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Soil properties and metal accumulation by earthworms in the Siena urban area (Italy)

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ABSTRACT

This paper reports the results of a study focused on the metal (Cd, Co, Cr, Cu, Ni, Pb, Sb, U and Zn) distribution in soils and uptake and accumulation by earthworms *Nicodrilus caliginosus* (Savigny) from urban, peri-urban, green-urban and non-urban zones of Siena municipality (central Italy). The main goal was to define the influence of soil properties and metal soil contents on the uptake of these contaminants by earthworms. Data indicated that Cd, Cu, Pb, Sb and Zn soil contents increased in the following order: non-urban < green-urban < peri-urban < urban soils, suggesting that vehicular traffic affects the distribution of these metals. Pb and Sb were the main soil contaminants and their highest enrichments were found in urban sites where stop-and-go traffic occurs. Concentrations of these traffic-related metals in earthworms showed a distribution pattern similar to that in soil, suggesting that soil contamination influenced the uptake of Cd, Cu, Pb, Sb and Zn by *N. caliginosus*. There were significant positive correlations between Cd, Pb and Sb earthworm concentrations and their soil contents. The lack of correlation for Cu and Zn could be due to the physiological regulation of these elements by earthworms. Statistical analysis pointed out that the uptake and accumulation of Cd, Cu, Pb, Sb and Zn by earthworms were affected by some soil physicochemical properties such as the organic carbon and carbonate contents that are able to rule the bioavailability of metals in soils.

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1. Introduction

In recent years, the interest for urban areas has increased due to environmental and health risks associated with the contamination by toxic substances such as metals (i.e., Cd, Pb and Sb). In the urban settings several human activities, such as industrial plants, vehicular traffic, home heating and waste incineration, spread metals in the atmosphere, seriously affecting air quality. Atmospheric dry and wet depositions are the major pathway of these contaminants to the surface soils, and contribute to the change of soil geochemistry as a result of fractionation and accumulation of metals through sorption and precipitation/coprecipitation reactions. The extent and features of these reactions depend on the chemical forms of the elements and the physicochemical properties of the soil (Essington, 2004; Sparks, 2003).

The mobile and available fraction of metals in soil can be partly transferred to the hydrosphere (surface waters and groundwater) and biosphere (plants and animals). Therefore, soil acts as both a sink and source of metals in urban areas, thus affecting their biogeochemical cycle in ecosystems.

Many studies dealt with the metal distribution in soils of urban areas, highlighting that the highest concentrations of these inorganic contaminants are generally located close to the main roads, as well as in residential areas and commercial districts (Cicchella et al., 2008; Imperato et al., 2003; Yang et al., 2011). Several researches also used some plants as bioaccumulators of metals and bioindicators in order to monitor levels of contamination of urban environments (Dong-Sheng and Peart, 2006; Malizia et al., 2012; Rucandio et al., 2011). Furthermore, few researchers studied metal uptake and accumulation in soil organisms, such as earthworms in urban areas (Kennette et al., 2002; Pizl and Josens, 1995).

Earthworms are soil-dwelling organisms commonly used in environmental and ecotoxicological studies. Several authors report that earthworms are able to accumulate metals such as Cd, Pb and Zn, and there is a fairly consistent correlation between the concentrations of these contaminants in earthworm tissue and those in soil (e.g., Dai et al., 2004; Hobbelen et al., 2006). Earthworms are one of the few soil organisms available as biological indicators of metals contamination in urban environments, therefore these organisms are considered by several authors (e.g., Lanno et al., 2004; Suthar et al., 2008) as bioindicators of soil quality providing important information for the assessment of environmental risks.

Moreover, earthworms are a source of food for terrestrial organisms, especially birds, reptiles and small carnivorous mammals (Spurgeon and Hopkin, 1996). The consumption of earthworms by

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predators may determine a transfer and accumulation of metals in the food chain (Edwards and Bohlen, 1996), and provides a route through which these contaminants may be transferred to higher trophic levels.

Uptake and accumulation of metals by earthworms depend on both total concentrations and chemical form of these contaminants in soil and the soil properties (e.g., Peijnenburg et al., 1999). In fact, mobility and bioavailability of metals in soil and their uptake by soil organisms are affected by many factors, including physicochemical properties such as pH, cation exchange capacity, organic carbon and carbonate contents (Lanno, 2003; Morgan and Morgan, 1988; Peijnenburg, 2002).

The present study investigated the relationship between soil contamination by metals and their accumulation in the earthworm *Nicodrilus* [=*Allolobophora* (Eisen) = *Aporrectodea* (Oerley)] *caliginosus* (Savigny) (Minelli et al., 1995). The research was carried out in the Siena municipality (central Italy), where vehicular traffic is the main potential source of contamination, since other human sources (e.g., industrial and waste emissions) are rather limited or absent.

