas redes alimentares e podem ser amostrados usando métodos relativamente baratos, fáceis e rápidos. Eles têm sido utilizados como indicadores ambientais para diversos fins, como gradientes de sucessão ecológica, poluição, entre outros. No entanto, pouco é mencionado sobre a bioacumulação na América do Sul. Nosso objetivo é apresentar os resultados de um teste realizado com um pequeno marsupial da espécie *Gracilinanus microtarsus* (J. A. Wagner, 1842), no qual foi concluído que o conteúdo de metais pesados encontrados nos tecidos do animal apresentou diferenças quando comparado a outro indivíduo da mesma espécie proveniente de uma área sem influência de metais pesados. Com esses resultados, procuramos incentivar a comunidade científica a realizar mais pesquisas nessa linha, pouco mencionada na literatura da América do Sul e incipiente para o Brasil.

Palavras-chave: Bioacumulação de metais pesados. Didelphimorphia. Ecologia. *Gracilinanus microtarsus*.

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INTRODUCTION

Brazil has suffered from an increasing impact of mining activities in recent years, such as the collapse of dams in Minas Gerais state, thereby affecting important watersheds of the Southeastern and Northeastern regions of Brazil, such as the Doce River and São Francisco River basins (Cionek *et al.*, 2019; Thompson *et al*., 2020). In addition to rupturing dams, mining activities can release contaminants through the acidic drainage of wastes, erosion, and surface runoffs (Fan *et al*., 2014; Vosoogh *et al*., 2016).

Small mammals are potential elements to evaluate the mining impacts because they occupy key positions in food webs (Kaufman *et al.*, 1998) and can be sampled using relatively inexpensive, easy and quick methods (Graipel *et al.*, 2003; Astúa *et al.*, 2006; Hice & Velazco, 2013). These animals have been used as environmental indicators (Machado *et al.*, 2013) for a wide range of purposes, such as changes in agriculture (Fischer *et al.*, 2011), ecological succession (Briani *et al.*, 2004), and altitude gradients (Moreira, J. *et al.*, 2009), and seed dispersal (Horn *et al.*, 2008; Andreazzi *et al.*, 2009). Rodents and marsupials are the most diverse terrestrial group of mammals in numbers of species and sub-species (Costa *et al.*, 2005; Paglia *et al.*, 2012). Small mammals act in a fundamental way in the trophic chain, contributing to the control and dispersion of plant species, serving as prey for larger predators, and controlling smaller species (invertebrates, other mammals, reptiles and birds) (Redford & Eisenberg, 1992).

We can also take measurements aiming to understand bioaccumulation processes from these mammals, since most species of these groups are omnivores and habitat generalists, therefore they are able to acquire different doses of chemical elements (metals or not) from various food sources (Hunter *et al.*, 1989). Heavy metals can enter food chains by plants and soil fauna, and in heavy metal accumulation areas, small mammals can present a high concentration of heavy metals in their kidney and bone tissues, and is usually correlated with its diet (Ma, 1989), mainly composed small invertebrates (Santori *et al.*, 1997, 2012; Finotti *et al.*, 2012). Many herbivores are less affected than carnivores/omnivores as the heavy metals are absorbed in derisory amounts by them (Ma *et al.*, 1983). However, earthworms and other soil invertebrates present high accumulation potential when they reside in heavy metal accumulation sites (Ma *et al.*, 1983).

The mining impacts in Brazil are disastrous and the contamination means in environments must be evaluated, including bioaccumulation in animals. Thus, herein we present data from a heavy metal accumulation test using the Brazilian gracile opossum *Gracilinanus microtarsus* (J. A. Wagner, 1842) as an experimental model. As a result, we encourage the scientific community to perform more studies on little mentioned line of research in the literature and incipient in Brazil.

MATERIAL AND METHODS

We performed an experimental test with two individuals of the *Gracilinanus microtarsus* species to analyze the bioaccumulation of heavy metals, one collected in a mining pit and the other in a natural environment. The first one (CMUFLA 2474) was captured in a mining area with high cadmium (Cd) and lead (Pb) contents, located in Vazante municipality (17° 55’ 23” S, 46° 48’ 56” W – 650 m), Minas Gerais state, Brazil (Figure 1).

