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ORIGINAL ARTICLE

ENVIRONMENTAL PARASITOLOGY: SOIL ANALYSIS FOR GEOHELMINTH LARVAE CONTAMINATION IN ILHÉUS, BAHIA

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ABSTRACT

The study of parasitic soil contamination is important for monitoring biohazards in densely populated areas or those with high anthropic activities. The present study quantified geohelminth larvae in two estuaries in the urban area of Ilhéus, Bahia; one to the north, formed by the Almada River and another to the south, in the Pontal Bay. The collections were performed following standard methodology considering climactic conditions and local tide tables; the Rugai method with modifications was used to analyze soil sediments. Morphological results showed a significantly higher number of *Strongyloides stercoralis* larvae in the estuary of the Almada River from September to December, coinciding with higher temperatures and lower rainfall and humidity. However, there was significant variation in climatic conditions and in the classification of anthropic activity interfering in the frequency and diversity of soil-transmitted helminth larvae, which justifies its monitoring to ensure environmental health in areas frequented by residents and tourists in Ilhéus, Bahia.

KEY WORDS: Environmental health; environmental monitoring; environmental medicine; helminths; soil.

INTRODUCTION

Brazil is vastly diversified in regard to geography, climate, cultural and socioeconomic features that may influence the distribution of parasitic diseases, mainly in urban areas which offer the inhabitants poor infrastructure, as well as inadequate access to health (Gazzinelli et al., 2012; Confalonieri et al., 2014).

It is well known that parasitic diseases do not spread randomly throughout the population since there are predisposing factors that increase risks and vulnerabilities. An example of this is the direct influence of climatic conditions on parasite diseases, possibly interfering in the maintenance or

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establishment of parasite biological cycles with human health implications (Patz et al., 2000; MacPherson, 2005; Atehmengo & Nnagbo, 2014).

Another important factor is the anthropogenic impact on the environment, which has altered the diversity, frequency, and incidence location of different parasites (Zarlenga et al., 2014). Anthropogenic actions linked to inadequate waste management, sewage discharge in estuaries and demographic growth increase the possibility of inadvertent human exposure to biohazards (Gebreyes et al., 2014). These characteristics are commonly observed in Brazilian coastal cities, especially in Ilhéus, located in the south of the State of Bahia, where there seems to have been inefficient urban planning (Fontes & Sousa, 2010).

Ilhéus is a coastal city with an estimated population of 182,350 inhabitants, bordering the Una municipality to the south, Itacaré in the north, Itabuna in the west, comprising the Southern Micro-region of the State. Leisure tourism is an important industry due to the beauty of the rivers and beaches in the region (Carvalho, 2010; IBGE, 2014). The major economic activity is cocoa farming and processing (Instituto Nossa Ilhéus, 2013).

Large cities featuring beaches and rivers as tourist attractions must offer bathing with minimum human exposure to contaminants. In this sense, measures to control contamination, not only of river and beach water, but also the soil, should be targeted by public management in sanitation and health education.

According to Brener et. al. (2008), several studies carried out in Brazil to evaluate soil contamination of beaches, public squares and playgrounds presented a prevalence ranging from 0.6% to 100%. The study of soil sediment contamination has deserved the attention of clinical pathology researchers, especially in highly urbanized areas, or in locations with intense anthropic activity. It is based on this understanding that this work aimed to identify the presence of geohelminth larvae in soil sediment samples from different urban areas and estuaries and to verify the influence of the climate and anthropic activities on larvae counts in the soil of the municipality of Ilhéus, Bahia.

MATERIAL AND METHODS

Collection Areas

The definition of the collection areas (Figure 1) took into account the hydrography of the districts that make up the Almada River estuary (i.e. Vila Juerana, Samabaituba, and Urucutuca), and the beaches most frequented by bathers and residents of the urban area of Ilhéus: Marciano beach, Malhado and São Miguel (North Coast) (latitude 14 ° 4700S, longitude 39 ° 0030W); and the beaches of Avenida, and Cristo (Pontal Bay) (latitude 14 ° 5000S, longitude 39°0300W).

