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The ground beetle *Parallelomorphus laevigatus* is a potential indicator of trace metal contamination on the eastern coast of Sicily

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ABSTRACT

Carabids are generally considered to be non-specialized predators, and they have been considered useful ecological indicators. They can play a key role in clarifying the route of contaminants in food webs because they are predators of small invertebrates and, in turn, part of the diet of several vertebrates. The Mediterranean species *Parallelomorphus laevigatus*, which so far has not been studied from an ecotoxicological point of view, is an excellent ecological indicator in sandy coastal environments.

We investigated the accumulation of trace elements in Ionian populations of *P. laevigatus* and evaluated the transfer of metal through the food chain of the coastal ecosystem. We analyzed 15 metals, including 11 essential metals (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, V and Zn) and four toxic metals (As, Cd, Hg and Pb). Significant differences were found in metal concentration in animal tissues among sites.

Our results support the existence of defense mechanisms for the studied species.

High values of As, Cd, Cr, Pb, Ni, and Hg detected in the beetles from the control site can be explained by both the emission sources from the nearby industrial plants and the intense agricultural activity.

The present paper shows increasing Hg concentrations in the simplified trophic web of sandy beaches and confirms the capability of this pollutant to biomagnify. Moreover, the high value of biomagnification factor (BMF) points to the severe pollution level in this protected area.

1. Introduction

The trophic transfer of toxins through the food chain has increasingly become the focus of ecotoxicologists. One of the reasons for this special interest is that our species actively participates in food webs, and like any consumer, man must depend on food intake to survive.

Many studies have been primarily carried out in aquatic environments, where potentially harmful substances, such as pesticides and heavy metals (i.e., mercury and hydrocarbons), are often released (e.g., Metcalf et al., 1971; Gerlach, 1981; Nriagu, 1979).

Later investigations in terrestrial systems have clarified the fundamental role of soil as a sink for metals and other chemicals. Some groups of invertebrates belonging to the meso- and macrofauna have been proposed as bioindicators (Hopkin, 1989, 1990). Saprophagous animals, such as woodlice, springtails, millipedes and earthworms, are appropriate organisms to evaluate the effects of toxic substance accumulation in the soil (Hopkin et al., 1985; Gräff et al., 1997; Kale, 1988). Moreover, these invertebrates are situated at the lowest levels of terrestrial food webs, thus, they are food for several animals and a transference route for the biomagnification of contaminants in food webs (Andréa, 2010).

Among the predators that live and feed on the soil surface or within the first few centimeters of soil, the carabid beetles are considered useful ecological indicators (den Boer, 1977; Brandmayr et al., 2005; Butovsky, 1997; Butovsky et al., 1999; Lagisz and Laskowski, 2008; Schirmel et al., 2015; Simon et al., 2016). In fact, they play a key role in clarifying the route of contaminants in food webs, as they are predators of small invertebrates and, in turn, part of the diet of amphibians, reptiles, birds and small mammals (Butovsky, 2011). These animals have been studied in forest, grassland, agro-ecosystems, and even roadside habitats, to evaluate the impacts of man on terrestrial ecosystems (e.g., Andrews and Cooke, 1984; Beyer et al., 1985; Butovsky, 1994, 1995a, 1995b; Emetz and Kulmatov, 1983; Emetz and Zhulidov, 1983; Jelaska et al., 2007; Novak, 1989; Purchart and Kula, 2007; Roth, 1993; van Straalen and van Wensem, 1986).

The distribution of this group of insects in all types of terrestrial habitats makes it excellent for ecotoxicological analysis. Generally, carabids are characterized as poor accumulators of heavy metals (Kramarz, 1999; Heikins et al., 2001), probably because they have a series of detoxification enzymes (Kramarz, 1999; Stone et al., 2002). In

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addition, it was found that carnivorous ground beetles incorporate higher concentrations of non-essential metals, such as Cd and Pb (van Straalen et al., 2001), and that non-specialized predatory ground beetles accumulate less Cu and Zn than specialized predators or parasitoids (Butovsky and van Straalen, 1995).

The Mediterranean species *Parallelomorphus laevigatus* (Fabricius) (homotypic synonym of *Scarites* (*Parallelomorphus*) *laevigatus* Fabricius, 1792) is a ground beetle that has not been studied from an ecotoxicological point of view. An excellent ecological indicator for sandy coastal environments (Zanella et al., 2009), it lives on sandy beaches and spends the daylight hours in an individual burrow in the sand near the shoreline. The beetle goes outside after dark and spends the night hunting mainly *Talitrus saltator* (Alicata et al., 1982; Caltabiano et al., 1984). *P. laevigatus* is widespread on the Atlantic coasts of the Mediterranean and Morocco, and along the Mediterranean basin and the western coast of the Black Sea (Magistretti, 1963). It has been the subject of several eco-ethological investigations, in different coastal sites of eastern and southern Sicily, since 1981(Caltabiano et al., 1981, 1984; Conti, 1994; Conti et al., 2004; Costa et al., 1982a, b, c; Costa et al., 1986). Thus, it was possible to identify all the details of this beetle's extraordinary ability to orient itself (Costa et al., 1986). The shoreline can be a rather hazardous habitat because often animals are in danger of falling into the water, dragged by the surf or wind, but effective behavioral adaptations allow *P. laevigatus* to overcome this danger. Indeed, it is able to float and quickly land thanks to a specialized swimming technique (Caltabiano et al., 1981); in addition, it reaches the shore with the shortest path, which is perpendicular to the coast line (Ferguson, 1967). During daytime, the beetle uses the solar azimuth as an orientation cue to maintain the correct direction to land according to a y-axis path (Costa et al., 1982a). It is also able to compensate for the apparent motion of the sun (Costa et al., 1982b). Nocturnal tests have also demonstrated the ability of *P. laevigatus* for lunar orientation (Costa et al., 1982c) and a sensitivity to the magnetic field (Conti, 1994).

