

Sulfur isotope fractionation and sequential extraction to assess metal contamination on lake and river sediments

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Abstract

Purpose The present study investigated lake and river sediments affected by metals from an acid mine drainage (AMD) from a former uranium mine. The role of bacterial sulfate reduction in the immobilization of contaminants was evaluated, and the analyses of acid volatile sulfide (AVS) and sequential extraction were performed. Consequently, the potential mobility and bioavailability of contaminants were established. **Materials and methods** Sulfur isotopic fractionation ($\delta^{34}\text{S}$), AVS, and sequential extraction procedure were used to assess the sulfate bacterial reduction and the availability of contaminants in the environment at six sampling stations. **Results and discussion** The $\delta^{34}\text{S}$ indicated that bacterial reduction is a key process in the natural attenuation of contamination in the Águas Claras reservoir, precipitating metal sulfides. According to the USEPA criteria, adverse biological effects are expected for sample S1 (inside the reservoir) which is likely to be toxic, while for sediment S4 (in the river), the toxicity is uncertain. The other samples were classified as non-toxic, likely because of the decreased solubility of zinc sulfide. A decrease in the concentration of the contaminants downstream of the reservoir was observed. The predominance of U (0.4 %) in the labile fraction and the elevated concentrations of Zn (0.5 %) and Mn (0.7 %) in the sediments inside the

reservoir raises concerns regarding the availability of these contaminants in the environment.

Conclusions The main environmental impact appears to be concentrated in the Águas Claras reservoir, whereas the Antas creek does not seem to be affected by the AMD process. Although the bacterial sulfate reduction is effective in its production of sulfides capable of immobilizing the contaminants, the presence of Zn and U in the labile and reducible fraction is a matter of concern due to its long-term bioavailability. Thus, continuous monitoring of the redox potential of the waters and sediments, mainly in the reservoir, is recommended in order to assess and possibly prevent later dissolution of sequestered contaminants.

Keywords Acid volatile sulfides · Bacterial sulfate reduction · Metals · Sediments · Sulfur isotopes

1 Introduction

Acid mine drainage (AMD) is a critical environmental issue caused by chemical and bacterial oxidation of pyrite (FeS_2) and other sulfide bearing minerals exposed to atmospheric conditions during the mining of metal ores and coal (Taylor et al. 1984; Balci et al. 2007). As a result, the generation of acidic waters containing a wide array of toxic metals and sulfur species, mainly sulfate, eventually reaches rivers, groundwaters, and lakes and has adverse effects to both flora and fauna. In particular, dissolved and sedimentary sulfur species and their transformations play an essential role in the hydrochemistry of the waters, since the content of dissolved metals can increase or decrease through interaction with them (Knöller et al. 2004; Sarmiento et al. 2009). Sulfate from the AMD can be reduced to sulfide due to bacterial action and can be assessed through isotopic analyses. In general, the sulfates

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with lighter sulfur isotopes are more readily metabolized because their bonds are weaker (Seal 2003). As a consequence, a distinct sulfur isotopic signature in sulfide is produced by a depletion in ^{34}S with values as high as 46‰ (Rees 1973; Habicht and Canfield 1997) or even depletion up to 75‰ as reported by Brunner (2005). Once formed, the sulfide can react with divalent metals to form insoluble sulfides, such as FeS, ZnS, and CuS, decreasing the chemical activity and thus the bioavailability of these contaminants (USEPA 2005). In addition, the metastable iron monosulfides (FeS) can be a precursor of pyrite (FeS_2) that is a fairly stable sulfide which is capable of adsorbing contaminants. Therefore, the assessment of sulfur isotopic fractionation, the quantification of the sulfides, and the determination of the solid phases along with their association with the contaminants are essential for the following reasons: firstly they provide information about distribution, mobility, and bioavailability of the contaminants, and secondly, they indicate the occurrence of natural attenuation processes (Morillo et al. 2008; Sarmiento et al. 2009; Byrne et al. 2010; Charriau et al. 2011).

In Brazil, AMD occurs in a former uranium (U) mine and contains radionuclides and other elements, which are precipitated from the acid water by liming. Due to the inefficiency of the acid water treatment, contaminants overflow from the settling ponds and end up at the Águas Claras reservoir, leading to a concern about their migration to the environment, mainly to the Antas creek (Rodgher et al. 2013). The multiplicity of interactions between the contaminants and the sediments requires a broader approach in order to understand the fixation and/or transport of a wide range of elements, including U. Within this framework, a combination of different techniques was used to elucidate the bio-availability of the most hazardous contaminants present in surficial sediments from the Águas Claras reservoir and Antas creek. Sulfur isotopic fractionation was used to assess the occurrence of the bacterial reduction process. Additionally, the sediment's toxicity was predicted based upon the interactions of toxic metals with the sulfide and organic matter. Finally, the availability of contaminants was assessed by a sequential extraction procedure that indicated the immobilization of contaminants due to sorption, occlusion, and co-precipitation. These data help to infer the mobility and bioavailability of contaminants in the sediments, which can in turn be useful for the decision-making related to remediation of the area.