Aims of the study were: (i) to assess the influence of vehicular inputs on metal distribution in soil; (ii) to determine which soil properties among pH, organic carbon and carbonate contents, cation exchange capacity, iron and manganese oxides and grain size, mostly affect the uptake of metals by earthworms; (iii) to define the relationships between earthworm uptake and accumulation and metal concentrations in soil. This study was conducted in urban and peri-urban environments and considered main soil properties as possible factor of influence of metal levels in earthworm tissue. Moreover, the research addressed the presence of some metals such as Sb and U, which are less investigated in these kinds of studies.

2. Materials and methods

2.1. Study area

The study area encompasses the territory of Siena municipality and includes the urban centre of the city as well its peri-urban, green-urban and non-urban zones.

Siena is a tourist city with a resident population of about 55,000 inhabitants. Traffic is estimated on average at about 50,000 vehicles a day and mainly flows along a ring road in the peri-urban sector of the city (Comune di Siena, 2005). This ring road allows the entry and exit of vehicles in and from the urban centre, and is connected to the important national road axes towards Florence, Rome and Perugia. Since 1965, the historic centre of Siena is a limited traffic zone (LTZ).

From the geological point of view, the study area extends in the northern sector of the Siena Basin, a NW–SE oriented tectonic depression where the deposition of clayey–silty–marly to sandy–marly marine sediments of the Neogene–Quaternary succession occurred during Pliocene.

2.2. Sampling strategy and procedures

Taking into account the features of vehicular traffic in the study area, 30 sampling sites were identified as follows: (i) urban sites ($n = 11$) close to the most important urban roads outside the limited traffic zone of city; (ii) peri-urban sites ($n = 9$) close to the ring road and its branches; (iii) green-urban sites ($n = 4$) located in green parks within or immediately outside the historic centre of Siena; (iv) non-urban sites ($n = 6$) placed in uncultivated terrains in rural zones faraway from both the urban centre and the peri-urban zone. Soil samples (20 cm deep) were collected in the 30 selected sites. Each soil was a composite sample consisting of three sub-samples collected a few meters apart. In the urban and peri-urban sampling sites, soil was collected near the edge of the roadway within a distance of 2 m.

In 15 sampling sites, 150 (10 for each sites) mature (clitellated) specimens of earthworm *N. caliginosus* were collected. This species, adapted to live also in conditions modified by human activity, was selected due to its common presence in the study area. The individuals of *N. caliginosus* live permanently in horizontal tunnels dug in the upper 20–25 cm of soil, feed mainly on soil organic matter and belong to the group of endogeic earthworms. Several factors, such as body size, physiological or seasonal conditions, may influence the contents of chemical elements in earthworm tissues. Consequently, only mature individuals were collected in this study and the sampling period was restricted to one month. The sampling activity was carried out in September 2007 for both soils and earthworms.

2.3. Laboratory treatment

In the laboratory soil samples were dried at +40° C and manually sieved through a metal-free 2 mm sieve. The soil fraction <2 mm was homogenized by quartering. Quartering procedure was performed by separating this soil fraction into four equal portions (quadrants), combining two of the four quadrants diagonally and rejecting the other two. This procedure was repeated until the amount of about 100 g was obtained. Finally, this aliquot of soil sample was powdered in an agate mortar using a mechanical pulverizer. The soil samples were solubilised by acid digestion: 1 mL HF, 2 mL HNO_3, 2 mL HCl and 1 mL $HClO_4$ (ultrapure reagents) were added to 200 mg of powdered soil; then the mixture was processed in Teflon bombs using a Milestone Ethos 900 microwave lab station (EPA, 1996). The solution was filtered and diluted with ultrapure water to a final volume of 100 mL.

In the laboratory earthworms were identified and rinsed with deionised water to clean them of adhering soil particles. Then earthworms were placed in plastic Petri dishes (two individuals in each dish) containing Whatman® No. 1 filter paper and a few drops of deionised water to maintain moisture, and kept at 18 °C for 72 h. In order to prevent coprophagy, the filter paper was changed daily. The individuals were subsequently depurated for an additional day without filter paper but with a few drops of deionised water to allow the complete egestion of gut contents. The earthworms were killed by freezing (−80° C) and freeze-dried for 48 h. The dry weight of sampled individuals ranged from 101 to 256 mg (146 ± 32 mg as mean ±SD).

The freeze-dried earthworms were digested individually with an acid mixture of 3 mL HNO_3 70% and 1 mL H_2O_2 30% (ultrapure reagents) in Teflon® bombs placed in a Milestone Ethos 900 microwave lab station. Solution was filtered and diluted to 50 mL with ultrapure water.

2.4. Determination of soil properties

Soil particle size distribution as percent content of the sandy, silty and clayey fractions, was determined by the hydrometer method. Soil pH (pH_{H_2O}) was measured in a 1:1 (w/v) soil/water mixture following the EPA method 9045/D. The procedure of Hendershot and Duquette (1986) was used to determine the effective cation exchange capacity (CECe) measuring the Ca, Mg, K, N and Al concentrations in a mixture obtained by the reaction of 2 g of soil and 20 mL of a 1 M NH_4Cl solution. The method of Walkley and Black (1934) was used to determine the organic carbon (C_{org}) content in soil. The carbonate content as percentage content of calcium carbonate (%$CaCO_3$) was determined with De Astis calcimeter.

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