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SHORT RESEARCH AND DISCUSSION ARTICLE

Phthalate pollution in an Amazonian rainforest

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Abstract Phthalates are ubiquitous contaminants and endocrine-disrupting chemicals that can become trapped in the cuticles of insects, including ants which were recognized as good bioindicators for such pollution. Because phthalates have been noted in developed countries and because they also have been found in the Arctic, a region isolated from direct anthropogenic influence, we hypothesized that they are widespread. So, we looked for their presence on the cuticle of ants gathered from isolated areas of the Amazonian rainforest and along an anthropogenic gradient of pollution (rainforest vs. road sides vs. cities in French Guiana). Phthalate pollution (mainly di(2-ethylhexyl) phthalate (DEHP)) was higher on ants gathered in cities and along road sides than on those collected in the pristine rainforest, indicating that it follows a human-mediated gradient of disturbance related to the use of plastics and many other products that contain phthalates in urban zones. Their presence varied with the ant species; the cuticle of *Solenopsis saevissima* traps higher amount of phthalates than that of compared species. However, the presence of phthalates in isolated areas of pristine rainforests suggests that they are associated both with atmospheric particles and in gaseous form and are transported over long distances by wind, resulting in a worldwide diffusion. These findings suggest that there is no such thing as a "pristine" zone.

Keywords Phthalates · Pollution · Tropical rainforests · Ants · DEHP

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Introduction

Of all of the pollutants found across the globe, phthalates (mainly di(2-ethylhexyl) phthalate (DEHP)) are some of the most widely distributed. Phthalate esters are used in many industrially made products, such as cosmetics, pesticide carriers, insect repellents, vinyl, cables, tubing, films, paints, adhesives, PVC, and inks. They are also used as plasticizers (i.e., to make plastics more flexible). Because phthalate esters do not chemically bind to plastic polymers, they migrate to the surface of the polymer matrix where they may more easily leach into the air, water, or food. They have been detected in the air (including in aerosols), water, soil, different sediments, and animal tissue, including that of humans (Teil et al. 2006; Alves et al. 2007; Babich and Osterhout 2010; Williams et al. 2010; Gaudin et al. 2011; Salapasidou et al. 2011; Choi et al. 2012; Huang et al. 2013).

Hundreds of scientific papers and many newspaper articles have chronicled the effects of endocrine-disrupting chemicals (EDCs, mainly phthalates, and bisphenol A), which have been associated with human pathologies (e.g., negative effects on the male reproductive tract, breast and testicular cancers, disruption of the neuroendocrine system, allergies, and asthma) (Saillenfait and Laudet-Hesbert 2005a, b; Desdoits-Lethimonier et al. 2012; Manzetti et al. 2014). Moreover, we know that the toxicity of certain pollutants is greater than previously thought and frequently results in transgenerational effects (e.g., in fish; Schwindt et al. 2014). Furthermore, the impact can be exacerbated by interactions between contaminants or "cocktail effects" (e.g., pesticide combinations on bees) (Vidau et al. 2011; Gill et al. 2012) or between contaminants and natural stressors, including malnutrition, osmotic perturbations, and global warming (Rhind 2009; Holmstrup et al. 2010).

Phthalate air pollution has both acute and chronic effects ranging from minor upper respiratory irritations to chronic respiratory and heart diseases, lung cancer, acute respiratory infections in children, and chronic bronchitis in adults. In addition, short- and long-term exposure to phthalate pollution has also been linked to premature mortality and reduced life expectancy (Kampa and Castanas 2008) and transgenerational effects through epigenetic mechanisms (Doyle et al. 2013; Manikkam et al. 2013; Rissman and Adli 2014). Many reports have indicated that the phthalates found in dust in houses are associated with asthma and allergies in both children and adults (Ait Bamai et al. 2014).

Phthalates have been found on insect cuticles such as those of ants, crickets, and honey bees, something which has been taken as evidence of their ubiquity (Cavill and Houghton 1974; Kather et al. 2011; Lenoir et al. 2012); they can also become trapped in the wax of honey bee combs (Gómez-Ramos et al. 2016). DEHP and dibutyl phthalate (DBP) are toxic at high doses for *Folsomia candida* springtails, causing modifications in symmetry (Jensen et al. 2001; Kristensen et al. 2004). Phthalates deposited in large quantities on *Lasius niger* ant cuticle remained in dead, control individuals, while they were adsorbed and metabolized in less than 5 days and so returned to their basic level, in live individuals (Lenoir et al. 2014). At doses corresponding to chronic exposure levels, phthalates reduce ant queen fecundity and stimulate an immune response in workers (Cuvillier-Hot et al. 2014).

