Assessment of trace elements pollution and its environmental risk to freshwater sediments influenced by anthropogenic contributions: The case study of Alqueva Reservoir (Guadiana Basin)

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Abstract

The Guadiana Basin (SW Iberian Peninsula) is affected by acid mine drainage (AMD), a consequence of ancient mining activities in the Iberian Pyrite Belt (IPB). Consequently, the sediments at the Alqueva reservoir (SE Portugal) in the Guadiana Basin are potentially contaminated by trace elements, which makes important: (i) to characterize the status of trace elements pollution of the sediments; (ii) to evaluate the mobility and the bioavailability of As, Cd, Cu, Cr, Pb and Zn; and (iii) to assess the environmental risk associated with the total and bioavailable concentrations of trace elements, using the sediment quality guidelines (SQGs) and the risk assessment code (RAC). Metal enrichment factors (EF) and geoaccumulation indexes (*I_{geo}*), determined taking into account the regional background levels, revealed that, among the metals analyzed, Cd contributed to the highest pollution levels followed, by Pb and As. Despite the trace elements contamination of the Alqueva sediments, the sequential extraction showed that most of them are found in the oxidizable and residual fractions, which indicates that they are sparingly bioavailable, with exception of Cd (acid-labile fraction) and Pb (reducible fraction). Based on the RAC, Cd was the only metal that presented a high risk, while Pb, As and Zn showed a medium risk. Moreover, the SQGs revealed the existence of certain areas of extremely high risk, particularly related to high concentrations of total As and, in less extent, of Pb and Cd, associated with AMD, wastewater discharges and runoff of plant protection products from agricultural fields located near the reservoir.

Keywords: Freshwater sediments; Enrichment factor; Geoaccumulation index; Sequential extraction procedure; Risk assessment code, Alqueva reservoir

1. Introduction

Pollution of the natural environment by trace elements is a world-wide problem. With origin in natural sources (*e.g.* weathering of soil and rock, erosion, forest fires and volcanic eruptions) and anthropogenic activities (*e.g.* industrial effluents, mining and refining, agriculture drainage, domestic discharges and atmospheric deposition), these elements continuously enter to the aquatic systems, posing serious threat due to their hazardous characteristics (Carman et al., 2007; Chon et al., 2010; Davutluoglu et al., 2011). In fact, one of the most serious environmental issues concerning trace elements, which distinguish them from other toxic pollutants, is that they are resistant to biodegradation and have potential to bio-accumulate and become biomagnified, increasing the exposure of aquatic communities and human populations through the trophic chain (Gao and Chen, 2012; Subida et al., 2013).

As the principal compartment of trace elements accumulation, the assessment of sediments quality plays an important role in the good ecological and chemical status of water (Borja and Heinrich, 2005), which is the principal goal of the European Water Framework Directive (WFD) (ECC, 2000). Notwithstanding, this legal document does not mention the sediments as a compartment to be specifically investigated, this matrix constitutes one of the most important source of water contamination by trace elements, as well as, an important carrier of these hazardous substances within the rivers, reservoirs and other waters (Sekabira et al., 2010; Yaun et al., 2014). Consequently, the preservation of this compartment is an important step to maintain the full quality of the water body.

The environmental risk of sediments and its quality could be assessed through: (i) pollution indexes, such as the enrichment factor (EF) and the geoaccumulation index (I_{geo}), that characterized the trace element anthropogenic contributions and the contamination levels, comparing the metal enrichment and the unpolluted reference concentrations

(background levels) (Christophoridis et al., 2009; Delgado et al., 2010; Mil-Homens et al., 2007); (ii) sediment quality guidelines (SQGs), which relate the pollution status of sediments, considering the total trace elements content, with their adverse effects in aquatic organisms, for instance using the threshold effect level (TEL) and the probably effect level (PEL) (Caeiro et al., 2005; Díaz-de-Alba et al., 2011; Saleem et al., 2013); (iii) and methodologies that evaluate the ecotoxicological risk, taking into consideration the mobility/availability of the pollutant, such as, the risk assessment code (RAC; classification that correlates the percentage of the metal more available, with the risk to the aquatic species) (Delgado et al., 2011; Passos et al., 2010; Yuan et al., 2014). Hence, despite the use of total trace elements content as a criterion to assess their possible risk to the aquatic ecosystem, it provides insufficient information about the mobility, bioavailability and consequently toxicity, of these hazardous substances to the aquatic and human populations (Gu et al., 2014; Hooda, 2010; Sundaray et al., 2011). Accordingly, the speciation of metals in sediments is therefore a critical factor in assessing their potential environmental impacts (Peng et al., 2004) and can be determined with the use of sequential extraction procedures. Of the many existing schemes, the most widely accepted standardized method was proposed by the European Community Bureau of references (BCR sequential extraction procedure) (Ure et al., 1993) and improved in subsequent works (Rauret et al., 1999; Sahuquillo et al., 1999). The BCR sequential method presents advantages comparing with other methods, as high reproducibility and high recoveries percentages (Cuong and Obbard, 2006; Pueyo et al., 2008). Further, this procedure has already been applied to assess metal mobility in several types of solid samples, such as sediments (Davutluoglu et al., 2011; Díaz-de-Alba et al., 2011; Delgado et al., 2011; Passos et al., 2010), soils (Martley et al., 2004; Pérez-López et al., 2008; Rao et al., 2008), and sewage sludge (Alvarenga et al., 2007).

