



Original Articles

Influence of heavy metals on nematode community structure in deteriorated soil by gold mining activities in Sibutad, southern Philippines

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ABSTRACT

Ore mining is among the most environmentally destructive anthropogenic practices, particularly in developing countries. Correct assessment of its impacts on soil ecosystems requires an understanding of the response of soil food webs. Nematodes, often the most abundant invertebrates in soils, occupy various positions in food webs, and their assemblages are commonly used to reflect soil health. In October 2014, we collected soil samples from five sites of a small-scale mining area in Sibutad, southern Philippines, to assess the influence of mining activities on nematode assemblages. Two sites were considered undisturbed as there were no visible signs of mining, while the other three sites were disturbed. Nematodes were extracted live and identified to genus level using morphology-based identification. We analysed genus composition, genus and trophic diversity, and the life-history based maturity index. We measured soil environmental variables (pH, organic matter, granulometry and several heavy metals), and correlated variation in nematode genus composition to variation in these environmental factors. Small-scale mining activities had variable but generally non-significant impacts on soil properties, altered vegetation and caused increases in concentrations of Hg and Pb, but not consistently so in all impacted sites. The high patchiness in vegetation and heavy metal content were reflected in a high within-site variability of nematode assemblages. Total nematode abundance was significantly lowest in the most physically disturbed site, but not so in the most metal-polluted one, suggesting that abundance is not a good indicator of pollution status. Nematode genus composition significantly differed between disturbed and undisturbed sites. By contrast, only few differences between sites were found for diversity or maturity indices, demonstrating that genus composition was a better indicator of mining-related effects than many common indicator indices and highlighting that detailed assemblage analysis is required for a correct interpretation of moderate pollution effects on soil nematodes. Measured environmental variables together explained 60% of the variation in nematode assemblages in the area; the three 'single best' explanatory variables were the concentrations of Pb, Hg and N, but none of these by itself explained more than 8% of the variation in nematode data, while their combination explained 24%. Some genera of predacious and omnivorous nematodes, which are generally expected to be sensitive to both chemical pollution and physical disturbance (e.g., *Ironus* and *Eudorylaimus*), were most abundant in sites with elevated heavy metal concentrations, which can have repercussions for the interpretation of nematode-based indices such as the MI.

1. Introduction

Ore mining, both large and small-scale, is an important contributor to the economy in many developing countries. For instance, the Philippines is a major exporter of metallic minerals such as gold, copper, nickel and chromium (Hooley, 2005). In Sibutad, a

municipality in Mindanao, southern Philippines, gold mining activities have provided livelihood to local communities since the 1980's (Cortes-Maramba et al., 2006). Large-scale mining operations make use of advanced technology in the extraction of mineral deposits, whereas small-scale mining employs manual and fairly rudimentary techniques, which are often environmentally risky (Hinton et al., 2003).

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Small-scale mining produces about 80% of the Philippines' annual gold supply. However, these substandard routines, aggravated by lack of proper ecological monitoring, can result in deliberate and accidental disposal of wastes (van Straaten, 2000). Despite its economic contribution, it remains a highly polarized issue due to incidences of environmental degradation and health problems among exposed communities (Cortes-Maramba et al., 2006). Mining is associated with the rise of heavy metals in the environment (Getaneh and Alemayehu, 2006). Heavy metals are naturally deposited in rocks and can be released into the environment either by natural weathering or by artificial activities (e.g., digging, ore processing, etc.). They pose a threat because of their potential to bioaccumulate and interfere with various biological processes (Heikens et al., 2001). The gold extraction method by mercury (Hg), also known as amalgamation, is relatively popular among small-scale miners since it is inexpensive. Compared to other mineral extraction methods, amalgamation is easier to perform but potentially risky, and may cause environmental pollution due to improper handling and waste management (Israel and Asiro, 2002; Odumo et al., 2014). Hg is considered to be one of the most toxic elements naturally found in the environment even at very low concentrations (Göthberg and Greger, 2006), and its negative impacts on soil biota (Harris-Hellal et al., 2009) and soil processes are well-studied (Müller et al., 2002). In humans, Hg can induce damaging effects on reproduction, immune system, central nervous system and internal organs (Dietz et al., 2000).

