Integrative toolbox to assess the quality of freshwater sediments contaminated with potentially toxic metals

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ABSTRACT

The Guadiana Basin is a transnational basin, presenting historical contamination with potentially toxic metals (PTM), which origin can be both natural and anthropogenic. This study explores the use of a set of observational, chemical and ecotoxicological assays with *Heterocypris incongruens, Vibrio fischeri, Pseudokirchneriella subcapitata, Thamnocephalus platyurus,* identifying the most sensitive to be included in a toolbox to analyze the quality of freshwater sediments related to this type of contamination. The study included the analysis of a reservoir and streams sediments of Guadiana basin, in two consecutive years with different climate conditions 2017 (dry year) and 2018 (normal year). The results showed high chemical variability along the basin, with greater contamination with PTM in the reservoir sediments. The calculated Enrichment Factors (EF) indicated high anthropogenic contamination by Cd, followed by Pb (EF > 1.5). The geoaccumulation index (I*geo*) revealed that the sediments were severely polluted with Cd, and slightly polluted with Pb and Cu, inducing a higher sublethal toxicity to *Heterocypris incongruens*. Among the parameters evaluated, and after the use of multivariate statistical techniques, the toolbox for assessing sediments quality, in similar climate and geological conditions, should include the analysis of: meteorology, land use/cover in the area, granulometry, organic matter content, PTM concentrations, contamination indices (e.g., I*geo* and EF), and sublethal bioassays with *H. incongruens* (total sediment analysis) and *Vibrio fisheri* luminescence inhibition (pore water analysis).

1. Introduction

The importance of the sediments compartment in the aquatic ecosystem is unquestionable, and it is commonly accepted that the appropriate freshwater quality assessment is only possible if sediment analysis is also considered. Sediments are the habitat of several benthic species in lakes, reservoirs, and rivers, participating in their food webs (Burton, 2002). As a reservoir of hazardous substances, such as potentially toxic elements (PTM) and organic compounds, they act in the bioaccumulation and bioamplification in the food webs, as well as secondary sources of pollution to the aquatic ecosystems (Sahli et al., 2021).

Notwithstanding the above being accepted by the scientific community, in Europe, there is still a gap in guidelines for the protection of freshwater sediments. In fact, in several European countries such as Portugal, sediment quality assessment is not yet included in the river or reservoir management strategies.

Several authors have been working on proposing pollutants concentration limits, to ensure sediment health quality criteria (MacDonald et al., 2000; Macfarlane, 2003; USEPA, 2001). However, chemical concentrations are insufficient to demonstrate adverse environmental effects, to which toxic evidence is also needed. Indeed, in recent years, studies have been carried out and guidance manuals have been developed to identify the most promising bioassays to detect

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sediment-induced ecosystem disruption effects (Broccoli et al., 2021; Ingersoll and MacDonald, 2002; da Silva de Freitas et al., 2022). Researchers agree on the need to integrate tools from different areas of knowledge (physicochemical, biological/ecotoxicological) to improve the quality assessment of sediments, with a better response to different types of sediments, and to climate and land uses of each region/country (Broccoli et al., 2021; dos Santos Mendonça et al., 2021; Maradonna et al., 2020). The joint analysis of the results will allow a clearer interpretation of the most sensitive endpoints and the most important risk factors, considering the health of the sediments ecosystem.

One of the most important groups of contaminants that occur in sediments are PTMs, being their main accumulation compartment (Liu et al., 2021; Luo et al., 2021; Rani et al., 2021). Metals in freshwater sediments mainly derive from rock weathering, soil erosion, runoff from agricultural activities, wastewater, and atmospheric precipitation (Palma et al., 2015; Wang et al., 2021). Metals can be concentrated in sediments by adsorption to fine particles, organic matter, Fe/Mn oxides, and sulphides (Liu et al., 2021). However, sediments cannot fix metals permanently. In fact, under certain environmental conditions (such as, the decrease of pH, or the degradation of organic matter), the metals in sediments can be released, back into the overlying water column, and resulting in a secondary source of pollution (Boguta et al., 2022). In certain concentrations, metals are responsible for human diseases (Ehrlich et al., 2016; Huat et al., 2019; Soomro et al., 2019) and ecosystems unbalance (Rao et al., 2021; Varol et al., 2022), representing a real concern for health and environmental authorities.

The present study focused on the application of a set of observational and analytical parameters (chemical and ecotoxicological), to assess the impact of PTM on freshwater sediments during two consecutive years with marked climate differences, analyzing which parameters are the most effective to evaluate their quality and proposing an easy-to-use toolbox with the most sensitive ones. The study was carried out in the Guadiana Basin, a transnational Mediterranean basin, with a semi-arid climate. The set of parameters proposed initially included: (1) Land use/Land cover (LULC) analysis and meteorological indicators; (2) sediments characteristics: (a) physical parameters – granulometric parameters; (b) chemical parameters - quantification of PTM, as the Guadiana basin is part of a rich geological area, the Iberian Pyrite Belt (IPB), and within an intensive agriculture area; (3) degree of contamination using geochemical analysis through the Enrichment factor (EF) and the Geoaccumulation index (I*geo*); and (4) Environmental risk analysis, using: (i) sediments quality guidelines (SQG), through the Interim Sediments Quality Guidelines (ISQG), for Cu, Cd, Cr, Pb and Zn (CCME, 2002), and ERL (effect range low) for Ni, as proposed by Long et al. (1995), once Ni was not considered in the ISQG; (ii) biological indicators – to assess the toxicity of the sediments by developing an ecotoxicological risk analysis using the whole sediment and the pore-water, with species from different trophic levels (*Heterocypris incongruens, Vibrio fischeri, Pseudokirchneriella subcapitata, Thamnocephalus platyurus*), and lethal and sub-lethal ecotoxicological endpoints.

2. Materials and methods

2.1. Study area and sampling sites characterization

The Guadiana is a transnational basin with a total area of 67 200 km^2, being 11 580 km^2 in the Portuguese territory. The hydrological regime is torrential, with a dominance of intermittent rivers/streams. From the morphological point of view, the basin is divided into three distinct zones: High, Medium and Lower Guadiana (APA, 2016). The Lower Guadiana zone corresponds essentially to the Portuguese area of the basin and includes part of the Iberian Pyrite Belt (IPB), one of the world's most important provinces for volcanogenic massive sulphide deposits (Gomes et al., 2014). In the IPB, more than 80 deposits have been referenced, with an estimated 1700 Mt total reserves (massive sulphides and stockworks) containing 14.6 Mt of Cu, 13.0 Mt of Pb, 34.9 Mt of Zn, 46 100 t of Ag, 880 t of Au and significant amounts of other metals, such as Sn (Leistel et al., 1997).

The study was performed in eight sites located in the Portuguese sector of Guadiana Basin: four at the Alqueva reservoir (Lucefécit – Lf; Mourão – Mr; Al – Álamos; Alqueva-Montante – Mn), and the remaining four in streams of Guadiana (S_Lf – Lucefécit Stream; S_Al – Álamos Stream; S_Am – Amieira Stream; S_Zb – Zebro Stream (Fig. 1). For each sampling site, a drainage basin was calculated as the region where precipitation, surface and groundwater are collected and drain off to the sampling point. The drainage basins are deemed proper areas to examine associations between LULC and meteorological conditions with chemical and ecotoxicological indicators obtained at the sampling points, since all water in the area converges to that point. The drainage basins for each of the sites were obtained from the 1 arcsecond (30 m) resolution Shuttle Radar Topography Mission (SRTM) elevation data (Palma et al., 2021a). Fig. 1 shows the region of study comprising a total area of 8660 km^2, including the delimitation of the drainage basins for each of the sites, and the respective LULC classes.

The general characterization of the sediments at each sampling location is displayed in Table S1, adapted from (Palma et al., 2020a, 2021b), with sediments of reservoir mostly constituted by fine particles, higher amounts of organic matter (OM) and nutrients, compared to the sediments of the streams.

2.2. Land use/land cover (LULC) and meteorological characterization

Land use, land cover (LULC) data used is available from the 2018 CORINE Land Cover Pan-European inventory obtainable at the Copernicus Land Monitoring Service (https://land.copernicus.eu/; last accessed on 9 May 2022). The CORINE LULC inventory distinguishes 44 classes, spanning into the following five categories: (i) Artificial surfaces, (ii) Agricultural areas, (iii) Forest and seminatural areas, (iv) Wetlands, and (v) Water bodies. The minimum mapping unit and width are 25 ha and 100 m, respectively. The geometric accuracy is lower than 10 m and thematic accuracy greater than 85% (Kosztra et al., 2017). Open data available from the Portuguese National Geographic Information and from the Directorate-General for Energy and Geology (DGEG) were also considered. These data consist of areas licensed by the DGEG, in force, for the exploration of mineral masses in mainland Portugal and of degraded mining areas, in mainland Portugal, which are under concession for environmental recovery (https://www.dgeg.gov.pt/; vector data are available through https://snig.dgterritorio.gov.pt/; last accessed 28 June 2022). The CORINE LULC classification was combined with the DGEG information and grouped considering that some of the classes may constitute potentially toxic metal sources, yielding 11 classes (Table S2). The class distribution in each of the drainage basins considered (Fig. 1) is shown and analyzed in section 3.1.2.

The meteorological data was obtained from the E-OBS dataset (Cornes et al., 2018) of the Copernicus Climate Change Service, based on observations from identified and recognized European ground-based networks of weather stations, most of them managed by the European national weather services (https://climate.copernicus.eu/; last accessed on 6 May) The E-OBS is provided with a daily resolution, over a regular horizontal grid of 0.1° × 0.1°, from 1 January 1950. The meteorological variables used were the daily minimum, maximum and mean air temperatures, as well as precipitation. The air temperatures were measured 2 m above the ground, in accordance with the World Meteorological Organization (WMO) standards. The daily accumulated precipitation corresponded to the height of the equivalent liquid water. Daily tem-perature and precipitation data, for the period 1 January 2017 to 31 December 2018, were averaged for the areas corresponding to each of the drainage basins considered (Fig. 1; results analyzed in section 3.1.1).

