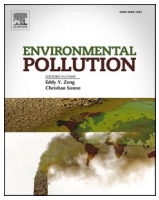
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Ants as bioindicators of urban traffic pollution

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A B S T R A C T

Ants can serve as valuable bioindicators due to their ecological ubiquity, stable presence due to perennial nests, and direct interaction with soil and airborne particulate matter. This study investigates the potential of the arboreal ant species *Crematogaster scutellaris* as a sentinel organism for traffic-related magnetic pollution in urban environments. Sampling was conducted across 30 sites in the city of Parma (Italy) characterized by varying levels of vehicular traffic, alongside four control sites. Magnetic susceptibility measurements and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) were employed to quantify and characterize magnetic particles accumulated on the ants' exoskeleton and surrounding soil. Results revealed a strong correlation between vehicular traffic intensity and the quantity of magnetic particulate matter deposited on ants (Spearman's $\rho = 0.825$, $p < 0.001$), while soil samples showed no significant relationship ($\rho = 0.167$, $p = 0.346$). SEM-EDS analysis identified iron oxide-rich particles doped with heavy metals such as Zn, Cu, and Ti on the ants' surface, particularly concentrated on the thorax. These particles exhibited morphologies consistent with traffic-derived ultrafine particulate matter. Soil samples, collected at 5 cm depth, predominantly contained clay minerals with minimal anthropogenic influence. The relationship becomes linear on a logarithmic scale ($R^2 = 0.66$, $p < 0.001$); each additional 1000 vehicles per day was associated with a 1.41-fold increase in ant magnetic susceptibility, corresponding to an approximately 700-fold increase across the traffic gradient sampled. The study proposes a novel biomonitoring protocol integrating magnetic and morphological analyses to assess urban air quality, offering a scalable alternative to traditional fixed monitoring stations.

1. Introduction

Ants (Hymenoptera, Formicidae) are a widespread and ecologically significant group, comprising over 14,000 described species that play a key role in terrestrial ecosystems across a broad spectrum of anthropogenic impact, from pristine natural habitats to densely populated urban agglomerations. Their remarkable diversification, shaped by a wide array of evolutionary strategies and ecological adaptations, enables even hundreds of species to coexist within the same habitat (Parker and Kronauer, 2021). Ants occupy a broad variety of ecological niches, often exhibiting local diversity that exceeds that of other social insects (Hölldobler and Wilson, 1990; Lach et al., 2010; Rico-Gray and Oliveira, 2008; Grasso et al., 2015; Parker and Kronauer, 2021). Due to their biological, ecological, and behavioural traits, including their abundance, species richness, year-round activity, and permanence of the colonies, ants have gained increasing recognition as reliable bioindicators of environmental quality, ecological disturbance, and pollution (Andersen and Majer, 2004; Castracani et al., 2015; Blinova and Dobrydina, 2019; Okrutniak and Grześ, 2021). To understand whether ants could serve as effective indicators of air quality, it is crucial to first consider the nature and health impact of airborne pollutants, in particular magnetic particles. Within the field of urban ecology, air pollution is widely recognized as one of the most critical drivers of both environmental degradation and public health concerns. Among the many pollutants generated by human activities, fine particulate matter (PM) stands out as one of the most harmful and pervasive components of urban air pollution. A fraction of PM consists of magnetic particles, primarily composed of iron oxides such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), often with solid solution phases that include Ni, Cr, Pb, and Cu (Chaparro et al., 2024). These particles originate from diverse anthropogenic sources such as fossil fuel combustion for residential heating, high-temperature industrial processes, construction and demolition activities, improper waste disposal, and for the most part vehicular traffic (Cui et al., 2024; Sager, 2020). In particular, the mechanical abrasion of brake pads, tires, and road surfaces represents a continuous and diffuse source of ultrafine magnetic dust (Yoo and Lee,

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2024). The magnetic properties of these particles not only make them effective environmental tracers of pollution, but also underscore their chemical reactivity and toxicological potential (Thakur et al., 2025). According to the World Health Organization (WHO), approximately 7 million deaths in 2016 were primarily attributed to pulmonary diseases linked to particulate matter exposure (Roser, 2023). However, the health risks associated with magnetic particles extend far beyond the respiratory system (Schraufnagel, 2020). Owing to their ultrafine size and high surface-area-to-volume ratio, these particles can penetrate deep into the lungs, translocate into the systemic circulation, and even reach the brain (Maher et al., 2016). Their redox-active surfaces catalyze Fenton-type reactions, producing highly reactive oxygen species (ROS) capable of causing oxidative damage to lipids, proteins, and nucleic acids (Imlay, 2003; Lim and Kim, 2024; Maher et al., 2016). A growing body of in vitro and in vivo evidence has demonstrated that magnetic particles can induce oxidative stress, mitochondrial dysfunction, systemic inflammation, and neurotoxicity, with potential links to neurodegenerative conditions such as Alzheimer's disease (Cohen et al., 2004; Maher et al., 2020; Agarwal et al., 2026). The spatial dispersion of magnetic particles is highly heterogeneous, even at small urban scales, and is influenced by local emission sources, atmospheric conditions, urban structure, and land use. As a result, traditional air quality monitoring systems, typically based on fixed sampling stations, may not be sufficient to fully capture the complexity and variability of air pollution in specific ecological contexts. These stations also face practical limitations: high operational costs, fixed location requirements due to size and power supply needs, and limited ability to detect the most dangerous ultrafine particle fractions that reach deep into the respiratory system. To overcome these constraints, bioindicator-based approaches have gained traction as integrative, cost-effective, and ecologically meaningful alternatives (Rocha et al., 2010). Recent research has proposed the use of magnetic measurements on bioindicators such as tree leaves and terrestrial isopods to monitor local accumulation of traffic-related magnetic particles (Mantovani et al., 2018; Winkler et al., 2022; Tribaudino et al., 2022). Within this context, ants have emerged as especially promising sentinel organisms for environmental assessment (Andersen and Majer, 2004; Parikh et al., 2021; Akhila and Keshamma, 2022). Their ubiquity, nest fidelity, and direct interaction with soil and airborne particulate matter allow them to reflect site-specific contamination, including magnetic particle deposition. Ground-level foraging behaviour and relatively stationary nest locations make ants highly suitable for long-term, localized monitoring. Moreover, their species richness and abundance across different urban gradients enable comparative analyses among sites with varying levels of anthropogenic disturbance. Due to their ecological and behavioural characteristics, ants can be sensitive indicators of both temporal and spatial changes in metal accumulation (Grześ, 2010). Additionally, they offer a unique opportunity to assess the biological impact of pollution at different levels of organization, among individuals within a colony, between colonies of the same species, and across different species with varying ecological roles. In this study, we explore for the first time the potential of ants as bioindicators of environmental pollution caused by traffic-related magnetic particles. Our methodology combines magnetic susceptibility analysis with scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) to detect and characterize magnetic particles accumulated on ants. Sampling was conducted across multiple sites in the city of Parma (northern Italy), each characterized by different levels of vehicular traffic. This integrated analytical approach allowed us to assess the relationship between traffic intensity and magnetic particle loads, providing the basis for a novel biomonitoring protocol for evaluating urban air pollution.

