Environmental Pollution 240 (2018) 574–581

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Ants and their nests as indicators for industrial heavy metal contamination☆

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ARTICLE INFO

Article history:
Received 12 January 2018
Received in revised form
25 April 2018
Accepted 28 April 2018

Keywords:
Bioindicator
Biomarker
Formica lugubris
Melanisation
Monitoring

ABSTRACT

Ants accumulate heavy metals and respond to pollution with modification in species composition, community structure, altered behaviour and immunity. However, the levels of heavy metals in ants' nests and explicit individual-level responses towards heavy metals have not been revealed. We found that red wood ants *Formica lugubris* accumulate high and correlated values of such heavy metals as *Al*, *Cd*, *Co*, *Cu*, *Fe*, *Ni*, *Pb* and *Zn* both in ants and nest material near cobalt smelter in Finland. Relative differences in metal concentrations were higher in nests than in ants. The highest values were obtained for elements such as *Co* (36.6), *Zn* (14.9), *Cd* (9.7), *Pb* (8.5), *Cu* (7.4), *Ni* (6.4), *As* (4.7), *Cr* (2.9) and *Fe* (2.4) in nest material, and *Co* (32.7), *Cd* (6.3), *Pb* (6), *Fe* (2.8), *Ni* (2.9) and Zn (2.1) in ants. In industrial and reference areas, ants have no differences in size, but differed in dry and residual body mass. In polluted areas, *F. lugubris* had less melanised heads, but not thoraxes. The sensitivity of cuticular colouration in red wood ants subjected to heavy metal pollution might be related to metal-binding properties of melanins. The overall results are useful for the improvement of biomonitoring techniques using ants as indicators of industrial contamination and for further discovery of novel ecotoxicological biomarkers.

1. Introduction

Anthropogenic heavy metal contamination of aquatic and terrestrial ecosystems remains one of the key environmental concerns globally despite of the recent technological advances in pollution management. Moreover, in less developed countries the pollution levels are currently on the rise (Gong and Barrie, 2005; Järup, 2003; Tchounwou et al., 2012). The main sources of heavy metal dispersal into environment are industry, transport, refuse burning, power generation (Agarwal, 2009) and e-waste (Robinson, 2009). Once being released, pollutants penetrate into water, soil or air in particulate or gaseous form. Further they are accumulated in the organisms and transferred through food chains, producing variable adverse effects. Therefore, an improved and timely implemented ecotoxicological risk assessment programmes may prevent further ecosystem poisoning.

With proper methods of preselection and testing, terrestrial invertebrates are reliable bioindicators and biomonitors of environmental, ecological and biodiversity changes, including metal contamination (McGeoch, 1998; Gerlach et al., 2013). Terrestrial invertebrates demonstrate considerable variations in an ability to accumulate heavy metals: high in Isopoda and low in Coleoptera (Heikens et al., 2001; Nummelin et al., 2007). The major driver factors for this ability are behaviour, physiology and diet (Gall et al., 2015). The organisms that are effective at detecting risks for the ecosystem – sentinel species (Berhet, 2013) – are especially useful as bioindicators. So, who are sentinels? To meet the requirements, a species must live permanently in a studied site, possess sufficient population range, be easy to collect and identify, be able to tolerate stress for several years and it must have well known biology for proper differentiation between true signal and background noise (Berhet, 2013). Ants (Formicidae) are widespread, abundant, sufficiently sensitive and at the same time tolerant, highly involved into food chains and do not normally migrate, except army ants (Hölldobler and Wilson, 1990). They accumulate both essential and toxic heavy metals and respond to pollution with alterations at different organisational levels (Stary and Kubizňáková, 1987; Rabitsch, 1995; Eeva et al., 2004; Del Toro et al., 2010). Not surprisingly, when satisfying all the criteria for sentinels and accumulating metals, ants should be proper indicators for industrial heavy metal contamination.

☆ This paper has been recommended for acceptance by Wen-Xiong Wang.

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https://doi.org/10.1016/j.envpol.2018.04.134
0269-7491/

Several studies confirmed ants as a good bioindicator group for various pollution types (Andersen, 1997; Andersen et al., 2002; Kaspari and Majer, 2000), including soil-heavy metal contamination (Ribas et al., 2012; Gramigni et al., 2013; Khan et al., 2017), aerial phthalate pollution (Lenoir et al., 2014) and land rehabilitation (Majer, 1983; Khan et al., 2017). However, most research was focused on metal levels in ants, not in nests (but see Migula and Głowacka, 1996). Recently it has been confirmed that ant nests do not buffer heavy metal pollution (Jílková et al., 2017). Nevertheless, the available information about quality and quantity of metals in ant nests is still limited. As stated by Andersen et al. (2002), ants will not be widely used as bioindicators in practical land management until the development of simple and efficient protocols for their use. Therefore, revealing the levels of heavy metals in nest material in ant hills will simplify biomonitoring frameworks. Selection of appropriate entities to measure is an essential demand for effective monitoring programmes (Lindenmayer and Likens, 2010). Further search for sensitive responses in ants at different levels of their biological organisation allow the creation of practical environmental indicators and biomarkers of pollution.

Ants tolerate heavy metal pollution better than the many other invertebrate species (Folgarait, 1998; Grześ, 2010). However, pollution may affect ants' biodiversity (Grześ, 2009a) and abundance (Khan et al., 2017), community structure (Grześ, 2009a; Belskaya et al., 2017) and colony size (Eeva et al., 2004). In addition, environmental pollution in some ant species results in an increased frequency of small workers (Grześ et al., 2015b), disturbed immunity (Sorvari et al., 2007) and altered behaviour (Sorvari and Eeva, 2010). The effects may not be only direct. For example, arsenic pollution affects a number of tree species in southern Brazil, which in turn is crucial for the arboreal ants (Ribas et al., 2012).