This work was developed in the sediments of the Alqueva reservoir (the biggest artificial lake of the Iberian Peninsula) in the Guadiana Basin, which is one of the most important rivers of the Iberian Peninsula and drains the western part of Iberian Pyrite Belt (IPB), one of the world's most important metallogenic sulphide provinces, where mining dates back to the Third Millennium B.C. (Delgado et al., 2011; Nocete et al., 2005). Associated with these mining areas are acid leachates containing metals, metalloids and sulphates, which constitute the acid mine drainage (AMD). Presently, the mining activity is limited to a small number of active mines (Neves Corvo and Aljustrel), but the environmental impact of the AMD still exists, due to the some abandoned mines which need to be rehabilitated (Matos e Martins, 2006), and several authors have reported AMD as an important anthropogenic source of trace elements in the Guadiana Basin (Fernández-Caliani et al., 2009; Guillén et al., 2011; Nieto et al., 2007). On the other hand, the intensive agricultural activity and the discharge of untreated or inefficiently treated domestic wastewater may constitute other sources of metals in this water body (Palma et al., 2014a; 2010; Silva et al. 2011).

Previous studies developed at the Alqueva reservoir have characterized the textural structure and organic content of the sediments, the levels of nutrients, the total contents of As, Cd, Cr, Cu, Ni, Pb, Zn, Fe and Mn; and some ecotoxicological effects (Palma et al., 2014a, 2014b). Currently, it is important to understand, which amounts of trace elements are arising from anthropogenic sources, as well as their mobility and bioavailability in the sediments, and try to correlate these results with the toxicological risk for the reservoir and for the populations. In this scenario, the main aims of the present study were: (1) to characterize the status of pollution of the sediments, in terms of potentially toxic trace elements contents, based on the specific geochemical regional backgrounds, and using as pollution indexes, the EF (which estimates the anthropogenic impact, on sediments, of each of the element analyzed) and the *Igeo* (based on geochemical data, that makes possible to map the areas

according to their pollution degree); (2) to investigate the mobility and the bioavailability of the most potentially toxic elements (As, Cd, Cu, Cr, Pb and Zn); (3) to assess the environmental risk associated with the total and bioavailable trace elements contents in the sediments, using available SQGs (TEL and PEL) and the RAC; (4) to identify, among the trace elements analyzed, which are those with higher risk for the aquatic communities.

The results of this study, in combination with the outcomes obtained in previous ones, are intended to help the water resource managers and regulatory authorities to establish priority actions aimed at achieving the chemical and ecological status objectives, outlined in the Water Framework Directive. Further, this research intended to demonstrate the usefulness of diverse tools to estimate the real environmental risk of pollutants, which could become an innovative and useful approach to be applied in risk assessment of water environments.

2. Materials and methods

2.1. Study area and sampling sites characterization

The Alqueva reservoir is located in southern Portugal, along 83 km of the main course of the Guadiana River Basin. The river is divided from the morphological point of view in five distinct sections (Batista et al., 2012; Feio, 1951): Alto Guadiana or Ruidera lagoons, the Miocene aged Mancha plain, the “Monte do Toledo”, the Central Extremadura and, finally, the Portuguese section, where the Alqueva reservoir is located.

The lithology of the Guadiana Basin, which influences both sediment composition and grain size distribution, is dominated by greywacke, schist, and a volcano-sedimentary complex with polymetallic sulphide complexes of Cu, Pb and Fe-Mn and carbonate rocks (Mil-Homens et al., 2007).

The hydrologic regime of the Alqueva reservoir reflects the regional expression of the Mediterranean climate, which is characterized by dry and hot summers as well as mild and wet winters with concentrated rains (Morales, 1993). Further details about its physical and hydrodynamics characteristics can be found elsewhere (Palma et al., 2014a; 2010).

Five sampling sites were established at the Alqueva reservoir: three upstream, Ajuda (Aj; 38°46'28.56"N, 7°10'47.00"W), Alcarrache (Ac; 38°19'1.53"N, 7°19'51.10"W), Álamos (Al; 38°20'30.00"N, 7°34'40.00"W), and two at the middle, Mourão (Mr; 38°23'60.00"N, 7°23'25.80"W) and Lucefécit (Lf; 38°33'6.32"N, 7°17'52.86"W), of the reservoir (Fig. 1), selected taking into account previous studies (Morais et al., 2007; Palma et al., 2010).