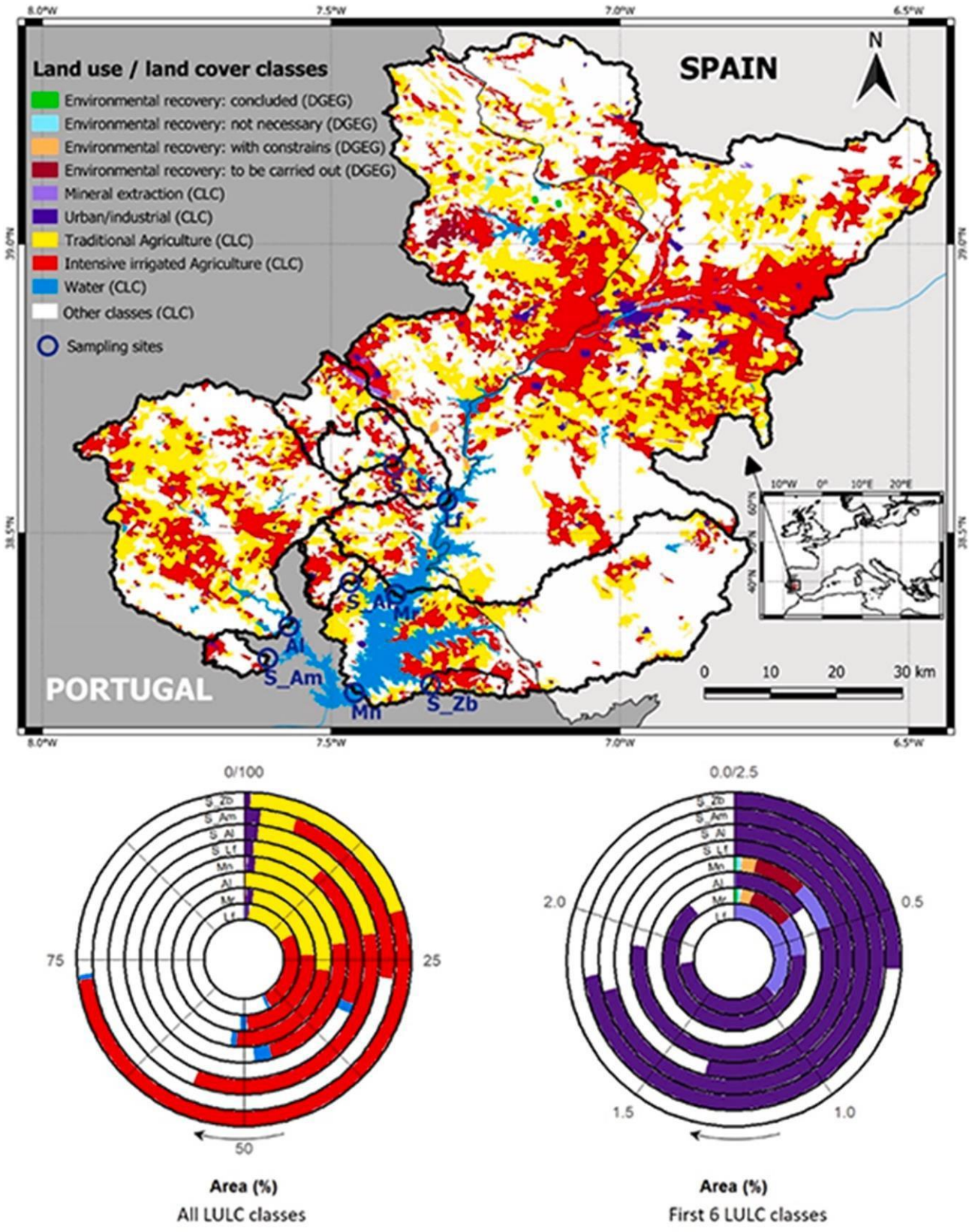

Fig. 1. Map illustrating the region considered for the study in the south of Portugal, encompassing Alqueva reservoir (in deep sky blue) and the respective LULC distribution. The blue circles indicate the sampling sites and the respective drainage basins are delimited by the black lines. Polar plots showing the percentage areas corresponding to each class (bottom left) and only to the first six classes indicated in the legend (bottom right). Each LULC class corresponds to a color, as illustrated in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.3. Sampling campaigns

The study comprised 12 sampling campaigns, with bimonthly collections during 2017 (drought year) and 2018 (normal climate year). The temporal analysis was developed considering the seasonal classification for the Alentejo region: wet period comprising the months of January, March and November, and dry period that includes May, July and September (APA, 2016).

At each sampling point, five litters of surface sediments (collected to a depth <10 cm) were gathered using a stainless steel van Veen grab of 0.05 m^2. In Al, Mn, Mr and Lf (reservoir sampling points), sediments were collected in locations with different water columns depths (26, 65, 45 and 20 m, respectively), using platforms in the middle of the reservoir, while the sediments from the streams were collected along the banks. The sediment samples were packed in polyethylene bags and transported to the laboratory, refrigerated at 4 °C, after which they were preserved according to the standards recommendations (USEPA, 2001). The pore-water for the indirect ecotoxicological assays, was obtained until 72 h after the sampling, through centrifugation at 8000 rpm for 30 min, at 4 °C (USEPA, 2001). The whole-sediment and the pore-water to be used in the ecotoxicity tests were stored at −20 °C during a maximum period of one month before analysis.

2.4. Sediment analysis

2.4.1. Potentially toxic metals concentrations

Potentially toxic metals concentrations (Cd, Cr, Cu, Ni, Pb, Zn and Fe) were determined according to ISO 11466 (ISO, 1995), by atomic absorption spectrometry, with flame or electrothermal atomization, using a Varian equipment (SpectrAA 220FS and 220Z and 110Z), according to the methodology described in Palma et al. (2014). The quality control of the analysis was performed by evaluating blanks, triplicates, recovery rates, and relative deviations with standard reference materials (Palma et al., 2015).

2.4.2. Degree of contamination

The analysis of the degree of contamination by PTM in rich geological areas must be performed taking into consideration their background levels in the area, to distinguish the concentrations of metals of natural origin from those of anthropogenic sources. Background metal levels in the Guadiana Basin were obtained from previous studies (Delgado et al., 2008, 2010; Palma et al., 2015). In sediments, this kind of analysis is done by determining pollution indexes, such as the EF and the I*geo*. The EF is used to estimate the anthropogenic impact of each element, analyzed using a normalized element, which must be correlated with fine particles and have large concentrations in the area. In the present study, Fe was the element selected for the normalization (justified by *Spearman* correlation, Table S2). The EF was calculated by the equation (1) (Christophoridis et al., 2009; Liu et al., 2003).

$$EF = \frac{[X/N]sample}{[X/N]\ background} \quad (1)$$

Where, $[X/N]_{sample}$ and $[X/N]_{background}$ correspond to the ratios of mean concentrations (mg kg^{-1}, dry weight) of the target metal [X] and the normalizing element [N] in the sediment sample and in the regional background (Delgado et al., 2008, 2010), respectively. Enrichment Factors between 0.5 and 1.5 indicate that the PTM is mostly provided from natural origin; EF > 1.5 indicate that an important portion of the PTM is provided from biota or anthropogenic action (Zhang and Liu, 2002).

The I*geo* is used to assess the contamination by comparing the current and regional background concentrations of the metal in the sediments (Hanif et al., 2016). The I*geo* is calculated using the following equation (eq. (2); Loska et al., 1997):

$$Igeo = \log 2 \left(\frac{Cn}{1.5\ Bn} \right) \quad (2)$$

where Cn is the measured concentrations of metal (n) and Bn is the geochemical background concentrations of the metal. The factor, 1.5, is the "background matrix correction value". I*geo* values are classified into seven levels (Duodu et al., 2016): $Igeo \leq 0$, unpolluted; $0 < Igeo \leq 1$, slightly polluted; $1 < Igeo \leq 2$, moderately polluted; $2 < Igeo \leq 3$, moderately severely polluted; $3 < Igeo \leq 4$, severely polluted; $4 < Igeo \leq 5$, severely extremely polluted; $5 > Igeo$, extremely polluted.

2.4.3. Ecotoxicological analysis

Ecotoxicological analysis were performed in the whole-sediment and the extracted pore-water, using a set of representative bioindicator species from different trophic levels. The whole-sediment toxicity was assessed using *Heterocypris incongruens*, a cosmopolitan ostracod species, with a sensitivity quite like the amphipod crustacean *Hyalella azteca* and the midge larva *Chironomus riparius* (Belgis et al., 2003). The pore-water ecotoxicity was assessed using aquatic organisms, the bioluminescent bacterium *Vibrio fisheri*, the crustacean *Thamnocephalus platyurus* and the microalgae *Pseudokirchneriella subcapitata*. The results for the selected endpoints allowed the characterization of the levels of lethal and sublethal toxicity of the sediments for these species.

For all bioassays, a test with a reference substance was performed, as a positive control. The sensitivity of the organisms was in accordance with the followed protocols. The control groups had a survival rate above 90%.

2.4.3.1. Toxicity assessment of whole-sediment. The mortality and growth inhibition, of neonates of the ostracod crustacean *H. incongruens*, were performed according to the Ostracodtoxkit F standard operating procedure (Creasel, 2001). Test organisms were obtained from cysts, incubated for hatching at 25 °C under continuous illumination, 52-h before the test. After the first 48-h, neonates were pre-feed with *Spirulina* powder. The control and five replicates per sediment sample (without dilution), each with 10 neonates, were performed. After a 6-days exposure period, survival, and final length (in μm) were recorded. Toxicity was expressed in mortality, as acute endpoint, and growth inhibition as chronic criterion. Measurements of length were only performed when mortality was lower than 30%. The length increment was calculated based on equations (3) and (4) adapted from Creasel (2001):

$$\%\ \text{Growth inhibition} = 100 - (L_{\text{increment test sediment}} \ / \ L_{\text{increment control sediment}})\ x100 \quad (3)$$

where $L_{increment}$ is the mean length increment, calculated with the formula:

$$L_{increment} = L_{end} - L_{start} \quad (4)$$

where:

L_{end}: is the mean length at the end of the test (in μm)

L_{start}: is the mean length at the start of the test (in μm)

2.4.3.2. Toxicity assessment of pore-water

2.4.3.2.1. Luminescence inhibition of V. Fischeri. Luminotox® was used to evaluate the luminescence inhibition of the marine bacterium *V. fischeri* (NRRL B-11177), according to the protocol "DR LANGE luminescent bacteria test" following ISO 11348–2 (1998). Tests were carried out using the sediment pore-water samples and their dilutions with a 2% NaCl solution (50, 25, 12.5, 6.25 and 3.125%, v/v). Two replicates per treatment were used. The inhibition of the bacteria natural light emission was measured against a nontoxic control (2% NaCl solution), at a temperature of 15 ± 0.5 °C. For each sample, bioluminescence was measured before and after the desired incubation period (30 min). The EC_{50} (%), the concentration of each sample (%; v/v) that

reduced 50% of the bacterial luminescence, was determined.