2. Materials and methods

2.1. Sample collection

Sampling was carried out in selected sites across the city of Parma (population: ~200,000; urban area: ~260 km^2), with the aim of evaluating magnetic particle contamination linked to vehicular traffic. Traffic intensity across different areas was assessed using official databases provided by the Parma City Administration, which include data from 57 monitoring stations equipped with magnetic sensors capable of detecting vehicles with an engine displacement of at least 50 cm^3 (~80 kg). Traffic intensity at each site was expressed as average daily traffic (ADT), i.e. the mean number of vehicles per day recorded by the corresponding monitoring station. Data from one survey campaigns were collected: inductive-loop sensors operating continuously from 12/09/2022 to 23/12/2022. To identify suitable bioindicator organisms, field surveys were conducted at each site to assess the presence of the arboreal ant species *Crematogaster scutellaris* (Olivier, 1792). This species is monomorphic, meaning that foraging workers show minimal size variation, which facilitates standardized sampling (Seifert, 2018). It is widely distributed in the Western Mediterranean and commonly inhabits both natural and urban environments. *C. scutellaris* is known to form large colonies, with foraging activity occurring both at ground level and in the tree canopy (Seifert, 2018) and has been previously employed to assess metal bioaccumulation in polluted environments (Gramigni et al., 2011, 2013). Out of the 57 surveyed sites, colonies were detected in 30 locations (see Fig. 1). Additionally, four control sites located in areas completely free of vehicular traffic were included for comparative purposes. In each area, the colony closest to the traffic monitoring station was selected for sampling. At each site, 50 foraging workers were collected from the same colony. Ants were sampled directly from the tree trunks where their nests were located, in proximity (min 10 m; max 68 m) to the traffic sensors. Collected individuals were immediately stored in magnetically clean plastic vials to avoid contamination and kept at a constant temperature of 20 °C until laboratory analysis. Simultaneously, soil samples (50 g) were collected near each nest. To standardize collection, the surface layer was removed, and soil was sampled at a depth of approximately 5 cm using plastic tweezers and spatulas, one set of tools per sample, to prevent cross-contamination. All samples were collected within a restricted time frame between May 22 and May 28, 2023, during a consistent time window from 11:00 a.m. to 3:00 p.m., in order to minimize the effects of meteorological variability on particle deposition and foraging behaviour.

2.2. Magnetic analysis

Magnetic susceptibility was measured on all the 34 ant samples and on the corresponding soil samples. Each sample was placed in cellulose-based containers, sealed with a thin layer of high-temperature-resistant silicone (up to 220 °C), and dried in an oven at 30 °C for 24 h to eliminate residual moisture. Once dried, the samples were transferred into non-magnetic gelatin capsules (diameter: 5 mm; length: 10 mm), and the mass of each capsule was measured using an analytical balance with a precision of 0.1 mg. The alternating current (AC) magnetic susceptibility was measured using the MAGLAB2000 system (Oxford Instruments). This instrument applies a low-frequency alternating magnetic field, and measures the sample's response through inductive coils, ensuring high sensitivity and resolution. AC susceptometry is particularly well-suited for detecting small amounts of magnetic particles embedded in a large, weakly magnetic matrix. This is due to the significantly higher low-field magnetic susceptibility of ferromagnetic and ferrimagnetic particles, such as particles of magnetite and maghemite, compared to paramagnetic and diamagnetic minerals or substances. As a result, magnetic susceptibility measurements offer a fast and cost-effective method for analysing the magnetic contribution of particulate matter.

