



# *Papillifera papillaris* (O.F. Müller), a small snail living on stones and monuments, as indicator of metal deposition and bioavailability in urban environments



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## ABSTRACT

Although mosses, lichens and vascular plants are widely used as biomonitors of metal deposition in urban environments, these organisms also accumulate soil and rock dust particles making difficult the recognition of metal sources and their bioavailability to consumers. Based on the assumption that a stationary, widespread and omnivorous animal species, scarcely interacting with soil and inhaling fine particles, could be a much more reliable indicator of metal deposition and bioavailability in urban environments, we analyzed for the first time the elemental composition of soft tissues and excreta of a pulmonate gastropod, *Papillifera papillaris* (O.F. Müller), commonly dwelling on stone walls and monuments in Italian and Mediterranean urban environments. Snails and some moss species were collected in 2014 and 2015 from stone- and brick-walls at the side of roads with different traffic intensity and in control sites. Results showed that the soft tissues of *P. papillaris*, purged of the gut contents, accumulated much higher Cd, Cu, Mn, Pb, and Zn levels than mosses from the same walls and concentrations of these metals were significantly higher at the side of roads with higher traffic intensity. Mollusk excreta had very high concentrations of Al, Cr, Fe, Ni and Pb, suggesting that a large proportion of elements associated to soil or stone particles, or with the black crusts scraped from walls, is not absorbed in the digestive tract. Surprisingly, *P. papillaris* tissues accumulated much higher Mn concentrations than those reported in literature for other species of terrestrial gastropods.

Although preliminary, this study indicates that *P. papillaris* is a very promising species to monitor metal deposition pattern and bioavailability in urban environments and to evaluate the possible metal transfer along food chains.

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## 1. Introduction

Airborne particulate matter (PM) is among the most problematic pollutants in terms of harm to health in Europe (EEA, 2014) and the International Agency for Research on Cancer (IARC) has recently designated it as a Group 1 carcinogen. The analysis of several European cohorts has shown that air pollution by PM contributes to increase lung cancer incidence (e.g., Hamra et al., 2014) and natural-cause mortality (Beelen et al., 2014), even at concentrations well below the European annual mean limit value ( $25 \mu\text{g m}^{-3}$  for  $\text{PM}_{2.5}$ ). Fine PM containing metals, polycyclic aromatic hydrocarbons (PAHs) and other toxic chemicals mainly originate from anthropogenic activities. In urban environments, the main near-ground sources are vehicles (fuel combustion, tyre and brake wear),

road wear, dust re-suspension, the burning of biomass or fossil fuels for domestic heating and cooking (EEA, 2014). Although heavy metals are among potentially toxic pollutants associated to airborne particles, European air quality target values have been established only for As, Cd, Ni and Pb; furthermore, most monitoring stations in urban environments measure the concentrations of particles based on size (aerodynamic diameter of  $\text{PM}_{10}$ :  $\leq 10 \mu\text{m}$ ; or  $\text{PM}_{2.5}$ :  $\leq 2.5 \mu\text{m}$ ) and not their chemical composition. Atmospheric concentrations of potentially toxic contaminants are highly variable in space and time and the different sensitivity of living organisms to the complex mixture of air pollutants (bioindicators) or the capability of certain species to accumulate persistent atmospheric pollutants at easily detectable concentrations (bioaccumulators) are very useful to achieve information about their biological effects or spatial deposition patterns (e.g., Bargagli, 1998). As a rule, lichens, mosses and tree leaves are among the most used biomonitors for large-scale or long-term biomonitoring of airborne metals in urban environments. However, the chemical

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composition of tree leaves also reflects the root uptake of elements, and although lichens and mosses lack roots and are largely dependent on atmospheric deposition for water and nutrients, they also adsorb rock and soil dust particles, making difficult the assessment of element contribution from anthropogenic sources and their bioavailability (e.g., Bargagli, 1995). In urban environments, stationary and widespread animal species having scarce interactions with soil are probably more reliable biomonitors of airborne trace metals than mosses and lichens. In the past, some surveys have been performed with urban birds such as the house sparrow (*Passer domesticus*; e.g., Kekkonen et al., 2012) or the pigeon (*Columba livia*; e.g., Frantz et al., 2012), with lizards (e.g., Loumbourdis, 1997), and with soil invertebrates such as terrestrial isopods (e.g., Gál et al., 2008), and mollusks. Although mollusks (particularly bivalves) are among the most used organisms for large-scale and long-term monitoring of metals in aquatic ecosystems, in terrestrial ecosystems their use as biomonitors is rather neglected. Some species of pulmonate gastropods are rather common in urban environments, have a suitable biomass for the analysis of pollutants, are easy to sample and through different routes (food, contact and, to a minor extent, inhalation) they can accumulate high metal concentrations (e.g., Dallinger and Wieser, 1984; Rabitsch, 1996; Pihan and de Vaufléury, 2000; Scheifler et al., 2006; Boshoff et al., 2013; de Vaufléury, 2015). In general, concentrations of some heavy metals in gastropods reflect the pollution levels in their food and habitats, and being eaten by birds and mammals, land snails and slugs contribute to the metal transfer through terrestrial food chains. Most terrestrial gastropods live in contact and move on soil, eat plants and dirt and use the ground for egg-laying, embryo development, shelter and hibernation; thus, their chemical composition mainly reflects the metal bioavailability in soil and plants, and only indirectly the metal burden in atmospheric deposition. Thus, to monitor metal deposition in urban environments it would be better to expose terrestrial snails in plastic cages without contact with the soil, as performed by Regoli et al. (2006) in the city of Ancona (Italy). However, this approach implies rather short exposure periods, has some logistic constraints and does not allow to evaluate the spatial pattern of metal deposition in urban areas.