Because phthalates are transported everywhere in the atmosphere above developed countries (Choi et al. 2012; Blanchard et al. 2013) and because they have been found in the Arctic (Xie et al. 2007), a region isolated from direct anthropogenic influences, they appear to be widespread. To verify this, we hypothesized that their presence in isolated pristine Amazonian rainforests would provide strong evidence that the planet's atmosphere is thoroughly polluted by these compounds.

Ants are present everywhere, are found in almost every part of the food web, and constitute the most abundant animal taxon in tropical ecosystems (Longino et al. 2014; see also Basset et al. 2015 for tropical insect diversity). Consequently, ants represent important bioindicators based on the degree to which they have been contaminated by pollution. So, we compared the phthalate pollution levels of ants from isolated pristine rainforest in French Guiana, far from any human activity, with areas having increasing levels of anthropogenic perturbation, including urban areas, where plastics and many products containing phthalates (e.g., detergents, building materials, and furniture) are in constant use. However, because phthalates are rapidly degraded by microbial activity and abiotic processes (i.e., hydrolysis, photocatalytic oxidation, and photolysis) (Staples et al. 1997; Zhou et al. 2005; Yuan et al. 2010; Huang et al. 2013; Manzetti et al. 2014), the levels recorded are likely much lower than those associated with the original source of contamination. We also aimed to identify the various phthalates present because, due to concerns over their safety, the most frequently used (i.e., DBP, diisobutyl phthalate (DiBP), and DEHP) are progressively replaced by heavier molecules, which have already been found in soft plastics produced in Asia (Barušić et al. 2015; AL, personal observation).

Materials and methods

We collected ants from various sites in French Guiana in November 2013 (Fig. 1). The CNRS Nouragues research station (40° 05′ N, 52° 40′ W, 121 m asl) was an important sampling location in our study because it is situated in an isolated, uninhabited, and protected area ≈90 km from the coast and can be reached only by helicopter (or by pirogue then a 4-h hike). We collected ants near the station, where human activity may have served as a source of pollution (i.e., different materials have been used to construct shelters, and plastic has been brought in as a result of the provision of food and research materials—see Suppl. 1). We also collected ants in the rainforest far from the station and on the summit (397 m) of the inselberg near the station (see Suppl. 2). Exposed to the elements, it harbors sparse vegetation. We also sampled ants near the Petit-Saut hydroelectric dam (4° 59′ N, 53° 08′ W) as well as in the forest of *Crique Plomb*, including the dirt road which crisscrosses the forest over 10 km from the road leading to the dam from Route N° 1, and in other forested areas along this road. We also collected ants in and near the cities of Sinnamary (5° 22′ N; 52° 57′ W), Kourou (5° 09′ N; 52° 38′ W), and Cayenne (4° 56′ N; 52° 20′ W).