2.2. Sampling

Sampling was carried out approximately every two months from February 2011 to November 2012 (12 sampling campaigns). The wet season included the months of November (Nv), February (Fb) and April (Ap), and the dry season included the months of June (Jn), July (Jl) and September (Sp) (ARHAlentejo, 2011).

At each sampling location, 5 L of surface sediments (<10 cm) were collected, gathered using a stainless steel van Veen grab of 0.05 m². The sediments at Lf, Mr, Ac, and Al were collected at a water column depth of 25, 48, 37, 27 and 30 m, respectively. All the samples from Aj were collect in the shore. The sediment samples were packed in polyethylene bags and transported to the laboratory, in a cooler at 4°C, where they were preserved and stored until the analysis following the requisites for sediments conservation (USEPA, 2001).

2.3. Trace elements analysis

Total trace elements concentrations in the sediment were determined after digestion of the samples with aqua regia according to ISO 11466 (1995). Flame atomic absorption spectrometry (FAAS) or electrothermal atomic absorption spectrometry (ETAAS) were used in the analysis, using a Varian apparatus (SpectrAA 220FS, 220Z and 110Z). The methodology was described in Palma et al. (2014a).

2.4. Sequential Extraction Procedure

The sequential extraction procedure applied in this study was the modified BCR sequential extraction method described by Rauret et al. (2000). The basis of the sequential extraction procedure is that each successive reagent dissolves a different component, so that the resulting elemental distribution can be used to infer information about the mobility and bioavailability of a specific element, or the potential risks posed to the environment (Passos et al., 2010). This method is based on the sequential extraction of samples: first step, with acetic acid 0.11 mol L^{-1} , to liberate exchangeable/acid-extractable metals (Fraction 1: water soluble, exchangeable and bound to carbonates metal); second step, with hydroxylamine hydrochloride, to solubilize metals associated with the reducible phases (Fraction 2: bound to Fe and Mn oxide); third step, with hydrogen peroxide, to release oxidizable contaminants, afterwards extracted with 0.1 mol L^{-1} ammonium acetate (Fraction 3: bound to sulfides and organic matter); fourth step, by digestion with aqua regia, according to ISO 11466 (1995), to liberate the residual forms (Residual). Extracts of each step were analyzed in order to assess total trace elements concentration (As, Cd, Cu, Cr, Pb and Zn) through flame atomic absorption spectrometry (FAAS) or electrothermal atomic absorption spectrometry (ETAAS),

using a Varian apparatus (SpectrAA 220FS, 220Z and 110Z). Analyses were performed on independent triplicates of the samples and blanks were measured in parallel for each set of determinations.

The study integrated two groups of BCR analysis: the first, with samples of the wet period (April 2012), and the second, with samples of the dry period (July 2012), with the purpose of understand if the seasonality may change the mobility and bioavailability of the trace elements. The temporal variability is very important, mainly in regions characterized by a semi-arid clime, where the river discharges may range from zero, in the dry season, to high rates, during the wet season. These adjustments affect all the physical, chemical and biological parameters, with implications in the water and sediments quality of the reservoir. Further, this particular hydrology makes the tributaries to the reservoir very sensitive to anthropogenic pressure, with serious implications in the status of water body (Moraes et al., 2007; Serafim et al., 2006).

The analytical quality control of the results comprised: (i) the analysis of two geological certified reference materials, Buffalo Lake sediment (SRM 2704), from NIST (National Institute of Standard Technology) and Soil-7 from IAEA (International Atomic Energy Agency), to validate the aqua regia extraction procedure, used to quantify total trace element concentration; and (ii) an internal check of the results of the BCR, performed by comparing the sum of the four steps with the analysis of total trace element concentration. The recovery percentage of the sequential extraction was calculated using Eq. (1):

$$\text{Recovery (\%)} = [(\text{Step 1} + \text{Step 2} + \text{Step 3} + \text{Step 4}) / \text{total concentration}] \times 100 \quad (1)$$

2.5. Metal pollution indexes

Anthropogenic contributions of the selected trace elements in the sediment samples can be estimated from the element enrichment relative to the widely accepted background (average continental crust abundance or geochemical regional background values).

The enrichment factor (EF) estimates the anthropogenic impact on sediments of the trace elements, using a normalizer element, such as Al, Fe, Li or Sc (Aloupi and Angelidis, 2001; Schiff and Weisberg, 1999). These normalizer elements are tracers of the natural, metal-binding phases and are ideally not influenced by anthropogenic inputs. Furthermore, their application as a normalizing factor implies large concentrations in the studied environment (Abraham et al., 2008; Christophoridis et al., 2009) and a significant correlation to the fine-grained sediment fractions, as for the other metallic elements (Liu et al., 2008). The EF is calculated using the following equation (eq. (2); Loska et al., 1997):

$$EF = \frac{[X/N]_{\text{sample}}}{[X/N]_{\text{background}}} \quad (2)$$

Where, $[X/N]_{\text{sample}}$ and $[X/N]_{\text{background}}$ refer respectively, 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.