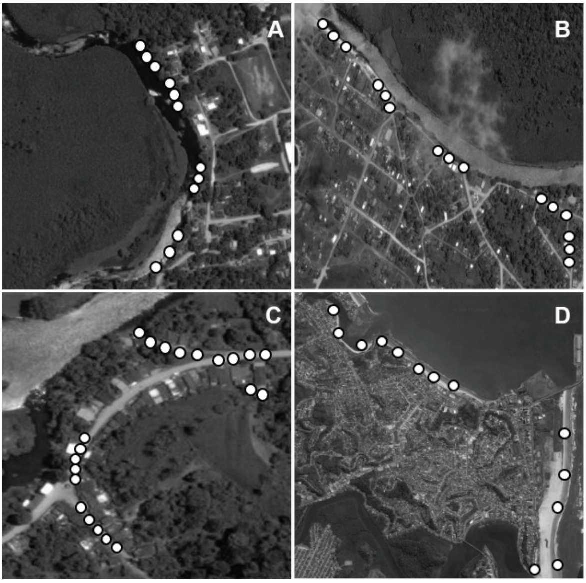

Figure 1. Collection areas in Ilhéus: Almada River: A) Vila Juerana, B) Sambaituba, C) Urucutuca and D) North Coast and Pontal Bay. The circles show the areas of soil sediment collection. Source: Google Earth.

Collection, Processing and Sample Analysis

The climatic information and tide tables were available in the GOES Global WV system, and feasibility of access to river, estuary and beach areas were consulted before each soil sample collection. The work was carried out during nine consecutive months (2012-2013), on dry Saturdays, Tuesdays or Wednesdays from 9:00 am to 1:00 pm. The samples were collected at each of the previously selected sites during the spring/summer and autumn/winter seasons. The collected material was packed in plastic bags and processed, following the methodology described by Cáceres, et al. (2004). Briefly, a sample of approximately 500 g of each soil was collected randomly on beaches and district community areas of the municipality. Each field was divided into two subareas, each of which was marked for sediment removal from five distinct points (five subsamples), where the harvests were performed by zigzagging the surface, then at a depth of 10 cm and 20 cm. Each subarea generated three composite samples with 500 g total weight. The samples were sent to the Parasitology Laboratory of the State University of Santa Cruz (UESC), where they were weighed and processed according to the adapted Rugai method (Carvalho et al., 2005). Briefly: 100g of the blended composite samples was immersed in a sedimentation cup containing water at 45ºC. The modification of the method was in the fact that the material was left to stand for 24 hours, followed by further centrifugation at 2000 g for ten minutes in conical plastic tubes to favor greater migration and concentration of the geohelminth larvae. Then, the supernatant was discarded and at least five slides were prepared per sample – until the end of the pellet – with Lugol staining and analyzed by five independent subjects under an optical microscope with an objective lens at 4x, 10x or 25x when necessary. The criteria used to differentiate the rhabditiform and filariform larvae from *Strongyloides stercoralis, Ancylostoma* spp. and environment larvae was displayed in an atlas with several images containing differential morphological aspects such as the proportion of buccal vestibule, esophagus size, presence or absence of genital primordia, sheath and tail end of each larva as previously published elsewhere (Leite, 2005).

Assessment of Anthropic Activity

A non-structured questionnaire consisting of twelve questions was used to observe and record anthropic activity subjectively. This was answered by the researcher in the field during the scheduled collection day, who noted different aspects found in each collection area such as: sewage, presence of pets and errant animals, seashore degradation, adult or children flow, ambiance, estuary preservation, vehicle flow, leisure activities, occupancy, habitation, and fishery.

