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To cite this version:

M. Chatelain, François Nold, Jérôme Mathieu. Metal pollution drives earthworm biodiversity in urban lawns. Science of the Total Environment, 2024, pp.169867. 10.1016/j.scitotenv.2024.169867. hal-04385393

HAL Id: hal-04385393
https://hal.sorbonne-universite.fr/hal-04385393v1
Submitted on 10 Jan 2024

Metal pollution drives earthworm biodiversity in urban lawns

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ABSTRACT

Urban soils represent hotspots of metallic trace elements (MTEs) pollution. Despite the critical impact of soil organisms on soil ecosystem services, there is limited understanding regarding the effects of MTE levels in urban soils on these organisms. This is particularly surprising considering that earthworms, key organisms for soil ecosystems, are commonly used in MTE toxicity tests. This research investigates the impact of MTE pollution on earthworm communities in lawns within the city of Paris. In this study, we sampled a comprehensive array of earthworm communities, totalling 965 individuals from 13 distinct species belonging to *Lumbricus*, *Aporrectodea*, *Allolobophora* and *Octolasion* genera. These communities were collected from three different locations within 18 parks. At these sites, we assessed the concentrations of eight metals and metalloids in the soil (As, Cd, Cr, Cu, Zn, Ni, Pb and Hg), along with selenium concentrations and eight fundamental soil parameters, to examine the association between earthworm communities and soil attributes. Median MTE concentrations exceeded recommended statutory limit values by approximately 20% (0.6 mg/kg Cd), 30% (36.8 mg/kg Cu), 40% (122.0 mg/kg Zn), and up to 90% (0.6 mg/kg Hg and 99.7 mg.kg Pb). Nevertheless, these concentrations exhibited considerable variability both between and within parks, correlating with variations in earthworm community structures. Specifically, our results highlight that Cu concentrations in the soil explain about 6% of the variation in the assemblage of earthworm species. Our findings underscore the importance of considering MTE pollution levels to enhance our comprehension of earthworm distribution in urban environments and its effects on the ecosystem services provided by urban lawns.

Keywords: urbanization, soil pollution, heavy metals, trace elements, mercury, arsenic, species composition, community assemblage

1. INTRODUCTION

Soil contamination stands out as a major concern when assessing threats to soil resources in both Europe and globally (COM, 2021; FAO, 2020). Metallic trace elements (MTEs), along with mineral oils, are the most common contaminants in European urban soil. While lithogenic sources predominantly influence MTE concentrations in soils, urban areas have become hotspots for MTE pollution due to anthropogenic sources such as vehicle emissions, industrial discharges, construction activities, improper waste disposal, and the use of metal-containing products (Alloway, 2013; Tóth et al., 2016). Despite this, the ecology of urban soils represents only 2-3% of overall research in the field of soil ecology, and this proportion has not shown significant growth over time. Furthermore, many studies on urban soils exclusively focus on investigating physicochemical conditions and biogeochemical processes, neglecting the exploration of the biota present in those environments (Guilland et al., 2018). Nevertheless, urban soils can host abundant and diverse organisms (Joimel et al., 2017), which provide crucial services such as supporting vegetation development or regulating water flow (O'Riordan et al., 2021). Determining the role of MTE pollution in the distribution of soil organisms remains a challenging yet pivotal task for creating safer, more resilient, and sustainable cities and human (United Nations, SDG 11).

Urban areas are the recipient of various MTEs such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), copper (Cu), zinc (Zn), and nickel (Ni). MTEs concentrations in urban soils can greatly vary within the urban matrix (Appleton and Cave, 2018; Bityukova et al., 2000; Cachada et al., 2013), for instance increasing in densely populated areas, industrial sites and along high-traffic roads (Cicchella et al., 2020). However, data on MTE levels in urban environments remain geographically biased and are sometimes inadequately reported (e.g. 45% of the 174 studies on European urban soils did not provide data on standard deviation (Binner et al., 2023)). Furthermore, only a limited number of studies have explored the connection between MTE pollution and soil fauna communities, and the results obtained from these investigations vary considerably (Fiera, 2009; Pižl and Josens, 1995; Santorufo et al., 2012; Tóth et al., 2023; Yu et al., 2022). The scarcity of research in this area stands in stark contrast to the abundant literature available on MTE toxicity in soil organisms, particularly earthworms (Sivakumar, 2015; Yadav et al., 2023).

Earthworms are considered keystone organisms because of their profound impact on soil ecosystems, influencing soil structure, organic matter dynamics, and the activities of other organisms. Their activity affect soil aggregation and porosity, leading to significant consequences for water, nutrients and gas flow, as well as soil erodibility. Additionally, they are associated with positive effects on plant growth and immunity (Bertrand et al., 2015; Blouin et al., 2013). The importance of earthworms in temperate environments has long been recognized, resulting in a substantial number of studies aimed at identifying the dominant biotic or abiotic factors influencing earthworm distribution (Phillips et al., 2019). These studies consistently demonstrated that earthworms can detect MTEs in soils and escape from contaminated to clean soil (e.g. Chatelain, 2023; Langdon et al., 2005; Lowe et al., 2016; Lukkari and Haimi, 2005). However, MTE sensitivity (e.g. survival, reproduction) varies between species (Chatelain, 2023; Langdon et al., 2005, 2001; Lukkari et al., 2005). More specifically, previous research indicates that epigeic species may exhibit lower sensitivity to MTEs (Langdon et al., 2005; Lowe et al., 2016; Lukkari et al., 2005). This could be attributed to their proximity to the soil surface, exposing them to a variety of conditions that may not all be conducive to optimal growth. Consequently, they need to be resilient enough to withstand such environmental variability (Tomlin, 1992). Furthermore, invertebrates show a reduced sensitivity to xenobiotics as body size increases (Baird and Van den Brink, 2007; Ippolito et al., 2012). Finally, different earthworm species may be more or less exposed to MTEs in the soil, depending on their ecological traits, particularly their position in the soil and dietary habits. Epigeic earthworms, for example, inhabit parts of the soil with abundance organic matter, typically at or near the soil surface, where they feed on leaf litter, decaying plant roots or dung. Consequently, their diet may contain lower concentrations of MTEs compared to the diet of endogeic earthworms, which also consume soil where MTE can accumulate. Moreover, MTEs are expected to be less available at the soil surface than at deeper horizons utilized by endogeic and anecic earthworms (Bravin et al., 2009; Van Herwijnen et al., 2007). Supporting this observation, the limited number of studies that have explored the impact of MTE levels on earthworm communities on site have shown a change in community composition, along with a decline in earthworm richness, density, and/or biomass as MTE contamination increases near mining sites (Huang et al., 2021; Lévêque et al., 2015; Nahmani et al., 2003). The potential existence of comparable relationships between soil MTE pollution and earthworm communities in urban environments has yet to be investigated (Rutgers et al., 2016; Tóth et al., 2020).

To address this gap, we sampled earthworm communities within the lawns of 18 parks in Paris (France), where we measured soil physicochemical properties, including the concentrations of eight metals and metalloids. Based on earthworm species assemblage in grasslands in the northern part of France, we expected to encounter up to 17 earthworm species and communities to be composed of seven to nine species, dominated by the species *Aporrectodea caliginosa*, *Allolobophora chlorotica*, *Aporrectodea longa*, and *Aporrectodea icterica* (Decaëns et al., 2008). The 18 parks included in this study were created between 1840 and 2007, with soil imported from market-garden or agricultural lands. Due to variations in park age and soil origin, differences in soil physicochemical characteristics were anticipated between parks. Additionally, local renovations and varying proximity to roadways led us to expect variations in physicochemical characteristics within parks as well. Therefore, we hypothesised that (i) earthworm species assemblage differ between and within parks in response to variations in MTE levels, (ii) earthworm abundance and richness decrease with increasing MTEs concentrations in the soil and (iii) the association between earthworm community composition and MTE levels in the soil depends on soil physicochemical characteristics affecting MTE availability in the soil.

2. METHODS

2.1 Study sites

To investigate earthworm response to soil physico-chemical characteristics, earthworms and soils were sampled from lawns at 18 parks in Paris (Figure 1). Three distinct locations, each situated between 40 and 370 meters apart, were sampled at every park, resulting in a total of 54 sampling sites. The selection of parks aimed for a relatively even distribution across the city, intending to encompass a range of park sizes and ages (see Supplementary Material); soil was always sampled from open areas, away from trees and bushes. As parks have been created or renovated, soil has always been imported. The thickness of the material varies between 0.30m and 2.00m, depending on the type of plant cover (lawns, flowers, shrubs, trees). The oldest gardens have soil imported from the inner suburbs of Paris (often sandy, calcareous market-garden soils, frequently contaminated with MTEs by irrigation with wastewater and/or sewage sludge); while the most recent gardens have soil imported from locations further away from the city (often silty, non-calcareous agricultural soils, only slightly contaminated with MTEs). However, partial renovations or extensions were carried out in certain parks, resulting in the introduction of new soil to specific areas (see Supplementary Material).