In order to verify if the marsupial collected in the mining area had higher concentrations of cadmium and lead than other marsupials of the same species which were not exposed to these heavy metals, quantitative analyzes of cadmium and lead were carried out from tissues of both the animal test and from other individuals (frozen) from the Federal University of Lavras (UFLA) Mammalian Collection (CMUFLA), herein referred to as control samples. The animal control was collected in Itutinga (21° 17’ 53” S and 44° 39’ 28” W – 969 m), Minas Gerais state. We used sherman traps (250 x 80 x 90 mm) arranged in linear transects with baits composed of a mixture of peanut butter, cod liver oil, sardines, cornmeal, banana

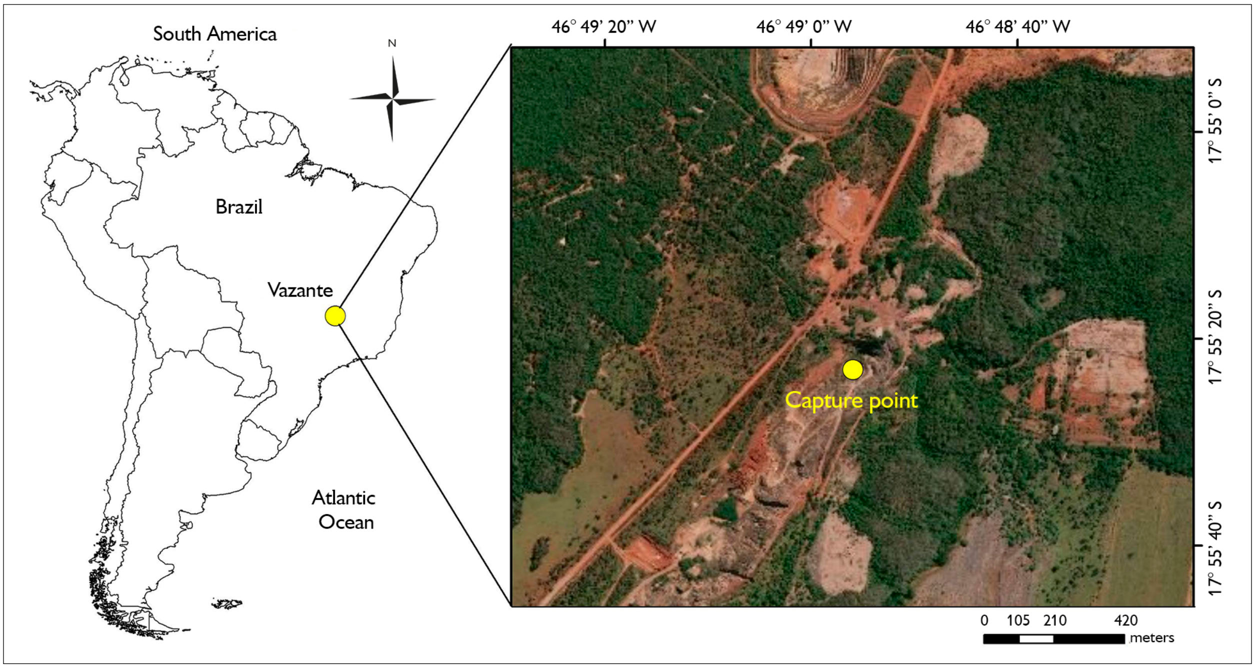

Figure 1. Study area map in Vazante municipality, Minas Gerais state, Southeastern Brazil. Map produced by the authors, 2020.

essence and mashed pineapple on small pieces of banana to capture both individuals (test and control). The species were identified following Gardner (2008).

The test animal came from a wildlife monitoring study and was captured in a forest area adjacent to a mining plant. We found the individual dead inside one of the Sherman traps after nine months of performing a capture-recapture program. The individual was taxidermized and its soft tissues and bones were divided into sub-samples (Table 1).

After obtaining the wet weight, the control and experimental samples were oven dried at 60 °C for 12 h, and then weighed again to perform the heavy metal analysis, in which each sub-sample had a minimum weight of 0.05 g. This material was placed in Teflon® PTFE tubes with five milliliters of HNO_3 (nitric acid) and microwaved at a pressure of 0.76 MPa and a temperature of 175 °C for 10 min. Cd and Pb contents were determined by atomic absorption spectrophotometry using a PerkimElmerAAnalyst 800® flame atomizer (similar to Carvalho *et al.*, 2013).