2 Methodology

2.1 Study area and sampling

The study area surrounds a former U mine at Poços de Caldas Plateau (Minas Gerais State, Brazil) whose activity was ceased in 1996 leaving behind 44 t of waste rocks that

generate AMD. The waste rocks consisted of gibbsite, muscovite, kaolinite, and microcline as well as pyrite and sphalerite. The acid mine water contains mainly sulfate (2400 mg L^{-1}), F (100 mg L^{-1}), U (8 to 12 mg L^{-1}), Mn (up to 170 mg L^{-1}), and Zn (41 mg L^{-1}) and has a pH of ~ 3.7 (Ladeira and Gonçalves 2007; Rodgher et al. 2013), and it is neutralized with limestone (CaCO_3) and lime (CaO) at the water treatment unit. The treated water is released into the Águas Claras reservoir whose waters join the Antas creek. Sediment samples were taken in August 2011 from the reservoir and the Antas creek (Fig. 1). In the Águas Claras reservoir, the sediments were collected using a van Veen grab while in the Antas creek a cylindrical collector was used. Samples were placed in plastic containers and stored at -4°C before analysis. The sampling locations were defined by the Brazilian Environmental Agency and were labelled S1, S2, S3, S4, S5, and S6. The procedure was repeated in August 2012, and the samples were lyophilized and submitted for isotopic analysis. These samples were taken from the same sampling stations but named S1B, S2B, S3B, S4B, S5B, and S6B.

2.2 Determination of organic carbon, total sulfur, acid volatile sulfide, simultaneously extracted metals, and toxicity of the sediments

The total sulfur and the organic carbon (f_{oc}) were determined using a LECO CS230 (Leco Corporation, St. Joseph, USA). Samples were dried at 40°C and 2 mL of HCl (4 M) was added to eliminate the inorganic carbon. The samples were dried again and the residual organic carbon determined. The acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) determination was in accordance with the EPA 821-R-91-100 (USEPA 1991) procedures. Wet sediments were weighed and placed in flasks with 100 mL of Milli-Q water, which was deaerated by bubbling N_2 . After 20 min, 20 mL of HCl (6 M) was added and the reactor was under continuous flow of nitrogen for the following 2 h, while the volatilized sulfides were trapped in flasks with 0.5 M NaOH. The acid slurry was filtered in a $0.45\text{-}\mu\text{m}$ polyacetate membrane, and the metals in the filtrate (Fe, Mn, Cu, Cd, Pb, Ni, Zn) were determined by flame atomic absorption spectrometry (Varian AA 240FS, Mulgrave, Australia). Detection limits were 0.03 mg L^{-1} for Mn and 0.1 mg L^{-1} for all the other elements. Sulfide was determined by the colorimetric method with methylene blue using a UV–VIS spectrophotometer (Varian Cary 50, Mulgrave, Australia). The calibration curve for the determination of sulfides was obtained with solutions of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ in concentrations between 0.2 and 1.2 mg L^{-1} . The percentage of recovery was estimated based on a standard solution of Na_2S , yielding results ranging between 84 and 92 %.