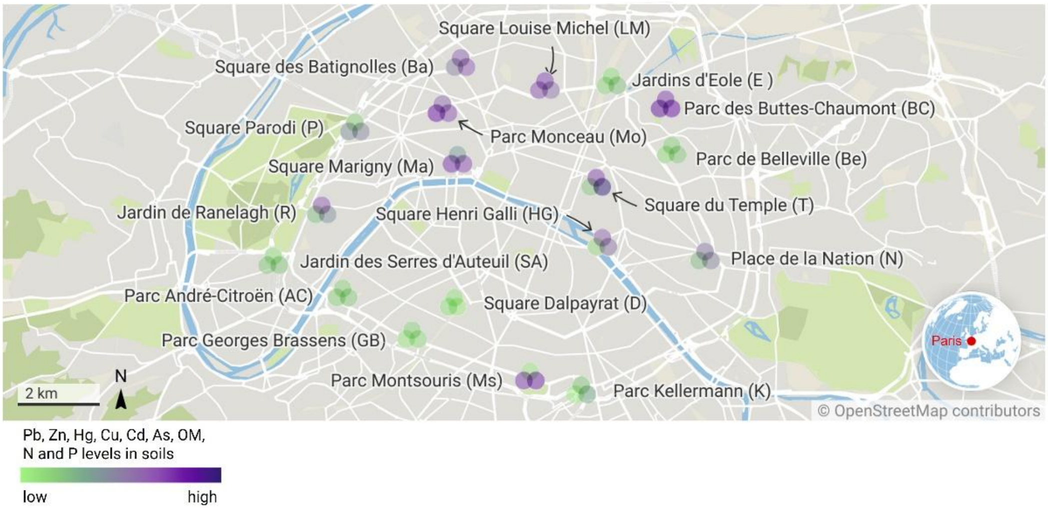


Figure 1. Sampling sites – Map highlighting the location of the 18 Parisian parks where earthworms and soils were sampled. They were sampled at three different locations per park, symbolized by the three circles. The colour of the circles represents the coordinates of the sampling sites on the first dimension of the cluster analysis conducted on the 16 soil physico-chemical variables (see section 4.1).

2.2 Earthworms' communities & soil sampling

Free-living earthworms were collected from February to May 2016, a time when they are particularly active, at each 54 sampling point. At each point, one 30 x 30 x 30 cm block of soil was extracted using a spade. The topsoil (from the surface to 5 cm below the surface) and the subsoil (from 10 to 30 cm below the surface) were separated before being manually searched for earthworms. Earthworms were kept in small terrariums (12 cm x 10 cm x 8 cm) to be transferred to the laboratory for identification. For the purpose of soil physico-chemical analyses, at least 1 L of soil was sampled from the topsoil and from the subsoil, separately.

2.3 Earthworm identification

Earthworms were identified in the lab using morphological characteristics (Bouché, 1972; Sims and Gerard, 1985). The species names used herein are conformed to the Fauna Europaea web site (http://www.faunaeur.org/index.php). Species were categorized within the four functional groups: epigeic (*Lumbricus castaneus*, *Lumbricus rubellus rubellus*, and *Lumbricus rubellus castanoides*), epi-anecic (*Aporrectodea giardi*, *Lumbricus terrestris*, *Lumbricus herculeus*, and *Aporrectodea longa*), endogeic (*Aporrectodea caliginosa*, *Aporrectodea rosea*, and *Aporrectodea icterica*,) and intermediate (*Allolobophora chlorotica*, *Octolasion lacteum*, and *Octolasion cyaneum)* (Bottinelli et al., 2020). For each 54 sample of 27 dm^3 each, we calculated species abundance (*i.e.* the number of individuals per species), juvenile abundance, (*i.e.* the number of juveniles, whatever the species), earthworm abundance (*i.e.* the total number of individuals, including juveniles), and functional group abundance (*i.e.* the number of individuals per functional group). We also calculated species richness (*i.e.* the number of different species, excluding juveniles), and species frequency (*i.e.* the percentage of sampling sites where each species occurred).

2.4 Soil preparation & physicochemical analyses

Each sampling site (n=54) was characterised by 16 physico-chemical characteristics. These encompass the concentrations of eight metals or metalloids (As, Cd, Cr, Cu, Zn, Ni, Pb and Hg), as well as the concentration of selenium (Se) – another trace element of health concern. The set of characteristics also includes basic soil parameters, specifically pH, electrical resistivity (i.e. how much the soil conducts electric current, which varies with salt content), total carbonates (CO_3), organic nitrogen (ON), soil organic matter (SOM), assimilable phosphorus (P), and the carbon to nitrogen ratio (C/N). These parameters were measured separately in both the topsoil and subsoil. Analyses were carried out by the Laboratory of Agronomy of the Paris City (France) using the methods summarised in Table 1.

2.5 Statistical analyses

Statistical analyses were performed using R software (version 4.1.3).

Soil characteristics – To compare soil chemical characteristics between the 54 sampling points, we performed multiple linear models ('lm' function) with one of the 32 soil physico-chemical characteristic as dependant variable and the park as explanatory variable. In addition, we performed a cluster analysis on soil physico-chemical characteristics per sampling site ('kmeans' function in the 'stats' package). The optimal number of clusters was determined using within cluster sums of squares method, aka elbow method ('fviz_nbclust' function in the 'factoextra' package). The results of cluster analysis were visualized using the 'fviz_cluster' function ('factoextra' package). To determine the soil physico-chemical characteristics underlying those clusters, the coordinates of each sampling point on the two-dimensional space were extracted and we performed correlation test between these coordinates and each 32 physico-chemical characteristics ('cor.test' function in the 'rstatix' package).

Earthworm abundance & richness – To investigate the association between earthworm total abundance and soil physico-chemical properties, we performed multiple linear models ('lm' function) with the log-transformed number of earthworms sampled at each sampling point as the dependent variable and one of the 34 soil physicochemical characteristic as the explanatory variable. Because earthworm abundance and richness are expected to vary between parks due to different histories and location, the park was added as covariate. Because soil pH and content in organic matter is known to influence MTE bioavailability (Nahmani et al., 2007), hence toxicity, pH or SOM and their interaction with the MTE concentrations were added as explanatory variables in models including one of the MTEs as explanatory variable. For those models, we performed a backward stepwise selection using the AIC.

When interaction terms were retained in the models, simple slope analyses using Johnson-Neyman intervals were performed using the ‘sim_slopes’ function of the ‘interactions’ package (Long, 2019). To identify different responses between species and age categories, we run similar models (i) for each species present in at least 10% of the sampling sites, (ii) on adults only (whatever the species) and (iii) on juveniles only (whatever the species). We used similar models to measure the association between earthworm richness (the number of species at each sampling point) and soil physico-chemical properties. The analysis comparing earthworm responses to soil physico-chemical properties among functional groups was discontinued due to the predominant representation of endogeic species among the sampled earthworms’ communities.

Species composition – Differences in the taxonomic composition of earthworm communities were tested using a linear constrained ordination method based on Hellinger distances (tb-RDA) on log-transformed abundance data using the ‘rda’ function of the ‘vegan’ package (Oksanen et al., 2013). Only the six species that were present in at least 10% of the 54 sampling sites were included. The global model included the 32 soil physico-chemical characteristics as constraining variables. The soil characteristics that were the most important to explain the compositional changes were identified by forward selection using the ‘ordistep’ function (‘vegan’ package). The variance explained by the constrained ordination and by each of the components was tested by Monte Carlo permutation test (‘anova’ function in the package ‘vegan’). Variation partitioning was calculated using the ‘rdacca.hp’ function of the package of the same name (Lai et al., 2022). The contribution of each selected constraining variable to the components of the ordination was fitted by linear contribution scores using the ‘envfit’ function. The ordination diagrams were created with the ‘ordiplot’ function from ‘vegan’, which coordinates were extracted for use in ‘ggplot2’ (Wickham et al., 2016).