Table 1. Sub-samples obtained from the animal test and respective references for the quantitative analysis of cadmium and lead.

Sub-samples	References
1	Liver, heart, spleen and kidneys
2	Intestine and stomach
3	Thoracic and pelvic limbs
4	Ribs
5	Vertebra

RESULTS & DISCUSSION

The results showed that the specimen captured near the mining area had high levels of cadmium and lead when compared to the control samples. These values showed differences between some of the sub-samples (1 to 5 – Figures 2 and 3). The cadmium and lead contents present in the test animal are highly discrepant in relation to those found in the control samples.

The results demonstrated the potential of small terrestrial mammals as a bioindicator, in this case using the Brazilian gracile opossum of the *Gracilinanus microtarsus* species. Lead accumulation in trialkylated form (Leite, 2006) can affect organs in the different body systems such as endocrinology and cardiovascular, among others. Furthermore, this heavy metal can also accumulate in bone tissues (Moreira, F. & Moreira, J., 2004). In the case of Cadmium, interaction with the liver results in oxidative stress, competing with zinc for protein binding sites and causing DNA strand breaks, or inhibiting the pathway associated with repair of base-pairing DNA (Lauer, 2007). Moreover, Cadmium negatively affects reproduction, and causes cardiotoxicity, hepatotoxicity, and neurotoxicity (Tomza-Marciniak *et al.*, 2019). Critical levels reported in the literature are 15 μg/g of lead in mammals in general (Ma, 2011) and 30-60 mg/kg of wet weight and 105-210 mg/kg of dry weight of small mammals for cadmium (Cooke, 2011). It is reiterated that these values were analyzed in large animals, such as cattle, monkeys, dogs and rats in Ma (2011) and the values to Cooke (2011) were found with captive wild European species studied in the laboratory with chronic oral doses equivalent to those encountered by wild small mammals in contaminated habitats. Therefore, the levels found in our research are considered high in relation to these critical levels.