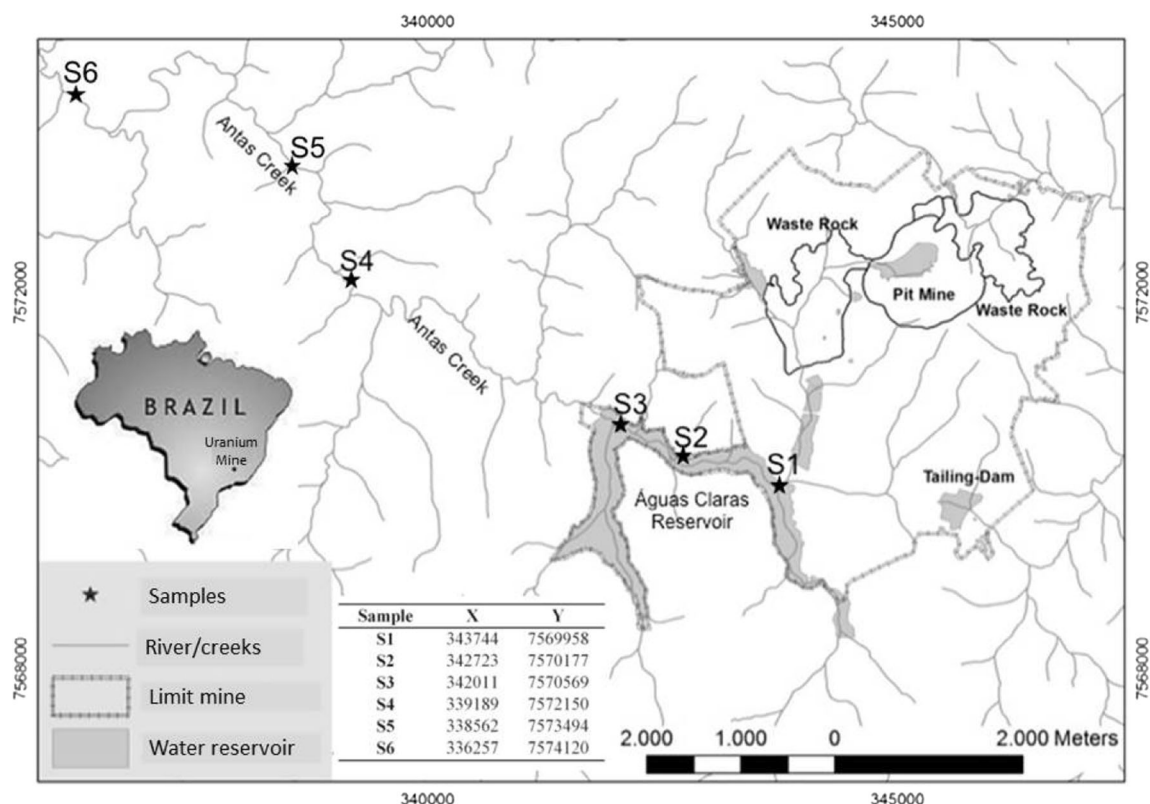


Fig. 1 Overview of the study area showing Águas Claras reservoir, the Antas creek, sampling stations (S1, S2, S3, S4, S5, and S6), and the UTM coordinates (southern hemisphere, zone 23)

The toxicity of the sediments was estimated by the difference of $\Sigma\text{SEM} - \text{AVS}$. If the $\Sigma\text{SEM} - \text{AVS} \leq 0$, the absence of toxicity can be assured; however, predicting which sediments with $\text{SEM} - \text{AVS} > 0$ will be toxic is less accurate. In this case, the organic carbon was considered as additional binding phase as many dissolved metals readily bind to dissolved organic carbon forming complexes that do not appear to be bioavailable (Di Toro et al. 2005; USEPA 2005); this is a standard procedure to derive concentrations of metal mixtures in sediment, which are protective of the presence of benthic organisms (USEPA 2005). This approach was chosen because it accounts for the varying biological availability of chemicals in different sediments and allows for the incorporation of the appropriate biological effects concentration. The toxicity criteria for the sediments based on AVS, SEM, and organic carbon (f_{oc}) are shown in Table 1.

2.3 Isotopic analysis

For the isotopic analysis, three distinct sedimentary sulfur compounds were extracted from the sediments and analyzed separately: (i) the sulfur present in the AVS, (ii) the elemental and pyritic sulfur (chromium reducible sulfur (CRS)), and the (iii) water-soluble sulfate. In order to obtain the AVS and CRS fractions, the freeze-dried sediment samples were reacted with HCl (6 M) under nitrogen gas flow, liberating AVS as H_2S

(Fossing and Jorgensen 1989). Subsequently, the remaining slurry was boiled for 2 h after the addition of acidified CrCl_2 solution, and CRS was carried out in the flow as H_2S . In each step, the produced sulfide was trapped in a zinc acetate solution and the ZnS precipitate was converted to Ag_2S by addition of 0.1 M AgNO_3 solution. The water-soluble sulfate fraction was obtained by shaking the freeze-dried sediment with distilled water for 4 h, followed by filtration. The filtrate containing the soluble sulfate was acidified with HCl 6 M to a pH between 2 and 3 and heated for the elimination of CO_2 , and then the BaCl_2 solution was added leading to the precipitation of sulfate as BaSO_4 . The stable isotope ratios of sulfur ($^{34}\text{S}/^{32}\text{S}$) were determined at UFZ—Centre for Environmental Research, Leipzig-Halle, Germany, using continuous-flow isotope ratio mass spectrometry (DeltaS, ThermoFinnigan, Bremen, Germany) with a reproducibility of $\pm 0.4\%$. The sulfur isotope measurements were reported in a delta notation ($\delta^{34}\text{S}$) as part per thousand (‰) deviation relative to the Vienna Canon Diablo Troilite (VCDT) standard. NBS 127 was used as a primary reference material for normalization of the raw data against a standard reference.

2.4 Sequential extraction

The sequential extraction procedure was based on the modified Bureau Communautaire de Références (BCR) (Rauret et

Table 1 Toxicity criteria for sediments based on AVS, SEM, and f_{oc} . (adapted from USEPA 2005)

Limit values ($\mu\text{mol/g}_{\text{OC}}$)	Predicted biological effect
$\Sigma\text{SEM} - \text{AVS} \leq 0$	Sediment is non-toxic
$\left[\frac{\Sigma\text{SEM} - \text{AVS}}{f_{oc}} \right] < 130$	Sediment toxicity is not likely to be caused by Cd, Cu, Pb, Ni, Ag, and Zn
$130 < \frac{\Sigma\text{SEM} - \text{AVS}}{f_{oc}} < 3000$	Sediment toxicity is uncertain due to Cd, Cu, Pb, Ni, Ag, and Zn
$\frac{\Sigma\text{SEM} - \text{AVS}}{f_{oc}} > 3000$	Sediment toxicity is likely to be caused by Cd, Cu, Pb, Ni, Ag, and Zn