An EF value of 1 indicates a predominantly natural origin for the element in the sediment, while an EF value greater than 1.5 indicates enrichment due to either natural causes (biotic input) or anthropic actions (Zhang and Liu, 2002). EF values lower than 1, reflect remobilization and loss of that element in relation to the normalizing element, or overestimation of the contents of the normalizer element (Mil-Homens et al., 2006).

The I_{geo} enables the assessment of contamination by comparing the current and regional background concentrations of the metal in sediments (Loska et al., 2004). It is calculated using the Eq. (3):

$$I_{geo} = \log_2 \left(\frac{C_n}{1.5 B_n} \right) \quad (3)$$

Where C_n is the measured concentration of the metal in the sediment samples and B_n is the regional geochemical background value (Lide, 2005). The factor 1.5 is introduced to minimize the effect of possible variations in the background values that may be attribute to lithogenic variations. I_{geo} values were interpreted as: $I_{geo} \leq 0$ – practically uncontaminated; $0 < I_{geo} < 1$ – uncontaminated to moderately contaminated; $1 < I_{geo} < 2$ – moderately contaminated; $2 < I_{geo} < 3$ – moderately to heavily contaminated; $3 < I_{geo} < 4$ – heavily contaminated; $4 < I_{geo} < 5$ – heavily to extremely contaminated; and $I_{geo} > 5$ – extremely contaminated (Loska et al., 2004; Iqbal and Shah, 2011).

2.6. Risk assessment of sediments contamination with trace elements

2.6.1. Sediments quality guidelines (SQGs)

This approach is based on the relation between measured concentrations of the trace elements and observed biological effects, such as mortality, growth or reproduction of living organisms. Two types of SQGs developed for freshwater ecosystems (MacDonald et al., 2000) were applied in the present study: (a) the threshold effect level (TEL), the concentration below which adverse effects upon sediment dwelling fauna would be infrequent/rare; and (b) the probable effect level (PEL), concentration of the chemical above which adverse effects are frequent to occur.

2.6.2. Risk assessment code (RAC)

The RAC is defined as the fraction of metal exchangeable and/or associated to carbonates (% Fraction 1; F1). When RAC is less than 1% the sediment is of no risk to the aquatic environment. Percentages of 1-10% indicate a low risk, 11-30% medium risk, and 31-50% high risk. Above 50% the sediment poses a very high risk, and is considered very dangerous for the aquatic organisms (Singh et al., 2005).

2.7. Statistical analysis

All statistical analysis was carried out with the software Statistica 6.0 (StatSoft, Inc., 2001).

The Spearman rank correlation (R), applied to variables with a non-normal distribution, was used to evaluate the associations between the texture of the sediments and their trace elements distribution, with a level of significance $p < 0.01$.

3. Results and discussion

3.1. Pollution status of the sediments

To evaluate the anthropogenic influence on the trace elements quantified in the Alqueva sediments, the EF values were determined.

The variability of trace elements in sediments can be natural or influenced by anthropogenic sources. Trace elements from these sources usually accumulate in fine fractions of the sediments; this fact justified the use of a normalizer element in order to

compensate the dependency of the trace elements concentrations on the variations of the grain size, and/or on the mineralogy of sediments (Aloupi and Angelidis, 2001). The good correlation between Fe and the fine-grained sediments fraction ($r = 0.43$; $P < 0.01$), the strong and significant associations ($P < 0.01$) between Fe and all the potentially toxic elements evaluated (see Table 1) and the abundance of this metal in the Alqueva sediments, justified its choice as a normalizer element. This element was already used with this purpose in several studies developed in surface sediments (Díaz-de-Alba et al., 2011; Herut et al., 1993; Salem et al., 2013). Another factor to consider for the establishment of pollution indexes is the geochemical background. This value is essential to distinguish whether an element present in the environment appears naturally or, on the contrary, is influenced by anthropogenic activities (Liu et al., 2003). In the present study, the local geochemical backgrounds used for the Guadiana Basin were estimated by Delgado et al. (2008).

Figure 2 showed the EF indexes, for each metal, in the five sampling sites, at the wet and dry period. In general, the EF values showed an accentuated variability among locations, being Lucefécit and Sra Ajuda, the areas that presented higher EF values, meaning that were the most influenced by anthropic activities. Nonetheless, the great EF values determined for Sra. Ajuda may have been influenced by the lithology and the textural composition of its sediments. Previous results (Palma et al., 2014a) showed that the sediment samples from this area are totally composed by sand particles, with little capacity to adsorb trace elements, and with low concentrations of Fe. Hence, this deficiency in Fe can inflate the EF values when comparing with the regional geochemical backgrounds. Despite the low variability between results from different seasons, in the wet period, the EF values were higher for most of the elements, except for Cr and Mn.