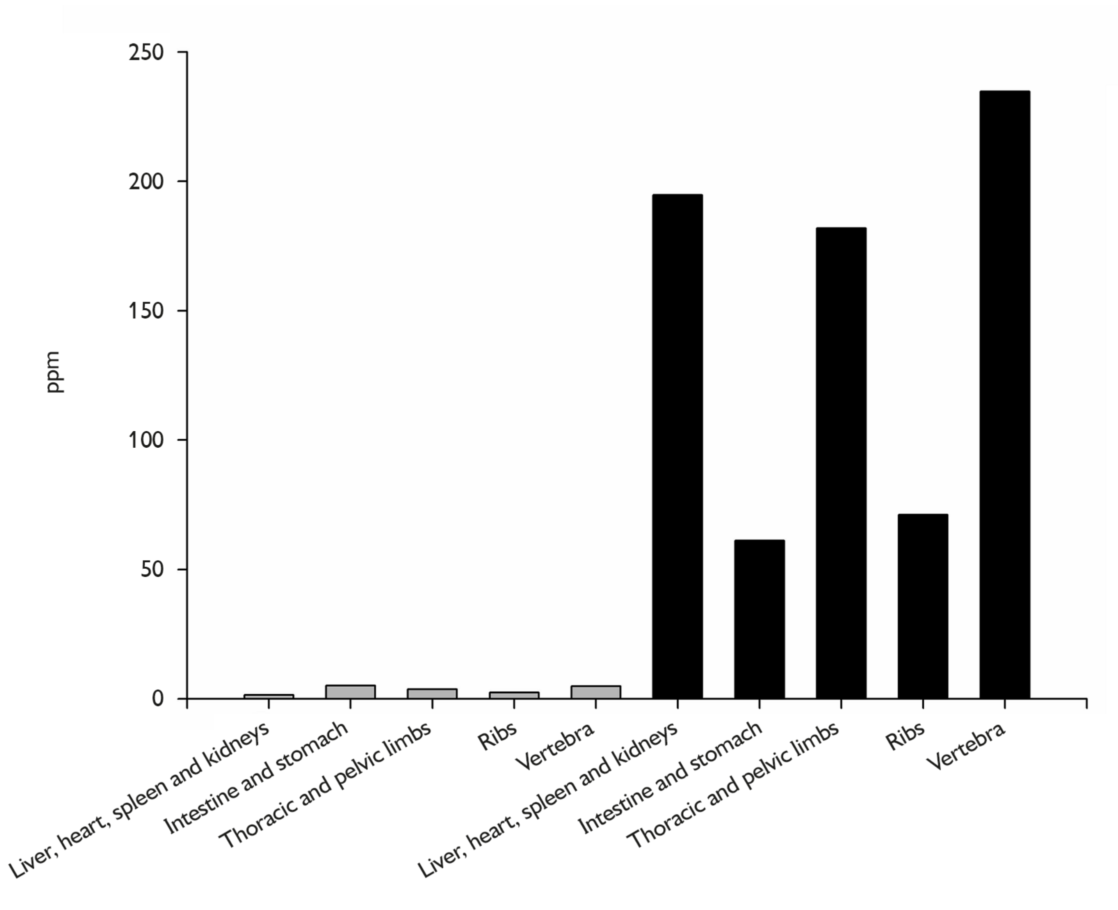

Figure 2. Cadmium contents accumulated in tissues of small terrestrial mammals in ppm. The gray bars represent the sub-samples from the control animal, and black bars represent the sub-samples from the animal test.