3. RESULTS

3.1 Soil characteristics

Soil physico-chemical characteristics varied significantly between the 54 sampling points; the concentrations of assimilable phosphorous (P), total carbonates (CO_3), mercury (Hg), lead (Pb), copper (Cu), cadmium (Cd) and zinc (Zn) in both topsoils and subsoils varied the most between sampled soils (Figure 2). Importantly, despite the wide range of Hg, Pb, Cu, Cd and Zn concentrations, the soil of the 18 Parisian parks were in general characterised by high concentrations of these five MTEs, for which median concentrations were ca. 20% (Cd), 30% (Cu), 40% (Zn) and 90% (Hg and Pb) higher than the recommended statutory limit values (Table 3). The majority of the soil physicochemical characteristics were more variable between the 18 Parisian parks than within each park; between 24% and 84% of the variability among sampling points was attributed to variations between parks; only the C/N ratio and resistivity in the subsoil, and chromium concentrations in the topsoil were not significantly different between parks (Table 2). However, the cluster analysis revealed that soil composition within sites can be highly variable: the three samples per park had comparable soil composition in only half of the parks (Figures 1 and 3). Variations in Pb, Zn, Hg, Cu, Cd, soil organic matter (SOM) and organic nitrogen (ON) concentrations in the topsoil and subsoils, and in assimilable phosphorous concentrations (P) in the topsoil, mainly accounted for the dissimilarities between the three clusters (Figure 3). Consistently, several chemical characteristics were strongly correlated. This was particularly notable for concentrations of Pb, Zn, Hg, Cu, Cd and P (Figure 4).

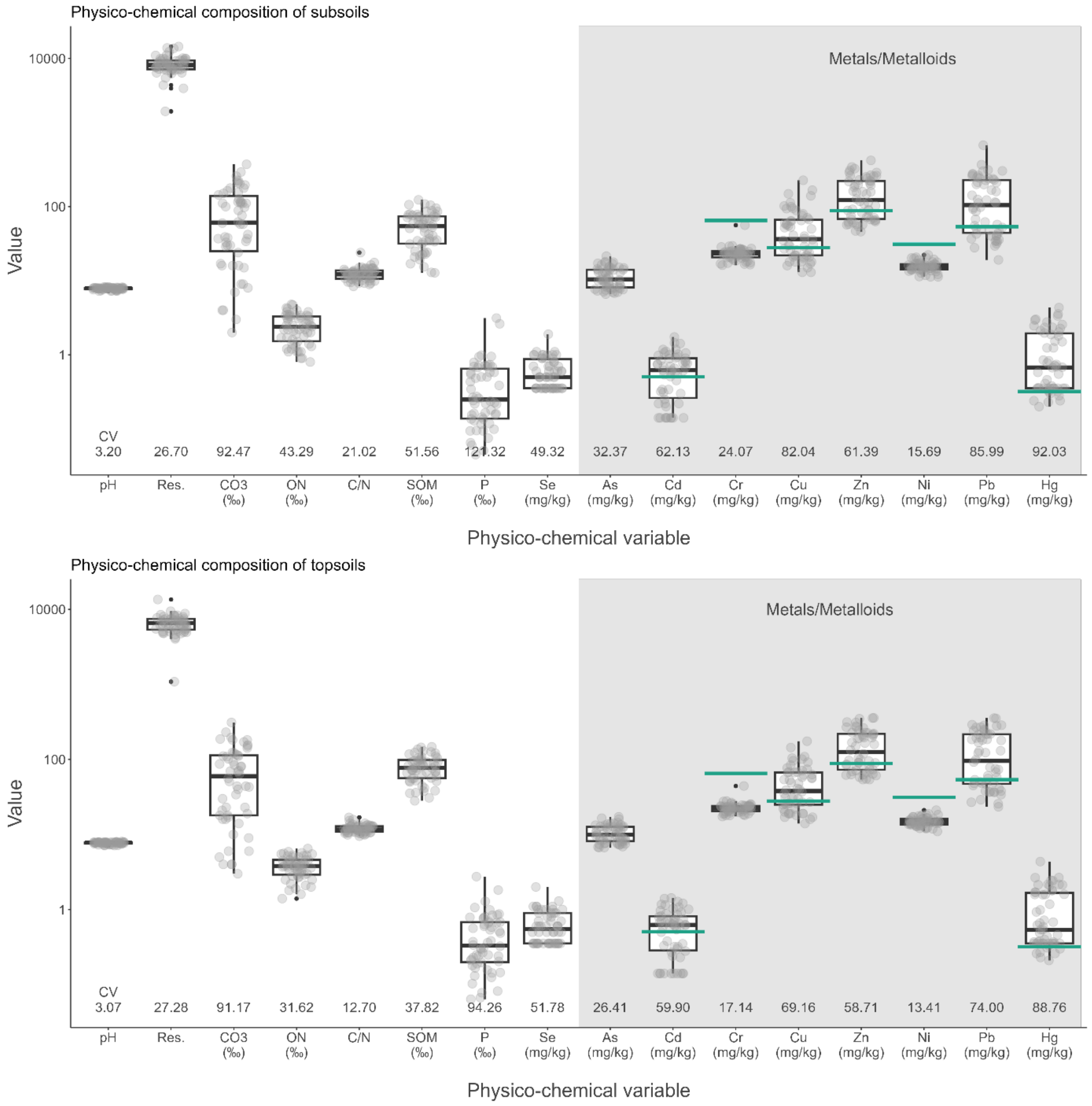


Figure 2. Soil physico-chemical composition – Boxplots highlighting the distribution of each of the variables measured to characterise the physico-chemical composition of the topsoils (top) and subsoils (bottom) of 18 Parisian parks. For each variable, we indicate the coefficient of variation (CV) and for each metal/metalloid, we indicate the recommended statutory limit value (see Table 3).

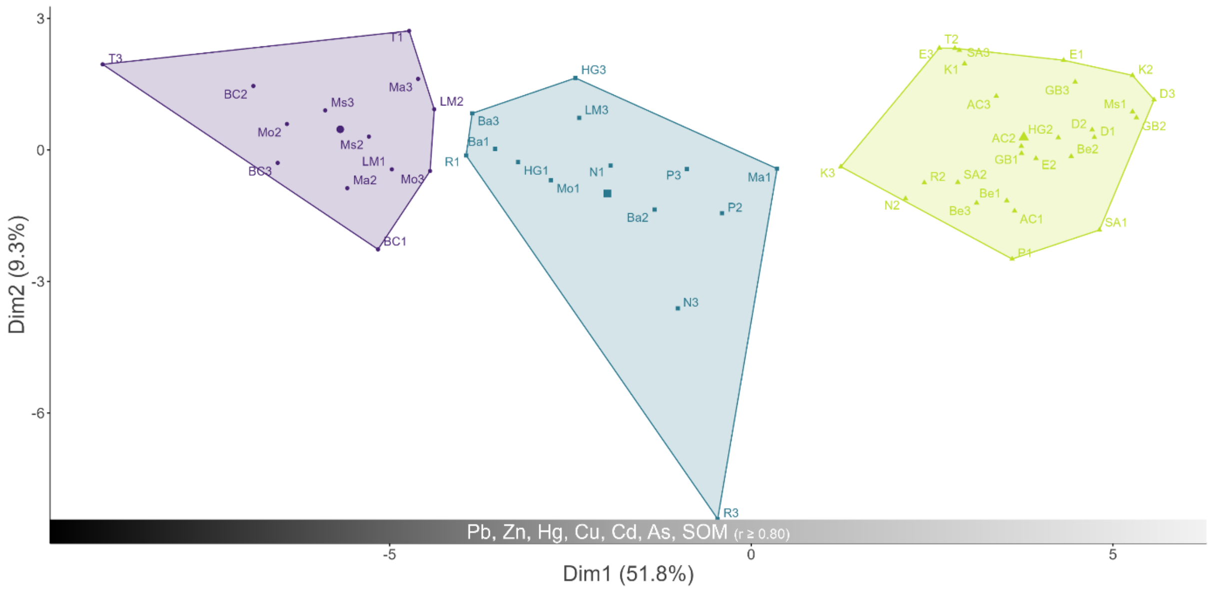


Figure 3. Physico-chemical composition-based similarities between sampled soils – K-means clusters computed from the 16 physico-chemical variables measured in the topsoil and the subsoil at the 54 sampling sites (*i.e.* three sites per each of the 18 parks). Pb, Zn, Hg, Cu, Cd, As, and SOM concentrations mainly contributed to Dim1 (|r|≥80; see Supplementary Material).

R: Jardin de Ranelagh, E: Jardin d'Eole, N: Place de la Nation, AC: Parc André-Citroën, Be: Parc de Belleville, BC: Parc des Buttes-Chaumont, SA: Jardin des Serres d'Auteuil, GB: Parc Georges Brassens, K: Parc Kellermann, Mo: Parc Monceau, Ms: Parc Montsouris, Ba: Square des Batignolles, D: Square Dalpayrat, T: Square du Temple, HG: Square Henri Galli, LM: Square Louise Michel, Ma: Square Marigny, P: Square Parodi.