Before the 1980's, our sampling area in Sibutad, was predominantly covered with cogon grass (*Imperata cylindrica*), economically unproductive and had only few inhabitants. The discovery of gold deposits in the 1980's caused an influx of miners, with an estimated peak of 10,000 in the early 2000's. Although the number of active miners has been gradually decreasing since, a few hundreds are still operating around the mountain sides. Hence, disturbance impact in small-scale mining areas in Sibutad may be caused by past and/or existing mining activities. In practice, small-scale miners use ball mills to grind rocks into fine particles, from which the gold is extracted by amalgamation and blowtorching, which results in the formation of wastes (e.g. Hg and tailings). The lack of proper waste storage can cause Hg and tailings to end up in the soil or river, and finally into Murcielagos Bay, a semi-enclosed bay adjacent to the mined sites. At present, there are approximately 500 small-scale miners in the area of Sibutad who can potentially release 120–360 kg of Hg per year (Perez et al., 2007). Previous studies have revealed elevated Hg levels in humans (Cortes-Maramba et al., 2006) as well as in marine organisms from Murcielagos Bay (Lacastesantos, 2000), whereas information on Hg effects on terrestrial animals or plants from the area is lacking. Our initial inspection showed that the river bed of the sampling area was largely composed of thick, dark-brown clay sediments and the water appeared very turbid. Preliminary river water analysis revealed a Hg content of ca. $50 \mu\text{g L}^{-1}$ (our own unpublished data), which is 5 times higher than the permissible limit for wastewater discharge by EPA, i.e., $10 \mu\text{g L}^{-1}$ (USEPA, 2001), and 25 times higher than the current water quality criterion for the protection of public health by the Philippine government, i.e., $2 \mu\text{g L}^{-1}$ (www.emb.gov.ph/wp-content/uploads/2016/04/DAO-1990-34.pdf). The high Hg content of the water is most probably caused by the discharges from small-scale mining activities upstream. Mercury concentrations higher than the allowable level proposed by UNEP (2013) are generally expected to be toxic, and in Sibutad where Hg disposal is a problem, Hg levels in soils may have exceeded the 'permissible' limit. Aside from heavy metal pollution, other activities such as burning of vegetation, digging, construction of physical structures (e.g., tunnels, processing plants, etc.) may also affect soil structure, organic matter content and soil pH, which can in turn influence the biological activity of soil biota such as nematodes (Sánchez-Moreno et al., 2006b).

Nematodes are important biological components in the soil ecosystem due to their functional roles in organic matter decomposition

and nutrient cycling (Freckman, 1988; Yeates, 2003); their abundance and community composition are widely used as ecological indicators in several different environments (Bongers and Ferris, 1999; Neher, 2001; Shao et al., 2008). Nematode responses to pollution range from sensitive to very tolerant, with substantial differences between species (Kammenga et al., 1994; Monteiro et al., 2018). Therefore, changes in the nematode assemblage structure and function can be used to assess pollution effects or disturbances in soil, and can be measured by diversity and ecological indices, as well as through a detailed analysis of their taxonomic composition (Fiscus and Neher, 2002).

The present work was conducted to assess whether nematode assemblage structure reflects the impacts of small-scale mining in the southern Philippines. Specifically, this research aimed to a) determine the extent of pollution, particularly that of Hg, and other disturbances (e.g., burning of vegetation, digging, etc.) caused by small-scale mining activities in soils in a small-scale gold mining area; b) assess whether the nematode assemblage structure differed between locations with different degrees of mining-related impact; and c) determine whether such mining impacts are better revealed by particular nematode-based (diversity and maturity) indices or by nematode genus composition.

2. Materials and methods

2.1. Study site and sampling

The area of Sibutad is situated in the northwestern part of Mindanao, southern Philippines, with an average annual temperature of 27.4°C and precipitation of 2310 mm, the latter distributed fairly evenly throughout the year. Our sampling area is situated on a slope of mountain and covers approximately a distance of 1.2 km (between Site 1 and 5) towards Murcielagos Bay (Fig. 1). Some parts of the area have been subjected to 'physical' disturbances such as land clearing, excavation of mountain slopes, open-cast and underground mining, construction of small processing plants and habitation by a few individuals, while other areas have been chemically contaminated owing to mining and ore processing.

Soil samples were taken in October, 2014. We divided the study area into five sampling sites – S1, S2, S3, S4 and S5 (Table 1). Five replicate soil samples, each composed of 3 composite samples, were randomly collected with approximate interdistances of 8–10 m from each of the sites. S1 and S2, 300 m apart from each other, were characterized by the absence of inhabitants and mining activities, albeit S1 appeared to have a more diverse vegetation than S2. Mining-related activities and/or local communities were manifest in S3, S4 and S5, thus we *a priori* referred to them as 'disturbed' sites as opposed to the 'undisturbed' (reference) sites, S1 and S2. Perennial grass species (e.g., *Paspalum conjugatum*) generally characterized the disturbed sites (S3, S4 and S5) due to their relatively fast colonizing ability after disturbance episodes. S3, the uppermost part (in terms of altitude) of the area, was marked by intense mining activities with the presence of a community of miners (< 30 ind.), two ball mill plants, and the site's close proximity to the excavated areas. S4 had the largest human population (> 40 ind.), who were not engaged in mining operations but hosted one ball mill plant. S5 was also inhabited (< 5 ind.) and located about 0.25 km from Murcielagos Bay. An active ball mill plant was found near S5, which was situated at an elevated ground a few meters away (ca. 20 m) from this site. Although we cannot rule out the possibility that the undisturbed sites had previously been impacted by mining-related disturbances due to lack of information of the past mining activities, the present Hg and other heavy metal levels were used to assess the impacts of local mining activities since their operation in the 1980's.

2.2. Soil properties

Five replicate composite samples, each consisting of 500 g (a composite of 3 samples combined), were collected from the upper 5 cm

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