2.4.3.2.2. Growth inhibition of the green microalgae P. subcapitata. Microalgae were exposed to pore-water samples for 72 h and algae growth was determined based on OECD 201 (2006). At the beginning of the test, *P. subcapitata* (100 μL of an inoculum with 3–5 × 10^4 cells mL^{-1}) was exposed to the respective diluted samples (12.5, 25, 50, 75, 100%; v/v; 900 μL of total volume) performed with MBL medium (negative control) in a 24-well microplate. Test vials were randomly incubated in an orbital shaker for 72 h at 21 ± 2 °C and with a constant luminous intensity (60–120 μE m^{-2} s^{-1}, equivalent to 6.000–10.000 lx). The concentration of the algal biomass at the beginning and after 72 h of exposure was calculated by counting the number of cells in a Neubauer chamber.

The average specific growth rate for a specific period was calculated from equation (5):

$$\mu_{i-j} = \left(\ln B_j - \ln B_i\right) / t_j - t_i \quad (5)$$

Where: μ_{i-j} is the average specific growth rate from time i to j; t_i is the time for the start of the exposure period; t_j is the time for the end of the exposure period, B_i is the biomass concentration at time i, and B_j is the biomass concentration at time j.

The inhibition of algal growth was estimated from equation (6):

$$\%\, I = [(\mu_c - \mu_t) / \mu_c] x\, 100 \quad (6)$$

Where: % I is the mean percentage of inhibition for specific growth rate; μ_c is the mean value for the growth rate in the control, and μ_t is the mean value for the growth rate in the water samples.

3.1.1.1.1. Mortality of the crustacean T. platyurus. The mortality assay with the crustacean *T. platyurus* was adapted from Persoone (1999). The test with *T. platyurus* was developed using the THAMNOTOXKIT F™ kit. Larvae of the shrimp *T. platyurus* (<24 h, obtained from the hatching of cysts) were exposed to different dilutions of sediment pore-water samples (12.5, 25, 50, 75 and 100%; v/v), during 24 h. Four replicates per dilution were performed. Organisms were not fed during the test. The number of dead organisms was used as endpoint and the EC_{50} (%), concentration (%; v/v) causing lethality on 50% of the exposed organisms, was calculated.

2.5. Statistical treatment of data

In the *V. fischeri* bioluminescence inhibition test, the EC_{50} (%) values were determined using LUMISsoft 4 Software™. The EC_{50} (%) values for the *T. platyurus* mortality were determined using the probit analysis (Finney, 1971). Data of sublethal bioassays were checked for homogeneity of variance by the Kolmogorov–Smirnov test (Sokal and Rohlf, 1995) and, when possible, subjected to one-way analysis of variance (ANOVA). Data that do not satisfy the assumption for ANOVA were analyzed non-parametrically using Kruskal–Wallis ANOVA by ranks test. Whenever significant differences were found ($p < 0.05$), a post hoc *Dunnett's* test was used to compare treatments with the control, for a p value of 0.05 as the significant level (Zar, 1996).

The *Spearman* rank correlation coefficients were used to evaluate the associations between the LULC, meteorological conditions, chemical indicators, ecotoxicological indicators (with the respective endpoints: EC_{50}, growth inhibition), with a level of significance $p < 0.05$.

All the variables were explored and described using descriptive statistics (mean ± standard deviation) and multivariate statistical analysis ((factor analysis (FA) and cluster analysis (CA)), using ((90 samples x 2 groups of observational parameters (LULC classes and climate conditions) x 9 chemical indicators (Clay, OM, pH, Cd, Cr, Cu, Ni, Pb, Zn) x 4 ecotoxicological indicators)). Multivariate analysis was used to identify the most important parameters that explain the variability of sediment quality, allowing their integration in an easy-to-use toolbox to assess the sediments quality. Furthermore, this statistical methodology can also be used to prove correlations previously established between the potentially toxic elements and the observed toxic effects. Data were normalized (log x; x = mean value) prior to multivariate statistical analyses, avoiding misclassification due to differences in data dimensionality (Simeonov et al., 2003).

All statistical analyses were performed with the STATISTICA 7.0 (Software™ Inc., PA, USA, 2007).

3. Results and discussion

3.1. Land use/land cover (LULC) and meteorological characterization

Fig. 1 characterizes the basin in terms of LULC classes, resulting from the information of the CORINE LULC classification and of the DGEG information in areas with environmental recovery or areas with exploration of mineral masses (Table S2), showing their distribution in each of the drainage basins. A relevant class that may be correlated with the existence of PTM in the area, because of the use of fertilizers and pesticides, is Intensive Irrigated Agriculture, which occupies at least 20% of each drainage basin, reaching more than 50% in the case of S_Zb (53%). Other important classes, that can be made accountable for an increase of PTM concentrations in the hydrographic basins, are Urban/Industrial and Mineral Extraction, both with low percentages in the area, but very significant as point sources of pollution. The distribution of classes as Urban/Industrial, or related with Mineral Extraction and environmental recovery, are presented in the lower right polar plot of Fig. 1 with a more appropriate scale to show their occupancy values (0–2.5%), evidencing that the drainage basin of the streams did not present mineral extraction class. Relatively to the locations in the Alqueva reservoir, Alamos present the lowest occupation for these classes, with a small area of Mineral Extraction and Urban/Industrial (0.11 km^2 and 4.67 km^2, respectively over a total area of 1279 km^2). Lucefe´cit is the location for which the drainage basin presents the highest percentage area of mineral extraction (3.80 km^2 in a total of 424.78 km^2), followed by Mr (0.23%), and Mn (0.19%), corresponding to an area of 13.70 km^2 over total areas of 5910.25 km^2 and 73 330.14 km^2, respectively. In addition, these sites have the highest occupancy of Environmental Recovery processes (with restrictions: 0.07% and to be carried out: 0.26%), which can be one of the major environmental problems in terms of PTM pollution. Traditional Agriculture generally occupies areas between 10 and 20%, except for S_Am (only 4%), which is dominated by natural vegetation, particularly the Agro-silvo-pastoral system (included in the class "Other"), the most important ecosystem in the western Mediterranean region (Godinho et al., 2016). The LULC class "Other" is also significative in the other drainage basins, with high percentage in S_Lf (67%), followed by Lf (57%) and Mn (52%), occupying less than 50% in the rest of the sites.

Fig. 2 displays the time series data of mean, maximum and minimum air temperatures, as well as accumulated precipitation, on a daily basis, for each of the eight drainage basins under study. The top panels of Fig. 2 illustrate the location of each drainage basin inside the study area and for each of these basins, air temperature (left plot) and precipitation (right plot) are represented for the two-year period considered ((y axis, from January 2017 (bottom) to December 2018 (top)). The mean temperature and accumulated precipitation are coded by the superior and inferior color scales, respectively, and represented by the colored bars in the plots. In the temperature plots, the left end of the horizontal bars indicates minimum air temperature, and the right end denotes the maximum values of air temperature. Conversely, the right end of the bars in the precipitation plots, conveys the daily accumulated precipitation values, with the left end of the bars in the origin (zero, corresponding to no precipitation).

During 2017, the south of Portugal (area of study included) suffered a severe meteorological drought with very low precipitation amounts (January 2017 to February 2018 as shown in the precipitation plots of Fig. 2) and high temperatures during a long period (April to November 2017 as illustrated in the temperature plots of Fig. 2). This drought lasted until March 2018, when heavy precipitation affected the area and

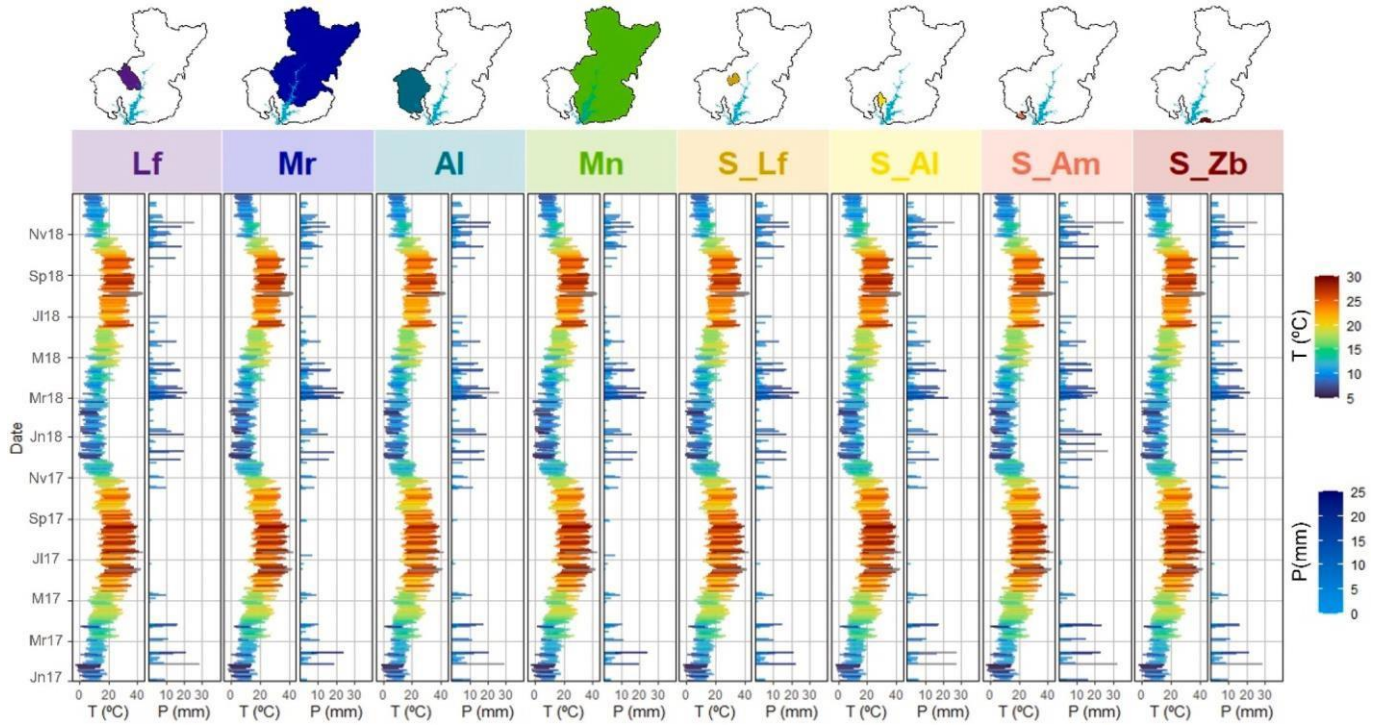

Fig. 2. Daily mean, maximum and minimum air temperatures, as well as precipitation values for each drainage basin considered (top representation), during the two-year period. Daily mean temperatures are indicated by the color scale. Daily minimum and maximum temperature values are represented by the beginning and end of each bar, respectively. Daily accumulated precipitation is indicated by the bar length, as well as, the color scale. The corresponding drainage basins are shown by the filled areas on top of each group of temperature plus precipitation plots.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.).