al. 1999). The sequential fractions were named labile (E1), reducible (E2), oxidizable (E3), and residual (E4). Among all the constituents of the sediments, the elements Fe, Mn, U, and Zn were selected for investigation. Uranium analyses were carried out using a X-ray spectrometry (KeveX, model Sigma X9050, CA, USA) coupled with a radioactive source of americium (^{241}Am) and a Si(Li) detector with a detection limit of 10 mg L^{-1} . Samples below this detection limit were determined by neutron activation in the Triga Mark I IPR-R1 (General Atomics, San Diego, USA) reactor, and the detection limit was 0.1 mg L^{-1} . The other elements were analyzed by flame AA spectrometry (Varian AA 240FS, Mulgrave, Australia). The sum of the four fractions of U, Fe, Mn and Zn accounted for 82–116 % of the total concentration. Detection limits were 0.13, 0.03 and 0.08 mg L^{-1} for Fe, Mn and Zn, respectively. All reagents used for analysis were ACS grade (Merck), and reagent blanks were determined for each step of analysis.

3 Results and discussion

3.1 Sulfur isotopic composition of the sediments

The isotopic ratio ($\delta^{34}\text{S}$) is a useful tool to provide information about the occurrence and the extension of sulfur redox processes. In sediments, there are three main inorganic fractions associated with the redox processes: (i) the AVS produced by the precipitation of biogenic hydrogen sulfide with bivalent metal ions, forming the biogenic sulfide; (ii) the CRS is comprised mostly of pyritic sulfur originated either from the aging of the biogenic sulfide and/or from geogenic pyrite, and (iii) the SO_4 generated during the weathering of sulfide bearing rocks and mainly present in AMD. The $\delta^{34}\text{S}$ signatures of the three main sulfur compounds (SO_4 , AVS and CRS) for each sample are presented in Fig. 2. As shown in Fig. 2, the slight variation of the $\delta^{34}\text{S}$ signatures for the SO_4 throughout the reservoir and the creek indicates that this anion derives from the same sulfur source in all sediments. The average $\delta^{34}\text{S}$ value for the SO_4 for all samples is -3.03 ‰ and is within the values reported for the pyrite from the Poços de Caldas

Plateau that ranges from $+1.24$ to -3.63 ‰ (Waber et al. 1992). Considering that the SO_4 formed via oxidation of pyrite presents small or negligible sulfur isotope fractionation (Taylor et al. 1984; Balci et al. 2007), it may be concluded that the pyrite oxidation is the main source of sulfate. In the sediments S4B and S6B, no sulfate was detected, probably because the concentrations of dissolved sulfate and Ca^{2+} in the creek are below the saturation limit.

According to Fig. 2, the AVS isotopic signatures were $\sim 37 \text{ ‰}$ for the sediments S1B and S3B; the first and last sampling station inside the reservoir. These results are consistent with those reported in the literature where the dissimilatory reduction of sulfate produces sulfide depleted in ^{34}S up to 46 % compared to the initial isotopic composition of SO_4 (Rees 1973; Habicht and Canfield 1997). The sulfate-reducing process and the precipitation of divalent metals with the resulting sulfide is shown in Eqs. (1) and (2), respectively:



where the acetate CH_3COO^- represents dissolved organic carbon, although other organic compounds may be found. A

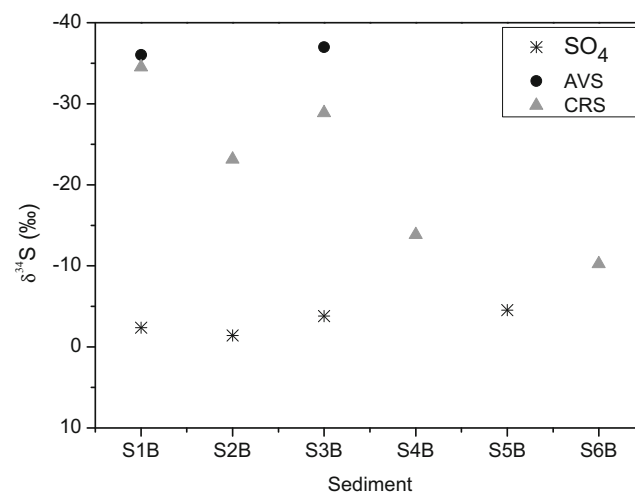
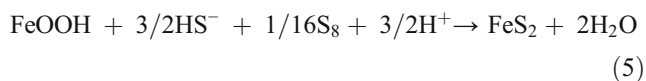


Fig. 2 Isotopic signatures of the fractions SO_4 , AVS, and CRS for the surface sediments

group of anaerobic microorganisms, mainly bacteria and archaea (Muyzer and Stams 2008), reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}) using organic electron donors through dissimilatory reduction. The sulfate reduction consumes protons, consequently increasing the pH and yielding sulfides which react with metals (e.g., Fe, Zn, Cu, Cd, Ni, and Pb), leading to the precipitation of insoluble metal sulfides. Most of the sulfate-reducing bacteria are neutrophilic, thriving at a pH between 6 and 8. However, the existence of acidophilic bacteria is also reported, offering the possibility to form insoluble metal sulfides without the need of prior neutralization of the AMD (Sánchez-Andrea et al. 2014).