Amongst the elements studied, Ni, Cr, Zn and Cu were those that, in the majority of the samples, presented values of EF between 1 and 1.5, which points out to a non-anthropic

influence. Similar results for Ni and Cr had already been reported by Delgado et al. (2010), in a study developed in the Guadiana river low basin, identifying the lithology of the basin as the main source of these metals in these sediments. Mil-Homens et al. (2014), in a study established in 15 estuaries within Portugal, also attributed the levels of Ni and Cr in the surface sediments of Guadiana estuary to the lithology of the basin. In general, the enrichment on As, in the sediments of Mourão and Lucefécit (EF_{Mr} 1.97; median value, $n=10$; EF_{Lf} 2.32; median value, $n=12$), and Pb (EF_{Mr} 2.01; median value, $n=11$; EF_{Lf} 2.35; median value, $n=12$), was attributed predominantly to anthropogenic activities, resulting from AMD from inactive mines. The anthropic enrichment of Mn (EF 2.41; median value, $n=59$), observed in all sampling sites, may be related with the atmospheric deposition, resulting from the application of plant protection products in the vineyards and olive crops existent in the vicinity of the reservoir. Cadmium was the trace element that presented the highest EF values ($7.3 \leq EF \leq 65$; min. and max. values, $n=59$), which points to the strong anthropic enrichment of the sediments with this element. Apart from the contamination through the AMD, Cd can also be released from several diffuse and point sources in the area, including phosphate fertilizers, road traffic and sewage sludge application.

The level of pollution in the sediments can be evaluated by comparing current metal concentrations with the regional background levels using the *Igeo* (Fig. 3). Overall, the *Igeo* determined for the selected metals revealed that all the sediments were moderately to heavily contaminated with Cd ($1.89 \leq Igeo \leq 3.57$; min. and max. values, $n=59$). With occasional exceptions, the sediments were characterized as unpolluted, for both seasons, except for As (Mourão and Lucefécit), Cu (Álamos and Lucefécit; wet period), Cr (Álamos) and Pb (Álamos, Alcarrache and Lucefécit) which were classify as “unpolluted to moderately polluted” with index ranging from 0 to 1. The *Igeo* index confirmed the assumption that the Ajuda EF values were influenced by the texture and low amounts of Fe that characterized its

sediments; once, with the exception of Cd, all the *Igeo* indexes determined in this location were lower than 0. Generally, the site with the highest contamination status was Lucefécit, which may be due to the high number of anthropogenic sources in the vicinity of this area, such as AMD, agriculture fields and wastewater discharges (untreated or with inefficient treatment), as well as, to its textural characteristics (100% of fine particles) and high amounts of organic matter (11 to 23%) (Palma et al., 2014a). In general, the pollution indexes found in the Alqueva sediments were similar to those determined in other reservoirs and Bays, like Mangla Lake (Salem et al., 2013), Algeciras Bay (Díaz-de Alba et al., 2011) and Deception Bay (Brady et al., 2014), and lower than values obtained in the Deûle river in Northern France (Louriño-Cabana et al., 2011), Nerbioi-Ibaizakabal river in Basque country (Ortiz de Vallejuelo et al., 2010), and some Portuguese rivers like Tagus and Sado (Mil-Homens et al., 2014).

3.2. Metal fractionation of sediments

Metal fractionation is an important factor affecting the potential toxicity of trace elements. Total concentrations of trace elements can provide fundamental information of the sediments contamination levels, of the pollution indexes and of the quality guidelines for the sediments environmental risk assessment. However, such information alone is insufficient to understand the mobility, bioavailability and toxicity of these elements in the sediments, once the environmental behaviour of trace elements depends largely on their chemical form and binding states. Hence, it is essential to integrate the knowledge about the mechanisms of the association of trace elements with the sediments, to develop a more correct and integrative interpretation about their environmental risk.

The BCR sequential extraction method was applied to the trace elements that could pose a risk for the ecosystem in study, i.e. Cd, As, Cu, Pb, Cr and Zn, with the purpose of increase the knowledge about their bioavailability. However, the results obtained for Cr will not be discussed, once, in most of the BCR fractions, the Cr concentrations were below the detection limit and the recovery percentages, of the sequential extraction, were very low. Regarding the other trace elements the recovery percentages showed a good agreement between the obtained and the certified values for the total trace elements concentrations of the certified reference materials (As, Cd, Cu, Pb and Zn) (mean relative errors <10%), being the average recovery obtained for each step of the BCR was $94 \pm 7\%$, for the samples of the dry period, and $97 \pm 8\%$, for the samples of the wet period. The chemical partitioning of trace metals in sediment samples is plotted in Figure 4. Each geochemical fraction was represented as the mean percentage of that fraction to the sum of all fractions. Of the all phases, the residual was the one that accounted the highest proportion for the trace elements analysed, with the exception of Cd and Pb. It was observed that Cd was the most mobile metal, in both periods: wet period: 51 to 88%; and dry period: 42 to 76%. The results are in agreement with reports that identified Cd as being among the easily removed and most labile metals (Sahuquillo et al., 2003), and also with its high affinity for secondary carbonates (siderite; capable of retaining trace elements) resulting from selective oxidisation of pyrite (Delgado et al., 2011). Furthermore, the high mobility of this element and the high amounts presented in the F1 BCR fraction had already been reported in sediments of Guadiana Basin (Delgado et al., 2011), and in sediments from Algeciras Bay (South of Spain) (Díaz-de-Alba et al., 2011). The results showed that Pb had the highest percentage at the reducible fraction (wet period: 53 to 87%; and dry period: 55 to 88%) followed by Cd (wet period: 10 to 34%; and dry period: 15 to 25%) and Zn (wet period: 2 to 23%; and dry period: 2 to 11%). The accumulation of hazardous trace elements in the reducible fractions was observed in various