ended the drought. The 2018, instead, was a normal year from a climatological point of view, with respect to temperature and precipitation values The longer extension of yellow to red colors during 2017 with respect to 2018, highlight the high temperatures that occurred over a long period during the year of 2017, roughly from April to November, along with very low precipitation during the whole year. Instead, the year of 2018 presents high temperatures between June and October and higher amounts of precipitation, especially from March on. Only very slight differences can be noted in the meteorological parameters, between the different drainage basins, which was expected given the proximity and overlap of some of these areas. Nevertheless, it can be noted that the highest air temperatures in both years were registered at the southernmost streams S_Am, S_Al and S_Zb, with the latter reaching a value of 44.7 °C on 4 August 2018. In general, mean air temperatures were higher in the southern streams. As for the precipitation, the highest amounts were obtained in the southwestern areas (S_Am, Al, S_Al and Lf), with highest values in S_Am of 32.7 and 36.1 mm in January 2017 and November 2018, respectively.

3.2. Concentrations of potentially toxic metals and degree of contamination of the sediments

The sediments along the Portuguese sector of Guadiana Basin showed physical and chemical differences (Table S1), being those from the streams most constituted by sand particles, with lower levels of OM and nutrients, comparing with the reservoir sediments, as discussed in previous studies (Palma et al., 2020a, 2021a). The pH of the Guadiana sediments was neutral to slight alkaline (Palma et al., 2021a), which can induce the immobilization of metallic elements in the sediment compartment, instead of their mobilization to the water column.

The concentrations of PTM followed similar spatial patterns, with the smallest amounts quantified in the streams (Fig. 3), which presented low levels of OM and coarse particles. In fact, the results evidence the high positive correlations among OM and all the PTM (Cu: $R = 0.68$, $p < 0.05$; Pb: $R = 0.64$, $p < 0.05$; Zn: $R = 0.58$, $p < 0.05$; Ni: $R = 0.50$, $p < 0.05$). The correlation among OM and PTM was already reported by other authors (Boguta et al., 2022; Varol et al., 2022). In the streams, S_Lf presented the highest concentrations of PTM, with Ni, Cu and Cd surpassing the guide levels for the Protection of Aquatic Life in most of 2018: Ni surpassed de ERL (effect range low), representing a range intended to estimate conditions in which an effect would be rarely observed (Long et al., 1995); while Cu and Cd surpassed the ISQG (Interim Freshwater Quality Guideline; CCME, 2002). At the reservoir, Al and Lf sediments presented the greatest concentrations of PTM, with levels of Cd, Pb above the ISQG (Interim Freshwater Quality Guideline; CCME, 2002) and Ni above the ERL guideline value (Long et al., 1995), in most of the study. Cadmium was the PTM that exceeded the guide value in all the sampling points of Guadiana Basin, at least once. Despite the great temporal variability, the results highlighted higher amounts of PTM in the sediments during 2018, with positive significant correlations for Ni and Cd (Table S3).

In regions of great geological richness, the origin of the PTM must be considered, since the quantities detected, even above the limit established for the protection of aquatic life, may be from natural origin. Thus, the determination of the enrichment factor (EF) for each metal, considering the background values, allows concluding the influence of anthropogenic actions on the observed PTM concentrations. The EF index of PTM in the Guadiana sediments, showed that the quantified concentrations of Ni and Cr were, in general, of natural origin ($EF_{Ni} < 1.48$; $EF_{Cr} < 1.45$), as was already observed on previous studies (Palma et al., 2015). Henceforth, Fig. 4 represents the EF for the PTM mainly influenced by anthropogenic actions ($EF > 1.5$; Cu, Pb, Cd and Zn). In the drainage basins of Lf, Mr and Mn, the LULC class that most contributed for the levels of PTM quantified was Mineral Extraction. In fact, despite the low percentage of this activity in all the drainage basins, its importance in the contamination/transport of metals is highlighted

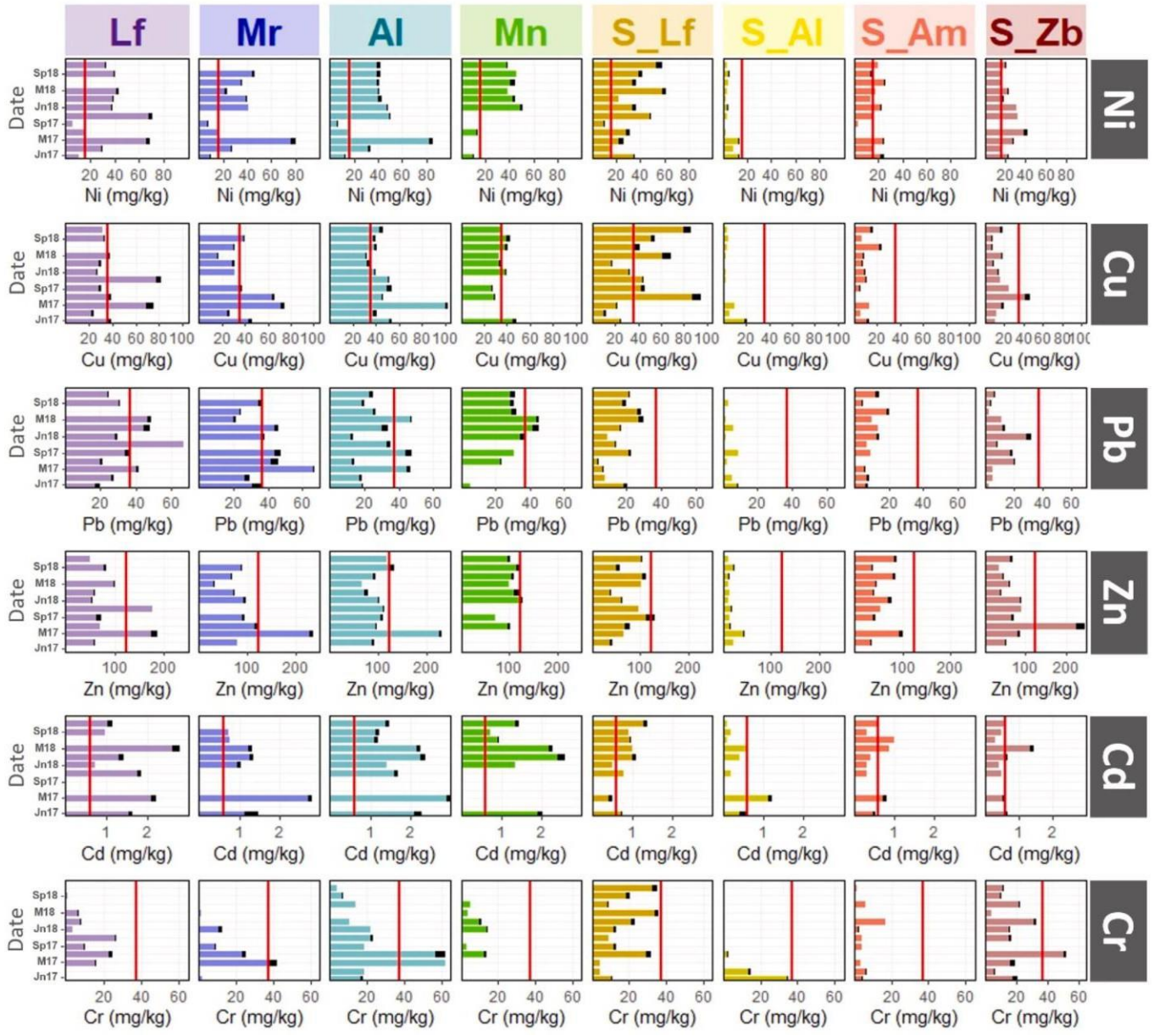

Fig. 3. Variability of potentially toxic metals on Guadiana sediments ((mean ± standard deviation (dark bar)). Red Line represents the Canadian Interim Freshwater (ISQG) for the Protection of Aquatic Life (CCME, 2002) for Cu, Pb, Cd, Cr and Zn; and ERL guideline value (effect range low) for Ni (ERL; Long et al., 1995). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

by the *Spearman* correlation results, which indicate significant positive correlations between PTM (excepting Zn) and Mineral Extraction (Table S3). In addition, agricultural activity is another class of LULC that may have influenced the presence of some of the PTM, as Cd, in the sediments of the Guadiana Basin, due to the high use fertilizers in intensive agriculture (Smolders, 2013). Agricultural activities can also be a LULC class of diffuse pollution for concentrations of Cu (traditional agriculture $R = 0.32$; $p < 0.05$; irrigated agriculture $R = 0.58$; $p < 0.05$; Cu is used in fungicide formulations applied to vineyards), which have been increasing over the years in the Guadiana Basin (Gomes et al., 2014; Palma et al., 2015). In fact, previous studies in 2011_2012, in the Alqueva reservoir, Palma et al. (2015) reported that the presence of Cu in the sediments of the reservoir, was explained mainly by anthropogenic activities.

The I*geo* index (Fig. 4) provides information on the sediment pollution status for each PTM. For Cd, the Guadiana sediments were moderately polluted in the streams, to severely polluted in the reservoir.

In fact, the temporal analysis over the years shows the increase in the state of pollution of the reservoir sediments with Cd (Palma et al., 2015), covering the entire Guadiana Basin. Further the mining activities, agriculture is another class of LULC that may have influenced the presence of Cd, in the sediments of the Guadiana Basin, due to the high use of phosphate fertilizers in intensive agriculture, formulations that naturally present Cd as an impurity in rock phosphate (Smolders, 2013; Roberts, 2014). The Alentejo region has a long history of cereals production, mainly wheat, which was produced before the water availability brought to the region by the Alqueva reservoir that allowed a diversification of crops, namely to irrigated crops. Phosphate fertilizers are a major source of trace metals to agricultural soils, especially Cd and, not only the rate of Cd:P in phosphate fertilizers used in Portugal were reported by Kabata-Pendias (2011) has the highest among the EU countries (3.1 g ha^{-1} $year^{-1}$) as, also, the application rates of P-fertilizers to cereals production in Portugal are, traditionally, very high (28 kg P_2O_5 ha^{-1} $year^{-1}$, values for 2010–2011; Smolders, 2013).