Despite multiple studies on ecosystem- and community-level effects, the explicit individual-level morphological responses to pollution have only rarely been studied. Several attempts failed to reveal the impact of pollution on body size (Eeva et al., 2004; Grześ et al., 2016) or on developmental instabilities (Rabitsch, 1997a; Grześ et al., 2015a). We assume individual-level responses in the traits, which are controlled more by environmental than genetic factors like body mass and colouration, may be more sensitive to environmental stress (Hill, 1995, 2011) by comparison to functionally important traits. Especially since it had been confirmed that pollution affects colouration in a taxonomically diverse range of species (Lifshitz and St Clair, 2016).

We assume that in the case of organic mound building ants, sampling ants with nest material will simplify biomonitoring. Then we suggest, that focusing an attention on individual-level responses in traits with flexible evolutionary function in metal-tolerant organisms will further help to reveal novel biomarkers of environmental pollution (Skaldina and Sorvari, 2017a). The wood ant *Formica lugubris* (Zetterstedt, 1838), which is quite common in benign and anthropogenically altered habitats of Central and Northern Europe, rarely was chosen as a model organism for revealing colony- and individual-level responses to industrial heavy metal contamination.

2. Materials and methods

2.1. Study area

We carried out our field sampling in Western Finland in one industrial area and one reference area without industrial activities in 10th June 2014 (Fig. 1). Kokkola Ykspihlaja industrial area (WGS84, 63°51: 23°3) harbours a metal smelter, cobalt factory, and sulphur dioxide factory. It has a known history of pollution with multiple heavy metals such as *As*, *Cd*, *Co*, *Cu*, *Fe*, *Hg*, *Ni* and *Zn* (Laita et al., 2008; Huuskonen et al., 2013). The reference area was located in the nearby Lohtaja region (WGS84, 63°59: 23°26) 21–25 km northeast of Ykspihlaja in a similar coastal sandy region. The forests in both areas are dominated by Scots pine (*Pinus sylvestris*) mixed with birches (*Betula pubescens*, *B. pendula*) and Norway spruce (*Picea abies*). The ground layer was dominated primarily by the scrubs *Vaccinium vitis-idaea*, *Calluna vulgaris* and various grass species (*Poaceae*).

2.2. Study species

The northern red wood ant *F. lugubris* belongs to the *Formica rufa* species group. It is one of the most common red wood ants in Central and Northern Europe, where it is particularly common in young successional stage forests and islands (Punttila, 1996; Sorvari, 2018). It appeared to be abundant in the study area in the coastal region of western Finland. In Europe, *F. lugubris* has highly variable social structure, from monogynous and monodomous to polygynous and polydomous (Bernasconi et al., 2005; Pamilo et al., 2016). In Finland, each colony of this species occupies typically one nest and has one or only few reproducing queens (Pamilo and Rosengren, 1983). Colonies of *F. rufa* goup species forage within 30 m of the colony, but sometimes their routes can reach more than 100 m (Savolainen and Vepsäläinen, 1988; Sorvari, 2009). A previous study related to heavy metal pollution and red wood ants, revealed a very low abundance of *F. lugubris* in the Harjavalta industrial area located in the southwest of Finland (Eeva et al., 2004). Rarely found in Harjavalta, this species was the most common representative of the *F. rufa* group in Kokkola and Lohtaja. We conducted sampling in summer time, as previously it has been shown that there is a seasonal variation in metal levels in red wood ants: lower in spring and higher during summer (Rabitsch, 1995). In addition, we collected samples within one day to avoid seasonal changes in body mass of similarly sized workers (Rabitsch, 1997b).

2.3. Sampling and preparation of ants and nest material

We studied 20 colonies of *F. lugubris* located from 0.4 to 25 km from the metal smelter and the cobalt refinery. Ten of the studied colonies were located close to the factories in the Ykspihlaja peninsula within 0.4–0.85 km from the factories. Ten colonies were 21–25 km away from the factories located in the Lohtaja region (Fig. 1). From each colony we took nest material samples with live ants from the top of the nest mounds, placed them into 0.75 L plastic containers and brought to the university campus in Kuopio. Different categories of red wood ant worker accumulate different levels of heavy metals (Maavara et al., 1994). For biomonitoring purposes, surface workers and foragers fit the best, as they take in the highest quantities of chemicals among all functional groups of ants (Maavara et al., 1994; Migula and Głowacka, 1996). Anyhow, they are the easiest group of worker ants to be collected by a non-specialist person. The ants were allowed to come out of the containers to larger, 5 L transparent closed containers. When the majority of ants had moved to the larger container, they were freeze killed. After all, two colonies had a limited number of workers, so we used ants from 18 colonies for morphological data analyses. Eight individuals from each of the nests were randomly selected for further morphometric and colour analyses ($N = 142$). The other ants (30–50 workers) were put in open plastic tubes and the nest materials in open paper bags and dried in an oven set to 55 °C for 48 h. The dried ants were pulverised with a plastic pestle in a plastic tube. An unused pestle and tube was used for each nest sample to avoid contamination. Dried nest material was sieved through clean 1.5 mm plastic sieves to get homogenous fine material samples and stored in 15 ml plastic with screw cap.