Statistical Analysis

The significance of the data collected was evaluated according to the nature of the variables for non-parametric tests (association tests or contingency table) and parametric tests (Student t-test, Tukey or Bonferroni) with a significance of 5%. In the percentage data, significance was assessed by the proportions test with 95% confidence interval (95% CI). The linear correlation between quantitative variables was used to verify the correlation power (r^2), maintaining a significance level of 5%.

RESULTS AND DISCUSSION

Quantity of Geohelminth Larvae in Soil Sediments

Under conventional microscopy, the results indicated higher quantity of geohelminths in the districts on the banks of the Almada River, being significantly higher for *Strongyloides stercoralis* ($p < 0.001$) when compared with *Ancylostoma* spp. demonstrating a potential risk of infection to local inhabitants. Therefore, in the comparison between the soil sediment samples in the Almada River area, there was a higher amount of *Ancylostomatidae* larvae than those obtained on the beaches of the North Coast (Figure 2). However, there was a significant difference in the counts of geohelminth larvae from each beach, in addition to an increase in environmental larvae (Figure 2). One of the factors associated with this variation may be the material composition of the sediment. In this sense, Cassenote et al. (2011) suggest that sand sediment must suffer a greater climatic influence (due to wind and rain) when compared to earth sediment, which may exert some influence on the contamination profile. However, on beaches, part of this sand is bathed by the sea, therefore favoring salinity increase, which hinders the development of parasitic larvae (Santiago & Gagliani, 2011).

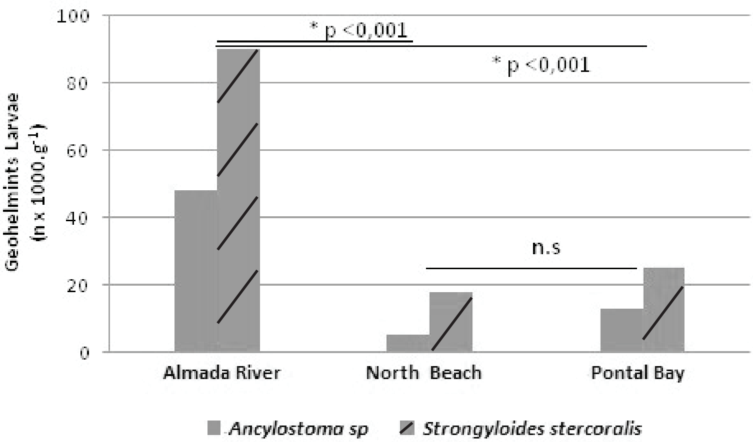

Figure 2. Quantity of geohelminth larvae in the areas of the Almada River (Vila Juerana, Sambaituba and Urucutuca), North Beaches (São Miguel, Marciano and Malhado) and Pontal Bay (Avenida and Cristo Beach) in Ilhéus, Bahia.
* Tukey test with significance of 5%; n.s: not significant.

In regard to the influence of depth on the distribution of the larvae, a larger amount was observed above the 20 cm depth, which can be explained by negative geotropism (Caceres et al., 2004, Carvalho et al., 2005, Santos et al., 2006). Thus, the larvae are displaced to more shallow areas, which activate and orient infective filariform larvae (L3) to penetration under the skin through direct contact, reaching lymphatic vessels or blood capillaries (Rey, 2008).

The results presented in this paper reinforce previous results obtained in the south zone of IIhéus, evidencing soil contamination as well as the possibility of human infections by geohelminths, on all the beaches. Caceres et al. (2004) stated that the degree of contamination was considered “medium contamination” due to the occurrence of parasites and different forms of litter in the beach soil, thus enabling the proliferation of microorganisms in the environment. The authors did not observe any relationship between the type of geohelminth and the depth of the larvae, despite a significant difference between the amounts of larvae in the different soil depths studied (Caceres et al., 2004). In a study by Hohlenwerger et al. (2011), hookworm larvae were noted in 43 samples (35.8%), from 19 sandy beaches in the city of Salvador, Bahia, using Ueno’s method (Ueno & Gonçalves, 1998). Whereas, Filho et al. (2011) obtained high positivity for protozoan cysts (62.5%), eggs (21.9%) and helminth larvae *S. stercoralis* (15.6%) and *Ancylostoma* spp. (3.1%) in wet sand samples from beaches in Paraíba. Santiago et al. (2011) reported a prevalence of enteroparasites in 36 positive samples for *Ancylostoma* spp. and *Ascaris* sp. From the São Vicente beach, with high levels of contamination for Itararé (41%), followed by Gonzaguinha (25%), and Milionários beaches (10%). The authors considered these locations as foci of infection by larvae constituting a public health hazard, evidencing the need for soil contamination control programs.