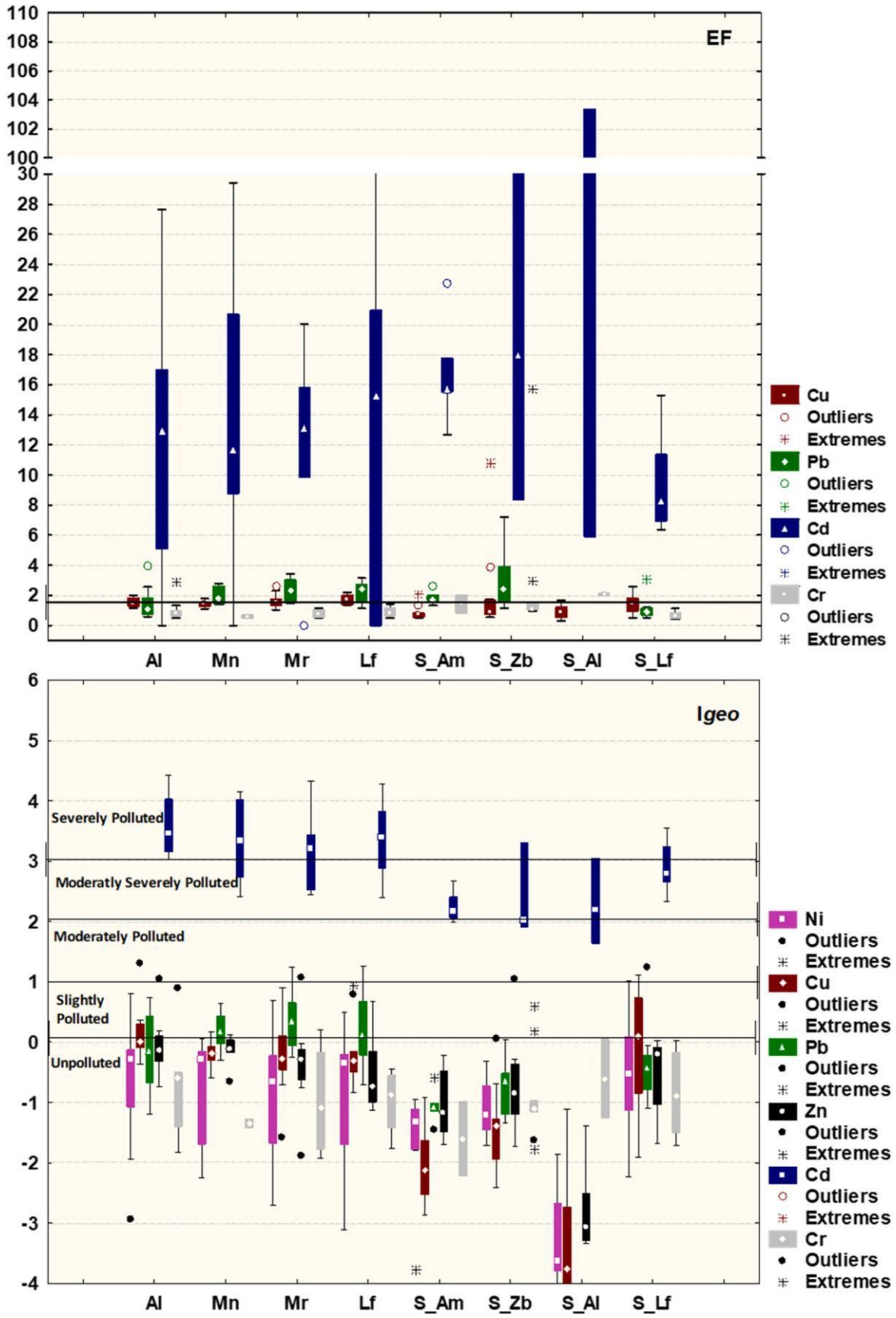

Fig. 4. Box-Whisker plots of the EF and I*geo* indexes calculated for the Guadiana sediments. The box shows the 25th and the 75th percentiles, and the whiskers represent the smallest and the largest indexes, while the line inside the box is the median. Sampling locations: Álamos (Al), Alqueva-Montante (Mn), Mourão (Mr), Lucefécit (Lf), Amieira Stream (S_Am), Zebro Stream (S_Zb), Álamos Stream (S_Al), Lucefécit Stream (S_Lf).

Relatively to the other PTM, the sediments were classified as unpolluted, except for Cu in Al and S_Lf, and for Pb in Al, Mn, Mr, and Lf, which were classified as "Slightly Polluted".

3.3. Ecotoxicological analysis

3.3.1. Toxicity of the whole-sediments

The lethal and sublethal toxicity of the sediments was evaluated with the bioindicator *H. incongruens*, using the mortality and the growth inhibition of the organisms, respectively.

Freshwater ostracods are reported as important bioindicators to assess anthropogenic stressors such as PTM, pesticides and hydrocarbons (Ruiz et al., 2013). Relatively to PTM, several studies in the literature reported its great sensitivity total Cr, Pb and Cd (Baran et al., 2015; Kudłak et al., 2011; Torokne and Toro, 2010). Further, several studies reported the sensitivity of the specie to some physical proprieties of the sediments, namely granulometry (Casado-Martinez et al., 2016). In fact, results evidenced the correlation between the presence of high percentages of clay and the inhibition of growth of the species (Table S3). The mortality percentages in the assays with *H. incongrues* (control and samples sediments) were always less than 10%, indicating that the organisms were in good condition and also that the hazardous substances existing in the sediments were in concentrations that did not induce considerable levels of acute toxicity.

Regarding the growth inhibition endpoint, the results showed that the reservoir sediments were more toxic towards the ostracod (inhibition rate reaching 80% in Al_2018) than the stream sediments (inhibition rate reaching 60% in S_Al_2017) (Fig. 5). The sublethal toxicity results agreed with the degree of pollution determined, with Al being the site that induced the greatest growth inhibition to ostracod, mainly during 2018, followed by Mn sediments. In fact, the I*geo* index indicated that Al had the most polluted sediments, being classified as severely polluted with Cd and slightly polluted with Pb, Cu, and, sporadically, with Zn. In fact, the statistical results highlight the significant positive correlations between the observed toxic effects (rate of growth inhibition of the species) and the presence of these PTMs (Table S3). In addition, the observed effect may also be related to the granulometry of the sediments, since Al presented a high percentage of clay particles in its structure. The temporal analysis allowed to conclude that the sediments from the reservoir collected in 2018 induced a higher toxic response (higher growth inhibition rate) to the organisms than those of 2017.

3.3.2. Toxicity of the pore-water

The pore-water toxicity was evaluated with species from different trophic levels, using distinct toxicological endpoints, the results were displayed in Table S4 and Fig. 6, evidencing that *V. fisheri* was more sensitive than *T. platyurus* to the analyzed sediment samples (Table S4). Spatial analysis showed that the bacteria was more sensitive to stream sediments, with the highest number of toxic samples occurring during 2018. The toxicity detected by the bacteria is likely to be correlated with hazardous substances other than PTM, as pharmaceuticals or pesticides already detected in the basin (Palma et al., 2020b, 2021b), since the streams had the lowest concentrations of PTM, generally below the guiding limits for the protection of aquatic life (CCME, 2002). In fact, the results indicated no significant correlation among *V. fisheri* and the presence of PTM in the sediments (Table S3). Thus, despite the *V. fischeri* bioassay being known to be highly sensitive for detecting PTM toxicity (Codina et al., 2000), being generally a good choice to evaluate this type of risk, that was not the case in this study. Other authors have already observed this kind of result, justified by the low concentrations of PTM in the sediments (Hwang et al., 2021). *T. playturus* was more sensitive to samples from the wet period of 2017 (Jn, Mr and Nv). In fact, the mortality of *T. platyurus* showed a significant positive correlation with the concentrations of Zn in the sediments ($R = 0.80$; $p < 0.05$), suggesting that this species is sensitive to this metal, as reported by (Zhang et al., 2022) and explaining the results observed in the first months of 2017 (see Table S4). The results obtained for the growth rate of the green microalgae *P. subcapitata* revealed, in general, great sensitivity to the sediments of the Guadiana Basin (Fig. 6). Growth inhibition was more pronounced during the wet period of 2018, with S_Zb and Lf being the areas with the highest toxicity. The Guadiana sediments induced greater sublethal toxicity to this species than others from other countries (Broccoli et al., 2021), but this cannot be explained by the concentrations of PTM alone, fact highlighted by the *Spearman coefficients*, without significant correlations between PTM and the inhibition of microalgae growth. In fact, the observed result may be correlated with the presence of other hazardous substances such as herbicides (very toxic to green microalgae; Ma et al., 2002), from agricultural activities, which are one of the main activities in the basin. The results supported this hypothesis, since the highest growth inhibition rates occurred in wet period of 2018, when precipitation increased, enhancing the run-off of hazardous substances, previously applied to the crops.