Commonly, iron is the most abundant divalent metal in the pore water of sediments and susceptible to sulfide precipitation; therefore, iron sulfides can be the predominant phase in AVS (Rickard and Morse 2005). Once the FeS is formed, three main mechanisms have been reported for the formation of neogenic pyrite: (i) a reaction in anoxic conditions (Eq. 3), (ii) a reaction with reduced sulfur or polysulfur (Eq. 4) and (iii) a direct precipitation of dissolved sulfides with iron oxides (Eq. 5) (Lesven et al. 2008):



The $\delta^{34}\text{S}$ values for CRS inside the reservoir, from -21 to -33 ‰, are between the AVS and the SO_4 values, which indicate the presence of primary sedimentary pyrite as well as neogenic pyrite formed by the bacterial reduction process. In contrast, the $\delta^{34}\text{S}$ value for CRS in the Antas creek were more enriched and closer to the sulfate isotopic value and thus to the $\delta^{34}\text{S}$ value of -3.03 ‰ for pyrite in the region. Therefore, inside the Águas Claras reservoir, the bacterial sulfate reduction is a significant process resulting in the precipitation of metallic sulfides and the formation of neogenic pyrite, capable of occluding contaminants in its structure.

3.2 Acid volatile sulfide and simultaneously extracted metals

Once the bacterial reduction of the sulfate was confirmed by isotopic studies, the next step was the assessment of the extension of the reduction process through the quantification of sulfides and their bonded metals (i.e., AVS–SEM) (USEPA 1991). The AVS fraction comprises the free sulfides (S^{2-}), iron sulfides such as FeS (mackinawite) and Fe_3S_4 (greigite) (Rickard and Morse 2005), and sulfides from bivalent metals such as Cu, Ni, Zn, Cd, and Pb. The SEM fraction is composed of the metals, normally Cd, Cu, Pb, Ni, Hg, Ag, and Zn, liberated from the sediment during the acidification. In the present work, the concentrations of Ni, Cu, and Cd were below the detection limit (0.1 mg L^{-1}) and considered not relevant. The results for AVS, metals extracted in the SEM, organic carbon (f_{oc}), and total sulfur (S_{Tot}) are shown in Table 2.

As shown in Table 2, there is a significant variability in the content of AVS in the sediments, ranging from 3 to 2969 mg kg^{-1} , which indicates different environmental conditions that may be unsuitable for the formation and precipitation of AVS phases. For instance, sediments S1 and S4 presented the lowest AVS concentrations, 150 and 3 mg kg^{-1} , respectively, as well as the lowest concentration of organic carbon, 5.584 and $10.214 \text{ mg kg}^{-1}$, respectively, and Eh of 66.4 and 58.8 mV, respectively. For sediment S1, the high content of total sulfur is associated with insoluble phases of sulfate, carried from the water treatment station, which contains ettringite ($\text{Ca}_6\text{Al}_2(\text{SO}_4)_3(\text{OH})_{12} \cdot 26\text{H}_2\text{O}$) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) (Gomes et al. 2012). The other samples inside the reservoir (S2 and S3) present high AVS, $\sim 3000 \text{ mg kg}^{-1}$, and the greatest amount of organic carbon. These results corroborate the bacterial reduction activity suggested by isotopic fractionation and indicate that the sulfide production is prolific, leading to precipitation of metallic ions and reducing the availability of metals. Furthermore, the bacterial reduction could be one of the reasons behind the decreasing sulfate content in the waters downstream of the reservoir, as reported in the Technical Report of the Water Committee (Comissão das Águas 2012). According to this report, the concentration of sulfate in the water at the station S1 was 290 mg L^{-1} while at station S2 the value was 135 mg L^{-1} .

Table 2 Organic carbon (f_{oc}), total sulfur (S_{Tot}), AVS, and metals extracted in the SEM procedure

	f_{oc} (g kg^{-1})	S_{Tot} (g kg^{-1})	AVS (mg kg^{-1})	Fe (g kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})
S1	5.6	11.6	151	34.5	7184	5845	24
S2	22.2	13.0	2665	42.5	184	1375	35
S3	23.6	9.9	2969	31.7	300	1561	37
S4	10.2	2.0	3	11.9	917	160	23
S5	25.5	4.2	133	21.1	2058	188	23
S6	21.4	3.2	58	17.9	789	101	30