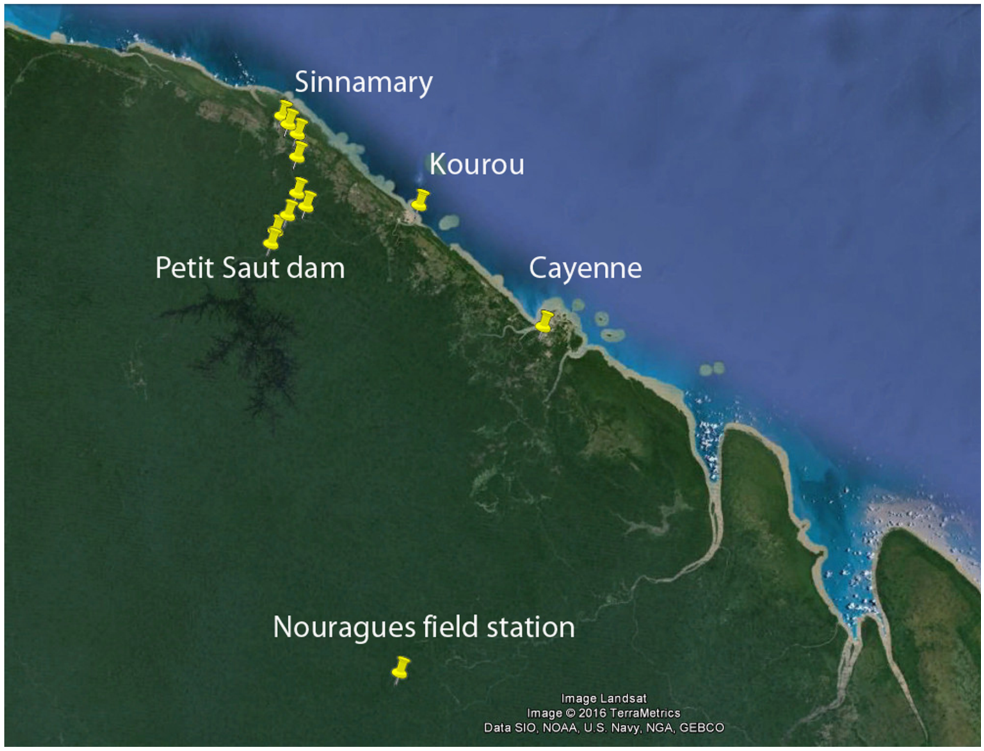

Fig. 1 Main ant sampling locations. Map created using Google Earth. A transect was established along the road between the Petit-Saut Dam and the city of Sinnamary. Other main places are Nouragues field station, Kourou, and Cayenne cities

Ants were captured with metal forceps and placed directly into glass vials containing hexane; they were never in contact with plastics and were left in the vials until the analyses were run. At that point, they were removed from the vials, and the solvent evaporated. Then, the extract was redissolved in 10 μL of hexane to which 2 μL of hexane containing 400 ng of eicosane (C20) was added as an internal standard (we verified that all the hexane used was phthalate free). We injected 2 μL of each redissolved extract into a Perkin-Meyer gas chromatograph-mass spectrometer (GC-MS) functioning at 70 eV and with a source temperature of 230 °C. The GC-MS was equipped with a ZB-5HT column (30-m L × 0.25-mm ID × 0.252 μm df; 5 % phenyl—95 % dimethylpolysiloxane). The following temperature program was used: 2 min at 80 °C, increased by 10 °C/min to reach 320 °C, and a 10-min hold at 320 °C (for a total of 36 min). An external mixture of phthalates is generally used to quantify phthalate acid esters (PAEs) (Teil et al. 2006). Eicosane is frequently used as the standard in hydrocarbon analyses, so we utilized it here to compare this study with previous ones (Lenoir et al. 2012, 2014; Cuvillier-Hot et al. 2014). We used ion 149, typical of phthalates, as the basis for our analyses of the phthalate peaks (Cao 2008; Valton et al. 2014; Barušić et al. 2015). This method is less sensitive but much more effective in differentiating phthalates from other hydrocarbons, particularly DEHP from 5MeC25 (Lenoir et al. 2014). We calculated the quantity of each compound relative to the eicosane internal standard. The threshold for DEHP quantification is 0.20 ng, so that, for small ants, we placed five workers in the extract vial. We analyzed a total of 243 samples.

Since the species ranged in size, the results were normalized and presented in terms of nanogram per milligram of dry weight (DW), as in Lenoir et al. (2014).

Data are presented as means ± standard errors (SE), and statistical analyses were conducted using ANOVAs and the Newman-Keuls post hoc test for multiple comparisons (R software).

Results and discussion

The different phthalates recorded

Guianese ants were contaminated with the same phthalates as their European and North African counterparts (Lenoir et al. 2012), notably DEHP, DBP, diisobutyl phthalate (DiBP), and benzyl butyl phthalate (BBP). DEHP, ubiquitous and noted in 95 % of the samples (Table 1), was found in higher quantities on *Solenopsis* (19.5 ng/mg DW vs. 0.9 for other ants) and accounted for 97 and 61.5 % of the phthalates found on *Solenopsis saevissima* workers and other ants, respectively. DEHP is also the most prevalent phthalate in the atmosphere in the Paris region (Teil et al. 2016).

We also found on Guianese ant cuticules two new phthalates, di(2-ethylhexyl) terephthalate ((DEHTP) = dioctylterephthalate (DOTP)) and diisononyl phthalate 35

Table 1 Different phthalates found on ants in French Guiana for *Solenopsis* and all other ant species (mean ng/mg DW ± SE, % of samples containing phthalates, % quantities related to the total amount of phthalates)