other studies showing that Fe-Mn-oxyhydroxides are important scavengers of these metals in sediments and that they play an important role in controlling their mobility in the environment (Álvarez et al., 2006; Davidson et al., 2006). The association of Pb to the reducible fraction (F2) already was observed in other surface sediments, such as in Guadiana estuary sediments (Delgado et al., 2011), in Jinjiang river (Wang et al., 2009) and in Taiku lake (Yuan et al., 2014). There were, however, differences in distribution patterns of Pb between the two periods of analysis. At wet period the concentrations of Pb were distributed mainly through F2 and residual (13 to 47%). Unlike April 2012, in June 2012, Pb was found mainly bound with F2 and F3 (1 to 13%) mainly in the locations with higher OM contents (Alamos and Luceférit; Palma et al., 2014a). The substantial amount of Pb in the oxidizable fraction (F3), in the dry period, indicates that the metals form complexes with humic substances and/or insoluble metal sulphides, which are important sinks of trace metals in sediments. Copper was the trace element that showed higher quantities at the oxidizable phase (F3), in both periods (dry period: 5 to 36%; and wet period: 34 to 65%), followed by Pb and Zn. These elements are strongly complexed to the sediment, and are only released following degradation of the organic matter or oxidation of sulphides to sulphates. Similar results for the mobility of Pb and Cu have been reported (Davutluoglu et al., 2011; Passos et al., 2010). Higher trace element contents were found in the residual fraction for Zn (wet period: 58 to 93%; and dry period: 74 to 85%), Cu (wet period: 58 to 85%; and dry period: 26 to 61%) and As (wet period: 59 to 91%; and dry period: 82 to 92%). These fractions are less bioavailable since the trace elements are strongly bound to silicates. Among these elements, As showed the greatest contents on the residual phase for both sampling periods, which indicates that this potentially toxic element is the less available to aquatic fauna, and consequently the less available to enter in the food chain. The relative content of trace elements in the residual phase can be used as a measure of the contribution of natural sources

and/or old anthropogenic contamination (Singh et al., 2005; Passos et al., 2010). On the contrary, the percentage of trace elements extracted in the most labile fraction (F1) indicates recent anthropogenic inputs, with an increment of the status of sediments contamination. Locations where trace elements are significant associated with the oxidizable or reducible sediment fractions are influence by pollution that is less recent (Passos et al., 2010). The order of mobility (from most to least bioavailable; having in consideration the percentage in the F1) was Cd>Pb>Cu>Zn>As, which points to Cd as the most bioavailable trace element at the sediments of Alqueva. High enrichments factors of Cd, obtained for all the areas analyzed, and high levels of sediments contamination, comparing with the background values, indicated that this trace element is the most important contaminant of the Alqueva sediments. Further, its high bioavailability, together with its high acute and chronic toxicity (Qu et al., 2013) and its bioaccumulation properties (Angeletti et al., 2014; Wang and Li, 2014), make this trace element, the metal with higher risk to the aquatic environment. On the other hand, its high bioavailability may increment the bioaccumulation processes, which may constitute a potential threat for the human populations that consume fish from the reservoir, once these processes might lead to concentrations of Cd exceeding the corresponding acceptable daily intake (ADI_{cadmium} : 0.057 to 0.071 mg kg⁻¹ day⁻¹; WHO 1972).

