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Multi-matrix quantification and risk assessment of pesticides in the longest river of the Iberian peninsula

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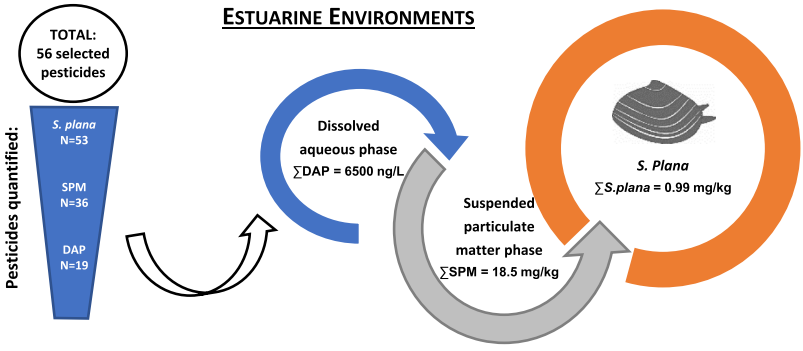
HIGHLIGHTS

- Number of detected pesticides followed the order: bivalves > particulate matter > water.
- Ecological risk assessed primarily for invertebrates.
- No human health risk identified through direct bivalve consumption.
- Some pesticides were above the 2013/39/EU Directive limits.
- Monitoring in various matrices is key to assess environmental and human pesticide pressure.

GRAPHICAL ABSTRACT



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ABSTRACT

The distribution of pesticides in dissolved aqueous phase (DAP), suspended particulate matter (SPM) and *Scrobicularia plana* soft tissues from the Tagus River estuary was determined to evaluate the chemicals pollution status and their hazard potential in this area. Samples were collected in 6 campaigns (December 2012–October 2013), from 3 strategical sites, and analysed via different extraction procedures followed by gas chromatography tandem mass spectrometry (GC–MS/MS) determination. The contamination profile among matrices (DAP, SPM, and soft tissue from bivalves (STB)) was marked by average concentrations of 345 ng/L, 0.51 mg/kg, and 0.02 mg/kg, respectively, with several samples above the 2013/39/EU Directive of environmental quality standards (EQS); no differences were observed between sex. A wider range of pesticides was present in STB ($n = 53$) than in SPM ($n = 36$) and DAP ($n = 19$) matrices. Sediment–water partition coefficient, bioaccumulation factor in both DAP and SPM fraction were estimated ranging between 2.5 and 4.4 and 0.008–2799, respectively. The spatial distribution of most pesticides and physicochemical parameters were consistent, indicating a pollution pattern primarily near the Trancão River mouth. Due to the presence of the target compounds, calculated risk quotients pointed out potential hazards for aquatic organisms, mainly to invertebrates. The estimated average daily intake, theoretical maximum daily intake, and hazard quotient of the studied pesticides–via bivalve ingestion–indicated

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no risk for human health, although it is important to note possible biomagnification processes that may happen along the estuarine food-chain.

1. Introduction

Contamination of aquatic environments is a worldwide problem, affecting, directly or indirectly, both human and wildlife (Serrano et al., 2012). Anthropogenic activities, such as industry and agriculture, distress the aquatic ecosystems via atmospheric pollution, effluent discharges and land use (Falconer, 2006; US Environmental Protection Agency (EPA), 2002). As a consequence of the agriculture activity, several million tons of fertilizers and pesticides are applied each year, contaminating both surface and groundwater (Schwarzenbach et al., 2006). By providing habitat for local and migratory fauna, the estuarine and coastal environments are highly impacted by the presence of these compounds (Barbier et al., 2010; Katagi, 2010; Pitarch et al., 2007), bringing deleterious effects to non-target organisms, such as birds, fish, aquatic invertebrates and plants (Köhler and Triebskorn, 2013; Osterberg et al., 2012; Scholz et al., 2012; Slaninova et al., 2009).

The Tagus River, chosen as case study, is one of the major freshwater sources of Europe and the longest river (1038 km) of the Iberian Peninsula (Ferreira et al., 2003). Due to an extensive surface of estuarine waters and vast mud and sandflats, and saltmarshes, it provides an ideal habitat for local and migratory waders (Catry et al., 2011; Instituto da Conservação da Natureza e da Biodiversidade, 2007). As a unique habitat, that includes a natural reserve of international relevance, it offers conditions for over 250 bird species and numerous benthic communities that include bivalves, crustaceans, and fishes, being of crucial interest to maintain the good quality of this ecosystem (Instituto da Conservação da Natureza e da Biodiversidade, 2007).