3.4. Parameters to include in an easy-to-use toolbox to assess freshwater sediments quality

3.4.1. Correlation of LULC classes, geochemical parameters and ecotoxicological data

To analyze the most effective parameters to integrate in a toolbox for

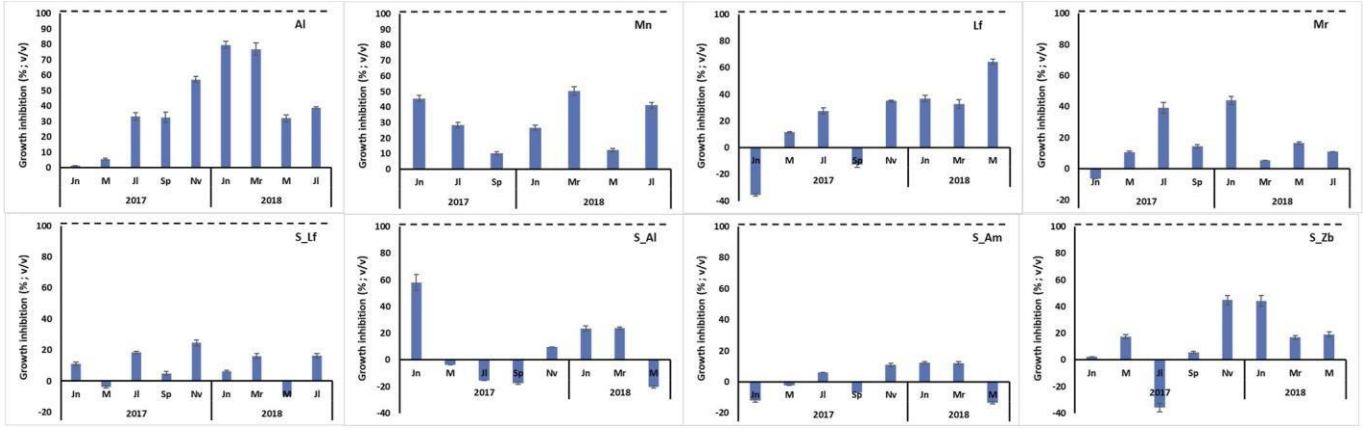

Fig. 5. Growth inhibition (%) of the ostracods *H. incongruens* neonates, after 6 days of exposure to sediments from the Guadiana Basin (mean ± standard deviation, n = 6). Sampling locations: Álamos (Al), Alqueva-Montante (Mn), Mourão (Mr), Lucefécit (Lf), Amieira Stream (S_Am), Zebro Stream (S_Zb), Álamos Stream (S_Al), Lucefécit Stream (S_Lf). Months of sampling: January (Jn), March (Mr), May (M), July (Jl), September (Sp), November (Nv).

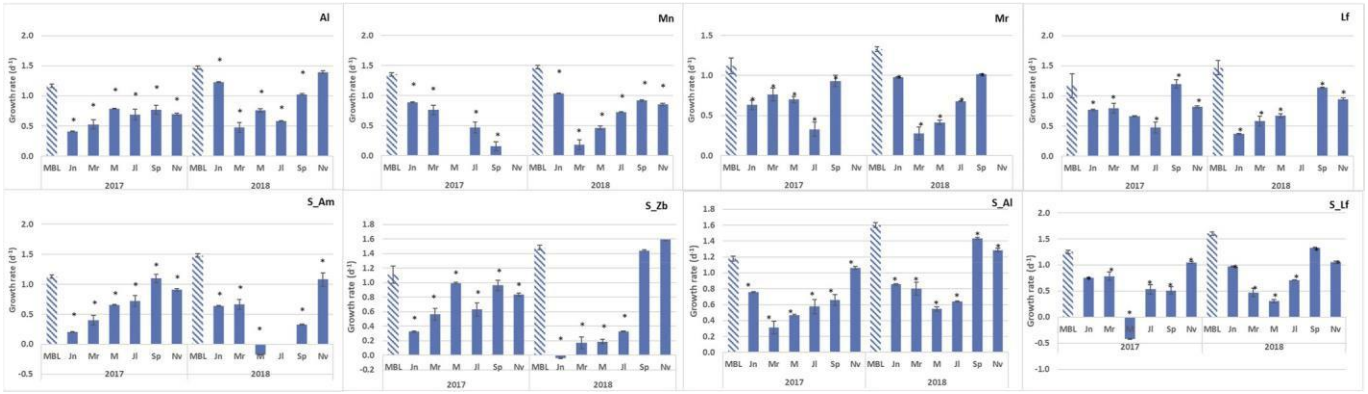

Fig. 6. Growth rate (d^{-1}) of the green microalgae *P. subcapitata* 72-h exposed to the different sediments samples tested (mean ± standard deviation; n = 6; *$p < 0.05$, *Dunnett's* post hoc comparison test with the control MBL (blue stripes). Sampling locations: Álamos (Al), Alqueva-Montante (Mn), Mourão (Mr), Lucefécit (Lf), Amieira Stream (S_Am), Zebro Stream (S_Zb), Álamos Stream (S_Al), Lucefécit Stream (S_Lf). Months of sampling: January (Jn), March (Mr), May (M), July (Jl), September (Sp), November (Nv). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sediment quality assessment, factor analysis (FA) and cluster analysis (CA) methods were used. The data were explored by year (2017: year of drought; 2018: year of normal climate), to understand if the climate conditions influenced the parameters that best explained the quality of sediments (Fig. 7).

The FA performed on the data from 2017 allowed the extraction of six factors with eigenvalue >1, accounting for 78% of the total variance. Factor 1 explained 30% of the total variance and presented factor loadings with positive absolute values > 0.50 for the variables: Mineral Extraction LULC class (0.54), clay content (0.68), OM content (0.58), PTM ((all of them with loadings between 0.63 (Cd) and 0.89 (Cu)), and growth inhibition of Ostrocod (0.53). Therefore, this factor reflects the link between the sediment properties and the metal pollution of the basin, identified by the bioassay with *H. incongruens*. All these parameters were projected in the positive quadrant of Factor 1. The Factor 2 with 15% of the total of variance was explained by Intensive Irrigated Agriculture (−0.88), Traditional Agriculture (−0.60) and mortality of *T. platyurus* (−0.57), which confirm that the mortality of the crustacean, induced by sediments from the Guadiana, was probably correlated with hazardous substances other than PTM, probably brought from agricultural activities. The CA applied to the 2017 samples (44 samples of the total), did not show similarities between those projected in the positive quadrant of Factor 1, and explained by metal pollution, which corresponded to 34% of the total samples. The two clusters formed (1 and 2), integrated samples from S_Am and Lf, influenced by Agriculture and Urban/Industrial classes.

The FA with the data from 2018 allowed the extraction of five factors with eigenvalue >1 that reported 77% of the total variance of the data. Factor 1 (34% of the variance) was positively correlated with Urban/Industrial LULC class (0.56) and luminescence inhibition of *V. fisheri* (0.52), and negatively explained by the same parameters correlated with metal pollution observed for 2017: clay (−0.62), OM (−0.80), PTM, excepting Cr, (−0.85 (Ni) to −0.77 (Cu)) and growth inhibition of ostracod (−0.52). Factor 2 was explained by intensive irrigated agriculture (−0.79) and traditional agriculture (−0.73) LULC classes, and by mortality of *T. platyurus* (−0.55), having as principal attribute the agriculture activities most identified by the *T. platyurus* bioassay responses. The 2018 samples (46 in the total), showed high similarities, allowing to project four clusters: cluster 1 (Al samples) and cluster 2 (Lf and Mr samples) mainly explained by metal pollution attribute; cluster 3, integrating samples from S_Am and S_ Lf, and cluster 4 (S_Al), were both mainly explained by the Urban/Industrial class, having high toxicity towards *V. fisheri*.

3.4.2. Toolbox proposal

The integrative analysis of all the results allowed to conclude that the toolbox for the assessment of freshwater sediments quality must include:

i Observational parameters: among the observational parameters used, those that showed greater importance in explaining the data related to the quality of the sediments were the CORINE LULC classification, allowing the correlation between the contamination indices and the sources of pollution.

ii Analytical parameters: (a) sediments granulometry and OM content are essential to understand the dynamics of hazardous substances in the sediments, the adsorption/desorption and leaching processes; (b) PTM quantification; (c) contamination degree indexes, both EF and I*geo*, must be used, to understand the type of contamination (natural or anthropic), given by the EF, and the degree of pollution, obtained by I*geo*. In both cases, we must have the PTM background of the area and the quantification of a normalized element, such as Fe or Al.

iii Biological parameters: The SQGs and the ISQG give us a general indication of the concentration of each PTM that can induce risk to the aquatic ecosystem, which is a limited information when an integrative assessment is needed. To understand the toxicological status and sediments, it is essential to include ecotoxicological parameters, that assess the impact of known and unknown substances. So, according to the data, among the tested bioassays, the toolbox should integrate the acute and chronic assay with *H. incongruens*, which was very sensitive and specific to detect PTM contamination in the whole sediment. Regarding the pore-water analysis, the data did not show clear evidence of which could be the best choice, although the *V. fisheri* bioassay was the one that detected the largest number of toxic samples. In fact, and despite *V. fisheri* being identified as a good bioindicator to detect toxic effect of PTM in sediments (Maradonna et al., 2020), the results showed some limitations of the assay, mainly when PTM concentrations were low, occurring a loss of bioindicator sensitivity.

4. Conclusions

The analyses of sediments are, nowadays, considered of crucial importance to understand the contamination processes in freshwater systems. Many of the studies carried out did not present an integrative approach, focusing mainly on chemical parameters. Presently, it is evident the need to include chemical and biological analysis to explain the action that sediments have in the water ecosystem. The question is, which parameters should be used to give the right answers? In the present study, a group of parameters were used to analyze the sediments, trying to identify the most sensitive ones to PTM's contamination. The study was carried out in a transnational basin (Iberian Peninsula), with Mediterranean climate conditions, which allow the

