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Ecotoxicological assessment of metal-polluted urban soils using bioassays with three soil invertebrates

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ABSTRACT

This study aimed at assessing the quality of urban soils by integrating chemical and ecotoxicological approaches. Soils from five sites in downtown Naples, Italy, were sampled and characterized for physical–chemical properties and total and water-extractable metal concentrations. Bioassays with *Eisenia andrei*, *Enchytraeus crypticus* and *Folsomia candida* were performed to assess toxicity of the soils, using survival, reproduction and growth as the endpoints. Metal bioaccumulation in the animals was also measured. The properties and metal concentrations of the soils strongly differed. Metal bioaccumulation was related with total metal concentrations in soil and was highest in *E. crypticus*, which was more sensitive than *E. andrei* and *F. candida*. Responses of the three species to the investigated soils seemed due to both metal contamination and soil properties.

1. Introduction

As an important part of environmental pollution, metal contamination is a widespread problem in several ecosystems. In urban areas, intensive human activities and traffic are the main sources of metals that pollute parks and roadside lawns. City green spaces provide benefits for human inhabitants (Sattler et al., 2010), and soil, being a vital constituent of the urban environment, performs numerous ecological, social and economic functions (Blum, 2005). Unfortunately, metals, because of their characteristics of duration, enrichment, toxicity and concealment (Dube et al., 2001), pose serious threats to urban soils. For these reasons, urban soil is a good indicator of the level and extent of metal accumulation in the surface environment.

Soil biota is continuously exposed to soil contaminants that can negatively affect organism physiology. The effects of metals on soil invertebrates are usually related to their concentration and bioavailability in the soil. However, metal toxicity varies among species, since organisms can selectively accumulate metals in certain tissues of their body to minimize the damage of reactive forms (Vijver et al., 2004). Metal bioaccumulation depends on metal and soil properties, biology of the organisms, climatic influences and on metal bioavailability, which represents the relevant exposure concentration for soil organisms (Frische et al., 2003). Therefore, soil chemical analyses are essential for the evaluation of soil pollution but give only limited information about soil toxicity. Ecotoxicity tests are a necessary complement to chemical analyses to evaluate the impact of metals on soil invertebrates (Xu et al., 2009). A correct analysis of the complex interactions between pollution and the environment requires the application of a multidisciplinary approach and the determination of different parameters, which can describe the effective contaminant exposure levels and their environmental adverse effects (Dagnino et al., 2008).

Earthworms, enchytraeids and collembolans are widely used in ecotoxicity tests, since they contribute to leaf litter decomposition and organic matter recycling. Feeding directly on decaying materials and soil fungi, they provide an earlier indication of ecosystem disturbance than predaceous soil animals (Cole et al., 2001; Didden and Römbke, 2001). In addition, these taxa are suitable ecotoxicity test organisms because they are easy to culture, have relatively short life cycles and are cost-effective (Fountain and Hopkin, 2005; Lowe and Butt, 2007).

In spite of their importance, little is known about the impact of the urban ecosystems on soil organisms. So far, most authors (Bradham et al., 2006; Ávila et al., 2009; Smith et al., 2010), focused on mining, artificially spiked or naturally contaminated soils. There is a great need for improving the knowledge about the effects on soil organisms due to metal accumulation in field soils, where the direct causality between measured compound concentrations and effects on the ecosystems is more complicated to understand.

This study aimed at assessing the toxicity of naturally contaminated soils by the integration of chemical and ecotoxicological

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approaches. In particular, five urban soils collected in downtown Naples (Southern Italy) were analyzed for soil properties (pH, water holding capacity, organic matter content) and total and water-extractable concentrations of Cu, Pb, and Zn. The measured metals were chosen among the main representative urban soil metal contaminants due to traffic pollution (Davis et al., 2001; Maisto et al., 2011). Earlier studies have shown that Cd, Cr, Ni, Sb, Hg also result from vehicular traffic but that their concentrations in Italian urban soils were rather low (Manta et al., 2002; Maisto et al., 2011). For that reason these metals are not expected to cause much effects on soil invertebrates, both at the individual and population level. To evaluate soil toxicity, *Eisenia andrei* (earthworm), *Enchytraeus crypticus* (enchytraeid) and *Folsomia candida* (collembolan) were exposed to the soils in order to assess the effects on survival, reproduction and growth according to ISO (1999) and OECD (2004a,b) standardized toxicity tests. Moreover, Cu, Pb, and Zn bioaccumulation was evaluated in the same organisms. Finally, the relationships among soil physical–chemical properties, soil metal concentration, soil metal availability and metal accumulation in the three soil-dwelling invertebrate species were investigated.

2. Materials and methods

2.1. Soil sampling

The soil sampling occurred in September 2010, at five sites (ACT, MIA, MAD, IOL, CAP) in downtown Naples, Italy. Detailed information about the soil sampling is given in Santorufo et al., 2012.