Phthalates (ng/mg DW)	Other species				*Solenopsis*			
	Mean	SE	% Samples	% Total	Mean	SE	% Samples	% Total
DBP	0.1	0.0	45.5	6.4	0.1	0.1	13.4	0.6
DiBP	0.04	0.0	25.5	2.9	0.01	0	12.4	0.1
BBP	0.4	0.1	39.1	27.6	0	0	0	0
DEHP	0.9	0.2	94.5	61.5	19.5	3.6	93.8	97.4
DINP	0.02	0	31.8	1.5	0.4	0.1	22.7	1.9
DEHTP	0.001	0.0	0.9	0.1	0	0	0	0
Total	1.4			100	20.0			100
n=	110				97			

isomers (DINP), which are recently being used instead of DEHP (Rastogi 1998; Abe et al. 2012). DEHTP can be passively transferred by simple contact between ants and fragments of plastic children's toys (A. Lenoir, unpublished results), explaining why it occurred on urban Guianese ants. DINP was detected in 22.7 % of *Solenopsis* and 31.8 % of other ants gathered around the Nouragues research station and in the cities of Cayenne (at the harbor), Kourou, and Sinnamary; it was also noted at the Petit-Saut field station and along the road to the dam. When present, DINP only represented 1 to 2 % of the phthalates. In the Nouragues research station, it was recorded in the pieces of flagging tape tied around trees to delimit parcels. DINP is found in toys, childcare products, PVC, flagging tape, and many soft plastics (Barušić et al. 2015). Its metabolites have been detected in human urine across the globe (Saravanabhavan 2012), and although it seems to be less toxic than the more common phthalates (Babich and Osterhout 2010), it was placed on California's official list of carcinogens (Tomar et al. 2013).

The plastic tubing used to delimit parcels at the Nouragues research station contains BBP and DEHP in small quantities, likely explaining their presence on ants. However, these compounds were also noted on ants gathered far from any human activity, such as the top of the inselberg.

Anthropogenic gradient of pollution

Classically, phthalate pollution levels increased from the rainforest to the cities regardless of the ant taxa tested, showing a relationship with human activity (Fig. 2). An ANOVA using

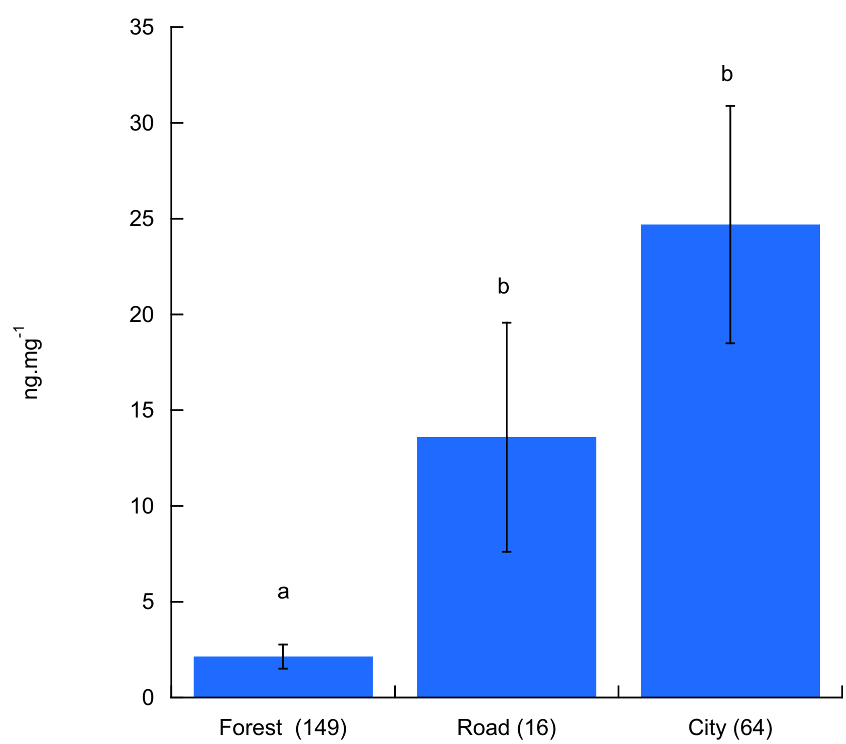

Fig. 2 Mean phthalate levels pooled from the cuticles of the different ant genera. Comparison between individuals gathered from the rainforest, along the roads, and in the cities (mean ng/mg DW ± SE). Statistical comparisons: ANOVA ($F = 24.31$; df 2; $p < 0.0001$) and Newman-Keuls post hoc test; *different letters* indicate significant differences at $p < 0.001$