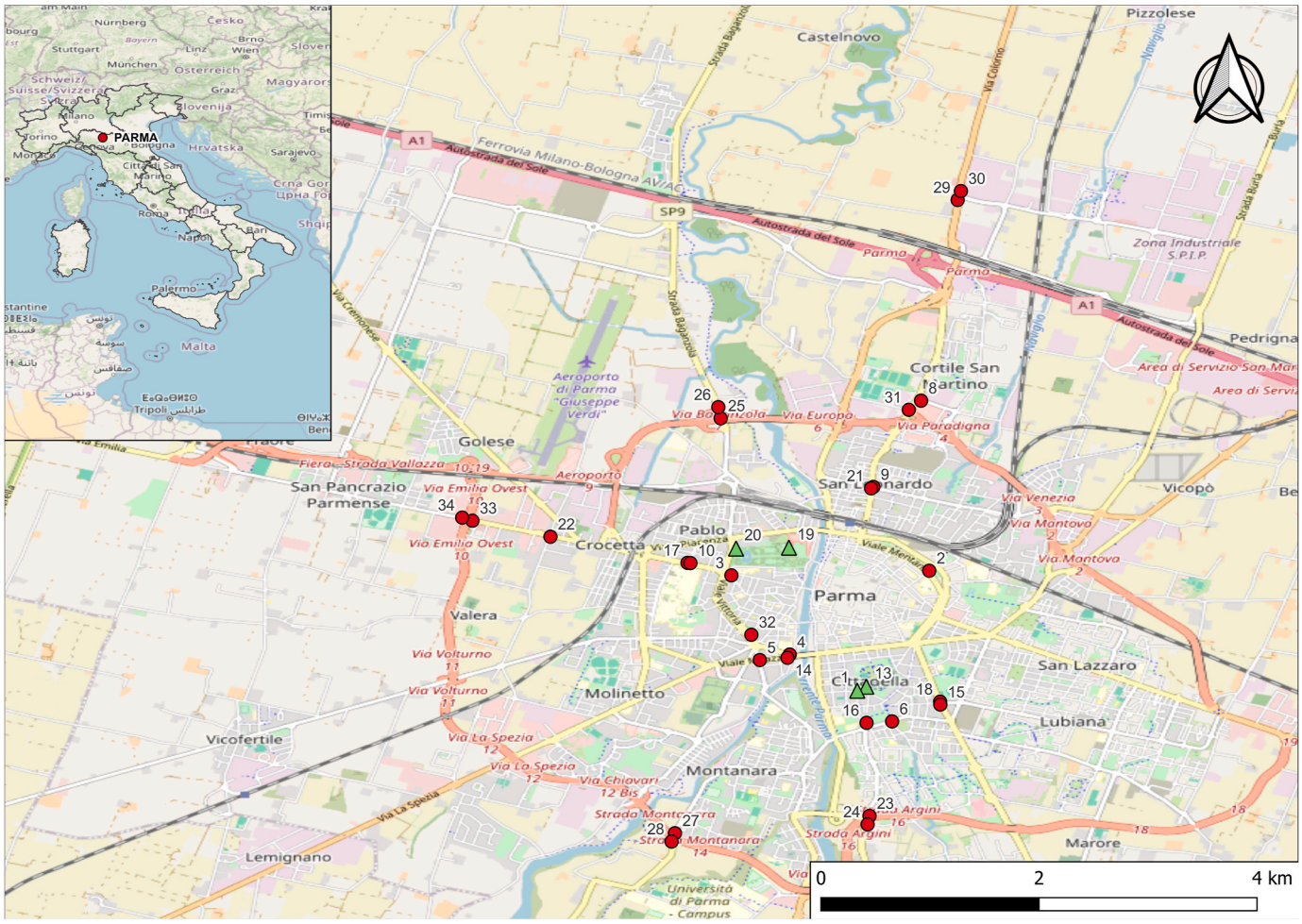


Fig. 1. Study area in Parma city (Emilia-Romagna, Italy). In the figure, the red dots (n = 30) indicate the sampling points in the presence of vehicular traffic, and the green triangles (n = 4) indicate the control points in the absence of traffic. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Measurements were performed using an AC field with amplitude of 2 mT and a frequency of 3 kHz. The background signal from the capsule and sample holder was independently measured and subtracted from each measurement. The obtained susceptibility values were normalized to the sample mass, yielding a mass-specific susceptibility that reflects the combined contributions of magnetic particles, along with the diamagnetic component of the biological matrix (i.e. ants). Measurements were repeated 15 times for each sample to improve the statistics and reduce the experimental error. The average values of the repeated measurements were used for the analysis. In total, 68 samples were analysed for this study, including both ant tissues and associated soil samples (Supplementary materials S1).

2.3. SEM-EDS analysis

SEM and EDS analyses were carried out using a Hitachi TM4000 Plus II Scanning Electron Microscope equipped with an integrated Oxford Instruments Energy Dispersive Spectroscopy (EDS) microprobe. Operating conditions for the EDS microprobe were set at 20 kV accelerating voltage, 1.2 mA beam current, with a counting time of 60 s per spot. For each sample, multiple analytical points and chemical mapping routines were performed. SEM imaging was conducted using both the backscattered electron (BSE) and secondary electron (SE) detectors to differentiate phases based on morphology, atomic number contrast, and surface texture. These images allowed identification and classification of magnetic and non-magnetic particulate matter adhering to the ants' exoskeleton. To quantify particle presence and surface distribution, image analysis was conducted using the open-source software Fiji/ImageJ (Schindelin et al., 2012). For each sampling zone (n = 34), two *Crematogaster scutellaris* were examined (n = 68), and the surface coverage of three body regions was analysed: head, thorax, and abdomen, using standardized fields of view of 0.5 mm^2 (Supplementary materials S2). In addition to particle quantification, EDS was used to determine the elemental composition of surface particles. Special attention was given to identifying the presence of iron oxides (indicative of magnetic particles), as well as non-magnetic components, including silicates, sulfates, and other metal-rich phases, potentially associated with combustion or industrial emissions (Buekers et al., 2010; Mitra et al., 2020).