Despite the high concentrations of organic carbon in the sediments S5 and S6, compared to S2 and S3, the content of AVS is much lower, i.e., of 133 and 58 mg kg⁻¹, respectively, which implies that bacterial reduction processes are not pronounced. This fact may be associated with the low content of sulfate in the waters of Antas creek (~35 mg L⁻¹), much lower than the values in the reservoir. In addition, the higher water flow in Antas creek results in an oxic environment, hindering the bacterial sulfate reduction and the formation of metals sulfides. Table 2 also shows the metals, Fe, Mn, Zn, and Pb, extracted in the AVS–SEM procedure after the dissolution of the biogenic metallic sulfides as well as the amorphous and crystalline phases of Fe and Mn. According to Table 1, the toxicity of the sediments that can affect the benthic organisms can be estimated by the difference (ΣSEM–AVS) which is based upon the chemical interactions of metal-sulfide and the equilibrium partitioning theory (Di Toro et al. 2005). When there is an excess of sulfide, the metals precipitate as insoluble phases, such as NiS, ZnS, CdS, PbS, CuS, and Ag₂S, whose preference for precipitation depends on their solubility product (*K*_{ps}). As a result, the metals are sequestered from the aqueous phase, what decreases their activity and, consequently, their bioavailability. The results for the assessment of toxicity based upon the difference (ΣSEM–AVS) and the normalized difference ((ΣSEM–AVS)/*f*_{oc}) for all the sediments are shown in Table 3. According to Table 3, the sediments S2 and S3 (inside the Águas Claras reservoir) and the sediments S5 and S6 (in the river) were classified as non-toxic, since the difference of ΣSEM–AVS were negative. On the other hand, the sediments S1 and S4 presented positive value, i.e., (ΣSEM–AVS)>0, and were normalized by the fraction of organic carbon (*f*_{oc}). According to these criteria, the sample S4 presented uncertain toxicity once the value of ΣSEM–AVS/*f*_{oc} is between 130 and 3000 μmol g_{OC}⁻¹. The sediment S1 presented (ΣSEM–AVS)/*f*_{oc}>3000 μmol g_{OC}⁻¹, which implies high risk of adverse biological effects mainly due to the high content of Zn. As will be discussed in the sequential extraction results below, the sample S1 was strongly affected by the overflow of sludge from the water treatment unit.

In conclusion, the results show that the soluble sulfate from the reservoir undergoes bacterial reduction producing sulfide. The S²⁻ can precipitate divalent metals, particularly Zn, producing highly insoluble metal sulfides which decrease the availability to the environment. Therefore, despite the high levels of total metals in most sediment, samples S2, S3, S5, and S6 were assessed as non-toxic. On the other hand, the sediments S1 and S4 present low amounts of sulfide; consequently, sample S1 is likely to be toxic, whereas for S4, the biological adverse effects are uncertain. Therefore, it is recommended to monitor the redox potential once its increase can promote the oxidization of sulfides as well as the degradation of organic compounds, accelerating the liberation of the adsorbed/complexed metals (Zhang et al. 2014). Furthermore, an increase in the redox potential can also inhibit the activity of anaerobic sulfate-reducing bacteria and thus hamper the production of sulfides.

3.3 Sequential extraction

The AVS procedure takes into account only the elements Cu, Zn, Pb, Ni, Cd, and Ag in the estimation of the toxicity of the samples. Consequently, other toxic metals present in sediments are not assessed by this method. However, as the study area presents other elements, such as U and Mn, additional methods should be used to identify their association with different mineralogical phases and, consequently, their availability to the environment. Thus, sequential extraction experiments were carried out and four elements were selected to be evaluated: Fe and Mn, because they are considered as a sink for contaminants (Filgueiras et al. 2002), and Zn and U because they are present in high levels in sediments, besides their toxicity. Figure 3 shows the sequential extraction results as well as the total metal concentration in the original samples. It is noted that the sample station S1 presents the highest concentration of Mn, Zn, and U (7417, 5285, and 3600 mg kg⁻¹, respectively) followed by samples S2 and S3 that are also enriched in U and Zn if compared to the sediments in the Antas creek. This enrichment is caused by part of

Table 3 Results of sediment toxicity based upon the difference (ΣSEM–AVS) and the normalized difference (ΣSEM–AVS)/*f*_{oc}

Sample	AVS (μmol g ⁻¹)	ΣSEM (μmol g ⁻¹)	<i>f</i> _{oc} (g _{OC} g ⁻¹)	(ΣSEM–AVS) (μmol g ⁻¹)	(ΣSEM–AVS)/ <i>f</i> _{oc} (μmol g _{OC} ⁻¹)
S1	4.70	89.40	0.006	84.70	15,168
S2	83.11	21.20	0.022	-61.91	-2785
S3	92.58	24.05	0.024	-68.53	-2913
S4	0.10	2.56	0.010	2.46	241
S5	4.15	2.98	0.025	-1.17	-46
S6	1.82	1.70	0.021	-0.13	-6