Climatic Influence

The climate from September to December presents increases in temperature and the ultraviolet index, intense variations in relative air humidity, and little rainfall, therefore, favorable to an increase in the number of larvae (154/42, F = 3.6) as noted in the Almada River area (Figure 3a, red arrow). On the other hand, a decrease in the number of larvae (84/63; F = 1.3) was observed in the samples from Pontal Bay when the climate variation was inverted, presenting a drop in temperature and the ultraviolet index, relative humidity and rainfall (Figure 3a, blue arrow). The increase in temperature and the decrease in relative humidity correlated significantly with the increase in the number of larvae found in the collection areas (Figure 3b). Despite being contradictory, it is important to state that the volume of rainfall accumulated in Ilhéus (1,260.4mm) was 40% lower than the expected total for the whole of 2012 (2,100.3mm). The rainy season in the city occurs from April to June, these being considered the rainiest months (Instituto do Meio Ambiente e Recursos Hídricos, 2012).

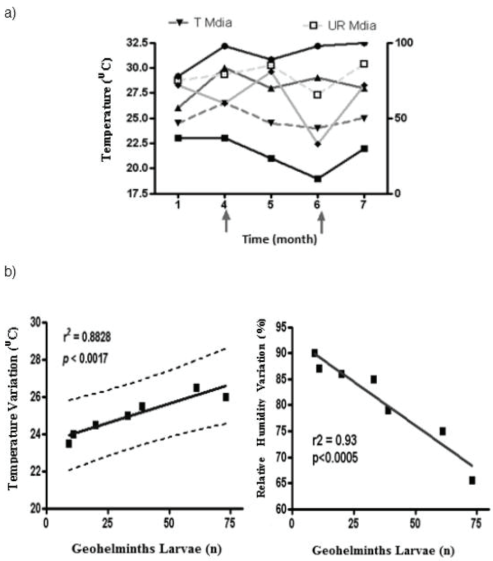

Figure 3. A) Climate variation in summer (arrow 4) and winter (arrow 6). T Mdia: Mean temperature (dashed upper line) and UR Mdia: Relative Humidity Mean (dashed botton line). B) Variation in the number of geohelminth larvae in the estuarine areas of Ilhéus, Bahia according to climatic influences. Linear correlation r2 with 5% significance.

According to data provided by the National Institute of Meteorology (INMET), May 2012 presented the highest rainfall index (411.5mm), exceeding by 17.7% the climatological average for the month (349.5mm). July and August confirmed their averages and the other months registered much lower indices than expected respectively. An atypical event occurred in April, one of the wettest months of the year, when the 2nd largest rainfall record (326.2mm) was expected, yet the rainfall verified was 15% (48.9mm) of that expected for the whole month. In June, July, September, and December the indices were close to normal. In August, October, and November, averages were higher than usual. In 2013, the rainfall in January (36.2mm), February (28.6mm) and March (38.4mm) was 73%, 80%, and 75%, respectively, increasing the monthly climate averages, due to the presence of a hot and dry air mass that covered most of Bahia (Instituto do Meio Ambiente e Recursos Hídricos, 2012). With this air mass losing strength, the rain became more intense and more frequent in April, yet the accumulation remained below average, again in May, considered the wettest month of the year, confirming our findings of an inverse correlation between climate alteration and the quantity of larvae in soil sediments, when samples were collected in two opposite rainy seasons as shown in Figure 3.