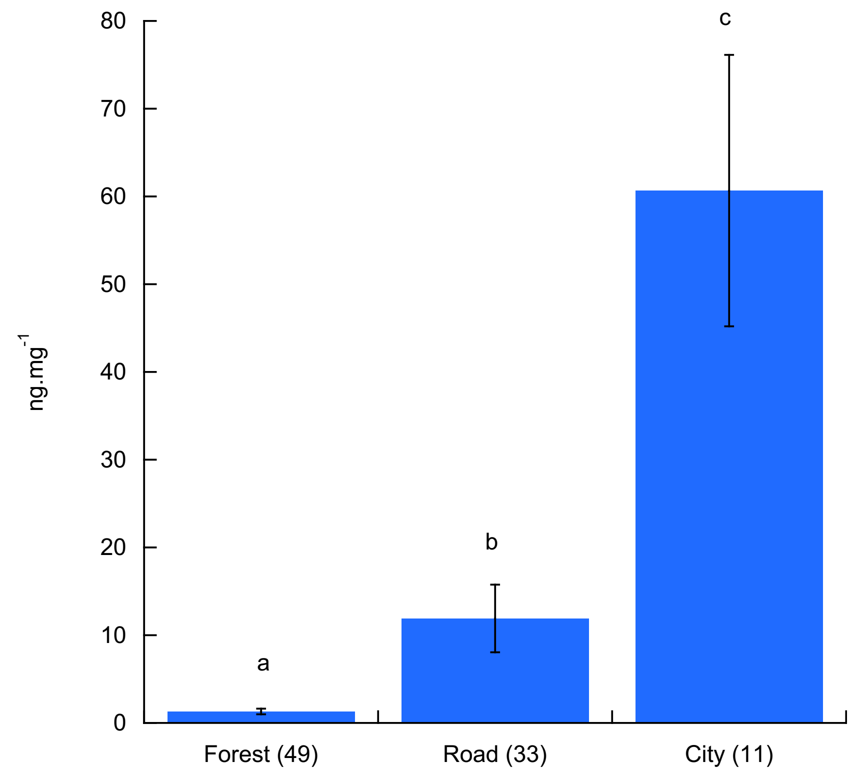

Fig. 3 Mean phthalate levels for the different ant genera in the different areas along the road from the Petit-Saut dam to the city of Sinnamary (mean ng/mg DW ± SE). Statistical comparisons: ANOVA ($F = 45.3$; $df = 2$; $p < 0.0001$) and Newman-Keuls post hoc test; *different letters* indicate significant differences at $p < 0.001$