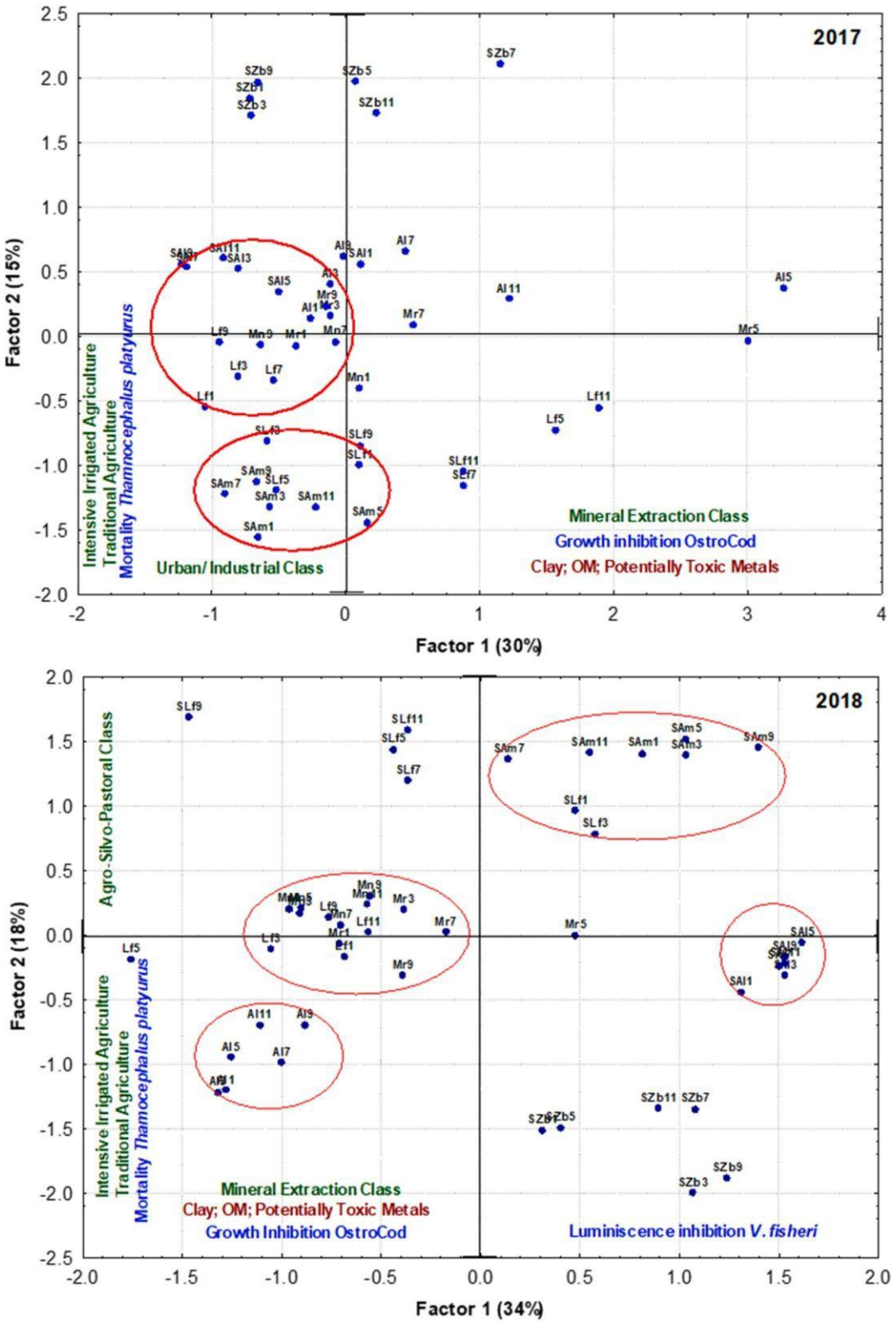

Fig. 7. Factor analysis plot showing the distributions of the LULC factors, physicochemical parameters and ecotoxicological indicators of Guadiana Basin sediments samples on the bidimensional plane defined by the first two principal components (PC). In the figure are displayed the samples scores and the statistically significant clusters obtained through the Square Euclidean distance. Samples were collected at Álamos (Al), Alqueva-Montante (Mn), Mourão (Mr), Lucefécit (Lf), Amieira Stream (SAm), Zebro Stream (SZb), Álamos Stream (SAl), Lucefécit Stream (SLf)., during the years of 2017 and 2018 in the months of January (1); March (3); May (5); July (7); September (9) and November (11).

transference of the conclusions to other basins with similar characteristics. Also, the results highlighted that the climate differences occurring in different years did not induce a pronounced variability in the organization of the data, allowing to assume that the proposal toolbox would be efficient in different climate conditions. Data analysis showed that, to be easier for the managing authorities to recognize existing problems in the basin area, and implement solutions, the toolbox must include three research fields: observational, analytical, and biological. The observational component should integrate the LULC class analysis; the analytical component the granulometry, OM, PTM quantification and degree of contamination indices; and the biological component the sub-lethal bioassay with *H. incongruens* and *V. fisheri*. Of course, this does not mean that the toolbox is closed to other parameters, it only means that they can be considered a basis for the quality assessment. Knowledge would increase with the inclusion of other chemical aspects, such as studies of metals bioavailability, or biological assays with benthic bioindicators, increasing the set of direct ecotoxicological studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114798.

References

APA (Agência Portuguesa do Ambiente), 2016. Plano de gestão de região hidrográfica 2016/2021. Parte 2. Caracterização e diagnóstico, Anexo IV.

Baran, A., Tarnawski, M., 2015. Assessment of heavy metals mobility and toxicity in contaminated sediments by sequential extraction and a battery of bioassays. Ecotoxicology 24, 1279–1293. https://doi.org/10.1007/s10646-015-1499-4.

Belgis, Z.C., Persoone, G., Blaise, C., 2003. Cyst-based toxicity tests XVI - sensitivity comparison of the solid phase *Heterocypris incongruens* microbiotest with the *Hyalella azteca* and *Chironomus riparius* contact assays on freshwater sediments from Peninsula Harbour (Ontario, Canada). Chemosphere 52, 95–101. https://doi.org/10.1016/S0045-6535(03)00186-3.

Boguta, P., Skic, K., Baran, A., Szara-Bąk, M., 2022. The influence of the physicochemical properties of sediment on the content and ecotoxicity of trace elements in bottom sediments. Chemosphere 287. https://doi.org/10.1016/j.chemosphere.2021.132366.

Broccoli, A., Morroni, L., Valentini, A., Vitiello, V., Renzi, M., Nuccio, C., Pellegrini, D., 2021. Comparison of different ecotoxicological batteries with WOE approach for the environmental quality evaluation of harbour sediments. Aquat. Toxicol. 237, 105905 https://doi.org/10.1016/j.aquatox.2021.105905.

Burton, G.A., 2002. Sediment quality criteria in use around the world. Limnology 3, 65–75. https://doi.org/10.1007/s102010200008.

CCME (Canadian Council of Ministers for the Environment), 2002. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Summary Tables. http://st-ts.ccme.ca/.

Christophoridis, C., Dedepsidis, D., Fytianos, K., 2009. Occurrence and distribution of selected heavy metals in the surface sediments of Thermaikos Gulf, N. Greece. Assessment using pollution indicators. J. Hazard Mater. 168, 1082–1091. https://doi.org/10.1016/j.jhazmat.2009.02.154.

Codina, J.C., Cazorla, F.M., Pérez-García, A., De Vicente, A., 2000. Heavy metal toxicity and genotoxicity in water and sewage determined by microbiological methods. Environ. Toxicol. Chem. 19, 1552–1558. https://doi.org/10.1002/etc.5620190611.

Cornes, R., van der Schrier, G., van den Besselaar, E.J.M., Jones, P.D., 2018. An ensemble version of the E-OBS temperature and precipitation datasets. J. Geophys. Res. Atmos. 123 https://doi.org/10.1029/2017JD028200.

Creasel, 2001. Ostracodtoxkit F™. Chronic "Direct Contact" Toxicity Test for Freshwater Sediments. *Standard Operational Procedure*. Deinze, Belgium.

da Silva de Freitas, A., de Oliveira Santos, A.D., da Cruz Félix, L., dos Santos, R.F., Bila, D. M., de Carvalho Aguiar, V.M., da Fonseca, E.M., Neto, J.A.B., 2022. Multiproxy analysis in contaminated sediments from niterói harbour (guanabara bay), Brazil. Mar. Pollut. Bull. 175 https://doi.org/10.1016/j.marpolbul.2022.113348.

Delgado, J., Nieto, J.M., Boski, T., Albardeiro, L., 2008. Determination of the regional background in Holocene sediments of the Guadiana river estuary (SW Spain). Geogaceta 44, 235–238.

Delgado, J., Nieto, J.M., Boski, T., 2010. Analysis of the spatial variation of heavy metals in the Guadiana Estuary sediments (SW Iberian Peninsula) based on GIS-mapping techniques. Estuar. Coast Shelf Sci. 88, 71–83. https://doi.org/10.1016/j.ecss.2010.03.011.

Duodu, G.O., Goonetilleke, A., Ayoko, G.A., 2016. Comparison of pollution indices for the assessment of heavy metal in Brisbane River sediment. Environ. Pollut. 219, 1077–1091. https://doi.org/10.1016/j.envpol.2016.09.008.

Ehrlich, S., Lambers, D., Baccarelli, A., Khoury, J., Macaluso, M., Ho, S.-M., 2016. Endocrine disruptors: a potential risk factor for gestational diabetes mellitus. Am. J. Perinatol. 33, 1313–1318. https://doi.org/10.1055/s-0036-1586500.

Finney, D.J., 1971. Probit Analysis Cambridge. UK Cambridge University Press.

Godinho, S., Gil, A., Guiomar, N., Costa, M.J., Neves, N., 2016. Assessing the role of Mediterranean evergreen oaks canopy cover in land surface albedo and temperature using a remote sensing-based approach. Appl. Geogr. 74, 84–94. https://doi.org/10.1016/j.apgeog.2016.07.004.

Gomes, A., Connor, S., Boski, T., Moura, D., Camacho, S., 2014. Geochemical characteristics of sediments along the margins of an atlantic-mediterranean estuary (the Guadiana, Southeast Portugal): spatial and seasonal variations. Rev. Gesta˜o Costeira Integr. 14, 129–148. https://doi.org/10.5894/rgci452.

Hanif, N., Eqani, S.A.M.A.S., Ali, S.M., Cincinelli, A., Ali, N., Katsoyiannis, I.A., Tanveer, Z.I., Bokhari, H., 2016. Geo-accumulation and enrichment of trace metals in sediments and their associated risks in the Chenab River, Pakistan. J. Geochem. Explor. 165, 62–70. https://doi.org/10.1016/j.gexplo.2016.02.006.

Huat, T.J., Camats-Perna, J., Newcombe, E.A., Valmas, N., Kitazawa, M., Medeiros, R., 2019. Metal toxicity links to alzheimer's disease and neuroinflammation. J. Mol. Biol. 1–26. https://doi.org/10.1016/j.jmb.2019.01.018.

Hwang, K., Lee, Junghyun, Kwon, I., Park, S.Y., Yoon, S.J., Lee, Jongmin, Kim, B., Kim, T., Kwon, B.O., Hong, S., Lee, M.J., Hu, W., Wang, T., Choi, K., Ryu, J., Khim, J. S., 2021. Large-scale sediment toxicity assessment over the 15,000 km of coastline in the Yellow and Bohai seas, East Asia. Sci. Total Environ. 792, 148371 https://doi.org/10.1016/j.scitotenv.2021.148371.

Ingersoll, Christopher G., MacDonald, Donald D., 2002. A Guidance Manual to Support the Assessment of Contaminated Sediments in Freshwater Ecosystems.

ISO (International Organization for Standardization), 1995. ISO 11466: Soil Quality—Extraction of Trace Elements Soluble in Aqua Regia. Geneve.

ISO (International Organization for Standardization), 1998. ISO 11348-2: Determination of Inhibitory Effect of Water Samples on the Light Emission of *Vibrio Fischeri* (Luminescent Bacteria Test). Part 2: Method Using Liquid-Dried Bacteria. International Organization for Standardisation, Geneve, Switzerland.