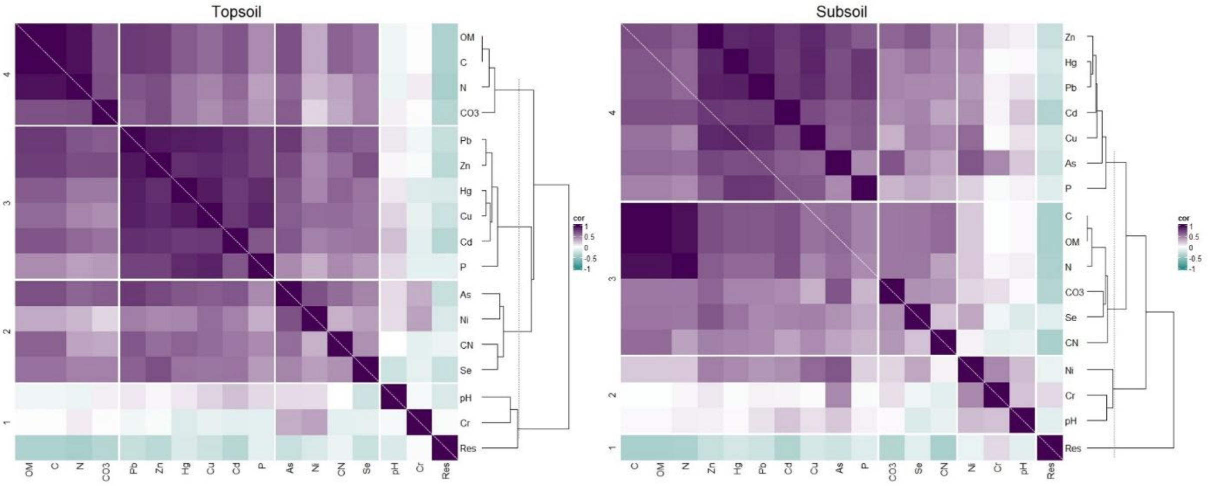


Figure 4. Covariance between physico-chemical variables – Heatmaps based on Pearson

correlation coefficients between each pair of soil physico-chemical characteristic in the topsoil and the subsoil.

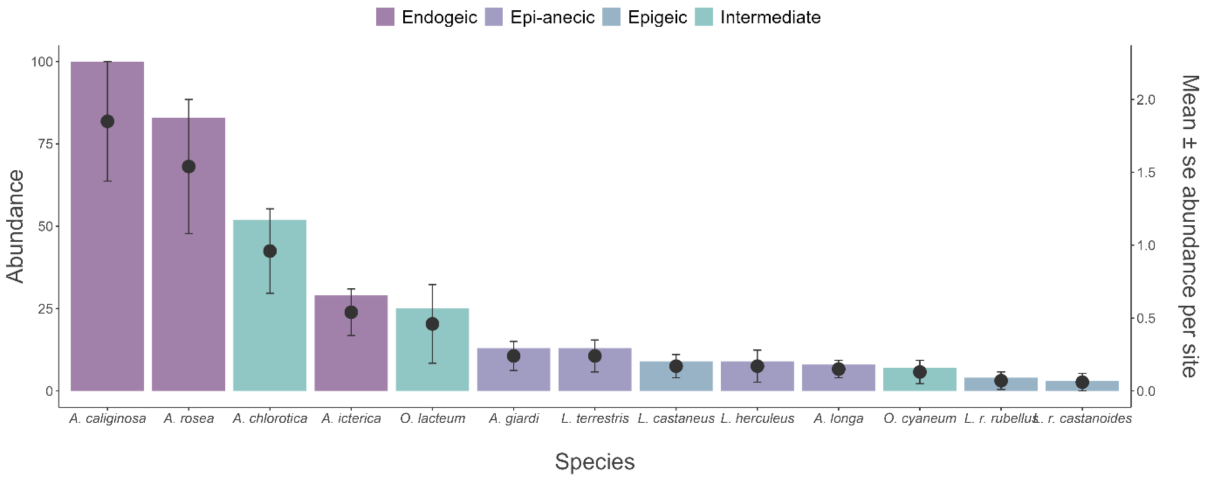
P: assimilable phosphorous, Cu: copper, Hg: Mercury, Zn: zinc, Pb: lead, As: arsenic, Cd: cadmium, Ni: nickel, SOM: soil organic matter, C:N: carbon/nitrogen ratio, Cr: chromium, ON: nitrogen, Se: selenium, CO3: total carbonates, Res: resistivity.

3.2 Earthworm communities

3.2.1 Species composition

A total of 965 earthworms were sampled. From them, 355 were sorted into 13 species; the remaining 610 individuals were juveniles. Overall, *A. caliginosa* and *A. rosea* were by far the most abundant and the most frequent species in the 18 Parisian parks, followed by *A. chlorotica* and *A. icterica* (Figure 5). Quite similarly, the variation in earthworm abundance between sampling sites was the highest in *A. caliginosa*, *A. rosea*, followed by *A. chlorotica* and *O. lacteum* (Figure 5). Endogeic, intermediate, epi-anecic, and epigeic earthworms accounted for 60%, 24%, 12% and 5% of the adults, respectively.

(a)



(b)

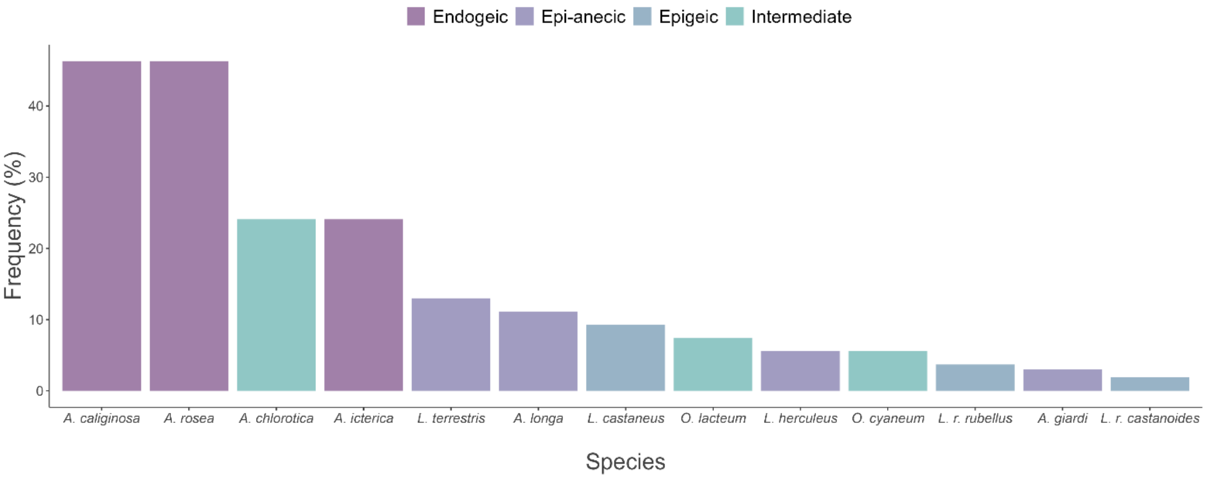


Figure 5. Species abundance and frequency – (a) Aggregate abundance (across all sites combined) and mean ± se abundance per sampling site (i.e. mean number of individuals per 27 dm^3 sample) per each 13 species sampled across the 18 Parisian parks and (b) percentage of sampling sites (*i.e.* frequency) where occurred each species of earthworms.

3.2.2 Variation in earthworm abundance

Earthworm abundance per site ranged from one to 74 individuals per 27dm^3, with a median of 13.5 individuals. Fifteen out of the 34 models explained a higher proportion of the variation in earthworm abundance than the model accounting for inter-park variations only (i.e. these models explained more than 58.6% of the variation in earthworm abundance between samples). However, the relationship between earthworm abundance and the physico-chemical variables was significant in only ten of the models. Earthworm abundance decreased with increasing P, Cu, Hg, Zn and Pb concentrations in the topsoil and the subsoil; the negative relationship between earthworm abundance and Hg concentrations in the subsoil tended to disappear in soils rich in organic matter (when SOM > 88.46 ‰); these variables explained between 4.8% and 10.4% of the variation in earthworm abundance (Figure 6a). Because P, Cu, Hg, Zn and Pb levels are highly correlated with each other (see cluster analysis in section 4.1), some of the relationships may result from covariations.