According to the Institute of Environment and Water Resources (INEMA), from the climatic point of view, one of the main causes for the rainfall decrease in 2012 was the cooler temperature of the Atlantic Ocean surface water near the coast of Bahia, thus reducing evaporation and rainfall. In addition, the continuous presence of hot and dry air masses over much of Bahia also caused a reduction in cloudiness and, consequently, in rain (Instituto Nacional de Meteorologia, 2012).

Climatic conditions such as temperature and rainfall have influences on soil flora, essential factors for the development and viability of the geohelminth free stage larvae (Rey, 2008). However, the rainfall regime is but one of several environmental factors influencing the amount of larvae in the soil (Tiyo et al., 2008). A study on parasite contamination of public squares in the eastern zone of the city of São Paulo by Mello et al. (2011), revealed the existence of a relationship between contamination, rainfall and temperature, since the highest index of fertile and infertile eggs, cysts and parasite larvae were noted during the rainy season (from October to March), highlighting the risk of parasitic contamination during periods of heavy rainfall and high-temperature indices.

Influence of Anthropogenic Activity

Figure 4 shows the factors associated with the various human activities identified in the Pontal Bay collection areas, evidencing the cumulative effect of the factors, demonstrating a difference in the classification of the anthropic activity among the areas. A general analysis of the results shows a greater variation in the anthropic activity on the Marciano, São Miguel, Malhado, Avenida and Cristo beaches. The most common anthropogenic characteristics observed were: flow of people (46% of the areas were considered crowded $p < 0.001$), poor sanitation (33% of the areas presented inadequate sewage or open septic tanks $p < 0.001$) presence of animals (40%) including vultures and dogs (30%), leisure activities (38%) and fishing (26.6%).

There is insufficient epidemiological data to assert categorically that beaches and river estuaries are sources of parasitic infection for humans. Nonetheless, the incidence of soil contamination by parasites of medical importance was noted (*Strongyloides stercoralis* and *Ancylostoma* sp) in soil samples from the municipality of Ilhéus, Bahia, mainly in the area on the banks of the Almada River, while not so intense in the North Coast and Pontal Bay. This result presented variations depending on climatic characteristics since an increase in temperature with lower relative humidity was related to a greater amount of geohelminth larvae in the soil. The other variables resulting from human activity in the collecting areas, revealing an impact on soil contamination by geohelminths were, especially, an increase in the flow of people, inadequate basic sanitation and the presence of animals.

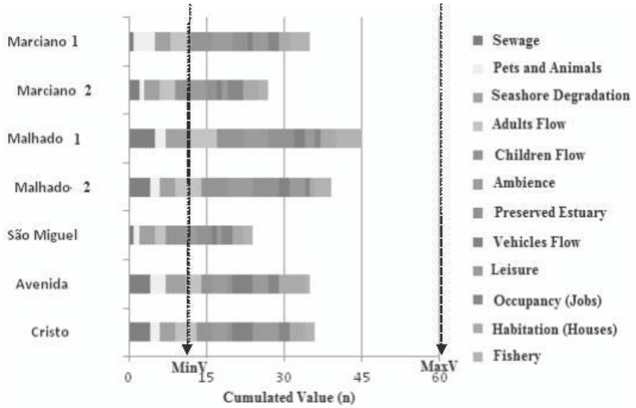

Figure 4. Sequential cumulative variation of anthropic activity in the different collection areas in the North Coast and Pontal Bay in the municipality of Ilhéus, Bahia. The intensity of each anthropogenic factor (depicted sequentially from Sewage to Fishery) was estimated (from 1 to 5 degrees of intensity) according to the perception of the researcher(s) at the time of sample collection. MinV: Minimum cumulated value: 12; MaxV: Maximum cumulated value: 60.

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