With the aim to find a reliable passive bioaccumulator of airborne metals in urban environments, we tested the easily identifiable small land snail *Papillifera papillaris*, which is naturally widespread on man-made stoneworks in Italian cities. To our knowledge there are no previous studies using *P. papillaris* as metal biomonitor. Our hypothesis is that a pulmonate gastropod living on stone manufactures (walls, monuments, and other architectural structures occurring in towns), feeding on organisms (cyanobacteria, algae, lichens and mosses) directly exposed to wet and dry deposition of pollutants, and inhaling the principal pollutants of urban air, can be a suitable tool to evaluate spatial distribution and bioavailability of airborne metals. Compared to soil, stones (marble, limestone or bricks) are much more homogeneous substrates; the black crust on their surface is formed by a sulfation reaction where metals and metal oxides act as catalyst, and it is known that the elemental composition of these crusts reflects temporal changes in atmospheric pollutant patterns (e.g., Barca et al., 2014; Ruffolo et al., 2015). Most studies with terrestrial gastropods have focused on the bioaccumulation of Cd, Pb, Zn, and Cu (e.g., Viard et al., 2004; Notten et al., 2005), but we planned to assess more elements to better differentiate between those from atmospheric deposition (including the atmophile Hg) and those mainly occurring in soil and rock dust particles, such as Al, Fe, Cr. Because there is evidence that mosses from stoneworks in urban areas are reliable biomonitors of metal deposition (e.g., Kosior et al., 2015), through the analysis of metal concentrations in mosses (*Hypnum cupressiforme*, *Homalothecium sericeum* and *Homalothecium lutescens*, and



Fig. 1. *Papillifera papillaris* and its habitat.

*Brachythecium rutabulum*) and *P. papillaris* (soft tissues and excreta) dwelling on the same walls, the main objectives of this preliminary survey were: (1) to compare the reliability of snails and mosses as biomonitors of metal deposition in urban and control walls facing roads with different traffic intensities; (2) to evaluate how the wall features, vegetation cover and traffic intensity affect the metal bioaccumulation; (3) to assess the potential role of *P. papillaris* as indicator of metal bioavailability and the potential transfer of these pollutants in urban food chains.

## 2. Material and methods

### 2.1. Mollusks

*P. papillaris* (O.F. Müller, 1774) syn. *Papillifera bidens* (Linnaeus, 1758) (Mollusca, Gastropoda, Pulmonata, Stylommatophora, Clausiliidae) has an ash-gray to grayish-brown shell, smooth or ribbed, 11–18 mm × 2–4 mm, with suture strongly papillated and a darker brown-red band below the suture running between the white dots (Fig. 1). Native to central Mediterranean areas (Italian peninsula, Sardinia, Corsica, Sicily), in Malta it occurs in open rocky habitats mainly below small shrubs and is known from there also as fossil in Quaternary deposits (Giusti et al., 1995). *P. papillaris* lives on all kinds of rocky substrata, including stone monuments, walls and ruins of buildings, presumably feeding on algae, lichens, mosses and other plants growing on stone surfaces. Largely synanthropic and apparently not negatively affected by human activities, this species has been passively dispersed along with plant material and stonework in many countries of the Mediterranean region (e.g., Albania, Spain, Balearic Islands, North Africa, Turkey) and is recorded from many archeological sites. Through marble manufactures transported from Rome in 1896, *P. papillaris* was introduced to England (Ridout-Sharp, 2005, 2010).

### 2.2. Sampling sites

Siena is a small medieval town in central Italy (55,000 inhabitants); the urban area covers about 10.4 km<sup>2</sup> in a hilly territory (from 166 to 414 m a.s.l.). The city center is delimited by medieval walls and has narrow streets where limited or no traffic is permitted. However, surrounding the historic center there are ring-roads where most of the traffic concentrates. Adult snails (30–50 individuals from each site) and moss samples (each being a composite of 5–10 subsamples from a 20 m<sup>2</sup> area) were collected by using

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