*g*_{OC} gram of organic carbon

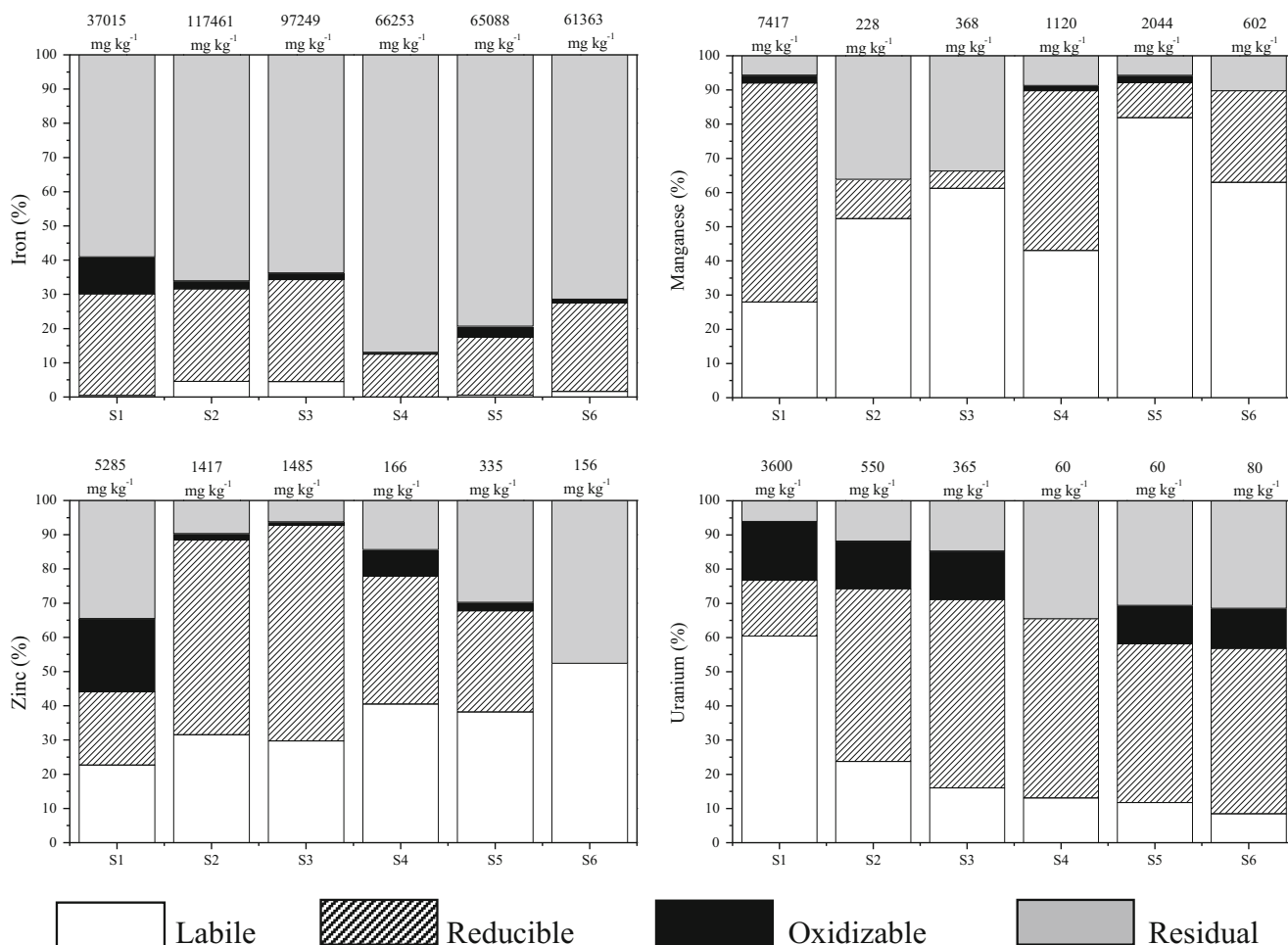


Fig. 3 Mn, Zn, U, and Fe partitioning in the sediments, according to mBCR procedure. The total concentration is displayed on the top of each column

the precipitate that overflows from the water treatment unit and settles at the bottom of the reservoir.

Iron As shown in Fig. 3, minor amounts of Fe were associated with the labile and oxidizable fractions. However, it was mainly extracted in the reducible and residual steps which are similar to the results reported in the literature (Morillo et al. 2008; Charriau et al. 2011). The reducible fraction (E2) comprises 12 to 30 % and consists of oxyhydroxides present in highly weathered environments (amorphous Fe). In the sediments, these amorphous phases are important sinks for contaminants, since they have a high capacity of adsorbing other metals in their structure (Filgueiras et al. 2002). The small portion of Fe extracted in the oxidizable fraction is associated with organic matter and sulfides. In all sediments, Fe was preferentially associated with the residual fraction comprising crystalline forms of hematite and goethite, in addition to aluminosilicates containing Fe (II and III) and pyrite.

Manganese High percentage of Mn (28 to 81 %) was extracted in the labile fraction (E1), most likely due to its association with the carbonate phases formed in the water treatment unit

as reported by Gomes et al. (2012). In the reducible fraction E2 (i.e., amorphous Fe oxides and crystalline Mn oxides; Filgueiras et al. 2002), it was extracted 5 to 64 % of the Mn. The highest extraction was for sample S1 which also presented the highest Mn content. The oxidizable fraction (E3) seems to be irrelevant as it represents at maximum of 2 % of the Mn. This is explained by the low content of Mn sulfides as well as the difficulty of Mn to form complexes with organic matter. The Mn present in the residual fraction, which comprises the phases resistant to the weathering and bonded to lithogenic minerals, ranged from 6 to 36 %.