2.4. Statistical analysis

Statistical analyses were performed using software R v 4.4.3 (R Core Team, 2024) and RStudio (v2024.12.1) (RStudio Team, 2024). Shapiro-Wilk tests (shapiro.test(.) function, stats package) showed that traffic intensity ($W = 0.935$, $p = 0.044$), the magnetic susceptibility of the ants ($W = 0.516$, $p < 0.001$) and the magnetic susceptibility of the soil ($W = 0.857$, $p < 0.001$) all deviated from a normal distribution. The association between traffic intensity and magnetic susceptibility was therefore assessed using Spearman's rank correlation coefficient (the cor.test(.) function, stats package). As ant susceptibility exhibits high variability, the relationship with traffic was modelled on a logarithmic

scale, following the approach adopted by Mantovani et al. (2018) and Tribaudino et al. (2022). Linear and quadratic models were compared by analysis of variance on nested models and by the Akaike Information Criterion, and residual normality and homoscedasticity were verified with the Shapiro-Wilk and Breusch-Pagan tests (bptest(.) function, lmtest package). The analysis among the surfaces covered by particles from different body parts was conducted by Kruskal-Wallis test (kruskal.test(.) function, stats package) with Dunn's post hoc (dunnTest(.) function, FSA package, applying the Bonferroni correction).

3. Results

3.1. Magnetic analysis

Fig. 2 presents the magnetic susceptibility data obtained for all ant and soil samples. The analyses conducted on 34 points show a clear difference in the distribution of magnetic susceptibility values between ants and soil samples. Susceptibility values for soil samples range from 9.5×10^{-8} to 1.3×10^{-6} m^3 kg^{-1} (median 3.0×10^{-7}) and appear randomly distributed with respect to the vehicular traffic data, reflecting the natural occurrence of ferromagnetic minerals (mainly iron oxides) in the soil. Instead, ant samples exhibited a broader range of susceptibility values from 0 (i.e. less than the sensitivity of the instrument around 1×10^{-8} m^3 kg^{-1} for analysed sample masses of about 100 mg) to 2.78×10^{-6} m^3 kg^{-1}. Notably, ants collected from control sites and areas with reduced vehicular traffic exhibit very low susceptibility values, in contrast to those from heavily trafficked sites, which show the highest values (Fig. 2). Spearman's rank correlation showed a strong positive association between traffic intensity and ant magnetic susceptibility ($\rho = 0.825$, $S = 1147.9$, $p < 0.001$, $n = 34$), whereas no significant association was found for soil ($\rho = 0.167$, $S = 5453.2$, $p = 0.346$). On the logarithmic scale the relationship between traffic and ant susceptibility is well described by a straight line: $\log_{10}(\chi) = -8.219 + 1.499 \times 10^{-4}\,V$, where V is average daily traffic ($F_{1,32} = 61.84$, $p < 0.001$, $R^2 = 0.66$). Back-transformed, the fitted slope corresponds to a multiplication of ant magnetic susceptibility by a factor of 1.41 for every additional 1000 vehicles per day, and by 5.6 for every additional 5000. The residuals satisfy the assumptions of the model (Shapiro-Wilk $W = 0.956$, $p = 0.19$; Breusch-Pagan $BP = 0.001$, $p = 0.97$) and no single site dominates the fit (maximum Cook's distance = 0.12). Adding a quadratic term does not improve the model (ANOVA $p = 0.31$; $\Delta AIC = 0.9$ in favour of the linear model). The same model applied to soil susceptibility gives a slope indistinguishable from zero ($p = 0.245$, $R^2 = 0.04$), confirming that the signal is specific to the ants. Traffic intensity alone leaves approximately one third of the variance in ant susceptibility unexplained, indicating that additional site-specific or biological factors likely contribute to the observed variability and warrant further investigation.

3.2. SEM-EDS analysis

SEM-EDS analyses were carried out on two *Crematogaster scutellaris* from each sampling site and from the soil. Back-scattered electron (BSE) images were acquired for all individuals, and the number of surface particles was quantified through grayscale image processing, normalized to the explored area. For a representative subset of samples, EDS spectra were also obtained to determine the chemical composition of the particles and to discriminate between soil-derived minerals and metallic fractions (Fig. 3c and d). In general, the brighter particles observed in the SEM images were identified as iron oxides. Chemical and morphological analysis revealed that two dominant types of particles occur on the ants' body: (a) clay particles, displaying flattened morphologies and sizes on the order of several micrometres, and (b) metallic particles. The clay particles have a silico-aluminous composition, occasionally containing Ca and Fe, indicating a strong likelihood of direct transfer from soil substrates and aggregates (Fig. 3e and f). The metallic particles, ranging from a few hundred nanometres to about 1 μm in diameter, were predominantly spherical, Fe-rich, and exhibited high contrast in BSE imaging due to their elevated mean atomic number; their morphology and chemistry are consistent with traffic-related magnetic particulates.

Each ant was examined by body region (head, thorax, and abdomen) and particle counts were performed separately for each sector (Fig. 4 a, b, c). This approach allowed us to evaluate whether particle adhesion occurred uniformly across the body surface or was preferentially associated with specific anatomical regions. Results revealed a statistically significant difference (Kruskal-Wallis: $\chi^2 = 11.68$, $df = 2$, $p = 0.003$). The area covered by particles shows a higher value on the thorax ($M = 0.012$ mm^2, $SE = 0.0022$) compared to the head ($M = 0.007$ mm^2, $SE = 0.0022$) and abdomen ($M = 0.010$ mm^2, $SE = 0.003$) (Fig. 4 d).