The relationships between earthworm abundance and soil physico-chemical properties were largely similar when considering adults only; only the relationships between abundance and Zn, Cu or Hg concentrations in the subsoil were marginally non-significant; the other variables explained between 7.9% and 17% of the variation in the abundance of adults (see Supplementary material). When considering juveniles only, the relationships between abundance and Cu or Hg concentrations in the topsoil and the subsoil, or P concentrations in the topsoil were conserved; they explained between 3.9% and 6.4% of the variation in the abundance of juveniles (see Supplementary material). Finally, the abundance of *A. longa* tended to decrease with increasing Cu concentrations in the subsoil (p=0.059). None of the relationships were significant when considering the other species alone.

3.2.3 Variation in species richness

Species richness per site ranged from zero to six, with a median of two species. Seventeen out of the 32 models explained a higher proportion of the variation in earthworm richness than the model accounting for inter-park variations only (*i.e.* these models explained more than 25.8% of the variation in earthworm richness between samples). However, the relationship between earthworm richness and the physico-chemical variable was significant in only six of the models (Figure 6b): earthworm richness decreased when increasing Pb, Hg, Cu, Zn and Cd concentrations in the subsoil but only when the subsoil was relatively poor in organic matter (when SOM < 65.07 ‰, 63.33 ‰, 58.71 ‰, 59.99 ‰ and 47.14 ‰, respectively). On the contrary, earthworm richness decreased with increasing Cr concentrations in the subsoil when the subsoil was relatively rich in organic matter (when SOM > 66.66 ‰). These variables explained 6.2% to 15.8% of the variation in earthworm richness. Moreover, earthworm richness tended to decrease with increasing P concentrations in the topsoil (P=0.074); this variable explained 4.6% of the variation in earthworm richness.

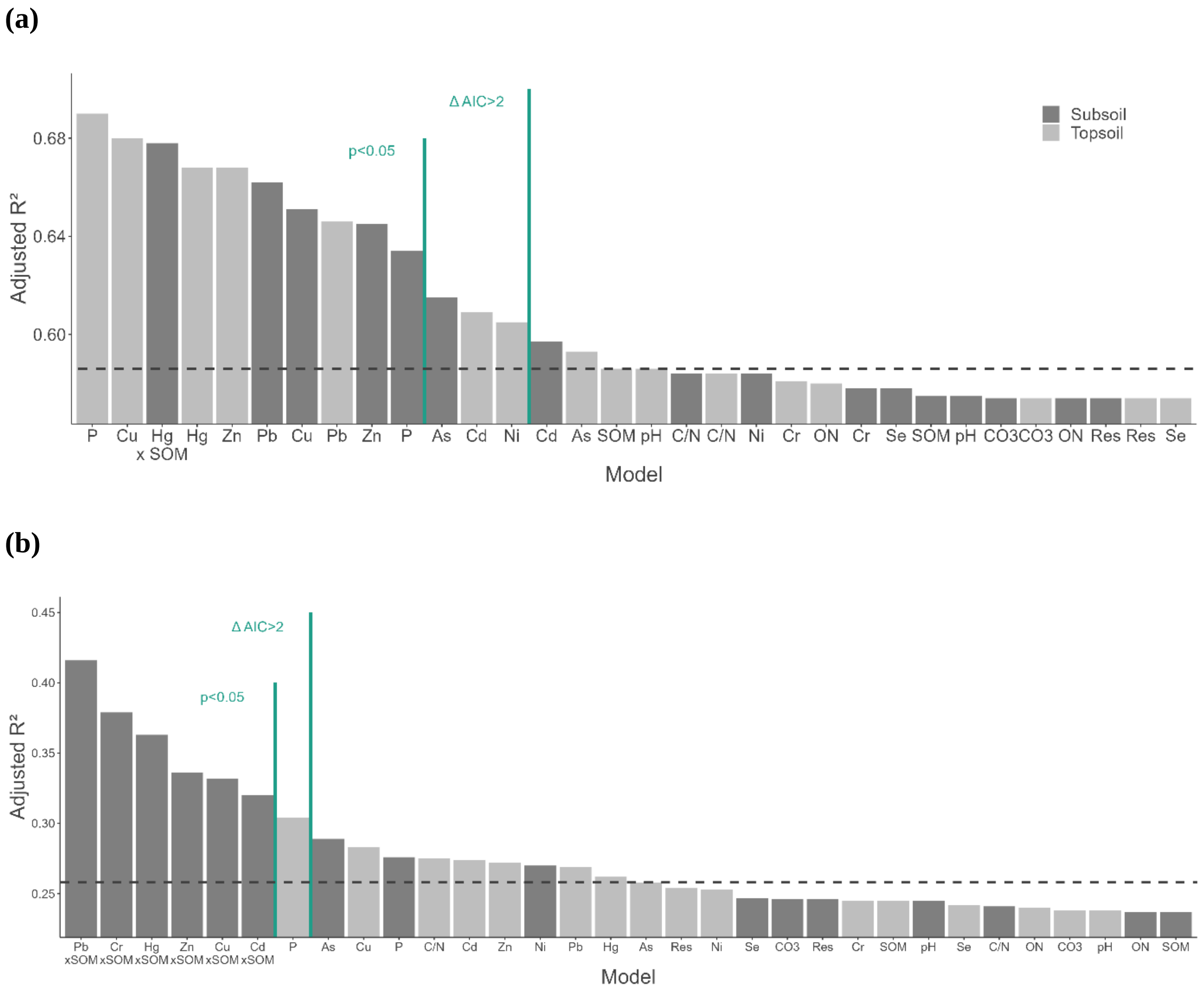


Figure 6. Best environmental variables explaining earthworm abundance and species richness – Adjusted regression coefficient R for each model with earthworm (a) abundance or (b) species richness as dependent variable and one of the 17 physico-chemical variables measured in the subsoil (in black) and topsoil (in grey) as explanatory variable. The park name was always added as categorical explanatory variable. We highlight the adjusted R of the model including only the park name as explanatory variable (R = 0.586 and R = 0.258, respectively), as well as the models with an AIC at least two points lower than the model including the park name only (Δ AIC > 2), and in which the association between earthworm abundance and the physico-chemical variable was significant (p-value < 0.05).

P: assimilable phosphorous, Cu: copper, Hg: Mercury, Zn: zinc, Pb: lead, As: arsenic, Cd: cadmium, Ni: nickel, SOM: soil organic matter, C:N: carbon/nitrogen ratio, Cr: chromium, N: nitrogen, Se: selenium, CO3: total carbonates, Res: resistivity.

3.2.4 Variation in community composition

The 16 physico-chemical variables measured in the subsoils and topsoils explained together 59% of the variation in earthworm community structure among the 54 sampling sites. However, only two variables significantly explained such variation, namely copper concentrations in the subsoil (F=3.20, P=0.020), and pH in the subsoil (F=2.43, P=0.030); they explained 5.7% and 4.3% of the variation in earthworm community structure, respectively.

The first and second components of the redundancy analysis explained 6% (F=3.79, P=0.003) and 4.1% (F=2.31, P=0.054) of such variation. RDA1 is strongly associated to Cu concentrations in the subsoil (r=-0.90), while RDA2 is strongly associated to the pH of the subsoil (r=-0.82). The ordination along RDA1 is driven by the variation in the abundances of *A. chlorotica*, and *A. longa*, which contributes to 78% and 16% to RDA1, respectively: the relative abundance of *A. chlorotica* and *A. longa* increased and decreased with increasing Cu concentrations in the subsoil, respectively (Figure 7). The ordination along RDA2 is driven by the variation in the abundances of *A. rosea*, *A. longa*, *A. icterica* and *A. caliginosa*, which contribute to 38%, 23%, 22% and 10% to RDA2, respectively: the relative abundance of *A. rosea* and *A. icterica* tended to increase, while the relative abundance of *A. longa* and *A. caliginosa* tended to decrease with increasing the pH in the subsoil, respectively (Figure 7).

These results are consistent with the distribution of each of the species (Figure 8). More specifically, while the median Cu concentration in the subsoil was 36.5 mg/kg, *A. chlorotica* were mainly present when concentrations were between 41 and 113 mg/kg. Because Cu, Hg, Pb, Zn, Cd, Ar, P, OM and N concentrations in the soil were positively correlated (see Figure 4), *A. chlorotica* occurred in soils with relatively high concentrations of these elements. On the contrary, *A. longa* mainly occurred in soils with low Cu concentrations in the subsoil (with concentrations between 16.5 and 23.5 mg/kg); interestingly, the physico-chemical characteristics of the soils where *A. longa* mainly occurred varied little (Figure 8). Finally, while the six species mainly occurred in slightly alkaline soils (with pH over 7), *A. icterica* and *A. rosea* also frequently occurred in moderately alkaline soils (with pH over 8) (Figure 7 & 8).