2.2. Physical–chemical analyses

Physical–chemical soil analyses were performed in triplicate, after mixing five of the 10 soil sub-samples for each site to obtain homogeneous samples. The soils were characterized for pH, organic matter content (OM), and water holding capacity (WHC) following the methods of Allen (1989). In order to measure total Cu, Pb and Zn concentrations, 0.1 g sieved (2 mm) and dried soil samples were digested with 2 mL of a mixture (4:1 = v:v) of HNO_3 (65%, p.a., Riedel-deHaën, Seelze, Germany) and HCl (37%, p.a., Baker Philipsburg, NJ, USA) at 140 °C for 7 h in a macrodestruction oven. The quality of the analysis was checked using ISE sample 989 (International Soil-Analytical Exchange) certified by Wageningen Evaluating Programs for Analytical Laboratories as reference material. Recoveries of Cu, Pb, and Zn were always within 10–15% of the certified concentrations. To measure water-extractable metal concentrations, an oven-dried soil:distilled water suspension (1:2.5 = v:v) was shaken for 2 h at 200 rpm and filtered over a 0.45 μm filter. The total and water-extractable metal concentrations were measured by atomic absorption spectrometry equipped with a graphite furnace (Perkin-Elmer 5100; Cu and Pb) or flame (Perkin-Elmer AAnalyst 100; Zn). For a detailed description of soil analyses see Santorufo et al. (2012).

The metal concentrations were reported in tables as $\mu g\ g^{-1}$ dry weight ($\mu g\ g^{-1}$ d.w.), and the sum of metal concentrations was expressed as toxic units (TUs) i.e. the ratio between measured and background metal concentrations in the soils. The background levels of total (4.53 μg Cu g^{-1} d.w.; 18.7 μg Pb g^{-1} d.w.; 21.3 μg Zn g^{-1} d.w.) and water-extractable metal concentrations (0.07 μg Cu g^{-1} d.w.; 0.02 μg Pb g^{-1} d.w.; 0.13 μg Zn g^{-1} d.w.) were measured in the natural standard soil Lufa 2.2 (Speyer, Germany).

2.3. Bioassays

E. andrei were cultured in a substrate of potting soil and peat, and fed abundantly with manure from healthy horses not treated with any pharmaceuticals for at least 6 weeks. The tests used adult earthworms, with fully developed clitellum, and followed OECD guideline 222 (OECD, 2004b). Each toxicity test had four replicate 850 mL glass test containers covered with aluminum caps containing approx. 300 g soil (dry weight), with 5 earthworms randomly assigned to each container. At the start of the test, each batch of 5 animals was weighed to determine the starting weights (1.96–2.33 mg; average ± s.e. 2.2 ± 0.04 mg; n = 10). After introduction of the earthworms, 5 g (dry weight) finely ground and moistened horse dung was introduced as a food source in a hole in the middle of the test soil. Once a week all test containers were opened to aerate the soils, correct for water losses and add additional food was provided if no food was visible anymore. After 4 weeks, surviving adults were collected, counted and weighed, and the soils were incubated for another 4 weeks to allow for hatching of the cocoons. After the second 4-week period, juveniles were extracted by placing the test containers in a water bath at 60 °C. All emerging juveniles were collected from the soil surface and counted.

E. crypticus was cultured in aqueous agar prepared from a Lufa 2.2 soil extract (1 l of soil mixed with 3 l of tap water), and fed with oat meal. Tests followed OECD guideline 220 (OECD, 2004a). Ten adult animals, with clearly visible clitellum, were introduced into 100 mL glass test containers containing approx. 30 g moist soil. Five replicate test containers were used for each soil. Test containers were closed with perforated aluminum foil, and a small amount of crushed oat meal was added for food on top of the soil. Once a week, water losses were compensated by weighing all test containers and additional food was added if no food was visible anymore. After 4 weeks, the enchytraeids were fixed by adding 10 mL ethanol to each test container. After 1 min the suspension was transferred to a plastic jar using 100 mL of distilled water. The enchytraeids were stained by adding 300 mL of a 1% Bengal rose solution. The samples were again shaken rigorously and incubated for 24 h at approx. 4 °C to achieve an optimal dying effect. Then the bright pink colored adult and juvenile enchytraeids were counted.

F. candida Willem 1902 were cultured in containers with a bottom of plaster of Paris and active charcoal (7:1 w:w). Granulated dry baker's yeast was given for food. To age-synchronise animals, adults from the culture were allowed to produce eggs for 48 h in freshly prepared culture containers. After removing the adults, the eggs were allowed to hatch and the juveniles were used in the experiments when they were 10–12 d old. Tests followed ISO guideline 11267 (ISO, 1999), using 10 replicate 100 mL glass test containers covered with a plastic caps. Test containers were opened twice a week to aerate the test soils, and once a week to correct water losses and add additional food if no food was visible anymore. After 4 weeks, the content of a test container was flushed with 100 mL water into a 300 mL glass beaker. Upon gently stirring, all animals came to float to the water surface, and by making a photograph the number of juveniles produced was counted. The number of surviving adults was counted by eye.

All toxicity tests were incubated in a climate room at 20 °C, and constant illumination.

The results of survival and growth were reported as percentages, whereas those of reproduction as number of juveniles produced. Bioassays were also performed in uncontaminated natural Lufa 2.2 standard soil (loamy sand soil; 1.93% organic carbon, pH-$CaCl_2$ 5.5) that was used as a control.

2.4. Metal bioaccumulation analyses

In order to measure the metal uptake from the soil by the test organisms, the body metal concentrations in surviving adults of each species were measured. At the end of the bioassays, the animals were collected. To ensure no soil particles remained attached to the surface of the animals, the earthworms and enchytraeids