3.3 Risk and quality assessment

There are several methodologies to assess the quality of sediment that may be used to evaluate the adverse effects of trace elements in the aquatic environment. One method is the RAC, which is based on the percentage of acid soluble fraction of metals (the most labile) determined with a sequential extraction procedure (Perin et al., 1985; Rodríguez et al., 2009). The RAC results (Table 2) showed that Cd represents a very high ecologic risk, in all

sampling locations, at both seasons. Zinc and As presented a medium risk in Luceférit at wet season ($F1=13.9$ and 16.32% , respectively) and Pb showed medium risk at Mourão and Ajuda in dry season ($F1=11.32$ and 12.02% , respectively). This ecologic risk was occasional, probably resulting from small variations in environmental conditions, such as pH or salinity mainly in the case of As, and/or of some point source pollution such as wastewater discharges, application of fertilizers and plant protection products, in the case of Zn and Pb. Copper was the only trace element that posed low risk, during the study. According to RAC, the most important stressor at the Alqueva reservoir was Cd, similar results in the Guadiana estuary have already been reported by Delgado et al. (2011). To overcome the limitations of the RAC approach, another assessment was made according to the SQGs based on the total amounts of contaminants established for freshwater ecosystems. The SQGs for the selected elements and the classification of the samples based on these guidelines are shown in Table 3. The analysis of the data, bearing in mind the SQGs (PEL and TEL) during the wet period suggested that the As and Ni were associated with frequently adverse effects (As: 57% of the total samples $>$ PEL; Ni: 43% of the total samples $>$ PEL); Cr and Zn may be associated occasionally with adverse biological effects, surpassing the respective PEL only in 7% of the total samples; Cu did not showed values greater than PEL value, during this period. In comparison, the data from the dry period revealed that As and Ni were the elements that surpassed PEL on 50 and 43% of the total of samples, respectively; the rest of the elements were in concentrations lower than TEL values, or between TEL and PEL limits. Overall, the samples from the wet period may be considered potentially more toxic than the samples from the dry period. Further, As and Ni were the trace elements that may be more associated with biological adverse effects at both seasons. Although the total concentrations of As, Cu, Cr and Zn, when compared with the SQGs of USEPA (Baudo et al., 1990; see Table 3) indicated that, the sediments of Alqueva are heavily polluted by As (80% wet samples; 79% dry

samples) and moderately polluted by Ni (60% wet samples; 55% dry samples), Cu (40% wet samples; 52% dry samples), Cr (37% wet samples; 48% dry samples), and Zn (43% wet samples; 48% dry samples); with the exception of Lucefécit, the EF and *Igeo* indexes point to a situation of little concern, probably due to the high background values estimated for the area. On the other hand, the BCR analysis showed that, on the whole, the higher concentrations of As, Cu and Zn are associated to silicates, being these elements probably less (bio)available to induce toxic effects on the aquatic species of the ecosystem. Cadmium results indicated that no sample is heavily contaminated with this element, and that PEL value was only surpassed in 13% of wet samples. Nevertheless, the pollution indicators point to this element as the most associated to anthropogenic contamination in the reservoir, and with concentrations much higher than the background regional value for the Guadiana Basin, being considered, in the present study and in all others developed in the Guadiana Basin, an important stressor for the ecosystem (Delgado et al., 2010; 2011; Mil-Homens et al., 2014). Further, the ecotoxicity assays developed in the sediments of the reservoir, showed a significant correlation between the acute toxic effects observed and the presence of Cd, mainly for samples of Lucefécit and Álamos (Palma et al., 2014b). In general, the Alqueva reservoir would be ranked as an area with moderate toxicological risk for most of the trace elements, except for Cd. The location more contaminated, and whose sediments showed a high probability of inducing toxic effects to the aquatic species, was Lucefécit. The temporal analysis revealed that the wet period presented more contaminated sediments than the dry period and with the possibility of developing more toxic effects.

4. Conclusions

This study allowed the identification of the trace elements that represent the most important contaminants and the main risk factors at the Alqueva reservoir. The results allowed the characterization of the status of metal pollution within Alqueva and the identification of the elements with higher bioavailability, which would probably induce higher ecotoxicological effects in the aquatic species, and are responsible for the bioaccumulation processes along of trophic chain. The results could be useful in developing effective management strategies to control trace element pollution at the Alqueva reservoir

In general, the pollution indexes indicated that the sediments were more contaminated, and more influenced by anthropogenic activities, at the wet period than at the dry period. Luceférit was the sampling location with higher contamination and where the concentrations of the trace elements were much higher than the regional backgrounds values. Among the trace elements studied, Cd presented the higher levels of contamination, with concentrations greater than the background value. Further, sequential extraction allowed us to identify Cd as the most bioavailable, pointing this element as a potential inducer of toxic effects in the aquatic species of this ecosystem (high concentrations at F1), followed by Pb, that was mainly associated to F2.

According to the RAC approach, Cd was identified as the most labile of the trace elements, again associating Cd to a “high risk” tag in all the sediments analyzed, while As, Pb and Zn pose sporadically a medium risk. Further, when total trace elements concentrations of the sediments were compared to SQGs, the sediments were classified as moderately to high polluted, for most of the elements, which may be associated mainly with AMD processes, in the case of As and Pb, and with wastewater discharges and runoff from plant protection products, in the case of Cd and Mn. In addition, the results reveal the existence of

certain areas of extremely high risk, particularly linked to high concentrations and bioavailability of Cd, and, in less extent, of As and Pb.