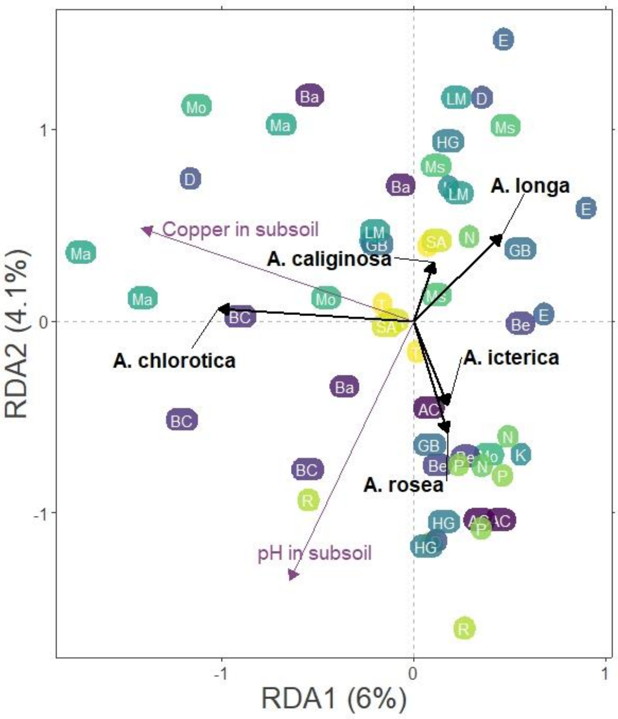


Figure 7. Species composition – Triplot representing the 1st (RDA1) and 2nd (RDA2) components of the redundancy analysis on earthworm species abundance at each 54 sampling sites. The sites are ordered based on their species composition. Different symbols highlight the 18 Parisian parks. The main species explaining the ordination are highlighted by vectors (*i.e.* the species contributing to 10% or more of the RDA components).

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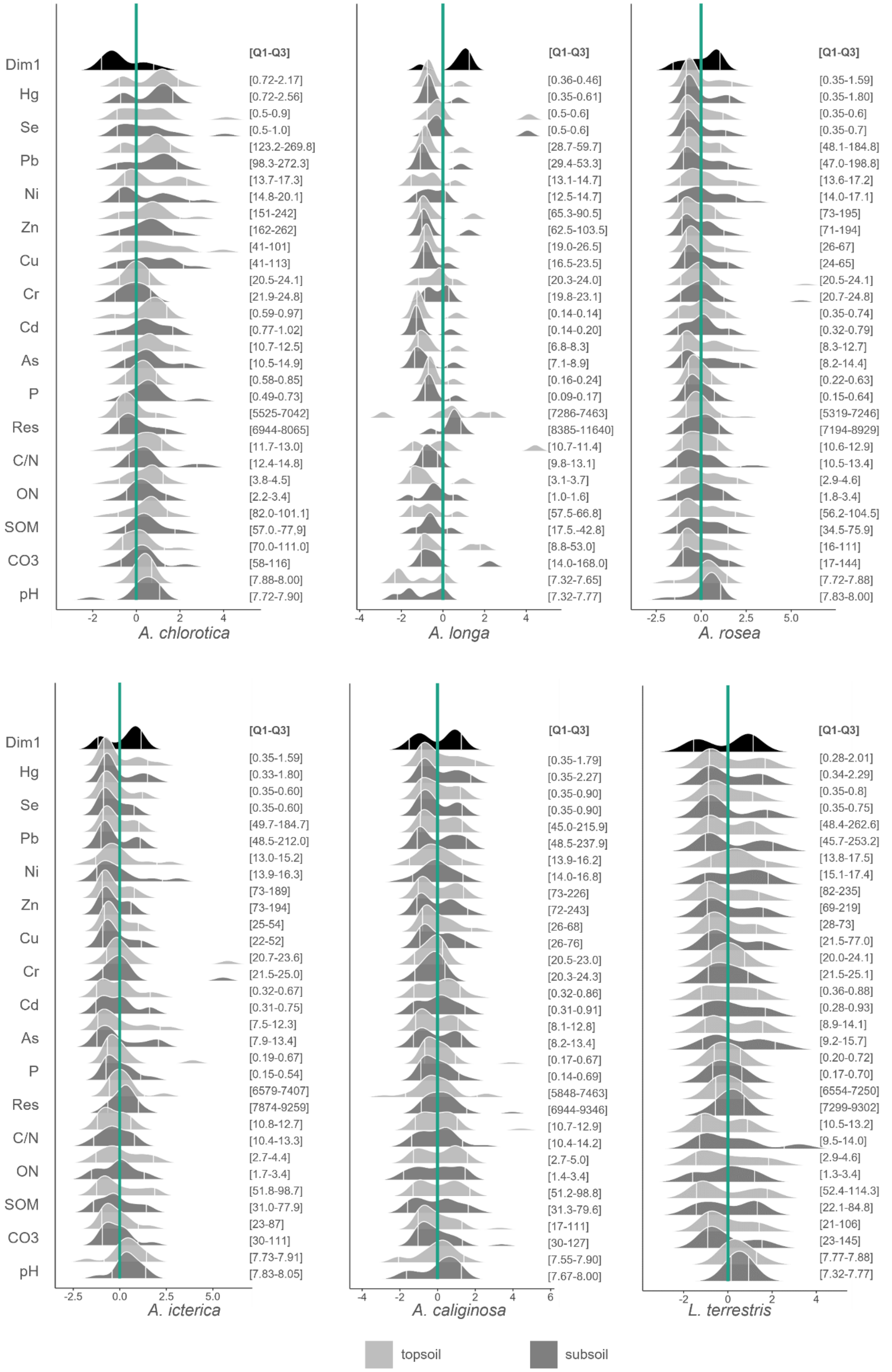

Figure 8. Species distribution – Density plots representing the distribution of the physico-chemical variables and of the composite variable dim1 in the soils where the focal species was present.

Concentrations were scaled and centred on the overall mean concentration. Therefore, values lower and higher than zero are relatively low and relatively high, respectively. For each physico-chemical variable, we also highlight the range of concentrations that includes 50% of the sites where the species was present (*i.e.* the 1st and the 3rd quartiles of the distribution). Those were calculated for the six earthworm species that were present in at least 10% of the 54 sampling sites.

Hg: mercury (in mg/kg), Se: selenium (in mg/kg), Pb: lead (in mg/kg), Ni: nickel (in mg/kg), Zn: zinc (in mg/kg), Cu: copper (in mg/kg), Cr: chromium (in mg/kg), Cd: cadmium (in mg/kg), As: arsenic (in mg/kg), P: assimilable phosphorous (in ‰), Res: resistivity (in in ohm.cm), C.N: carbon to nitrogen ratio, ON: organic nitrogen (in ‰), SOM: soil organic matter (in ‰), CO_3: total carbonates (in ‰).

4. DISCUSSION

A total of 965 earthworms belonging to 13 different species were sampled at three different sites within lawn of each of the 18 Parisian parks included in this study. At these sites, we measured the concentrations of eight metals and metalloids (As, Cd, Cr, Cu, Zn, Ni, Pb and Hg), along with selenium (Se), alongside basic soil parameters (pH, P, resistivity, C/N, ON, SOM, CO_3), and tested the link between earthworm communities and soil characteristics while accounting for variations between parks. Besides documenting on MTE pollution levels in the European city of Paris and their variations at short and very short scale, we were able to identify Cu, Hg, Zn and Pb levels, together with P and pH, as important abiotic parameters shaping earthworm communities in urban lawns.

4.1. Variation in MTE levels in urban lawns

Overall, the lawns in Parisian parks exhibit high levels of Hg, Pb, Zn, Cu, and Cd. Actually, median concentrations of these MTEs were 90% (Hg and Pb), 40% (Zn), 30% (Cu) and 20% (Cd) higher than the recommended statutory limit values (Mathieu et al., 2008). The mean concentrations observed in our study closely resemble those obtained in 2015 by Foti and colleagues across 30 urban lawns in Paris (Foti et al., 2017). These elements are characteristic of urban soils (Ajmone-Marsan and Biasioli, 2010; Cachada et al., 2013; Chatelain, 2023; Foti et al., 2017) where they accumulate due to various anthropogenic sources, including the combustion of fossil fuels, waste incineration, or the production of alloys (e.g. brass), paints, batteries, or tires (Binner et al., 2023). While our study did not specifically intend to pinpoint the origin and source of pollution, it is evident that older parks exhibited elevated concentrations of these MTEs. The observed pattern may be attributed, at least in part, to the soil's origin, as older parks were initially established using soil imported from the inner suburbs of Paris, frequently subjected to irrigation with wastewater and/or sewage sludge. Hg, Pb, Zn, Cu and Cd concentrations in the soil exhibiting a strong correlation within the finer scale of the urban environment, our results further suggest a shared emission source. Actually, the robust correlation observed between the concentrations of MTEs, particularly Pb, Cu and Hg, and the levels of assimilable phosphorus (P) – primarily emitted by vehicular traffic in urban areas - suggests that road traffic is likely the primary source of MTE pollution in urban lawns (Kuoppamäki et al., 2014). This conclusion is consistent with the findings of a previous study conducted in the same geographical region (Foti et al., 2017).