In natural soils, iron oxides are also common products of mineral weathering, especially in clay-rich substrates, where primary minerals

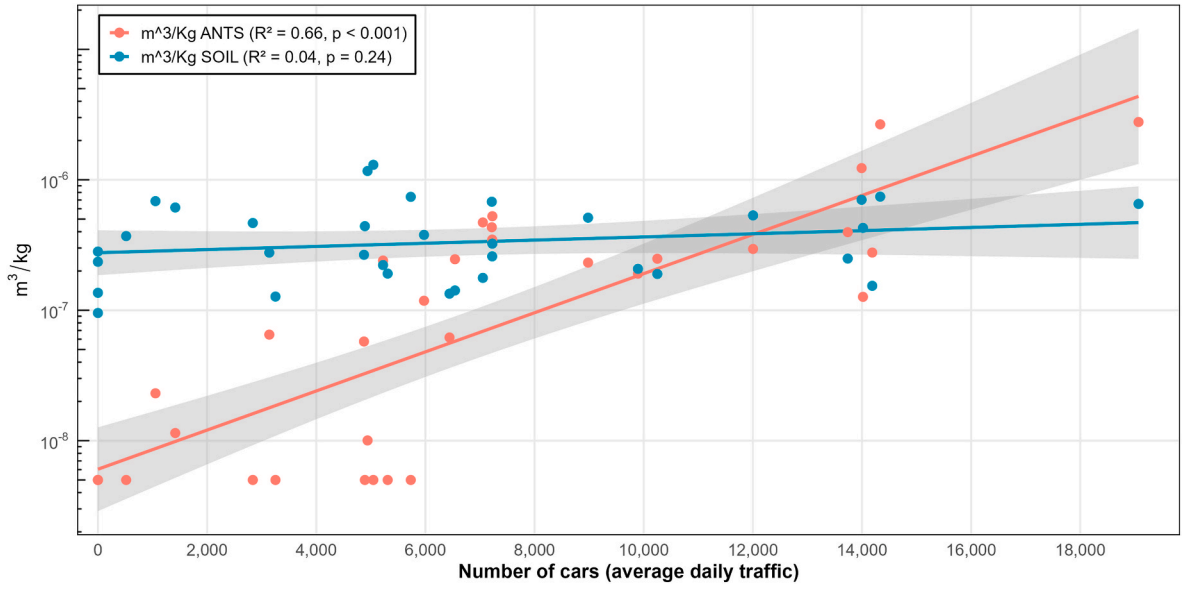


Fig. 2. Relationship between traffic intensity, expressed as average daily traffic, and magnetic susceptibility of ants (red) and soil (blue) at the 34 sampled sites. The vertical axis is logarithmic. Lines are ordinary least squares fits of log10-transformed susceptibility with their 95% confidence bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