Kabata-Pendias, A., 2011. Trace Elements in Soils and in Plants, fourth ed. CRC Press, Boca Raton (USA), p. 505.

Kosztra, B., Büttner, G., Hazeu, G., Arnold, S., 2017. Updated CLC Illustrated Nomenclature Guidelines. Wien, p. 124.

Kudłak, B., Wolska, L., Namie´snik, J., 2011. Determination of EC_{50} toxicity data of selected heavy metals toward *Heterocypris incongruens* and their comparison to "direct- contact" and microbiotests. Environ. Monit. Assess. 174, 509–516. https://doi.org/10.1007/s10661-010-1474-8.

Leistel, J.M., Marcoux, E., Thi´eblemont, D., Quesada, C., S´anchez, A., Almodo´var, G.R., Pascual, E., S´aez, R., 1997. The volcanic-hosted massive sulphide deposits of the iberian pyrite Belt: review and preface to the thematic issue. Miner. Depos. 33, 2–30. https://doi.org/10.1007/s001260050130.

Liu, W.X., Li, X.D., Shen, Z.G., Wang, D.C., Wai, O.W.H., Li, Y.S., 2003. Multivariate statistical study of heavy metal enrichment in sediments of the Pearl River Estuary. Environ. Pollut. 121, 377–388. https://doi.org/10.1016/S0269-7491(02)00234-8.

Liu, D., Wang, J., Yu, H., Gao, H., Xu, W., 2021. Evaluating ecological risks and tracking potential factors influencing heavy metals in sediments in an urban river. Environ. Sci. Eur. 33 https://doi.org/10.1186/s12302-021-00487-x.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manag. 19, 81–97.

Loska, Krzysztof, Jan, Cebula, Jacek Pelczar, D.W.J.K., 1997. Use of enrichment, and contamination factors together with geoaccumulation indexes to evaluate the content of Cd, Cu, and Ni in the rybnik water reservoir in Poland. Water. Air. Soil Pollut. 93, 347–365.

Luo, P., Xu, C., Kang, S., Huo, A., Lyu, J., Zhou, M., Nover, D., 2021. Heavy metals in water and surface sediments of the Fenghe River Basin, China: assessment and source analysis. Water Sci. Technol. 84, 3072–3090. https://doi.org/10.2166/wst.2021.335.

Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., Huang, Y., 2002. Toxicity of 40 herbicides to the green alga Chlorella vulgaris. Ecotoxicol. Environ. Saf. 51, 128–132. https://doi.org/10.1006/eesa.2001.2113.

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31. https://doi.org/10.1007/s002440010075.

Macfarlane, M., 2003. A Guidance Manual to Support the Assessment of Contaminated Sediments in Freshwater, Estuarine, and Marine Ecosystems in British Columbia.

Maradonna, F., Ancillai, D., Notarstefano, V., Valenti, A., Leoni, T., Carnevali, O., 2020. An integrated approach to evaluate port sediment quality: from chemical characterization to multispecies bioassays. Sci. Total Environ. 746, 141204 https://doi.org/10.1016/j.scitotenv.2020.141204.

dos Santos Mendonça, J.M., de Souza, I.S., de Medeiros, G.F., Mina, I.M.C.A.P., 2021. Geochemical and ecotoxicological evaluation of sediments of a semiarid estuary on the northeast of Brazil (Natal/RN). Reg. Stud. Mar. Sci 43, 101676. https://doi.org/10.1016/j.rsma.2021.101676.

OECD (Organization for Economic Cooperation and Development), 2006. Freshwater alga and cyanobacteria, growth inhibition test. In: OECD Guidelines for Testing of Chemicals Nº 201. Organization for Economic Cooperation and Development, Paris.

Palma, P., Ledo, L., Soares, S., Barbosa, I.R., Alvarenga, P., 2014. Integrated environmental assessment of freshwater sediments: a chemical and ecotoxicological approach at the Alqueva reservoir, 2 Environ. Geochem. Health 36, 9559. https://doi.org/10.1007/s10653-013.

Palma, P., Ledo, L., Alvarenga, P., 2015. Assessment of trace element pollution and its environmental risk to freshwater sediments influenced by anthropogenic contributions: the case study of Alqueva reservoir (Guadiana Basin). Catena 128, 174–184. https://doi.org/10.1016/j.catena.2015.02.002.

Palma, P., Fialho, S., Lima, A., Mourinha, C., Penha, A., Novais, M.H., Rosado, A., Morais, M., Potes, M., Costa, M.J., Alvarenga, P., 2020a. Switzerland Land-cover patterns and hydrogeomorphology of tributaries: Are these important stressors for the water quality of reservoirs in the mediterranean region? Water 12. https://doi.org/10.3390/w12102665.

Palma, P., Lima, A., Helena, M., João, M., Montemurro, N., Pérez, S., Lopez, M., Alda, D., 2020b. Pharmaceuticals in a Mediterranean Basin: the influence of temporal and hydrological patterns in environmental risk assessment. Sci. Total Environ. 709 https://doi.org/10.1016/j.scitotenv.2019.136205.

Palma, P., Penha, A.M., Novais, M.H., Fialho, S., Lima, A., Mourinha, C., Alvarenga, P., Rosado, A., Iakunin, M., Rodrigues, G., Potes, M., Costa, M.J., Morais, M., Salgado, R., 2021a. Water - Sediment Physicochemical Dynamics in a Large Reservoir in the Mediterranean Region under Multiple Stressors, pp. 1–23.

Palma, P., Fialho, S., Lima, A., Catarino, A., Costa, M.J., Barbieri, M.V., Monllor-Alcaraz, L.S., Postigo, C., de Alda, M.L., 2021b. Occurrence and risk assessment of pesticides in a Mediterranean Basin with strong agricultural pressure (Guadiana Basin: southern of Portugal). Sci. Total Environ. 794, 148703 https://doi.org/10.1016/j.scitotenv.2021.148703.

Persoone, G., 1999. Thamnotoxkit F™ - Crustacean toxicity screening test for Freshwater. Standard Operational Procedure 3–21 (Belgium).

Rani, S., Ahmed, M.K., Xiongzhi, X., Keliang, C., Islam, M.S., Habibullah-Al-Mamun, M., 2021. Occurrence, spatial distribution and ecological risk assessment of trace elements in surface sediments of rivers and coastal areas of the East Coast of Bangladesh, North-East Bay of Bengal. Sci. Total Environ. 801, 149782 https://doi.org/10.1016/j.scitotenv.2021.149782.

Rao, K., Tang, T., Zhang, X., Wang, M., Liu, J., Wu, B., Wang, P., Ma, Y., 2021. Spatial-temporal dynamics, ecological risk assessment, source identification and interactions with internal nutrients release of heavy metals in surface sediments from a large Chinese shallow lake. Chemosphere 282. https://doi.org/10.1016/j.chemosphere.2021.131041.

Roberts, T.L., 2014. Cadmium and phosphorous fertilizers: the issues and the science. Procedia Eng. 83, 52–59. https://doi.org/10.1016/j.proeng.2014.09.012.

Ruiz, F., Abad, M., Bodergat, A.M., Carbonel, P., Rodríguez-Lázaro, J., González-Regalado, M.L., Toscano, A., García, E.X., Prenda, J., 2013. Freshwater ostracods as environmental tracers. Int. J. Environ. Sci. Technol. 10, 1115–1128. https://doi.org/10.1007/s13762-013-0249-5.

Sahli, L., Belhiouani, H., Burga Pérez, K.F., El Hadef El Okki, M., Afri-Mehennaoui, F.Z., Férard, J.F., Mehennaoui, S., 2021. Assessment of freshwater sediment quality: potential ecological risk and ecotoxicity tests as complementary approaches. Chem. Ecol. 37, 219–233. https://doi.org/10.1080/02757540.2020.1853106.

Simeonov, V., Stratis, J.A., Samara, C., Zachariadis, G., Voutsa, D., Anthemidis, A., Sofoniou, M., Kouimtzis, T., 2003. Assessment of the surface water quality in Northern Greece. Water Res. 37, 4119–4124. https://doi.org/10.1016/S0043-1354(03)00398-1.

Smolders, E.L.L., 2013. Revisiting and Updating the Effect of Phosphate Fertilizers to Cadmium Accumulation in European Agricultural Soils. Final report KULeuven - DG Enterprise, Belgium.

Sokal, R.R., Rohlf, F.J., 1995. Biometry — the Principles and Practice of Statistics in Biological Research. Freeman, New York, DC.

Soomro, M.H., Baiz, N., Huel, G., Yazbeck, C., Botton, J., Heude, B., Bornehag, C.G., Annesi-Maesano, I., 2019. Exposure to heavy metals during pregnancy related to gestational diabetes mellitus in diabetes-free mothers. Sci. Total Environ. 656, 870–876. https://doi.org/10.1016/j.scitotenv.2018.11.422.

Torokne, A., Toro, K., 2010. Evaluation of the toxicity of river and creek sediments in Hungary with two different methods. Environ. Toxicol. 25, 504–509. https://doi.org/10.1002/tox.

USEPA, 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual. EPA Region V, Chicago. EPA-823-B-01-002.

Varol, M., Ustaoglu, F., Tokatlı, C., 2022. Ecological risks and controlling factors of trace elements in sediments of dam lakes in the Black Sea Region (Turkey). Environ. Res. 205 https://doi.org/10.1016/j.envres.2021.112478.

Wang, H., You, T., Gomez, M.A., Wang, Y., Li, S., Jia, Y., Shi, Z., 2021. Chemical speciation and ecological risk assessment of Cd, Pb and as in sediments: a case study in the Xijiang River basin, China. Environ. Earth Sci. 80, 1–14. https://doi.org/10.1007/s12665-021-09724-1.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall International Editions, New Jersey.

Zhang, J., Liu, C.L., 2002. Riverine composition and estuarine geochemistry of particulate metals in China - weathering features, anthropogenic impact and chemical fluxes. Estuar. Coast Shelf Sci. 54, 1051–1070. https://doi.org/10.1006/ecss.2001.0879.

Zhang, Y., Zhang, M., Yu, W., Li, J., Kong, D., 2022. Ecotoxicological risk ranking of 19 metals in the lower Yangtze River of China based on their threats to aquatic wildlife. Sci. Total Environ. 812, 152370 https://doi.org/10.1016/j.scitotenv.2021.152370.