Due to the ubiquitous presence of pesticides, monitoring studies involving different contamination "layers" are essential to enforce regulatory limits and warn for possible negligent agriculture and waste water treatment practices. To create an actual (since 2010) and a representative panel of pesticides used in Portugal, the European Commission database Regulation (EC No 1107/2009) (EU, 2009) and the Portuguese Regional Directorate of Agriculture and Fisheries (DRAP) were consulted. The biologic model used herein to study the bioaccumulation of pesticides, was the peppery furrow shell (*Scrobicularia plana*) which, beyond its commercial interest for human consumption, is also a crucial prey to higher trophic levels (Grilo et al., 2013).

In this vein, the main objectives of this work were: i) to evaluate the residual concentrations of 56 pesticides in DAP, SPM, and STB matrices collected in the Tagus River estuary during four seasons; ii) to identify possible ecological risks upon exposure to the maximum concentrations found in $\sum$ DAP, SPM fractions; iii) to infer about human health risks after consumption of local bivalves; iv) to evaluate the registered levels according to Directive 2013/39/EU; and v) to link the physicochemical water-quality parameters with pesticide concentrations found in the aqueous matrices.

2. Material and methods

2.1. Study area and sample collection

The Tagus is the largest river of Iberian Peninsula, ending in a large tidal estuary covering an area of 320 km^2. The estuary is located close to the Portuguese capital Lisbon, being formed by several channels, small islands, and mudflats, which provide optimum conditions for benthic communities that constitute an important source of food for higher trophic levels, such as crabs, fish, birds (local and migratory waders), and for humans too (Ferreira et al., 2003).

In this study three sampling sites were chosen, considering several factors, such as the margin side, the incidence of *S. plana* specimens, the degree of pollution previously identified by others (Rocha et al., 2015; Silva et al., 2012a; Silva et al., 2012b) and the location of the international Tagus natural park (Fig. 1). Thus, two selected sampling stations were on the south margin, close to the cities of Moita (S_1 - 38° 39′14.8″ N, 8°59′48.5″ W) and Alcochete (S_2 - 38°45′11.2″ N, 8°57′ 57.2″ W), and the other one was on the north margin, near the Sacavém city (S_3 - 38°47′47.6″ N, 9°05′46.1″ W) and close to the entrance of the Trancão River tributary into the Tagus River estuary (Fig. 1).

On each sampling site, the water samples were collected (once) at half meter depth, into pre-rinsed amber bottles, and kept refrigerated (~5 °C) during their transport to the laboratory. The animals (120 organisms *per* sampling site) were collected manually on the shore, at ca. 20 cm depth, and transported in their sediment. A total of six campaigns (December 2012, January, February, May, July and October 2013) were completed involving all four seasons; no occurrence of precipitation was observed during the sampling days.

2.2. Chemicals and reagents

2.2.1. Reagents

Methanol (MeOH), acetonitrile (MeCN), ethyl acetate (EtOAc) and hexane were purchased from Romil, with LC/GC grade, while the anhydrous magnesium sulfate ($MgSO_4$), sodium acetate (NaAcetate) and the Supelclean™ PSA SPE Bulk Packing were obtained from Sigma-Aldrich. The $MgSO_4$ was pre-heated (5 h/500 °C) to eliminate residual water and phthalates.

2.2.2. Pesticide standards and GC–MS/MS protectants

The pesticide reference standards, all with 98–99% of purity, were acquired from Sigma-Aldrich. With exception of Mix A (EPA 505/525, 500 mg/L) and Mix B (EPA 505/525, 500 mg/L), all other pesticides were purchased individually. All standard solutions were prepared individually in MeOH, to produce a final stock solution of 10,000 μg/L, and kept in dark at −20 °C, to avoid possible decay. The deuterated internal standards (IS) 4,4′-DDT-d_8 and atrazine-d_5 were used herein as surrogates. The GC–MS/MS protectants, 3-ethoxy-1,2-propanediol and D-sorbitol were acquired from Sigma-Aldrich.

2.3. Sample preparation and pesticides extraction

2.3.1. Water samples: dissolved aqueous phase (DAP) and suspended particulate matter (SPM)

Within 24 h, the water samples (500 mL) were filtrated through a 0.45 μm glass fibre filter, and the fractions DAP and SPM followed independent extraction procedures (Cruzeiro et al., 2015a; Cruzeiro et al., 2015b). The pH of DAP was adjusted to ~7, and the pesticides dissolved in this fraction were extracted by solid phase extraction (SPE) using preconditioned OASIS HLB cartridges (Waters©). Briefly, the cartridges were pre-conditioned with 5 mL of EtOAc, 5 mL of MeOH and 2.5 mL of ultrapure water (Cruzeiro et al., 2015b). The final extracts were eluted with 6 mL of EtOAc (Cruzeiro et al., 2015b).

The pesticides adsorbed to the particulate matter (SPM), which were retained by the above referred glass fibre filters, were soaked in 3 mL of EtOAc for 8 min in an ultrasonic bath (Axtor-Lovango, model CD-4820, 170 W); this procedure was done twice with the application of cooling devices to avoid temperature increase (Cruzeiro et al., 2015a).