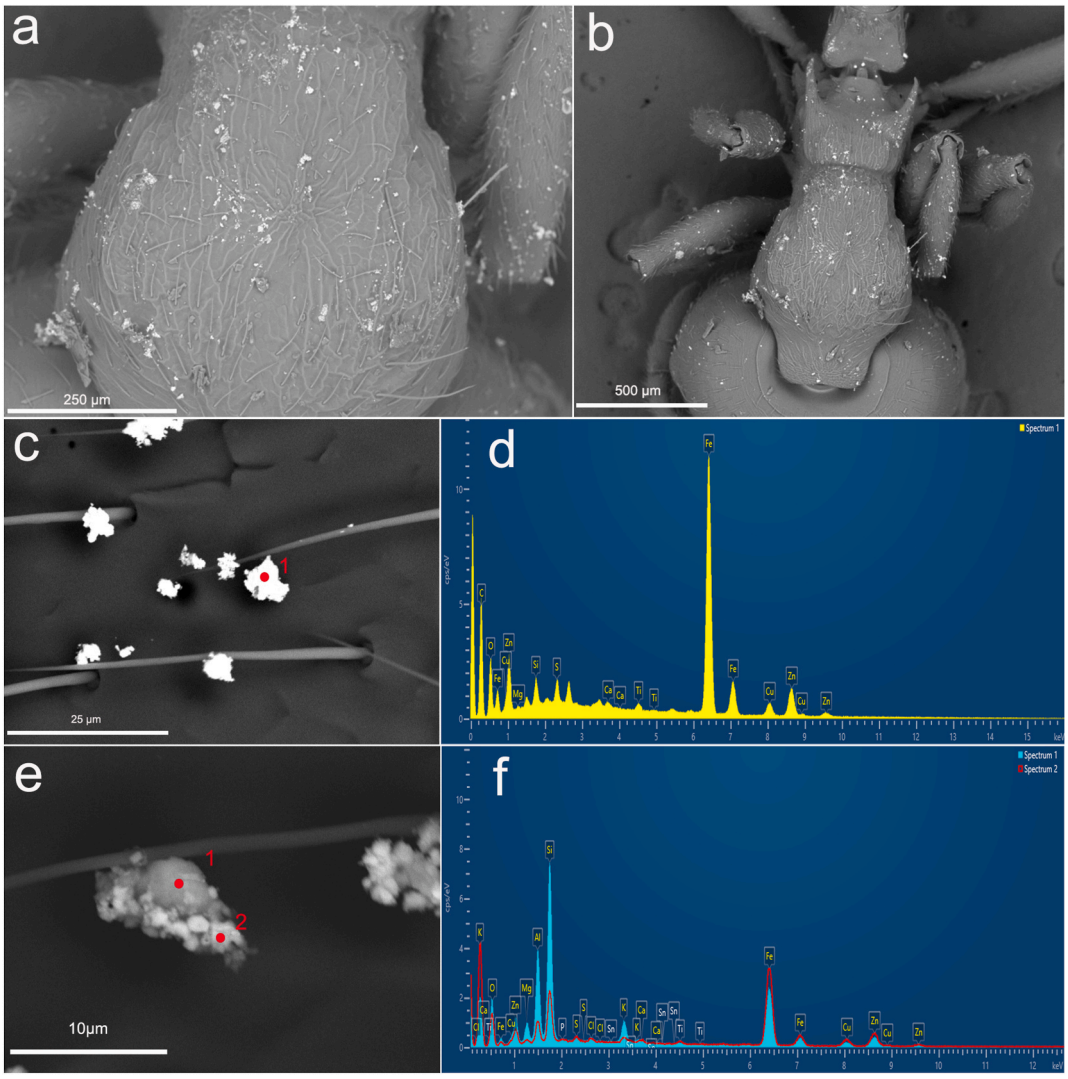


Fig. 3. SEM images at different magnifications showing numerous bright particles distributed across the ant's surface(a, b). Detail of an individual particle located between setae, selected for chemical analysis (Spectrum 1) (c). EDS spectrum of the analysed particle indicating a dominant iron (Fe) signal, consistent with iron oxide composition. The spectrum also reveals the presence of other elements such as zinc (Zn), copper (Cu), and titanium (Ti), suggesting that these iron-rich particles are doped with heavy metals(d); High-magnification SEM image showing two distinct zones within a particle cluster adhering to a hair (seta) (e). Spectrum 1 (brighter blue) corresponds to the darker central particle, identified as a clay mineral based on its high Al and Si content. Spectrum 2 (red) refers to the surrounding, brighter material, enriched in Fe, Cu, Zn, and other heavy metals, suggesting an iron-rich aggregate coating the clay granule (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

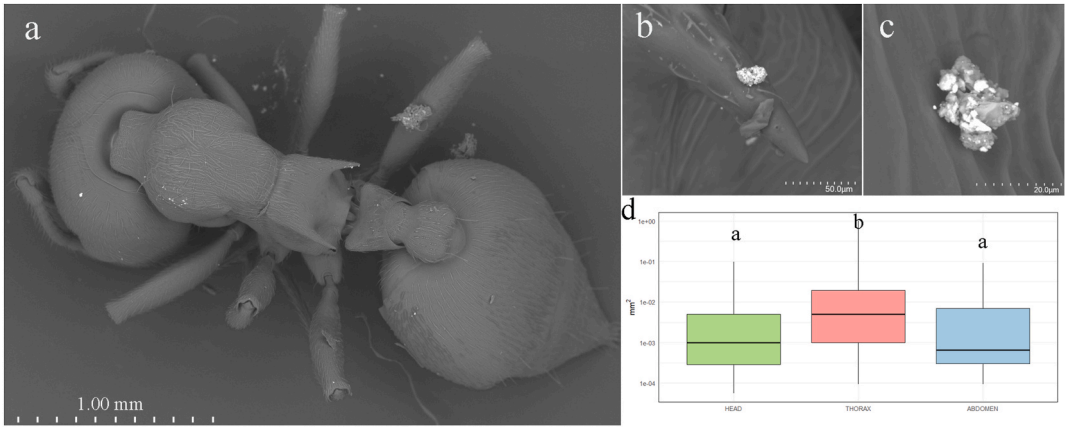


Fig. 4. SEM images of ant *Crematogaster scutellaris* (a) and the presence of metallic pollutants on the head and thorax (b, c). Graph showing the area covered by the particles in relation to the portion of the body. The bars with the same letter are not statistically different (Kruskal-Wallis, see text for further details).

are altered to form secondary oxides. However, anthropogenic particles typically exhibit distinct morphologies and size distributions compared to those of pedogenic origin. For this reason, the first step of our analysis was to evaluate the morphology of magnetic minerals within the soils. Magnetic susceptibility in soil samples showed nearly constant values across all sites, regardless of their proximity to traffic sources. This result is consistent with the fact that samples were collected at a depth of 5 cm below the surface, where background soil properties dominate over surface deposition. SEM-EDS observations confirmed that the soil matrix was largely composed of clay-rich phases, with characteristic morphologies (Fig. 5a–e) and chemical signatures corresponding to aluminosilicates containing Ca, Mg, K, and Fe (Fig. 5b–f).

In addition, a limited number of high-contrast particles of several hundred micrometres in size were detected under back-scattered electron imaging. In Fig. 5c and d a particle with a notable Ti content and a non-typical morphology of a Ti-bearing mineral suggest an uncertain origin. Although SEM provides projected geometric rather than aerodynamic dimensions, the observed particles, with sizes of several hundred micrometres, are substantially larger than traffic-related airborne magnetic particles, which are generally reported in the submicrometre-to-micrometre range and commonly display irregular, rough or frictional morphologies (Sagnotti et al., 2009; Winkler et al., 2020). Literature indicates that exhaust emissions are dominated by fine and ultrafine particles, whereas brake, and road wear generate a broader particle-size distribution and contribute substantially to PM_{10} (Thorpe and Harrison, 2008; Kukutschová et al., 2011; Grigoratos and Martini, 2015; Lopez et al., 2023). Therefore, these particles are unlikely to represent intact airborne PM_{10}, although coarse wear debris, resuspended road dust or post-depositional aggregates cannot be excluded.