Hg, Pb, Zn, Cu, and Cd are also the elements whose concentrations varied the most between soils. Generally, these elements exhibited greater variation across the 18 Parisian parks than within individual parks. However, in half of the parks, there were substantial differences in MTE pollution levels among the three sampled locations within each park. For instance, at Square du Temple, two of the sites displayed MTE concentrations (Hg, Pb, Cu, Cd, and Zn) at opposite ends of the spectrum: one site had MTE levels similar to those found in rural lawns, while another showed levels comparable to those measured near smelters (Chatelain, 2023; Lukkari et al., 2004); the third site showed intermediate levels of MTEs. This finding underscores the high heterogeneity of urban soils, emphasizing the importance of taking multiple samples when studying them. It also suggests that the soil biota's response to soil characteristics might vary at a very fine scale, leading to a strong structuration of communities within urban lawns.

4.2. Earthworm community response to MTE levels

Earthworm abundance per site ranged from one to 74 individuals per 27dm^3, with a median of 13.5 individuals. Consistent with patterns observed in proximity to MTE-rich mining sites, earthworm total abundance in urban lawns decreased with increasing MTE pollution levels in the soil (Huang et al., 2021; Lévêque et al., 2015; Mariet et al., 2020; Nahmani et al., 2003). More specifically, it was negatively associated with the levels of Cu, Hg, Zn and Pb. Our findings also align with the observations made in Brussels, Belgium (Pižl and Josens, 1995). Due to the positive correlation among the concentrations of these MTEs in the soil, it becomes challenging to ascertain whether the toxicity of these soils stems from the toxicity of individual MTEs or from synergistic effects within the MTE mixture. However, experimental studies demonstrated negative effects of all four MTEs on earthworm survivability and reproduction (Sivakumar, 2015; Yadav et al., 2023).

On the other hand, our research emphasizes that, among the physicochemical variables measured in this study, Cu concentrations in the soil have the most significant influence on the assemblage of earthworm species. While soils with elevated Cu levels also contained higher amounts of Hg, Pb, Zn, Cd, As, phosphates, organic matter and nitrogen, these additional factors did not enhance the explanatory power of our redundancy analysis. This suggests that the primary role in shaping earthworm communities lies with Cu concentrations. Specifically, the relative abundance of *A. chlorotica* increased in soils with a high Cu content. Interestingly, while Pižl and Josens concluded in their study that soil pollution levels in Brussels did not significantly shape the composition of earthworm communities, their results clearly show that the earthworm community at the site with the highest concentration of Cu in the soil (28.83 mg/kg) was dominated by *A. chlorotica* (Pižl and Josens, 1995). Although this outcome might imply that *A. chlorotica* has a higher tolerance to Cu pollution, earlier research indicated that this species is one of the most susceptible to MTE pollution (Chatelain, 2023; Nahmani et al., 2003; Spurgeon and Hopkin, 1996). For example, when subjected to soil containing a blend of Zn, Pb, Cu, Cr, and Ni, designed to simulate pollution levels found in urban environments, several *A. chlorotica* individuals died, while no casualty was measured in the two other earthworm species studied (Chatelain, 2023). Nevertheless, it does not exclude the possibility that this species is particularly tolerant to Cu. For instance, the presence of biliverdin contributes to the green coloration of this species (Satchell, 1967). Yet, in vitro studies highlighted the antioxidant and cytoprotective properties of biliverdin, predominantly due to their ability to scavenge free radical species (McDonagh, 2001). Moreover, compared to other earthworm species, *A. chlorotica* is rich in riboflavin-storing eleocytes (Mazur et al., 2011). Besides playing a key role in earthworm immunity, riboflavin may be involved in the storage and transport of Cu (Smith et al., 2006). Alternatively, the tolerance of *A. chlorotica* to Cu pollution may be attributed to its ecological niche. Specifically, this species predominantly inhabits the upper layer of the soil. In grasslands and lawns, it is situated amidst the roots of grass, where organic matter is abundant and the pH higher. This environment is also the place of intricate interactions among plants, microorganisms, and soil, which collectively influence the availability of MTEs (Bravin et al., 2009). Our findings underscore the necessity for additional comprehensive research dedicated to unravelling the intricate nuances of earthworm sensitivity to MTE pollution within urban environments. This inquiry is crucial for a more thorough understanding of earthworm distribution and for shedding light on acclimatization and/or adaptation mechanisms (Pauwels et al., 2013).

4.3. Organic matter and pH-mediated association between soil MTE levels and earthworm communities

Earthworms can be exposed to MTEs through both their skin and their digestive tract. Both pathways, dermal absorption and ingestion, contribute to the accumulation of MTEs in earthworm tissues. Soil pH plays a significant role in determining the solubility of MTEs in the soil, which in turn affects their bioavailability and their dermal uptake by earthworms. While experimental findings show variability, theoretically, in acidic soils, specific MTEs such as Pb and Cd tend to exhibit increased solubility, leading to a higher likelihood of absorption through the skin of earthworms (Bradham et al., 2006; Janssen et al., 1997; Morgan and Morgan, 1988; Nahmani et al., 2007; Spurgeon et al., 2006). Nonetheless, in our study, the associations between earthworm community composition and MTE concentrations were found to be unrelated to soil pH. This suggests that intestinal absorption, which is less influenced by soil pH, could be an important route of MTE uptake in nature (Oste et al., 2001). On the other hand, the lack of correlation with soil pH could be attributed to the limited variation in soil pH across the sites (ranging from 7.1 to 8.3), all falling within the classification of slightly alkaline. Nevertheless, our findings corroborated the influence of soil pH in shaping the structuration of earthworm communities (Rutgers et al., 2016). More specifically, the relative abundances of the species *A. rosea* and *A. icterica* increased with increasing soil pH. On the contrary, in less alkaline soils, *A. longa* and *A. caliginosa* dominated the communities.

The presence of organic matter in soils is also known to have a profound influence on MTE bioavailability, altering MTE speciation, pH, microbial interactions, physical properties, and binding competition (Hernandez-Soriano and Jimenez-Lopez, 2012; Nahmani et al., 2007). Hence, increased soil organic matter levels are typically linked to diminished availability of MTEs for soil organisms (Van Herwijnen et al., 2007). Consistent with these results, our study revealed that adverse impacts of soil concentrations of Pb, Hg, Cu, Zn, and Cd on earthworm richness were evident solely in soils with lower organic matter content (when SOM < 65.07 ‰, 63.33 ‰, 58.71 ‰, 59.99 ‰, and 47.14 ‰, respectively). More specifically, epi-anecic species and the uncommon intermediate species *O. cyneum* and *O. lacteum* were absent from soils exhibiting these chemical characteristics. Similarly, the inverse correlation between earthworm total abundance and subsoil Hg concentrations exhibited a tendency to weaken in soils with higher levels of organic matter (when SOM > 88.46 ‰). Surprisingly, earthworm species richness decreased with increasing Cr concentrations in the subsoil when the subsoil was relatively rich in organic matter (when SOM > 66.66 ‰). This finding contradicts the notion that organic matter, which provides electrons to the soil, aids in the conversion of Cr(VI) to the less soluble and less harmful Cr(III) (Choppala et al., 2013; Han et al., 2004). Despite the interactive effect of MTE concentrations and SOM on earthworm richness, SOM did not significantly explain the structuration in earthworm communities. This outcome could stem from the positive correlation between Cu and SOM, leading to SOM not contributing significantly to the variance explained by the multivariate analysis. Another factor may be that four of the six species most responsive to Pb, Hg, Cu, Zn, and Cd concentrations in low-SOM soil were excluded from the multivariate analysis. This exclusion was due to their relatively low occurrence, being present in less than 10% of the 54 sampling sites.