For all these reasons, it is extremely important perform an integrative analysis, with the application of different methodologies of risk assessment. Additionally, it is essential to develop SQGs for the classification of contamination, more adjusted to the European sediments, namely to sediments that comprise similar characteristics of lithology, climate and hydrodynamic.

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Captions

Figure 1. Map showing the five sampling stations at the Alqueva reservoir, adapted from Palma et al. (2014c).

Figure 2. Box-Whiskers plots of the EF values calculated for the Alqueva 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. The dashed area ($0.5 \leq EF \leq 1.5$) indicates that elements are provided from natural contributions.

Figure 3. Box-Whisker plots of the *Igeo* indexes calculated for the Alqueva 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.

Figure 4. Fractionation of metals (F1: Acid-labile fraction; F2: Reducible fraction; F3: Oxidizable fraction; R: Residual fraction), obtained by BCR method, in sediments of the Alqueva reservoir, in (a) April 2012, (b) June 2012.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1. Spearman's correlation coefficient matrix for the concentrations of selected metals (As, Cu, Cr, Cd, Pb, Zn, Ni, Mn and Fe) and grain-size from surface sediments samples collected in five sampling locations within Alqueva reservoir ((marked correlations in bold are significant at: $P < 0.01$; $n = 59$)).

	Sand (%)	Silt (%)	Clay (%)	As (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Mn (g kg ⁻¹)	Fe (%)
Sand (%)	1											
Silt (%)	-0.95	1.00										
Clay (%)	-0.89	0.87	1.00									
As (mg kg ⁻¹)	-0.58	0.59	0.42	1.00								
Cu (mg kg ⁻¹)	-0.63	0.54	0.51	0.74	1.00							
Pb (mg kg ⁻¹)	-0.49	0.49	0.40	0.80	0.85	1.00						
Zn (mg kg ⁻¹)	-0.44	0.33	0.29	0.63	0.87	0.76	1.00					
Cd (mg kg ⁻¹)	-0.49	0.39	0.38	0.63	0.77	0.75	0.76	1.00				
Cr (mg kg ⁻¹)	-0.39	0.31	0.37	0.47	0.68	0.59	0.70	0.66	1.00			
Ni (mg kg ⁻¹)	-0.49	0.38	0.35	0.64	0.92	0.80	0.91	0.73	0.72	1.00		
Mn (g kg ⁻¹)	-0.34	0.20	0.22	0.61	0.80	0.67	0.80	0.69	0.50	0.76	1.00	
Fe (%)	-0.56	0.38	0.39	0.54	0.81	0.65	0.79	0.68	0.59	0.84	0.66	1

Table 2. Evaluation of acid soluble fraction (F1) according to RAC-Criteria.

		Elements (acid soluble fractions; %)				
		Zn	Cu	As	Pb	Cd
April	Al	4.46	0.00	8.31	0.00	51.45
	Ac	1.46	0.00	2.28	3.43	72.73
	Mr	2.83	0.00	1.95	0.40	58.09
	Lf	13.90	0.00	16.32	0.00	66.86
	Aj	10.49	1.17	1.45	2.88	88.13
July	Al	2.15	0.22	0.77	4.92	66.67
	Ac	1.65	0.57	5.26	5.64	46.01
	Mr	2.76	0.98	3.28	11.32	41.67
	Lf	2.42	0.58	4.63	7.02	76.47
	Aj	3.79	0.92	1.51	12.02	69.23
RAC-Criteria		Medium risk	Low risk	Medium risk	Medium risk	High risk

Table 3. Percentage of samples integrated in each level of sediments classification and sediments quality guidelines (SQGs) during the wet and dry period, in Alqueva reservoir.

		As	Cd	Cr	Cu	Ni	Pb	Zn
Sediments Classification	non-polluted	<3	-	<25	<25	<20	<40	<90
	moderately polluted	3-8	-	25-75	25-50	20-50	40-60	90-200
	heavily polluted	>8	>6	>75	>50	>50	>60	>200
SQGs	TEL	5.9	0.6	37.3	35.7	15.9 ^a	35	123
	PEL	17	3.5	90	197	42.8 ^a	91.3	315
(%) Wet period	non-polluted	-	-	53	47	20	77	47
	moderately polluted	20	-	37	40	60	10	43
	heavily polluted	80	-	10	13	20	13	10
	<TEL	3	20	77	57	17	60	73
	≥TEL and <PEL	40	67	10	43	40	27	20
	>PEL	57	13	7	-	43	13	7
(%) Dry period	non-polluted	-	-	45	45	28	76	53
	moderately polluted	21	-	48	52	55	24	48
	heavily polluted	79	-	7	3	17	-	-
	<TEL	25	28	62	66	17	76	83
	≥TEL and <PEL	25	72	38	34	40	24	17
	>PEL	50	-	-	-	43	-	-

Sediments Classification (Baudo, 1999); ^a Long et al. (1995); TEL: threshold effect level; PEL: probably effect level.