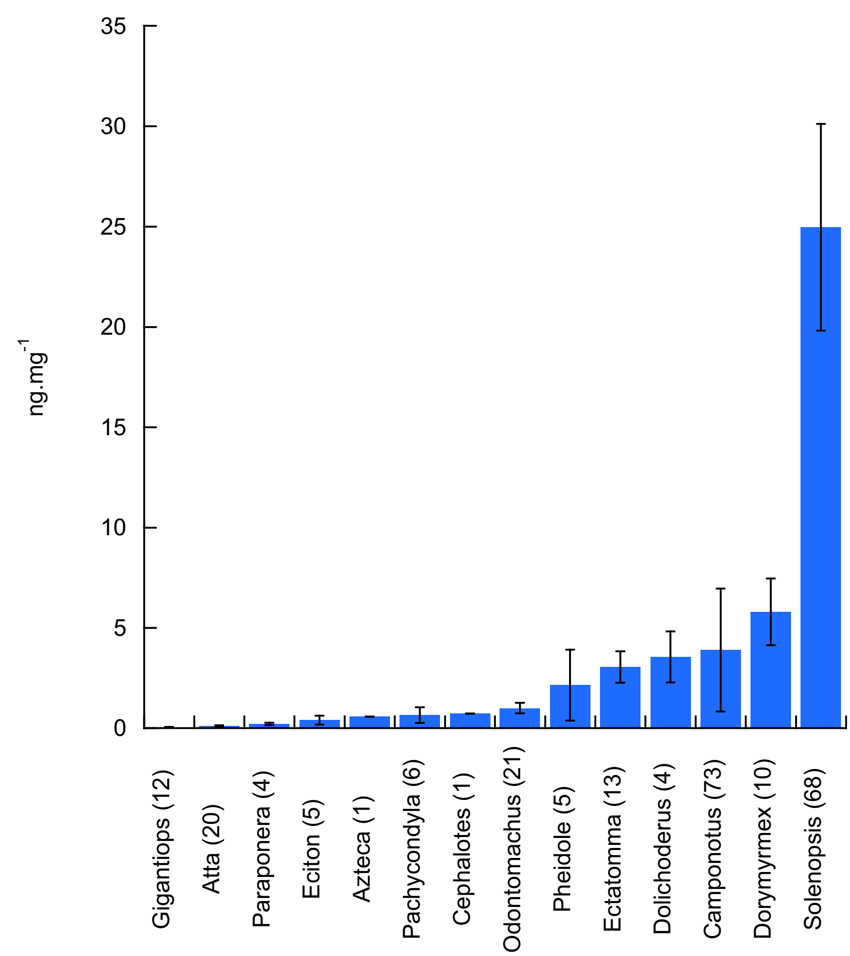

Fig. 4 Overall mean phthalate levels for the different ant genera (mean ng/mg DW ± SE). Statistical comparisons: ANOVA ($F = 6.576$; $df = 14$; $p < 0.0001$)

the full data set revealed that phthalate levels, which ranged from 0 to 200 ng/mg DW, differed significantly across ant genus (F = 6.57, df = 14, p < 001), areas (i.e., rainforests vs. roads vs. cities, F = 32.03, df = 2, p < 001), but not with altitude (F = 1.47, df = 1, p = 0.226). Overall, phthalate levels were significantly higher in urban areas (p < 0.001) and there was an increase, albeit non-significant, from road sides to cities (p = 0.45; Fig. 2). The same trend was noted for the data from ants sampled in the rainforest of Petit-Saut, along the road leading to the dam, and in the city of Sinnamary (ANOVA (F = 45.3; df = 2; p < 0.0001); here, all of the differences between areas were significant (Fig. 3a–c).

The cuticular phthalate levels observed for urban Guianese ants are similar to those noted for the ant *L. niger* in Europe (i.e., 2 ng/ant fresh weight, corresponding to 5 ng/mg DW) (Lenoir et al. 2012). Yet, a perfect comparison would require using the same species.

Phthalates were ubiquitous around the Nouragues research station, as they were found in ants from the camp, the forest, and the top of the inselberg. The levels were low, ranging from 0.5 (the top of the inselberg) to 2 ng/mg DW, and did not differ significantly between sites (p = 0.06, but near significance for the top of the inselberg, p = 0.055), so that human activity in and around the station is not likely responsible for the phthalate pollution noted deep in the rainforest and on the top of the inselberg.

Therefore, our hypothesis that phthalate pollution is globally ubiquitous is likely confirmed as, in addition to their presence in the Arctic (Xie et al. 2007), we found them in other areas isolated from direct anthropogenic influence, including parts of the Amazonian rainforest and the top of an inselberg. These results strongly suggest that contaminants arrive from the atmosphere both with air particles and in gaseous form (see Blanchard et al. 2014; Cecinato et al. 2012; Gao and Wen 2016; Teil et al. 2016; Xie et al. 2005). For example, in the Paris region, phthalate pollution ranges from 10 to 100 ng m^{-3} of total air and 80 % in the gaseous phase. It is more concentrated in urban areas compared to forest sites (Teil et al. 2016).

Variation in phthalate levels across ant genera

The levels of phthalate contamination varied between ant genera (Fig. 4), a pattern likely due to differences in cuticle composition (Vienne et al. 1995). *S. saevissima* had the highest levels but was not found at the Nouragues research station nor the rainforest (see Dejean et al. 2015). Yet, it did occur at all of the other sites, including along the dirt road of Crique Plomb which crisscrosses the rainforest at Petit-Saut. Phthalate levels noted on workers were low in the latter case and high in the cities with values of up to 180 ng/mg DW. Consequently, with its anthills interconnected by galleries forming huge colonies extending along several kilometers (Martin et al. 2011; Lenoir et al. 2016), *S. saevissima* appears to be a good bioindicator for gauging phthalate pollution in human-disturbed areas.

In conclusion, it appears that phthalates are universal contaminants and are probably major constituents of generalized anthropogenic pollution, which is a leading cause of human health problems. They may also be playing a role in the mass extinctions of the Anthropocene, which are affecting both vertebrate and, albeit less visibly, invertebrates (Dirzo et al. 2014). Phthalates are the major pollutants disseminated throughout the world in gaseous form and on atmosphere particles (Teil et al. 2016). Our results show that they are found in different levels on ant cuticle based on a gradient of urbanization, so ants can be considered good bioindicators due to their ubiquity and ease of sampling them. It is thus imperative to continue to study the pollution of ant populations, most particularly in tropical rainforests.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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