4.4. Conclusion

In this study, we demonstrated that the concentrations of Hg, Pb, Zn, Cu, and Cd in urban soils are key environmental factors influencing earthworm community structure at local levels. Given the widespread and highly variable distribution of these MTEs in soils, we believe that accounting for MTE pollution levels will improve our understanding of earthworm distribution within urbanised landscapes (Rutgers et al., 2016; Tóth et al., 2020). Importantly, our study underscores the significant heterogeneity in soil chemical characteristics within urban lawns, emphasizing the importance of examining these relationships at a very small geographical scale. By doing this, we can also gain insight into the differences in how soils perform essential ecosystem services, including water retention, atmospheric carbon storage, and the appearance of healthy lawns (Pavao-Zuckerman and Pouyat, 2017).

5. ACKNOWLEDGEMENTS

This work was carried out with the help of two master's students – Mégane Philippé and Aleksander Miedziejewski, and one bachelor's student – Neyla Turak, to whom we are very grateful. Moreover, we would like to thank the "Direction des Espaces verts et de l'Environnement" for allowing me to sample earthworms and soil in the parks in Paris. This work was supported by the Austrian Science Fund [grant numbers V-953].

During the preparation of this work the author(s) used ChatGPT in order to correct grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Chemical characteristic	Measurement method	Analyzer	Norm
Trace elements	Inductively coupled plasma atomic emission spectroscopy[1]	Thermo Electron – iCAP 7000 ICP-OES	NF ISO 22036
pH	pHmetry[2]	Skalar – pH meter SCHOTT Lab 860	NF ISO 10390
Electrical resistivity	Conductimetry[3]	Skalar – Conductivity meter WTW Inolab Cond 7310	NF ISO 11265
Carbonates	Carbonate content – volumetric method[4]	-	NF ISO 10693
Organic nitrogen	Element analysis through dry combustion[5]	Thermo Scientific - Organic elemental analyzer FLASH 2000	ISO 10694
Soil organic matter	Calculated from total organic carbon concentrations· element analysis through dry combustion[5]	Thermo Scientific - Organic elemental analyzer FLASH 2000	ISO 10694
Assimilable phosphorus	Joret-Hébert methodology : ammonium oxalate extraction followed by spectrophotometric analysis[6]	KPM – Wet chemistry platform SmartChem 200	NF X 31-161

Table 1. Methods used to measure soil chemical characteristics

[1] Soil samples were dried at 40°C, 2 mm sieved and 150 µm ground with a ball mill (MM400 Retsch). A total of 0.5 g per sample was then digested simultaneously in 1.2 mL HNO_3 (65%) and 3.5 mL HCl (37%) during 16h at room temperature, then 2h at 100°C. Ultrapure water was added to reach a final volume of 50 mL and left to decant during 8h. Total As (189.042 nm), Cd (228.802 nm), Cr (284.325 nm), Cr (284.325), Cu (324.754 nm), Zn (213.856 nm), Ni (231.604 nm), Pb (220.353 nm), Hg (184.950) and Se (196.090 nm) concentrations were determined using an inductively coupled plasma atomic emission spectrometer (iCAP 7000 ICP-AES, Thermo Electron, USA). The ICP-AES conditions were as follows: plasma power 1150 W, nebulize gaz flow 0.5 L/min, auxiliary gas flow 0.5 L/min. The ICP-OS was calibrated before performing measurements with the use of As, Cd, Cr, Cu, Ni, Zn, Pb and Hg standard solutions (1000 ± 2 mg dm^{-3} in 5%

HNO_3) from Chem-Lab. Each MTE was measured three times per sample. Relative standard deviation between the three measurements per sample were all below 10%. The blanks and certified reference materials (Internal Soil Reference Materials ISRM1 and ISRM2) were prepared and analyzed using the same methods as the samples. Calibration and blank samples were analyzed every ten measurements. Quantification limits were as follows: As: 4 mg/kg, Cd, 0.2 mg/kg, Cr: 5 mg/kg, Cu: 10 mg/kg, Zn: 25 mg/kg, Ni: 5 mg/kg, Pb: 10 mg/kg, Hg: 0.2 mg/kg and Se: 0.5 mg/kg. The concentration ranges for which the method is linear were as follows: As: 0-40 mg/kg, Cd: 0-10 mg/kg, Cr: 0-100 mg/kg, Cu: 0-1000 mg/kg, Zn: 0-500 mg/kg, Ni: 0-100 mg/kg, Pb: 0-1000 mg/kg, Hg: 0-40 mg/kg and Se: 0-40 mg/kg.

[2] pH was measured using a glass electrode, in a 1:5 (m/V) diluted suspension of soil in water. Measurement with a pH meter was done after 5 min. of agitation and after a minimum contact time of 2 hours but a maximum contact time of 24 hours.

[3] Conductivity was measured using a specific electrode, in a 1:5 (m/V) diluted suspension of soil in water. Conductivity was measured immediately after 30 min. of agitation.

[4] Hydrochloric acid was added to the soil sample to break down all carbonates. The volume of carbon dioxide released was measured using a Bernard calcimeter.

[5] Samples were previously ground to 150 µm, then weighed using a microbalance. They were combusted with oxygen and under helium flow at ca. 930°C. Carbon dioxide and nitrogen were separated by chromatography and quantified using a thermal conductivity detector. The organic matter content was calculated as 1.724 times the content in total organic carbon.

[6] Extraction of soluble forms of phosphorus was done by shaking the sample in an aqueous solution of 0.1 M ammonium oxalate (pH 7.0) with a sample/solution ratio of 1/25 (m/V). The solution was stirred during 2h and filtered. The determination of phosphomolybdic acid complex, reduced by ascorbic acid in sulphuric medium, was done by spectrophotometry.

Table 2. Between park variations in soil physicochemical characteristics – Outputs of the models (F-value, P-value and adjusted R²) testing whether the variation in soil physicochemical characteristics was higher between parks than within parks. We highlight in grey the variables that did not significantly differ between parks.

	Topsoils	Subsoils
pH	F=17.33, P<0.001, R²=0.84	F=9.53, P<0.001, R²=0.73
Resistivity	F=3.08, P=0.002, R²=0.40	F=0.55, P=0.908, R²=-0.17
CO_3	F=3.44, P=0.001, R²=0.44	F=3.04, P=0.002, R²=0.40
ON	F=4.60, P<0.001, R²=0.54	F=2.19, P=0.023, R²=0.28
C/N	F=2.31, P=0.017, R²=0.30	F=1.48, P=0.160, R²=0.13

SOM	F=4.27, P<0.001, R^2=0.51	F=2.22, P=0.022, R^2=0.28
P	F=2.67, P=0.007, R^2=0.35	F=4.53, P<0.001, R^2=0.53
Se	F=4.98, P<0.001, R^2=0.56	F=4.33, P<0.001, R^2=0.52
As	F=3.67, P=0.001, R^2=0.46	F=3.90, P<0.001, R^2=0.48
Cd	F=5.77, P<0.001, R^2=0.60	F=4.78, P<0.001, R^2=0.55
Cr	F=1.61, P=0.112, R^2=0.16	F=1.99, P=0.041, R^2=0.24
Cu	F=3.54, P=0.001, R^2=0.45	F=3.09, P=0.002, R^2=0.40
Zn	F=4.61, P<0.001, R^2=0.54	F=4.51, P<0.001, R^2=0.53
Ni	F=3.08, P=0.002, R^2=0.40	F=2.68, P=0.006, R^2=0.35
Pb	F=4.21, P<0.001, R^2=0.51	F=2.46, P=0.011, R^2=0.32
Hg	F=5.09, P<0.001, R^2=0.57	F=6.00, P<0.001, R^2=0.62

Table 3. MTE concentrations – Range and median concentrations of MTEs (in mg/kg) at the 54 sampling sites within the 18 Parisian parks.

	As	**Cd**	**Cr**	**Cu**	**Zn**	**Ni**	**Pb**	**Hg**
Range	6.6-21.5	0.1-1.7	16.2-56.2	13.0-227.0	46.0-421.0	11.0-22.3	19.1-675.7	0.21-4.36
Median	10.2	0.6	23.3	36.8	122.0	15.0	99.7	0.6
SLV	-	*0.51*	*65.2*	*28.0*	*88.0*	*31.2*	*53.7*	*0.32*

As: arsenic, Cd: cadmium, Cr: chromium, Cu: copper, Zn: zinc, Ni: nickel, Pb: lead; Hg: mercury. Median concentrations were calculated on the average concentration per sampling site in the topsoil and the subsoils. Recommended statutory limit values (SLV) are based on a note from the CIRE IdF from the 3rd of July 2006 (Mathieu et al., 2008). The note did not indicate any recommended SLV for arsenic. We highlight in grey the MTEs which median concentration was above the SLV.