4. Discussion

The results of this study support the potential of ants, specifically *Crematogaster scutellaris*, as bioindicators of traffic-derived magnetic particulate. This is primarily due to their ability to accumulate and

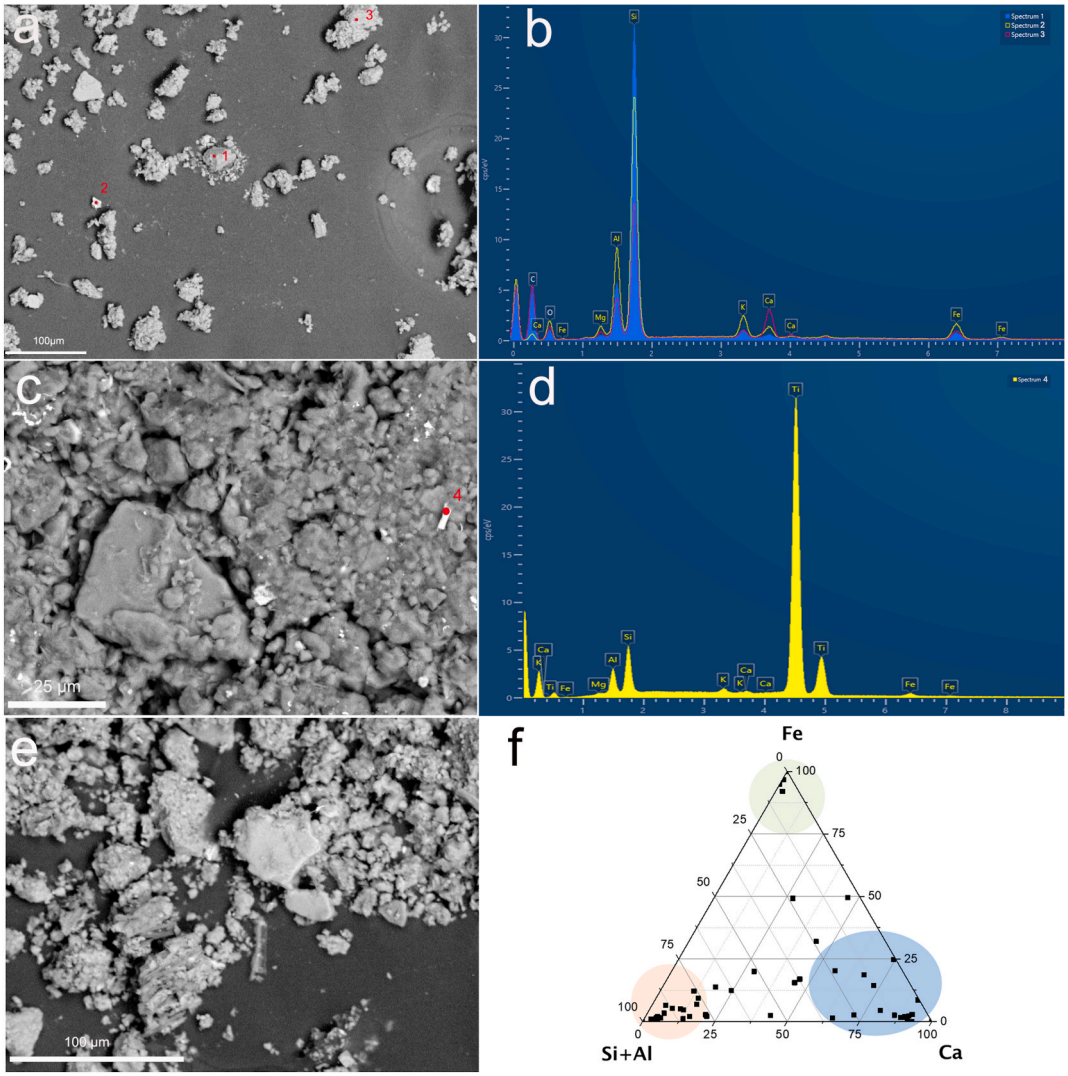


Fig. 5. *SEM micrographs and EDS analyses of the soil sample particles.* (a) Overview of the soil particle distribution, highlighting various morphologies and sizes. (b) EDS spectra of selected areas (Spectra 1–3) showing the typical chemical composition of aluminosilicates (clay minerals). (c) Higher magnification image showing the fine-grained matrix and a bright Ti-bearing particle of uncertain origin. (d) EDS spectrum of point 4 showing a notable Ti content. (e) Detailed view of platy particles, likely corresponding to phyllosilicates, consistent with their morphology observed in images (c, e). Ternary diagram showing the normalized chemical composition (wt%, total = 100%) of analysis points on ant samples. The plot represents the relative contents of Al + Si, Ca, and Fe. Blue-shaded areas correspond to aluminosilicate-rich compositions typically associated with clay minerals. Green regions indicate carbonate-rich points, mainly calcium-based, while orange areas represent iron oxide concentrations. The distribution highlights the mineralogical variability of particles adhering to or associated with the ants (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