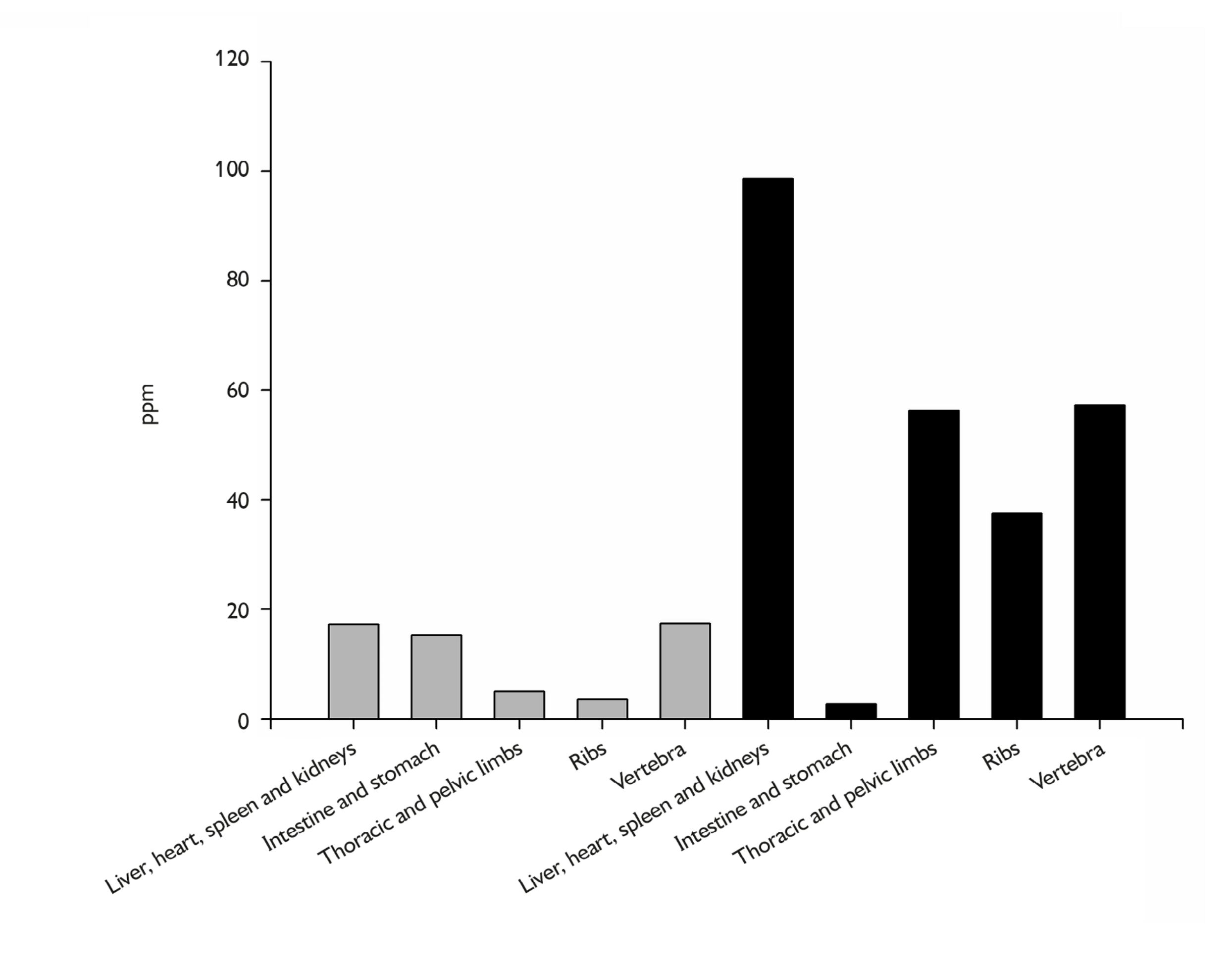

Figure 3. Lead contents accumulated in tissues of small terrestrial mammals in ppm. The numbers gray bars represent the sub-samples from the control animal, and black bars represent the sub-samples from the animal test.

These marsupials can bioaccumulate these two metals and disperse them in nature in different environments and in food chains. This can occur by the animal decomposing after death and/or when the animal is preyed upon by predators. Cadmium and lead are macronutrients. Only a few super-accumulating species can easily remove this metal from the soil [*e.g.*, the plant species *Gomphrena claussenii* Moq. – Carvalho *et al.* (2013)]. Nevertheless, some animals can retain these elements, but carnivorous and omnivorous animals accumulate more than herbivores when feeding on smaller animals such as earthworms or other soil associated invertebrates (Ma, 1989; Veltman *et al.*, 2007).

Some studies on bioaccumulation in Europe and Asia (*e.g.*, Hamers *et al.*, 2006; Veltman *et al.*, 2007; Wijnhoven *et al.*, 2007) found high cadmium values in the liver and kidney of herbivorous and carnivorous voles and shrews, correlating them with available soil elements. When studying sympatric species of small mammals in the north of France, Fritsch *et al.* (2010) verified different bioavailability response patterns, with age being a key factor in response to exposure to metals.

In addition, this is the first report of bioaccumulation of these two chemical elements in neotropical marsupials and has the secondary objective of presenting technical-scientific arguments to encourage more studies in this line in South America, mainly in Brazil (Zocche *et al*., 2010). Ferreira *et al*. (2014) mentioned that Brazil has many mining (or degraded) zones, presenting ecological implications of the expansion of these areas. However, studies in Brazil focusing on mining impacts related on fauna contamination are still incipient.

Bioaccumulation in mammals of medium and large size has already been shown, mainly for capybara and marine mammals (*e.g.*, Monteiro-Neto *et al.*, 2003; Ramm, 2015), both related to the bioaccumulation of metals such as silver, lead, copper, zinc and cadmium due to agricultural activities (agrochemicals) which are leached into lakes and rivers. There are promising initiatives for small mammals in Brazil (*e.g.*, Castro *et al*., 2018) with accumulation of Triphenyltin, and by Zocche *et al.* (2010) on the influence of heavy metals on blood cells in bats), however this study scope needs more attention by researchers, considering the increased contact with heavy metals and other toxic chemical elements from agrochemicals and mining activities in Brazil (for example, environmental disasters causing spillage of metal tailings in Mariana, in 2015, and Brumadinho, in 2019, in Minas Gerais state).

Our results raise questions about the bioaccumulation process from mining actvities: How much of the heavy metal bioaccumulation found in animals living in mining area surroundings is the result of the mining activities, and how much is result of an ecosystem established in an area which naturally has high concentrations of heavy metals? What is the concentration of heavy metals in surrounding soils and plants? What is the concentration of heavy metals in organisms consumed by the analyzed marsupial? Thus, these questions must be answered by future studies to clarify the environmental impacts of contaminating activities.

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