Zinc The mobility of Zn is evidenced by the high levels of extraction in the labile fraction E1 (20 to 50 %) indicating that it could be mainly weakly adsorbed, easily exchangeable, and/or co-precipitated with carbonates (Filgueiras et al. 2002). The labile fraction is also associated with the extraction of Zn precipitated as AVS, since the sample drying may result in the oxidation of the sulfides, increasing the amount of Zn extracted. Since metals in the labile fraction are considered readily and potentially bioavailable, the Zn associated to this fraction is of concern for the samples S1, S2, and S3, which

contain high levels of zinc (5285 to 2417 mg kg⁻¹). These results corroborate the findings in the AVS–SEM approach, in which sample S1 was classified as likely to be toxic due to the great amount of Zn which is highly bioavailable. Inside the reservoir, samples S2 and S3 have around 60 % of total Zn extracted in the reducible fraction, mainly in the form of Zn oxide and/or adsorbed in Fe oxyhydroxides since almost 30 % of total Fe is extracted in this phase. The association of Zn in Fe–Mn oxides/hydroxides confirms the known ability of this phase to scavenge Zn from the aqueous phase, and these phases are completely extracted during the AVS–SEM procedure. The sediment S1 was the only one to present significant amount of Zn associated with the oxidizable fraction. Since this sediment presented low contents of organic matter and sulfides, Zn was probably associated with other oxidizable phases such as CRS–Fe. Despite the elevated content of organic matter in the other samples, only 2 % of total Zn was extracted in the oxidizable fraction, indicating it is not significant as a binding phase. The Zn extracted in the residual fraction indicates the presence of sphalerite ZnS, a mineral found in the region, and in part contributes to the acid mine generation. The Zn inside the Águas Claras reservoir is mainly present in sediments due to reactions of precipitation, complexation, and/or adsorption, which enrich the levels of this element in the labile and reducible fractions.

Uranium The sample S1 presented the highest extraction of U in the labile fraction, ~60 %, most likely due to the presence of calcium diuranate (CaU₂O₇) that overflowed from the water treatment unit (Gomes et al. 2012). Minor amounts of U were extracted in the labile fraction in the other sediments, ranging from 2 to 23 %. For these samples, U can be non-specifically adsorbed on the surface of clays or on Fe and Mn oxyhydroxides. The presence of the U in the labile fraction is particularly worrying as its high concentration—3600 mg kg⁻¹ in sample S1, for instance—can pose risks to the environment. However, it is interesting to note that the U content in the labile fraction decreases with distance from upstream to downstream the lake, i.e., from sample S1 to S6. In all samples except S1, most of the U is associated with the Mn and Fe oxyhydroxides (step E2), most likely due to the sorption or occlusion of U in hematite (Fe₂O₃) and goethite (Fe₃O₄) (Duff et al. 2002). The lower extraction of U in step E2 for sample S1, ~15 %, is related to the low content of Fe in this sample. The U in the oxidizable fraction E3 is ~15 % and represents the U sorbed in the organic matter (Duff et al. 2002) and/or the U reduced into crystalline oxides (e.g., uraninite) in an effective mechanism of bioremediation. Sample S4, the only one without U in the oxidizable fraction, is also the one which presented the lowest level of total sulfur and organic matter. Significant contents of U in the residual fraction are found in sediments S4, S5, and S6, ~30 %. The U in these

sediments may be included in the crystalline iron oxides, silicates, and gibbsite or as uraninite; all of them are typical minerals of the region. On the other hand, the S1, S2, and S3 samples, collected inside the reservoir, showed a low content of U associated with the residual fraction (6 to 14 %), which reinforces the contamination of the lake by U solid phases with great bioavailability.

4 Conclusions

The results indicate that the Antas creek seems not to be affected by the AMD, while the environmental impact appears to be concentrated in the Águas Claras reservoir and this has been caused by an enrichment of metals in its sediments. Among the samples, point S1 was particularly affected by the discharge of treated effluent as shown by the elevated concentrations of Zn (0.5 %), Mn (0.7 %), and U (0.4 %). In addition, according to the AVS–SEM analyses, the sample S1 is likely to be toxic, probably due to the high content of Zn. All the other samples are unlikely to be toxic or present uncertain toxicity. The isotopic sulfur investigation in the Antas creek showed no evidence of bacterial sulfate reduction, and this is confirmed by the presence of geogenic pyritic typical of the geology of the region. Sequential extraction showed U, Zn, and Mn mostly present in the labile fraction and reducible phase. For this reason, special attention should be given to the sediments inside the reservoir to avoid the spread of the contaminants. Therefore, to prevent later mobilization of the metals it is recommended that the sediments remain in anoxic conditions, thus hindering the re-dissolution of the main scavenging phases.

It is worth mentioning that the present work is a contribution to the remediation of areas contaminated with metals, including radioactive and non-radioactive elements. The full characterization of the bioremediation process, as well as the definition of the chemical availability of the contaminants, could help determine the best remediation plan for such areas, which could be the complete removal of the sediments or the improvement of the bioremediation process. The enhancement of the bacterial sulfate reduction has the advantage of promoting the formation of metal sulfides as well as the reduction of the highly soluble U(VI) to a less soluble U(IV). In addition, the understanding of the natural processes provided by this investigation could lead to possible strategies for artificial immobilization of the metals.

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