retain magnetic and metallic particles on their body surface. Magnetic susceptibility analyses revealed clear differences between soil samples and ant samples. While soil values remained relatively constant across sites, largely independent of proximity to traffic sources, ant samples exhibited a much broader range of values, with the highest individual values exceeding the maximum recorded in any soil sample, although the median remained lower (Wong et al., 2006; Ajmone-Marsan and Biasioli, 2010). This variability suggests that ants act as dynamic accumulators of airborne particulate matter, providing a more direct reflection of anthropogenic emissions than soils, which are characterized by deeper, more stable background signals. Moreover, the soil analyses conducted beneath the exposed surface indicate that, despite the ants' ability to traverse the soil, the particulate pollutants they carry on their bodies derive from atmospheric contamination rather than from oxides naturally occurring in the soil (Chaudhari et al., 2012). Spearman's rank correlation showed a strong positive association between vehicular traffic and ant magnetic susceptibility, whereas none was found for soil. On a logarithmic scale the relationship is described by a straight line: susceptibility is multiplied by a factor of about 1.41 for every additional 1000 vehicles per day. The accumulation of traffic-derived magnetic particles by ants is therefore multiplicative rather than additive, and no threshold is observed. SEM-EDS analyses provided essential insights into the nature of the particles detected. In soils, most of the observed phases corresponded to aluminosilicates typical of clay minerals, with only limited evidence of large anthropogenic fragments. Their morphology and size were not consistent with the fine, traffic-derived magnetic PM typically associated with vehicular emissions. In contrast, ant specimens displayed two main categories of surface particles: (i) flat clay minerals likely transferred directly from substrates, and (ii) spherical, Fe-rich particles with high atomic number contrast, morphologically and chemically consistent with traffic-related magnetic particulates. EDS spectra revealed that these Fe-rich particles often contained additional elements such as Zn, Cu and Ti. Composition alone would not establish an anthropogenic origin; however the attribution rests on the convergence of morphology, size, and enrichment relative to the soils. Cu, Zn, and Sn were below the detection limit in all soil spectra but were detected in every particle analysed on the ants, whereas Fe and Ti were present in both the soil samples and the particles collected from the ants. Interestingly, particle distribution across ant body regions was not uniform. Moreover, analysis showed significantly higher numbers of particles deposited on the thorax compared to the head and abdomen. This may be explained by the thorax's larger surface area and its role in locomotion and interaction with the environment, making it more exposed to airborne deposition. Moreover, the presence of particle clusters combining clay mineral cores with Fe and heavy-metal rich coatings suggests complex aggregation processes, where natural soil particles may act as nucleation sites for anthropogenic pollutants. Finally, these results underline two important points. First, ants are capable of passively capturing airborne pollutants in a way that mirrors local traffic intensity, thus offering a biological proxy for environmental monitoring. Second, the combination of magnetic and SEM-EDS analyses provides a robust methodological framework to distinguish between natural and anthropogenic particles, strengthening the interpretation of ants as bioindicators (Underwood and Fisher, 2006; Skaldina et al., 2018; Ogrizek et al., 2022). Unlike soils, which dilute surface deposition signals with deeper mineralogical backgrounds, ants reflect short term and site-specific pollution conditions, making them particularly valuable for urban ecology and air quality assessment. While this study focuses specifically on traffic-derived magnetic particulate matter rather than on the full spectrum of atmospheric pollutants (e.g., gaseous pollutants such as NO_2 and O_3, non-magnetic PM fractions, or emissions from non-traffic sources), ant-based biomonitoring presents distinct advantages over conventional fixed monitoring stations. Fixed networks require substantial investment and infrastructure for each station, which inherently limits their spatial density and their ability to capture intra-urban variability; biomonitoring approaches, by contrast, have been shown to achieve considerably higher spatial resolution at a fraction of the cost (Moreno et al., 2003; Power et al., 2009). Because ants forage continuously and passively accumulate airborne particles on their cuticle, they integrate exposure over time and space in a way that instantaneous instrumental measurements cannot, offering a biologically meaningful, site-specific record of cumulative pollution that is otherwise difficult and expensive to obtain at fine spatial scale. Their ecological ubiquity, ease of sampling, and negligible operating cost make them particularly well suited to dense spatial screening across large urban areas, complementing the high-precision but spatially sparse data provided by fixed stations. As with other biomonitoring tools, some trade-offs remain: for instance, results are not yet standardized against regulatory instrumental thresholds and do not resolve short-term temporal variation or discriminate among all pollutant classes (Takano et al., 2024), but these are shared limitations of biomonitoring in general, and do not undermine the added value ants provide for high-resolution, low-cost mapping of traffic-related particulate pollution. Thanks to their presence and interactions, ants can constitute a valid bioindicator element for environmental monitoring (Schifani et al., 2023; Schifani et al., 2026). In conclusion, the study indicates that *Crematogaster scutellaris* could be a promising bioindicator of traffic-derived particulate pollution, both in terms of magnetic susceptibility and in the chemical nature of surface-adhering particles. Their sensitivity to vehicular traffic intensity, combined with their ecological ubiquity and ease of sampling, suggests that ants represent a promising and underutilized tool in environmental biomonitoring. However, the ant sampling, limited to a single time period, does not allow for the assessment of medium and long-term effects. Future analyses may enable the evaluation of potential variations in particulate deposition in relation to environmental variables and the presence and patrolling activity of worker ants. Future research should aim to quantify the direct relationship between particle load and health impacts on ant colonies, while also comparing their bioindicator potential with other organisms (e.g., plants, lichens, or other insects) (Mantovani et al., 2018). Such work would not only validate ants as bioindicators on a broader ecological scale but also contribute to the development of innovative biomonitoring networks.

CRediT authorship contribution statement

Daniele Giannetti: Writing – original draft, Methodology, Data curation, Conceptualization. **Antonio Verolino:** Writing – original draft, Methodology, Investigation, Data curation. **Luciana Mantovani:** Writing – original draft, Methodology, Investigation, Data curation. **Francesco Cugini:** Writing – original draft, Methodology, Investigation, Data curation. **Enrico Schifani:** Writing – review & editing, Data curation. **Daniele Pontiroli:** Writing – review & editing, Investigation. **Massimo Solzi:** Writing – review & editing, Supervision, Resources. **Donato A. Grasso:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.129010.

Data availability

The data are present in the supplementary materials

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