Frequent monitoring activity in the different sandy beaches of eastern Sicily has shown a progressive depletion of the population size of this species, in part due to human impacts (Conti et al., 2004). Moreover, it should be emphasized that the southern part of the Ionian coast of Sicily includes one of largest refining and petrochemicals industries in western Europe. Many studies have shown that this type of industrial activity (i.e., crude-oil production, transportations, and refining operations) releases several pollutants into the environment, both organic and inorganic (e.g., Mahajan, 1985; Cholakov, 2010). However, determinations of environmental pollution sources cannot exclude natural causes. Indeed, some of these sandy beaches are also influenced by the presence of Mount Etna, the tallest active volcano in Europe. It is well known that both volcanic eruptions and volcanic ash contain a large amount of toxic substances, including many trace elements, which are transported through the waterways to the sea.

The primary aim of this study was to evaluate the accumulation of trace elements in Ionian populations of *P. laevigatus*, as yet uninvestigated. Moreover, we sought to evaluate the metal transfer through the food chain of the coastal ecosystem. Since sandhoppers are the main food of *P. laevigatus*, and they have been thoroughly studied from an ecotoxicological point of view (Moore and Rainbow, 1987; Rainbow et al., 1989, 1998; Rainbow and Moore, 1990; Weeks and Rainbow, 1991, 1993; Fialkowski et al., 2000, 2003, 2009; Marsden and Rainbow, 2004; Ugolini et al., 2004, 2005, 2008; Rainbow, 2006; Ungherese et al., 2010a, 2010b; Conti et al., 2016), this study will generate useful contributions on this topic.

2. Materials and methods

2.1. Sampling method

Adult beetles were collected at four sites along the sandy, Ionian coastline of Sicily, during May, July and September 2013. Ten specimens per site and per sampling day were collected by hand within 10 m of the beach, close to the shoreline. Collections took place during 20:30 to 23:30, when the animals began their surface activity.

The specimens were placed individually in Falcon tubes containing sand from the collection sites and transported to the laboratory. Each individual was cleaned of sand and weighed 20 times, consecutively, using a *Sartorius* balance (model CPA225D), which allows the weighing of moving objects. Then, animal samples were stored at −18 °C.

2.2. Sampling areas

For this study we selected the sites **A**, **B**, **C**, and **D** along the sandy beaches of eastern Sicily (Fig. 1).

Site A was the Simeto Nature Reserve Oasis (37°24′28.605″ N, 15°5′30.046″ E), located south of the mouth of the Simeto, a major river in Sicily that runs through the volcanic territory of Mount Etna and reaches the sea a few kilometers south of the town of Catania. This reserve is very important ecologically because it is a wintering area for migratory birds. Unfortunately, the presence of many illegal houses and the proximity of a large industrial plant in Catania pose considerable risks to the natural conditions of this area, adding their negative effects to those caused by volcanic materials produced from Etna and transported to sea in the Simeto river.

Site **B** was the Agnone Bagni (37°19′55.353″ N, 15°5′41.247″ E), a seaside resort near the small town of Agnone Bagni, approximately 20 km south of Catania and 40 km north of Syracuse. The inshore waters of this area are frequently subject to the proliferation of the green algae *Ulva lactuca* due to a purification problem in the San Leonardo River. Because of this, bulldozers and mechanical shovels are often used to remove the algal mass deposited onshore by the tide.

Site **C** was the Marina di Priolo (37°8′54.473″ N, 15°13′20.581″ E), a sandy beach approximately 15 km north of Syracuse and just south of the Peninsula Magnisi, on a narrow strip of land located in the heart of the Augusta Bay known for the famous archaeological site Thapsos. This site lies only a few kilometers away from the small Priolo Bay, where the impressive industrial and petrochemical center of Priolo Gargallo (Syracuse) is located.

Site **D** was the Vendicari Nature Reserve (36°46′45.032″ N, 15°5′39.635″ E), located approximately 40 km south of Syracuse, which has been recognized as a wetland of international importance under the Ramsar Convention of 1971 because it includes a mixture of lagoons, sand dunes, rocky coastlines and sandy beaches. The Vendicari Reserve covers roughly 1512 ha, 575 of which constitute the zone A of integral reserve and 937 zone B (the so-called pre-reserve), which is dedicated to agriculture, tourism and sport. We selected it as a control area because it is located approximately 100 km from Mount Etna and considered by many researchers (e.g., Fasulo et al., 2012; Cappello et al., 2015) to be unaffected by the petrochemical contamination.

2.3. Metal analysis

Frozen samples were dried in an oven at 105 °C until they reached a constant weight. After being homogenized, they were mineralized with an *Anton-Paar Multiwave 3000* Microwave digestion system and PTFE vessels. Then, according to EPA Method 3052 (with a different amount of acid due to the best performance of the Anton-Paar system), we added 3.5 ml of HNO_3 and 1.5 ml of H_2O_2 to the digestion mixture. At the end of this procedure, the content of individual vessels was diluted with 2 ml of ultrapure water and filtered using *Whatman 40* filters. An aliquot of 500 µl, previously taken from the filtrate and diluted to 10 ml, was further diluted to 10 ml and then subjected to the experimental analysis. After suitable calibration through the internal standard method, analyses were conducted using a *Perkin Elmer ICP-MS*, model Nexion 300X (Perkin Elmer Inc. Waltham, Massachusetts,